SYSTEMATIC REVIEW ON THE THERAPEUTIC APPLICATION OF MESALAZINE IN ULCERATIVE COLITIS

(REVIEW WORK)

Submitted in partial fulfillment of the requirements for the degree of
Bachelor of Pharmacy

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SYSTEMATIC REVIEW OF THE THERAPEUTIC APPLICATION OF MESALAZINE IN ULCERATIVE COLITIS

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ABSTRACT

In 1977, mesalazine (5-aminosalicylicacid) was discovered as therapeutically active agent in treatment of ulcerative colitis (UC) and was launched in 1984 for topical and oral delivery.The drug belong to class of amino salicylate and act on the inflamed lining of the intestine (colon) so as to prevent the substance formation which causes inflammation. By this it provide relief from mild-to-moderate active ulcerative colitis.

As chemically mesalazine that is 5-aminosalicylicacid is active moiety of sulfasalazine it has proven to be the first line treatment in the UC by giving its effect locally in the colon and provides relief from inflammation of Ulcerative colitis.

Various formulation has been developed for increasing its efficacy starting with tablets formulation was developed with efficacy of reaching the drug to colon by pH dependent coating with polymer,Delayed release drug delivery to colon were further developed,Microbiallly triggered drug delivery to the colon which consist of (a) Prodrug approach,(b) Azo-polymeric approach,(c) Polysaccharide based approach. All this approach has proven to be efficacious but less effect was seen on colon and more concentration was getting absorbed in small intestine sometimes result in side effects. Further newly developed method were developed such as pressure controlled drug delivery system(PCDCS),CODESTM(A Novel colon targeted delivery system), Osmotic Controlled drug delivery to colon(OROSCT), Hydro gels(enema), Microspheres, Nanoparticles, Liposome's, Multi particulate Beads. This novel approaches are made to increase therapeutic efficacy and to give targeted delivery of drug to colon.

The main side effect of the drug is renal dysfunction, acute interstitial nephrotoxicity, some common side effect are rashes, headache, nausea, abdominal pain,etc. The purpose of review of articles is to determine the therapeutic application of mesalazine in ulcerative colitis by different formulation available.

INTRODUCTION

Mesalazine a drug which was discovered in 1977 as therapeutically active agent and launched in 1984 for topical and oral therapy.[4] Chemically it is 5-aminosalicylicacid (5-ASA) belonging to amino salicylate class and derived from sulfasalazine, molecular formula $C_7H_7NO_3$ (also known as mesalazine, 5-amino-2-hydroxybenzoicacid) and having molecular weight of 153.14 g/mol.

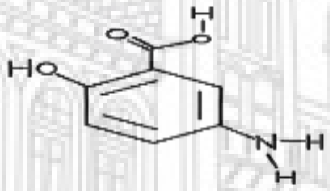
Mesalazine physically appears as odourless, white to pinkish crystals which in aqueous solution having pH approximately about 4.1, and a melting point of 283º C. It is soluble in

hydrochloric acid, slightly soluble in alcohol,water and is more soluble in hot water ,Insoluble in ethanol.

Mesalazine is an inflammatory agent which act locally on the colon , it act by inhibition of cyclooxygenase and prostaglandin production to give anti-inflammatory action in Inflammatory bowel diseases(IBD) such as Ulcerative Colitis. It is to be given by Oral and/or Rectal route in conditions like UC, diarrhoea, rectal bleeding, etc. Mesalazine being active locally does not have systemic action and in addition to anti-inflammatory it have analgesic, antipyretic and platelet inhibitory action as they act by blocking prostaglandin synthesis by inhibition cyclooxygenase which converts arachidonic acid to cyclic endoperoxides which are the precursors of the prostaglandins.

Mesalazine is absorbed up to 21% - 22% on oral administration in range of 2.4g or 4.8g tablet for 14days once daily and are eliminated mainly by renal route as metabolized product as N-acetyl-5-aminosalicylicacid and some drug are eliminated unchanged that is in parent form in urine.

Some of the side effects include acute interstitial nephrotoxicity, renal dysfunction, skin rashes, bloody diarrhoea, liver injury, nausea, abdominal pain, etc.[14]



MESALAZINE (5-aminosalicylicacid)

Physical properties	**Nature**	**Odour**	**Colour**	**pH**	**Melting point**	**Solubility**
	Crystals	Odourless	White to pinkish	4.1	283ºC	Soluble-HCL Slightly soluble-alcohol,water More soluble- Hot water Insoluble-Ethanol

Chemical properties	**Molecular formula**	**Molecular weight**
	$C_7H_7NO_3$	153.14 g/mol

MESALAZINE AND ITS FORMULATION:

SOLID DOSAGE FORM:

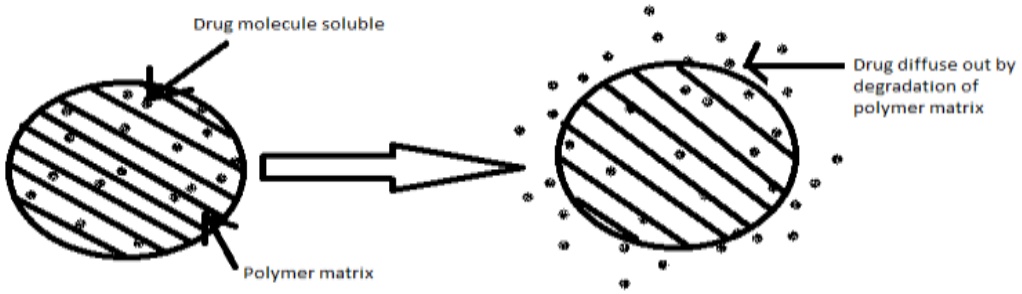
TABLETS AND GRANULES:

Delivery of the drug to colon that is targeting of drug to Colon and it is needed for the local treatment of the disease such as ulcerative colitis. There are two different route available for the treatment such as oral and/or rectal. Oral route is more convenient for the effective treatment of drug to colon as rectal route cannot be possible for proximal part colon treatment and also it is uncomfortable.

Although oral route is convenient but the conventional form the drug is normally dissolving in the GI that is gastric fluids and local treatment of drug to colon is not possible as systemic absorption takes place which some times results in side effect such as renal calculi, acute interstitial nephrotoxicity,etc.So to overcome side effects and to protect the drugs from the hostile environment of the GIT which include upper gastro intestinal tract, small intestine the the approach is carried out.The compression coated tablet of mesalazine is prepared which protect the drug from hostile environment of GIT and provide the drug for absorption on colon for its local effect.In this compression coating technique is carried out for core tablet by use of Guar gum alone and in combination with HPMC K4M and the tablet was evaluated for the hardness, friability,thickness,weight variation,etc.this method shown good release of drug as in invitro studies and no interaction between drugs and excipients,but it was not sufficient.[1]

As the main recommended dose is 4.8g in acute attack and 2.4g in maintenance of remission in divided doses.For this multiple doses upto 12 tablet per day required of low doses tablet which ultimately leads to patient noncompliance, Because of immediate release of drug at colon delayed release formulation lack efficacy, so Lialda® delayed release tablet with dose of 1200mg once per day was developed using Multi matrix system(MMX) to improve patient compliance. It was prepared by wet granulation with use of hydrophilic polymer (Carbopol®940) and hydrophobic polymer (Eudragit®RS), croscarmellose sodium to release the drug in pH -dependent manner, works in such a way that pH dependent gastro resistant film will disintegrate at pH 7 further the hydrophilic and hydrophobic layer will provide the drug to colon which results in better patient compliance and increased efficacy.[2]

The mesalazine matrix tablet was prepared with sodium alginate and compared with commercial Solofalk® tablet. The developed tablet was evaluated in-vitro and in-vivo in healthy volunteer and animal.The tablet were prepared by slugging method and average weigh about 600mg consisting of 250mg mesalazine.As sodium alginate is natural polymer with ph sensitive gel forming ability, the mesalazine alginate matrix tablet formulation can deliver the drug to the small intestine and colon and is the promising system in the treatment of ulcerative colitis.[3]



GRANULES:-

The drug was also formulated as Granules instead of tablets to provide high strength as compared with tablet, so the granules are filled in sachet. The efficacy of tablets and granules are compared and it was same. Compliance of patient increased as inconvenience of taking large amount of tablet were changed to once daily granules of mesalazine and was proven to be long term efficacious and safe for the treatment.[4]

The granules were prepared by combining the pH controlled release along with the extended release mechanism with an inner polymer matrix core in which mesalazine is embedded, and it has shown to provide continuous release of mesalazine in the entire colon as compared with tablets which provide active drug in the right colon and terminal ileum. The trial was carried on patient with mild to moderate active ulcerative colitis and result was known which ultimately showed increased therapeutic efficacy of mesalazine granules in UC.[5]

Oral mesalazine granules are important for treatment of ulcerative colitis if it is non adherence to mesalazine then the risk of relapse of disease is more, So for the confirmation of relapsing, We conclude two groups of patient first group has administered a tablet formulation and second group of patient has administered a granules formulation for six to eight week after a eight week the first group administered granules and vice-versa after a final result conclude that the granules formulation has better onset of action, patient compliance is better than the tablet formulation. We monitored that the none of the patient has adverse effect against granules or tablet formulation but the granules has good adherence and better long term acceptance therapy then the tablets.[6]

LIQUID DOSAGE FORM:

ENEMAS:

The drug mesalazine in liquid dosage form comes mainly in the form of enema and /or in suspension form. The enema usually comes in foam or gel based for the treatment of ulcerative colitis. As enema is difficult to administer and have low patient compliance study was conducted for the comparison of foam enema and mesalazine gel enema. The candidate for the study must be from age group of 18-70years with active ulcerative colitis disease. The study was conducted for the four weeks duration randomly candidate were given with 2g

mesalazine gel enema or 2gm mesalazine foam enema through rectal route as a single dose application study included various method such as clinical trial assessments, statistical method. The evaluation of the study was done on the basis of Endoscopic remission and clinical remission 76% and 69%. For gel and foam based accuracy was achieved respectively while for endoscopic remission 51% and 52% for gel and enema was achieved respectively. So it was concluded that the gel enema is better as compared to foam enema and they have better efficacy and tolerability as compare to foam enema.[7]

Mesalazine emulsion polymer (xylan) is water in water emulsion method based on micro particle without using harmful solvent, whereas some of the reports indicating the encapsulation of small molecule with the help of micro particle are produced with in this method or technique. The possible reason of rapid dissolution of the molecule from the discontinuous phase to continuous phase. In this method, polymer xylon containing drug that is mesalazine is produced with the help of double crossing approach which usually enhance the higher encapsulation rate was indicated. Formulation with spherical shaped microparticles are present was revealed in the result. At specific condition few of the formulation were reached upto 40% in just 12h in dissolution assay probably due to the crosslinking. So it was concluded that emulsion made with the double crosslinking method was effective with mesalazine encapsulation and many of the formulation can be made with the help of polymer carrier based technique.[8]

The suspension USP enema was prepared of mesalazine (5-aminosalicylicacid). As 4g of the drug is delivers up to the colon with mechanism of action which is not fully known but it appears to be a topical anti-inflammatory on colonic epithelial cells. The arachidonic acid which is produced from mucosal with the help of cyclooxygenase and lipoxygenase pathway helps to treat the patient. They usually poorly absorbed in the colon and excreted in the faeces, under specific condition 10 to 30% drug is recovered. And is been seen that mesalazine rectal suspension is not patient acceptable and it has shown many side effects which are serious to human body.[9]

SEMISOLID DOSAGE FORM:

PROBIOTICS:-

The high potent probiotic mixture is used to treat a ulcerative colitis, It has an faster onset of action then other formulation and minimal adverse reaction, The patient who are under the treatment with mesalazine tablet can take this probiotic supplement for the precaution of reemission or reoccurrence of disease, It helps to boost your immunity and provide effective against ulcerative colitis. The 144 people has taken for clinical trials to check the better onset of action, reemission of disease and treatment therapy the 71 people administered probiotic sachets and 73 people administered placebo sachets a morning and evening with cold water or yogurt and after the 8 week the result was found that the probiotic is better then the placebo as compared to onset of action.[10]

PELLETS:-

Reduction of patient compliance with multiple dosing of drugs(mesalazine) such as tablet, capsule or suppository are overcome with 1.5gm of sachet of pellets, It required a single dose on a day as compare to multiple dosing tablets. Clinical trials is performed on 24 healthy volunteers to be monitored a dosing compliance and better disease treatment with three layer enteric coated tablet compared with pellets at an end we get the bioavailability of mesalazine was 92% in pellets and tablet has 75% both the formulation has excretion rate is 26% but the bioavailability and patient compliance is better in micro pellets as compared to enteric coated tablet the onset of action was also delay in tablet.[11]

MICROSPHERE:-

Microsphere was prepared by the ionic gelation emulsification method using tripolyphosphate as cross linking agent, The coating material used in microsphere was polymer EUDRAGIT S100 it is used to prevent drug release in stomach the microsphere was evaluate by the drug loading, micrometric and in-vitro drug release method drug release of mesalazine from microsphere was found as an microsphere with coated eudragit S100 and chitosan, The coated drug is nor in release with gastric or nor in release with intestine it is released on site of affected in colon by its PH dependent nature.

As a end of conclusion we found that the microsphere encoated mesalazine drug provides a better bioavailability and better drug release property on the site of action.[12]

SUPPOSITORY:-

Mesalazine suppository is especially introduced for the paediatrics patient as compare to other patient, Because paediatrics are discomfort from oral and other routes so suppository is the formulation that to over comfort there incompliance by administered on rectal route.

Daily 500mg as two times a day or 1000mg once a day dose required for minimal effect of drug, Dose should be frequently monitored if it is overdose it cause adverse effect such as vomiting, diarrhoea, ulcer and rectal bleeding to overcome this cause dose should be monitored.

Suppository	Placebo
39 volunteers administered suppository	40 volunteers administered placebo
After 8 weeks suppository work better then placebo	After 8 weeks placebo work better in onset of action
Mesalazine suppository provide 80% effect on disease	Placebo drug provide 60% effect on disease
On first week suppository overcome rectal bleeding problem	On first week placebo has better onset of action

Caution: Do not administer an infant less than two years of age.[13]

CONCLUSION

From reviewing all of the above formulation it was concluded that to prevent adverse effect associated with the mesalazine formulation there should be close monitoring through out treatment should be carried out especially orally administered formulation so as to prevent side effect such as nephrotoxicity, renal dysfunction, bloody diarrhoea, rashes, etc.

Also it was concluded that the formulation for paediatric is sometimes difficult to administer oral mesalazine tab leads to patient non-compliance because of unpleasant taste, due to nausea. A formulation can be prepared such as suspension with flavouring agent for paediatric use which will include nano particles which are encapsulated with pH dependent coating, also the suspension will deliver the drug in alkaline pH that is in small intestine and not in stomach acidic environment. As the it will deliver the drug in small intestine the coating present on nano particles will protect the drug in alkaline environment of small intestine and degrades at pH of colon release nano particles which will be in the size range below 200nm especially up to about 15 nm so it will easily absorbed in the epithelial lining of the colon to give its local action.

REFERENCES:

1. Spandana Kodati*,Mrs Jaya,Dr.M.Chinna Eswaraiah.Formulation and invitro evaluation of compression coated mesalazine tablets for colon specific drug delivery.Int Res J Pharm. App Sci.,2012;2(6):1-11
2. Ahmed Abd Elbary[1] ,AhmedA.Aboelwafa[1,2] ,IbrahimM.Alsharabi[1] .Once Daily,High-Dose Mesalazine Controlled-Release Tablet for Colonic Delivery : Optimization of formulation variables using Box- Behnken Design.AAPS Pharm SciTech,Vol.12,No.4,December 2011(©2011)DOI : 10.1208/s/2249-011-9708-9
3. Fatmanur Tugcu-Demiroz [a] .Fusun Acarturk [a,] *,Sevgi Takka [a] et al.,Evaluation of alginate based mesalazine tablets for intestinal drug delivery.European Journal of Pharmaceutics and Biopharmaceutics 67(2007)491-497
4. Stephankarl Bohm[1] ,Wolfgang Kruis. Long-term efficacy and safety of once daily mesalazine granules for the treatment of active ulcerative colitis.Dovepress Clinical and Experimental Gastroenterology 2014:7
5. L.Leifeld*,R.pfutzer*,J.Morgenstem* et al., Mesalazine granules are superior to Eudragit-L-coated mesalazine tablets for induction of remission in distal ulcerative colitis-a pooled analysis.Aliment Pharmacol Ther 2011;34:1115-1122
6. Keiji Yagisawa[1],Taku Kabayashi[2],Ryo Ozaki[2] et al ., Randomized,crossover questionnaire survey of acceptabilities of controlled-release mesalazine tablets and granules in ulcerative coilitis patients. Intest Res 2019;17(1):87-93
7. P.Gion chetti ,[a] S.Ardizzone,[b] M.E.Benvenut et al.,A new mesalazine gel enema in the treatment of left sided ulcerative colitis: a randomized controlled multicentre trial.Aliment Pharmacol Ther 1999 ; 13:381-388

8. Bartolomeu S.Souza[1] ,Henrique R.Marcelino[2],Francisco Alexandrino , Jr[3] et al., Water-in-water Emulsion as a New Approach to produce mesalamine-Loaded Xylan-Based Microparticles.Appl. Sci. 2019,9,3519
9. https://dailymed.nlm.nih.gov/dailymed/fda/fdaDrugXsl.cfm?setid=d50f2c37-5675-49f3-bdd4-3b16d333ed40&type=display
10. Antonio Tursi,MD[1],Giovanni Brandimarte,MD[2],Alfredo Papa,MD,PhD[3] at al.,Treatment of Relapsing mild-to-moderate Ulcerative colitis with the Probiotics VSL#3 as Adjunctive to a Standard Pharmaceutical Treatment: A Double-Blind , Randomized , Placebo-Controlled study. Am J Gastroenterol 2010;105:2218-2227
11. I.R. Wilding*,C.Behrens, S.J.Tardif* et al., Combined scintigraphic and pharmacokinetic investigation of enteric-coated mesalazine micropellets in healthy subjects. ©2003 Black well publishing Ltd. Aliment Pharmacol Ther 17,1153-1162
12. Seema Badhana[1], Navneet Garud[2],*Akanksha Garud[1] . Colon Specific Drug Delivery of mesalamine using Eudragit S-100 Coated Chitosan microspheres from for the treatment of ulcerative colitis . International current pharmaceutical journal , February 2013,2(3) : 42-48
13. [Pr]Salofalk. Mesalazine suppositories 500mg and 1000mg . Aptalis Pharma canada Inc.
14. https://pubchem.ncbi.nlm.nih.gov/compound/Mesalamine

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Research Article

FORMULATION AND INVITRO EVALUATION OF COMPRESSION COATED MESALAMINE TABLETS FOR COLON SPECIFIC DRUG DELIVERY

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Abstract: The present study has been aimed at developing a compression coated tablets of Mesalamine with a view of minimizing the drug release in the physiological environment of stomach and small intestine and to ensure maximum drug release in the physiological environment of colon with an improved patient compliance, least side effects, better drug therapy and all aspects of an ideal drug delivery system. Mesalamine is one of the most popular drug used in the treatment of Inflammatory Bowel Disease (IBD). Here direct compression method was used for the preparation of Mesalamine core tablets and the Compression coating technique was used for coating of core tablets. Guargum alone and in the combination with HPMCK4M was used in the coating formulations. All the formulations were evaluated for the hardness, friability, thickness, weight variation, drug content, and *in vitro* drug release studies. In vitro drug release studies have in presence and absence of rat caecal content shown that guar gum in the combination with HPMC K4M is promising system for colon targeting

Key words: Mesalamine, Guar gum, HPMC K4M, colon targeting, compression coating tablets

1. INTRODUCTION

Colonic delivery refers to targeted delivery of drugs into the colon. Targete drug delivery in to the colon is highly desirable for local treatment of a several colonic diseases such as ulcerative colitis, crohn's disease and colonic cancer[1].

Now a days, various routes of administration have been explored for the effective delivery of the drug to the colon. The oral route is considered to be most convenient for the administration of drugs to patients. Rectal administration offers the shortest route for targeting the drug to the colon. However, reaching the proximal part of colon via rectal administration is difficult. Rectal administration can also be uncomfortable for patients and compliance may be less. Hence oral route is preferred route of drug administration[19].

Although oral delivery has become a widely accepted route of administration of therapeutic drugs, the gastro intestinal tract presents several barriers to drug delivery. On oral administration of conventional dosage forms drug normally dissolves in the gastro intestinal fluids and is absorbed from these regions of the gastrointestinal tract (GIT) which depends upon the physicochemical properties of the drug. It is a serious drawback in condition where localized delivery of drugs in the colon is required, or in conditions where drug needs to be protected from the hostile environment of upper GIT[9].To achieve successful colonic delivery, a drug needs to be protected from absorption and or the environment of the upper gastrointestinal tract (GIT) and then be abruptly released into the proximal colon, which is considered the optimum site for colon targeted delivery of drugs[9,26].

In the present study, Mesalamine is formulated in to the compressed coated tablet. This drug is selected because, Mesalamine is one of the most popular drug used in the treatment of Crohn's disease and ulcerative colitis[22].

2. MATERIALS AND METHODS

2.1 Materials:- Mesalamine, Guar gum, Hydroxypropylmethylcellulose K4M (HPMCK4M), Cross povidone, Microcrystalline cellulose, Talc, Magnesium stearate were obtained from Bright labs Hyderabad.

2.2 Method

Preparation of compression coated Mesalamine tablets

A. Preparation of Mesalamine core tablets

Core tablets of Mesalamine was prepared by Direct compression technique[4,15,39].. Cross povidone included in the formulation to obtain the Mesalamine tablets with fast disintegrating characteristics (disintegrating time < 1min). All the ingredients were weighed and thoroughly mixed and passed through a mesh no 60 to ensure complete mixing. The thoroughly mixed materials

were then directly compressed into tablets using 6 mm round, flat and plain punches on a single station tablet machine. Tablet quality control tests such as weight variation, hardness, friability, thickness, and dissolution in different media were performed on the core tablets. The composition of core tablets of Mesalamine given in table 1.

Table 1: Composition of core tablet

Ingredients	Quantity (mg)		
	C1	C2	C3
Mesalamine	100	100	100
Microcrystalline cellulose	38	35	32
Cross povidone	6	9	12
Talc	3	3	3
Magnesium stearate	3	3	3
Total Weight	150	150	150

B. Compression coating of core tablets[4,5,15,20,21,23,32]

The core tablets were compression coated with different quantities of coating material containing of Guar gum/ hydroxypropyl methylcellulose (HPMC) with different coat weights (i.e. the coat weights were either 200 or 175 mg). Microcrystalline cellulose was included in the coat formulations to impart enough hardness, since guar gum alone gave very soft coats. Half the quantity of the coating material was placed in the die cavity the core tablet was carefully placed in the centre of the die cavity and was filled with the other half of the coating material[20,21]. The coating material was compressed using 9 mm round, flat and plain punches. In this study, HPMC in combination with guar gum was used to enforce the mechanical resistance of the tablet during its transit in the GI tract. Tablet quality control tests such as weight variation, hardness, friability, thickness, and drug release studies in different media were performed on the compression coated tablet.

Table 2. Composition of guar gum coats used to cover Mesalamine core tablets

Ingredients	Quantity (mg) present in the coat formulation					
	F1	F2	F3	F4	F5	F6
Guar gum	160	150	140	120	110	100
Microcrystalline cellulose	35	45	55	50	60	70
Talc	3	3	3	3	3	3
Magnesium stearate	2	2	2	2	2	2
Total Weight	200	200	200	175	175	175

Table 3. Composition of Guar gum and Hpmc K4M coats used to cover Mesalamine core tablets

Ingredients	Quantity (mg) present in the coat formulation			
	F7	F8	F9	F10
Guar gum	80	85	90	95
Hydroxypropylmethyl cellulose (HPMC K4M)	20	15	10	5
Microcrystalline cellulose	70	70	70	70
Talc	3	3	3	3
Magnesium stearate	2	2	2	2
Total Weight	175	175	175	175

C. Determination of drug content

Core tablets of Mesalamine were tested for their drug content.Ten tablets were finely powdered, quantities of the powder equivalent to 100 mg of Mesalamine were accurately weighed, transferred to a 100 mL volumetric flask containing 50 mL Phosphate buffer pH 6.8 and methanol was added to ensure complete solubility of the drug. The solution was made up to volume with Phosphate buffer pH 6.8. The solution was suitably diluted and the absorption was determined by UV-Visible spectrophotometer at 333nm. The drug concentration was calculated from the calibration curve.

2.3. In vitro drug release studies:-

Drug release studies of Mesalamine core tablets

The core tablets containing 100 mg of mesalamine were tested in SIF (pH 6.8), for their dissolution rates. Dissolution studies were performed using USP dissolution test apparatus (Apparatus 2, 100 rpm, 37±0.5 °C). At various time intervals, a sample of 5 ml was withdrawn and replaced with equal volume of fresh medium. The samples were analyzed spectrophotometrically at 333 nm.

Drug release studies of compression coated Mesalamine tablets

The release of Mesalamine from compression coated tablets was carried out using USP basket -type dissolution apparatus at a rotation speed of 100 rpm, and a temperature of 37±0.5 °C.

For tablets, simulation of gastrointestinal transit conditions was achieved by using different dissolution media. Thus, drug release studies were conducted in simulated gastric fluid without pepsin (SGF, pH 1.2) for the first 2 h as the average gastric emptying time is about 2 h. Then, the dissolution medium was replaced with enzyme-free simulated intestinal fluid (SIF, pH 7.4) and tested for drug release for 3 h, as the average small intestinal transit time is about 3 h, and finally enzyme-free simulated intestinal fluid (SIF, pH 6.8) was used for 19 h to mimic colonic pH conditions.

Drug release was measured from compression coated mesalamine tablets, added to 900mLof dissolution medium. Samples withdrawn at various time intervals were analyzed spectrophotometrically at 333 nm. All dissolution runs were performed in triplicate.

Drug release studies in the presence of rat caecal contents[4]

Dissolution study procedure:

Drug release studies were carried out using USP dissolution rate test apparatus (Apparatus 1, 50 rpm, 37±0.5 °C) for 2 h in SGF (900 mL). Then the dissolution medium was replaced with SIF (pH 7.4) (900 mL) and tested for drug release for another 3 h. Then the dissolution medium was replaced with SIF (pH 6.8) containing rat caecal contents. The experiment was carried out with continuous CO_2 supply into the beakers to simulate anaerobic environment of the caecum. The drug release studies were continued for 24h (usual colonic transit time is 20–30 h).At different time intervals, 1ml sample was withdrawn and replaced with 1ml of fresh SIF (pH 6.8) bubbled with CO2 and the experiment was continued upto 24h. One milliliter of methanol was added to the samples to ensure solubility of finely suspended drug particles released due to the erosion of guar gum by caecal enzymes and the volume was made up to 10ml with PBS and the samples was analyzed for Mesalamine content at 333nm using a double beam UV spectrophotometer[4,15]

3. RESULTS AND DISCUSSION

The powder mixtures of different formulations were evaluated for angle of repose, bulkdensity, tapped density and compressibility index and their values were shown in Table 4.

Table 4: Characterization of core powder mixtures

Formulation code	Angle of Repose (^{0})	Bulk density (g/ml)	Tapped density (g/ml)	% Carr's Index
C1	27.91±0.13	0.35±0.03	0.42±0.21	16.6±0.54
C2	26.23±0.24	0.37±0.23	0.41±0.32	9.7±0.43
C3	25.34±0.32	0.34±0.43	0.40±0.12	15±0.76

The physical mixtures of the formulations C_1, C_2 and C_3 evaluated The apparent bulk density and Tapped bulk density values for core powder mixture is ranging from 0.34±0.43 to 0.377±0.23

and 0.40±0.12 to 0.42±0.21 respectively. The angles of repose and compressibility index (%) values are ranging from 25.34^0±0.32 to 27.91^0±0.13 and 9.7±0.43 to 16.6±0.54 respectively. These results show that the core powder mixture has good flow properties.

Table 5:Characterization of coating powder mixtures

Formulation code	Angle of Repose (^{0})	Bulk density (g/ml)	Tappeddensity (g/ml)	% Carr's Index
F1	28.23±0.23	0.38±0.12	0.46±0.23	17.3±0.34
F2	29.34±0.32	0.36±0.23	0.46±0.22	10±0.76
F3	30.71±0.35	0.39±0.87	0.45±0.54	13.33±0.65
F4	26.34±0.46	0.42±0.35	0.51±0.32	17.64±0.34
F5	29.23±0.13	0.46±0.45	0.55±0.32	16.3±0.23
F6	29.34±0.35	0.36±0.14	0.43±0.96	16.2±0.23
F7	28.78±0.84	0.39±0.45	0.47±0.65	17±0.21
F8	30.34±0.35	0.45±0.35	0.51±0.87	11.7±0.56
F9	29.56±0.86	0.47±0.23	0.55±0.67	14.5±0.11
F10	31.12±0.23	0.39±0.87	0.48±0.12	18.75±0.54

The physical mixtures of the coat formulations F1 to F10 evaluated (Table 5). The apparent bulk density and tapped bulk density values ranged from 0.36±0.23 to 0.47±0.23and 0.43±0.96 to 0.55±0.67 respectively. The results of angle of repose and compressibility index (%) ranged from 26.34^0±0.46 to 31.12±0.23 and 10±0.76 to 18.75±0.54 respectively. These results show that all the formulation exhibited good flow properties.

Evaluation of Mesalamine core and coated tablets

The tablets of different formulations were evaluated for Hardness, Weight variation, Friability, Drug content, and their values were shown in Table 6 & 7

Table 6. Physical properties of Mesalamine core tablets

Formulation Code	Hardness (Kg/cm^2)	Weight variation (mg)	Friability (%)	Drug Content (%)
C1	2.2±0.41	150.5±0.68	0.40±0.12	99.2±0.76
C2	2.4±0.21	152.65 ± 0.52	0.35±0.23	98.1±0.50
C3	2.3±0.11	148.25±0.20	0.45±0.34	99.5±0.30

Table 7: Physical properties of Mesalamine coating tablets

Formulation Code	Hardness (Kg/cm^2)	Weight variation (mg)	Friability (%)
F1	5.0±0.61	324.2±0.56	0.25±0.32
F2	5.2±0.35	326.4±0.12	0.33±0.21
F3	5.1±0.42	352.6±0.54	0.17±0.45
F4	4.6±0.70	351.2±0.45	0.64±0.78
F5	4.4±0.58	355.5±0.63	0.54±0.23
F6	4.8±0.46	356.7±0.22	0.58±0.87
F7	5.0±0.86	358.1±0.36	0.45±0.76
F8	5.2±0.46	359.6±0.74	0.38±0.32
F9	5.0±0.76	358.0±0.12	0.68±0.65
F10	5.5±0.62	362.5±0.56	0.46±0.34

The various formulations of tablets were evaluated. The percent drug content of the Mesalamine core tablets was found to be in the range of 98.1±0.50 to 99.5±0.30 of the labeled amount indicating uniformity of drug content in the formulation. The hardness of the core tablets of Mesalamine was found to be 2.2±0.41 to 2.4±0.21 kg/cm^2. The core tablets of Mesalamine were also found to comply with the friability test since the weight loss was found to be in the range of 0.35±0.23 to 0.45±0.34. The weight variation of core tablets was found to be in the range of 148.25±0.20 to 152.65 ± 0.52mg. The tablets thickness was found to be 2.2±0.12mm.

The hardness of the coated tablets of Mesalamine was found to be 4.4±0.58 to 5.5±0.62 kg/cm^2. The coated tablets of Mesalamine were also found to comply with the friability test since the weight loss was found to be in the range of 0.17±0.45 to 0.68±0.65. The weight variation of coated tablets was found to be in the range of 326.4±0.12 to 362.5±0.56mg. The coated tablets thickness was found to be 4.2±0.23 to 4.8±0.43mm.

Table 8:Percent drug release of Mesalamine core tablets(C1,C2,C3) in SIF (PH 6.8)

Time(min)	C1	C2	C3
0	0	0	0
5	52.34±0.45	50.65±0.43	55.67±0.54
10	65.43±0.43	68.45±0.54	72.34±0.34
15	85.67±0.23	88.45±0.43	95.76±0.12
20	92.23±0.54	94.23±0.56	98.32±0.32

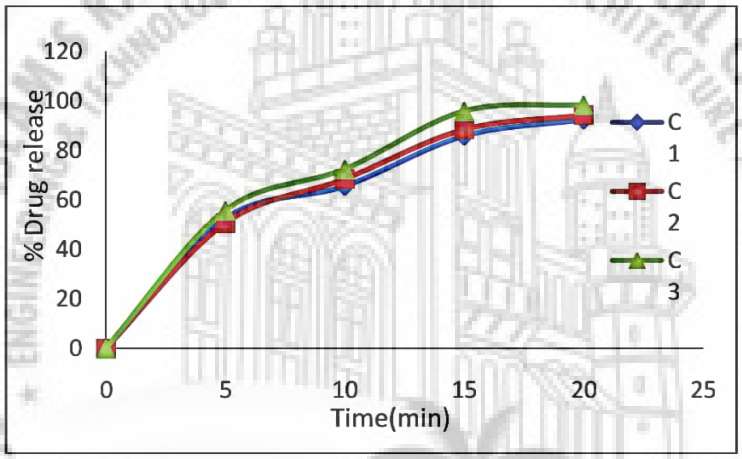

Figure 7: Dissolution profiles of Mesalamine core tablets(C1,C2,C3)

The dissolution results of Mesalamine core tablets in SIF (pH 6.8) were shown Figure7. The core tablets dissolved faster in SIF pH 6.8 and the C3 formulation reached 95% in 15min when compared to C1 and C2 formulations. Hence C3 formulation selected as a optimized formulation.

Table 9: Cumulative percent drug release of F1-F6 formulations containing guar gum

Time (hr)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	0.95±0.56	1.16±0.45	0.82±0.54	1.08 ±0.02	2.62±0.79	1.93 ±0.02
2	1.55±0.02	1.48±0.86	1.28±0.02	2.27±0.79	5.13 ±0.87	4.06±0.46
3	3.10±0.02	3.48±0.02	3.02±0.02	5.34±0.95	6.93±0.79	5.98 ±0.87
4	3.59±0.02	4.47±0.95	4.72±0.65	7.26 ±0.02	7.86±0.46	7.37±0.68
5	4.33±0.86	5.21±0.95	5.29±0.86	8.22 ±0.45	10.32±0.68	10.28±0.79
6	5.50±0.02	6.74±0.02	6.58±0.65	10.90±0.13	11.41±0.79	12.24±0.02

8	5.86±0.95	7.10±0.55	7.92±0.02	11.96 ±0.45	12.91±0.13	15.79±0.68
10	6.10±0.45	7.59±0.02	9.04±0.55	13.62 ±0.02	13.95±0.95	16.24±0.13
14	6.59±0.86	8.08±0.45	11.94±0.86	14.56±0.95	15.79±0.79	17.04±0.87
18	7.32±0.95	9.05±0.55	12.66±0.95	21.01±0.68	24.43±0.68	29.39±0.02
24	11.62±0.65	13.39±0.02	15.97±0.65	31.16 ±0.45	36.15±0.87	38.11±0.79

Data represents mean ± SD, n = 3

Table 10: Cumulative percentage drug release of F7-F10 formulations containing guar gum

Time (hr)	F7	F8	F9	F10
0	0	0	0	0
1	1.84±0.65	1.67±0.68	1.32±0.55	1.88±0.02
2	3.01±0.65	2.80 ±0.56	2.40±0.68	3.02±0.02
3	5.10 ±0.13	4.91±0.75	3.76±0.46	4.72±0.65
4	6.77±0.86	5.18±0.68	4.96±0.55	5.79±0.86
5	7.91 ±0.13	7.11±0.75	6.70±0.46	12.18±0.65
6	11.03±0.86	11.97 ±0.13	11.59±0.86	15.92±0.02
8	12.44 ±0.56	13.18±0.14	14.83±0.65	19.04±0.55
10	13.14±0.65	15.36 ±0.87	16.87 ±0.13	24.94±0.86
14	15.49 ±0.13	16.20 ±0.56	20.11±0.86	36.66±0.95
18	18.07±0.65	20.67 ±0.87	23.22 ±0.56	48.97±0.65
24	26.07±0.75	29.69±0.86	37.46±0.14	61.07±0.54

Data represents mean ± SD, n = 3

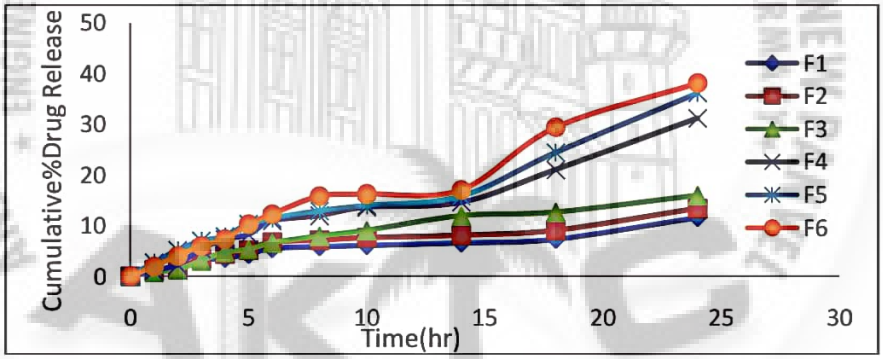

Figure 8: Dissolution profiles of F1-F6 formulations containing guar Gum.

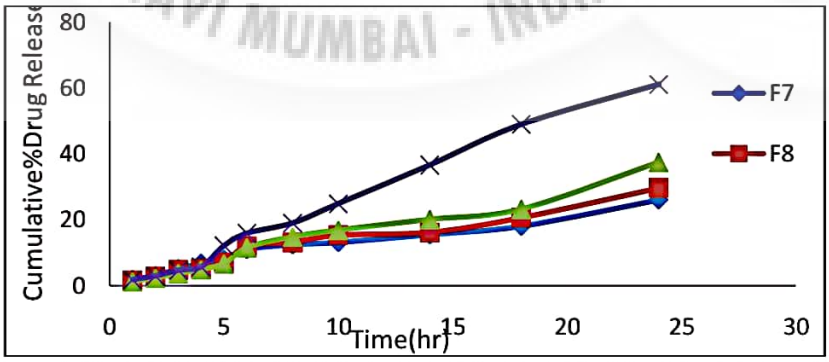

Figure 9: Dissolution profiles of F7-F10 formulations containing combination of guar gum and HPMC K4M

The cumulative mean percent of Mesalamine released from tablets coated with 200 mg coat weights of formulations containing varying amounts of guar gum (from F1 to F3) was found to vary from 4.33±0.86 to 5.29±0.86 after 5 h of testing in simulated gastric and intestinal fluids. The percent of drug released from tablets coated with 175 mg coat weights of formulations containing varying amounts of guar gum (from F4 to F6) was found to vary from 8.22 ±0.45 to 10.32±0.68 after 5 h of testing. The cumulative mean percent drug released from F7, F8 and F9 formulation containing different amounts of combination of guar gum and HPMC K4M was found to vary from 6.70±0.46 to 7.91±0.13after 5 h dissolution testing. Thus, guar gum in the form of coat is capable of protecting the drug from being released completely in the physiological environment of stomach and small intestine.

The drug release studies were further continued for 19 h by replacing the dissolution medium with SIF (pH 6.8). At the end of the experiment, the cumulative mean percent drug released from coat formulations F1, F2 and F3 was between 11.62±0.65 and 15.97±0.65 and the coats were intact. This indicates that the guar gum will not permit the release of the bulk of the drug core until the coat is broken. The percent drug release for the formulations F4, F5, and F6 was found between 31.16 ±0.45 and 38.11±0.79 after 24h study.

However, the tablets with coat formulation F10 found to release 12.18±0.65 after 5h and 61.07±0.54 after 24 h study. This may be due to lesser gum content of the coat which was unable to remain intact and not protecting the drug from being released.

This F10 was not studied further in rat caecal contents. The formulations F1andF2 were not studied in the rat caecal contents because very less amount of drug released after 24 h study. Even though F3 formulation releasing tiny amount (15.97±0.65 percent) of drug after 24 h, it was further studied in caecal contents to know the effect of coat thickness 200 mg coat weight compared with 175 mg coat weight.

Table 11: Cumulative percentage drug release of F3, F4, F5, F6 formulations in presence of rat caecal contents

Time (hr)	F3	F4	F5	F6
0	0	0	0	0
1	0.87±0.98	1.08 ±0.12	2.82±0.59	1.93 ±0.02
2	1.48±0.87	2.27±0.78	5.23 ±0.88	4.16±0.56
3	4.24±0.12	5.34±0.75	6.63±0.89	5.68 ±0.27
4	5.32±0.96	7.26 ±0.02	7.86±0.46	7.37±0.18
5	6.55±3.32	8.72 ±0.45	10.22±0.38	10.18±0.29
6	10.99±0.46	11.46±0.65	12.32±0.24	20.58±0.46
8	14.59±0.65	15.34±0.02	21.62±0.24	27.10±0.02
10	19.19±0.02	25.76±0.46	32.31±0.14	34.52±0.14
14	20.30±0.46	32.98±0.87	48.14±0.23	40.27±0.45
18	29.63±0.87	58.34±0.55	65.24±0.54	79.97±0.65
24	41.84±0.55	74.23±0.24	85.94±0.13	90.45±0.55

Data represents mean ± SD, n = 3

Table 12: Cumulative percentage drug release of F7, F8, F9 formulations in presence of rat caecal contents

Time (hr)	F7	F8	F9
0	0	0	0
1	1.94±0.65	1.67±0.43	1.12±0.65
2	3.11±0.55	2.80 ±0.54	2.10±0.28
3	5.60 ±0.53	4.71±0.85	4.60±0.54
4	6.27±0.76	6.28±0.28	5.56±0.75
5	8.23±0.44	7.65±0.55	6.85±0.95

6	17.24±0.87	15.23±0.45	18.79±0.46
8	28.23±0.46	23.32±0.45	27.29±0.55
10	39.23±0.68	39.23±0.78	44.89±0.46
14	44.54±0.55	55.32±0.36	68.43±0.87
18	68.07±0.87	76.47±0.45	83.12±0.65
24	86.52±0.46	91.95±0.32	96.53±0.46

Data represents mean ± SD, n = 3

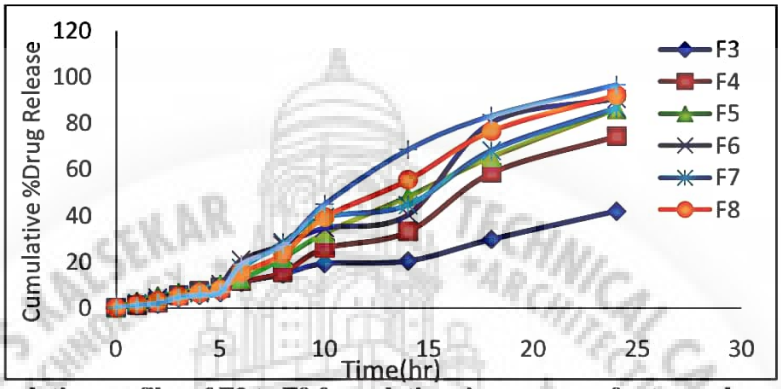

Figure 10: Dissolution profiles of F3 to F9 formulations in presence of rat caecal content

Dissolution results of compression coated Mesalamine tablets in rat caecal contents

The drug delivery systems targeted to the colon should not only protect the drug from being released in the physiological environment of stomach and small intestine, but also release the drug in colon after enzymatic degradation by colonic bacteria[39]. Hence, in vitro drug release studies were carried out for selected formulations in SIF (pH 6.8) containing 4% w/v of rat caecal contents. At the end of 24 h of testing which includes testing in simulated gastric and intestinal fluids, the percent drug released from Mesalamine tablets coated with coat formulation F3 was found to be only 41.41±2.68. The presence of higher amount of guar gum (200 mg) in the coat with resultant thicker coat might not have allowed the disintegration of the coat during the time period of testing. This also indicates that the drug will not be released unless the coat is broken.

The percent drug released from tablets coated with coat formulation F4,F5,F6, was found to be 74.23±0.24, 85.94±0.13, 90.45±0.55 respectively. The coat was completely degraded by the rat caecal enzymes thereby releasing the drug into the dissolution medium. Since the coat weight and thickness of coat formulations F4, F5 and F6 were lesser (175 mg) compared to coat formulation F3 (200 mg) the coat might have been completely hydrated and subsequently degraded by the caecal enzymes at a faster rate.

To improve the mechanical strength it was mixed with HPMC K4M polymer. The percent drug release from F7, F8 and F9 formulation at the end of 24 h study in presence of rat caecal content was found to be 86.52±0.46, 91.95±0.32, 96.53±0.46. Better release profile was observed in the system containing Guar gum and HPMC K4M[15,31].

It is evident from the results of the drug release studies in the presence and absence of rat caecal contents that the drug release occurred by the degradation of guar gum coats by the enzymes present in the caecal matter. And the tablet containing two polymeric systems shows much more promising release than the single polymeric system. From this we concluded that by taking single hydrophilic polymer also release can be retarded but addition of another polymer which can control its release is necessary. Thus the F9 formulation was considered better among other formulations to produce colon specific drug delivery of Mesalamine.

Kinetic results

The mechanism and kinetics of drug release of Mesalamine is determined by the application of korsmeyer-peppas model, higuchi's model, zero order and first order. Most of the tablet formulation follows the zero order release as their r^2 values are between 0.964 and 0.986.

The mechanisms of drug release was best fitted well with Korsmeyer–Peppas[3] models as their r^2 values in the range of 0.931-0.989.

Table 13:Drug release kinetics

Formulation Code	Zero order	First order	Higuchi	Korsmeyer & Peppas
F3	0.985	0.977	0.892	0.978
F4	0.974	0.919	0.827	0.989
F5	0.986	0.914	0.852	0.945
F6	0.964	0.886	0.839	0.961
F7	0.982	0.923	0.866	0.931
F8	0.979	0.915	0.852	0.943
F9	0.966	0.909	0.856	0.968

4. CONCLUSION

The study was undertaken with an aim to formulate and evaluate Mesalamine compression coated tablets to deliver the drug in the colon. The *invitro* dissolution studies shows that Guar gum and HPMC K4M in combination, in the form of compression coated tablets is capable of protecting Mesalamine from being released in the upper region of GI system, i.e. stomach and small intestine. The in vitro drug release studies indicated that formulation F9 was a promising system to provide targeting of Mesalamine to the colon. The release pattern of the above formulation was best fitted to Korsmeyer-Peppas model and zero-order model. FT-IR spectral studies showed that there is no interaction between the drug and excipients.

REFERENCES

1. Mohit Khandelwal, Ankit Ahlawat and Ram Singh. Polysaccharides and Natural Gums for Colon Drug Delivery.*The Pharma Innovation*,**2012**;1(1):9-13
2. Hemant H.Gangurde, Mayur A, Chordiya, Tamizharasi S, and Sivakumar T, A review on Diseases, approaches and evaluation parameterrs for colon specific drug delivery, *International Journal of Drug Research and Technology,***2012;** 2(3):239-262
3. Thiruganesh Ramasamy, Umadevi Subbaih Khandasamy ,Suresh Shanmugam and Himabindhu Ruttala, Formulation and Evaluation of Chondroitin Sulphate Tablets of Aceclofenac for Colon Targeted Drug Delivery, *Iranian Journal of Pharmaceutical Research,***2012**; 11(2):465-479
4. Mounika. B, Appa Rao. A, Prabhakar Reddy. V. Formulation and evaluation of compression coated meloxicam tablets for colon drug delivery**.** *International Journal of Pharmacy and Biological Sciences,***2012;**2(3):131-143
5. Bharani S. Sogali, Mohammed Yousuff and Shashank Nayak. Influence Of Natural Gums For Effective Colon Targeting Of Methotrexate For The Treatment Of Colorectal Cancer. *Int Journal of Pharmacy,***2012;**2(3): 498-506
6. M Manikandan,K Kannan, R Manavalan1, N Junior Sundresh. Design of Nanoparticles for Colon Target Drug Delivery – A Review. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* **2011;**2(4):128-139
7. Tarak Jayraj Mehta,A.D.patel,Mukesh R.Patel,N.M.patel. Need Of Colon Specific Drug Delivery System:A Review on primary and novel Approaches. *International journal of Pharma.Research & Development* ,**2011;**3(1):134-153.
8. Shailendra wasnik, poonam parmar. The design of colon-specific drug delivery system and different approaches to treat colon disease. *International journal of pharmaceutical sciences review and research,***2011**;6(2):167-177.
9. Jitender Mor.Recent Advances in colonic Targeted Drug Delivery Systems. *International Journal of Pharma Proffessional's Research,***2011;**.2(4):497-501.
10. K.Chandramohan, B.Rajkapoor, Development and *invitro* evaluation of Colon Specific Delivery System of Tinidazole, *jitps,***2011;**2 (4):111-121.
11. AO.Kabra, SS. Zavare and RS. Wanare, Hydrophilic polymers in formulation of sustained-release coated matrix tablets of 5-amino salicylic acid for targeting colon, International Journal of Research in Pharmaceutical and Biomedical Sciences,**2011;** Vol. 2 (1):224-230.

12. Negar Bashardoust, Josephine Leno Jenita, Parvin Zakeri-Milani. *Advanced Pharmaceutical Bulletin,* **2011;**1(2):87-92.
13. Wycliffe Omwancha, Chahinaz Kouba, Satish Yelamanchili, and Steven H. Neau. Colon-specific drug delivery using ethylcellulose and chitosan in the coat of compression-coated tablets. *Drug Development and Industrial Pharmacy*,**2011**;37(8):945–953.
14. Thiruganesh Ramasamy, Uma Devi Subbaih Kandhasami, Himabindhu Ruttala, Suresh Shanmugam, Formulation and evaluation of xanthan gum based aceclofenac tablets for colon targeted drug delivery. *Brazilian Journal of Pharmaceutical Sciences,* **2011;**47(2):300-311.
15. Fahima M. Hashem, Dalia S. Shaker, Mohamed Nasr, Ibrahim E. Saad, Reem Ragaey. Guar gum and hydroxy propyl methylcellulose compressed coated tablets for colonic drug delivery: *in vitro* and *in vivo* evaluation in healthy human volunteers. *Drug Discoveries & Therapeutics,* **2011;**5(2):90-95.
16. R.Vijaya Muthumanikandar , Sudeesh Edavalath, Saravanakumar K. Design and Evaluation of Mesalamine Tablet for Colon Specific Drug Delivery. *International Journal of Drug Development & Research,***2011**;3(3):197-212.
17. Pragnesh Patel, Anupkumar Roy,Vinod kumar SM, Martand Kulkarni. Formulation and Evaluation Of Colon Targeted Tablets of Ornidazole For The Treatment of Amoebiasis. *International Journal of Drug Development & Research,***2011**;3(1).
18. Poonam kushwaha, sheeba fareed and sanju nanda. Promising approaches to target drug delivery to colon. International journal of pharmaceutical sciences,**2010**;2(3):669-679.
19. Anil K.Philip, Betty Philip.Colon Trgeted Drug Delivery Systems: A Review on primary and novel approaches. *Oman Medical Journa,***2010;**25(2).
20. J.Josephine Leno Jenita, Vijaya k, Suma .R, Bincy Raj and Ayesha Siddiqca. Formulation and Evaluation of Compression Coated Tablets of Mesalazine For Colon Delivery. *International Journal of PharmTech Reasearch,***2010;**2(1):535-541.
21. Chickpetty S. M,and Raga Baswaraj. Studies on Design and In Vitro Evaluation of Compression-Coated Delivery Systems for Colon Targeting of Diclofenac Sodium. *International Journal of PharmTech Research,***2010;**2(3):1714-1722.
22. Nirav V. Patel, Jayvadan K. Patel, Shreeraj H. Shah, Jagruti J Patel. Design, development and in vitro evaluation of Mesalamine tablets containing Pectin and Chitosan for colon-specific drug delivery**.** *Int. J. Res. Pharm. Sci,***2010;**194-102.
23. Rajesh kumar.R, Ramasamy.P, VengadeshPrabu.K. Formulation and Evaluation of Guar gum compressed tablets for colon targeted drug delivery. *Journal of Pharmacy Research,* **2010;**3(7):1538-1540.
24. G.Kishore, Somashekar shyale, K.srikanth,V.R.M.Gupta.Development and Evaluation of Colon targeted tablets of Praiquantel and its β-Cyclodextrin complex to treat Schistosomiasis. *Journal of Pharmaceutical Science and Technology,***2010**;2(8):269-275.
25. Kinage Krishna, Nandgude Tanaji, Bhise Kiran, Deshmukh Pradeep. Studies on Development of oral colon targeted Drug Delivery Of Locust bean and Xanthan Gums *International Journal of Green Pharmacy,***2010**.
26. Tomuta,L.Vlase, Adina Popa,S.E.Leucuta. *Invitro –invivo* Evaluation of a Novel Drug Delivery System For Colonic Targeting.*Farmacia,***2010;**58(3):368-377.
27. Prabhakara prabhu, Nissara ahamed, Harish nairy matapady,Mohd. Gulzar ahmed, R. Narayanacharyulu, D. satyanarayana and EVS Subrahmanayam. Investigation and comparison of colon specificity Of novel polymer khaya gum with guar gum. *Pak. J. Pharm. Sci,***2010;** 23(3):259-265.
28. Raghavendra Rao NG, Pentewar Ram, Thube Ketan, Suryakar VB. Formulation and in vitro evaluation of gastric oral floating tablets of cefixime for controlled release. *RJPBCS,***2010;**1(3).
29. Sateesh Kumar Vemula, prabhakar reddy, Veera reddy. Different Approaches to design and evaluation of Colon specific Drug Delivery Systems. *International Journal of Phrmacy & Technology,***2009;1**(1):1-35.
30. Carien e. Beneke,Aalvaro m. Viljoen and josias h. Hamman . Polymeric plant-derived excipients in drugdelivery.*Molecules,***2009;**14:2601-2620.
31. A.V.Bhosale, Hardikar S.R, Naresh Patil1, Umang Patel, Yogesh Sumbe, Rajesh Jagtap. Formulation and In-vitro

Evaluation of Microbially triggered Ibuprofen Delivery for Colon targetting. *International Journal of PharmTech Research,* **2009;**1(2):328-333.

32. Soad A. Yehia,Ahmed H. Elshafeey,Ibrahim Sayed, and Ahmed H. Shehata. Optimization of Budesonide Compression-Coated Tablets for Colonic Delivery. *AAPS PharmSciTech,* **2009;**10(1).
33. Santanu Ghosh and B. B. Barik. Preparation and evaluation of aceclofenac sustained release formulation and comparison of formulated and marketed product. *International Journal of Medicine and Medical Sciences,* **2009;**1(9):375-382.
34. Naikwade Sonali R.,Kulkarni Pratibha P,Jathar Shripad R. Amrita N. Development of time and pH dependent Controlled Release Colon Specific Delivery of Tinidazole. *DARU,* **2008;**16(3):119-127.
35. Kishore Sahebrao Salunkh, Mohan Vinayak Kulkarni. Formulation and *Invitro* Evaluation of Dextrin Matrix Tablet of Ibuprofen for Colon Specific Drug Delivery. *J.Pharma.sci.,* **2008;**21(1):17-20.
36. Timucin Ugurlu, Murat Turkogulu,Umran Soyogul Gurer, Burcak Gurbu Akarsu. Colonic delivery of Compression coated nisin tablets using pectin/HPMC polymer mixture. *European Journal of Pharmaceutics and Biopharmaceutics,* **2007;**67:202-210.
37. J. Varshosaz, J. Emami and E. Jaffari. Comparison of hydrophilic natural gums and cellulosic polymers in formulation of sustained-release matrix tablets of terbutalin sulfate. *Reasearch in Pharmaceutical Sciences*,**2006;**1:30-39.
38. Gang Cheng, Feng An, Mei-Juan Zou, Jin Sun, Xiu-Hua Hao, Yun-Xia He. Time- and pH dependent colon-specific drug delivery for orally administered diclofenac sodium and 5-aminosalicylic acid. *World Journal of Gastroenterology*,**2004;**10(12):1769-1774.
39. Chellan vijaya Raghavan,Chithambaram Muthulingam, Joseph amaladoss josephine leno jenita and thengungal Kochupapy ravi. An *in Vitro* and *in Vivo* Investigation into the Suitability of Bacterially Triggered Delivery System for Colon Targeting. *Chem. Pharm. Bull,* **2002;50**(7):892-895.
40. Y.S.R.Krishnaiah, P.Veer Raju,B.Dinesh Kumar,P.B haskar.Development of Colon targeted drug delivery systems for Mebendazole. *Journal of Controlled Release,* **2001;**77:87-95.
41. Y.S.R.Krishnaiah,S.Satyanarayana,Y.V.R amaprasad, S.NarasimhaRao. Evaluation of Guargum as a Compression coat for drug targeting to colon.*International Journal of Pharmaceutics,* **1998;**171:137-146.

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Research Article

Theme: Advanced Technologies for Oral Controlled Release
Guest Editors: Michael Repka, Joseph Reo, Linda Felton, and Stephen Howard

Once Daily, High-Dose Mesalazine Controlled-Release Tablet for Colonic Delivery: Optimization of Formulation Variables Using Box–Behnken Design

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Abstract. The aim of this work was to statistically optimize a novel high-dose, mesalazine colonic delivery matrix system, potentially suitable for once daily administration, using simple wet granulation method. A hydrophobic–hydrophilic polymeric blend was used to manipulate drug release. A three-factor, three-level Box–Behnken design was used to construct polynomial models correlating the dependent and independent variables. Independent formulation variables were the percentages of the hydrophilic polymer Carbopol® 940, hydrophobic polymer Eudragit® RS, and the superdisintegrant croscarmellose sodium. The cumulative percentages of drug released at 6, 10, and 14 h were selected as dependent variables and restricted to 7.5–22.5% (Y_1), 42.5–57.5 % (Y_2), and 72.5–87.5% (Y_3), respectively. A second-order polynomial equation fitted to the data was used to optimize the independent formulation variables. Based on Box–Behnken experimental design, different mesalazine release profiles were obtained. The optimized formulation containing 5.72% Carbopol®, 9.77% Eudragit® RS, and 1.45% croscarmellose sodium was prepared according to the software determined levels. It provided a release profile which was very close to the targeted release profile, where the calculated values of f_1 and f_2 were 8.47 and 67.70, respectively, and followed zero-order release kinetics.

KEY WORDS: Box–Behnken; controlled release; Eudragit; mesalazine; optimization.

INTRODUCTION

The anti-inflammatory agent 5-aminosalicylic acid (5-ASA or mesalazine) is the recommended first-line therapy for the treatment of active symptoms, induction of remission, and maintenance of remission in patients with mild-to-moderate ulcerative colitis (1). Mesalazine acts topically on the colonic mucosa but when orally administered, it is extensively and rapidly absorbed in the small intestine, leading to little localization of mesalazine in the colon and hence, low efficiency with significant systemic side effects (2). Consequently, three methods have been commonly used for targeting of mesalazine to the colon: a pro-drug concept, enteric coating, and/or prolonged release of the drug through semipermeable membrane (3).

The recommended daily dose of mesalazine may reach 4.8 g in acute attack and 2.4 g in maintenance of remission in divided doses. Therefore, multiple daily dosing up to 12 tablets or capsules per day are required because of the low dosage strength of most currently commercially available mesalazine formulation (4). Reduced patient compliance and disease control are the results of these inconveniences of frequent daily dosing and the number of tablets or capsules required per day (5). Additionally, many traditional delayed-release formulations that lack any means for prolonging mesalazine release are characterized by the undesirable immediate release of mesalazine once they reach the colon. This leads to a relatively smaller amount of mesalazine delivered to the distal part of the colon, the area most commonly to be inflamed (6).

Lialda®, a delayed-release tablet (also known as Mezavant® XL in UK) with high-dose 1,200 mg mesalazine/tablet, was developed utilizing Multi-Matrix System (MMX) technology for the treatment of ulcerative colitis at a dosage of 2.4–4.8 g given only once daily with a view of improving patients compliance (7,8). The MMX technology involves incorporating mesalazine into a lipophilic matrix, which is itself dispersed as microparticles within a hydrophilic matrix. Then pH-dependent gastro-resistant film, designed to disintegrate when the pH is at least 7, was applied to delay the dissolution (5,9,10). The components of the MMX matrix are sodium-carmellose, sodium carboxymethyl-starch (type A), talc, stearic acid, and carnauba wax (11).

Factorial designs and analysis of the response surfaces are methods of experimental designs that could be used for the statistical optimization of pharmaceutical dosage forms (12). Box–Behnken statistical design is a type of response surface methodology that requires smaller number of experimental runs and is less time consuming than conventional formulation methods (13).

The current study is aimed at developing and optimizing a novel delayed-controlled zero-order release matrix tablet of mesalazine, suitable for once daily administration, employing a simpler method suitable for conventional tablets manufacture

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processes. The proposed method is based on a core matrix tablet, which mainly contains Eudragit® RS (hydrophobic polymer), Carbopol®940 (hydrophilic polymer), and croscarmellose sodium to manipulate drug release prepared by the traditional wet granulation technique followed by coating with pH-dependent polymer Eudragit® S.

In order to achieve this goal, computer-aided optimization techniques using three-factor, three-level Box–Behnken design was employed to investigate the effect of three formulation factors, namely, the contents of Carbopol®940, Eudragit® RS, and croscarmellose sodium on the cumulative percent of drug released and to statistically optimize the levels of these factors using mathematical equations and response surface plots in order to obtain the targeted dissolution profile for mesalazine.

MATERIALS AND METHODS

Materials

Mesalazine was kindly donated by Minapharm Pharmaceuticals (Cairo, Egypt); croscarmellose sodium by FMC BioPolymer (Brussels, Belgium); Carbopol® 940, Noveon Inc. (USA); polyvinylpyrrolidone K-30 (PVP), Fluka AG (Buchs, Switzerland); talc and magnesium stearate, Adwic, El-Nasr Pharmaceutical Chemicals Co. (Egypt); triethyl citrate, Alfa Aesar (Karlsruhe, Germany); and Eudragit® S 100 and Eudragit® RS PO, generously donated by Röhm Pharma, GmbH (Germany). All other chemicals and solvents were of analytical grade.

Methods

Compatibility of Mesalazine with Different Excipients

Differential Scanning Calorimetry. Thermal analysis by differential scanning calorimetry (DSC) was carried out using Shimadzu thermal analyzer (Shimadzu DSC 60, TA-60 WS, Japan) to investigate the compatibility between mesalazine and different excipients. The DSC thermograms of pure drug, individual excipients, and drug–excipient mixtures (1:1 *w/w*) were recorded. For each measurement a sample of approximately 6 mg was placed in an aluminum pan and scanned in the temperature range 30–350°C. A heating rate of 10°C/min was used, and the thermal analysis was performed under dynamic nitrogen atmosphere.

Fourier Transform–Infrared Spectroscopy. Fourier transform–infrared spectroscopy (FT-IR) spectra for the drug, the selected excipients and the drug-selected excipients powder mixtures (1:1 *w/w*) were recorded on FT-IR spectrophotometer (FTIR-8400S, Shimadzu, Japan) using the potassium bromide disk technique. The scanning range was 4,000 to 500 cm^{-1}.

Table I. Variables in Box–Behnken Design

Formulation variables	Level used (−1)	(0)	(+1)
X_1=Carbopol content (%)	0	4	8
X_2=Eudragit RS content (%)	1	5.5	10
X_3=Croscarmellose sodium content (%)	0	1	2
Responses variables	Constraints		
Y_1=release (%) after 6 h	$7.5\% \le Y_1 \le 22.5\%$		
Y_2=release (%) after 10 h	$42.5\% \le Y_2 \le 57.5\%$		
Y_3=release (%) after 14 h	$72.5\% \le Y_3 \le 87.5\%$		

Table II. The Composition and Observed Responses from Randomized Runs in Box–Behnken Design

Run	Factors X_1	X_2	X_3	Responses Y_1	Y_2	Y_3
1	0	5.5	2	6.810	32.717	53.345
2	4	5.5	1	9.642	71.949	96.120
3	0	5.5	0	3.014	11.340	20.540
4	8	10	1	2.015	13.599	44.660
5	4	1	2	17.210	65.420	95.134
6	8	5.5	0	4.520	20.240	49.210
7	4	5.5	1	10.500	66.392	90.320
8	4	1	0	12.100	63.227	93.497
9	4	5.5	1	9.012	69.170	92.650
10	8	1	1	7.896	39.029	83.505
11	4	10	2	11.020	44.660	84.296
12	4	10	0	2.010	6.558	15.604
13	0	10	1	3.510	12.210	18.260
14	8	5.5	2	4.320	46.886	92.098
15	0	1	1	16.010	60.120	92.227

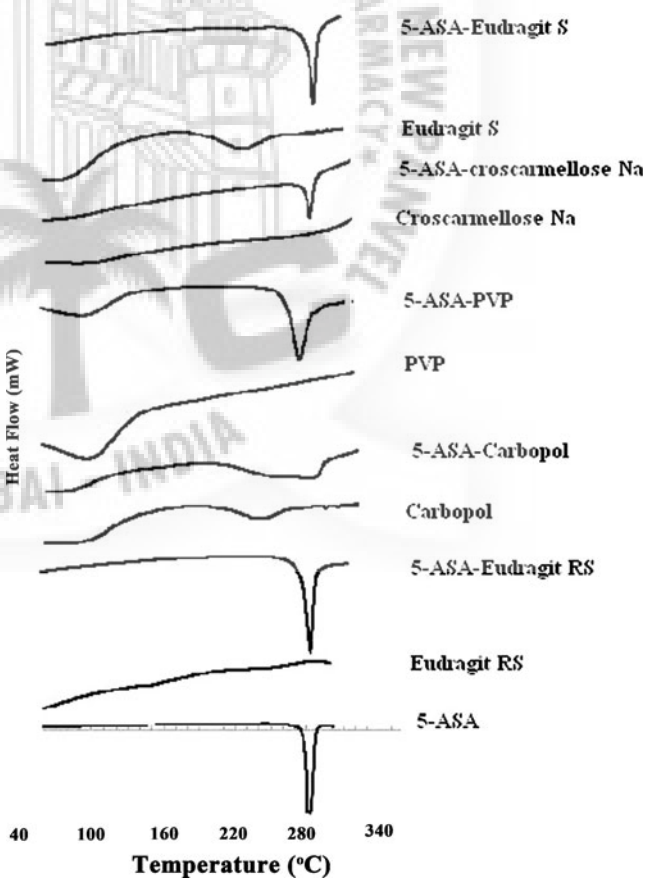

Fig. 1. DSC thermal scan of mesalazine, pharmaceutical excipients and their 1:1 *w/w* coground mixtures

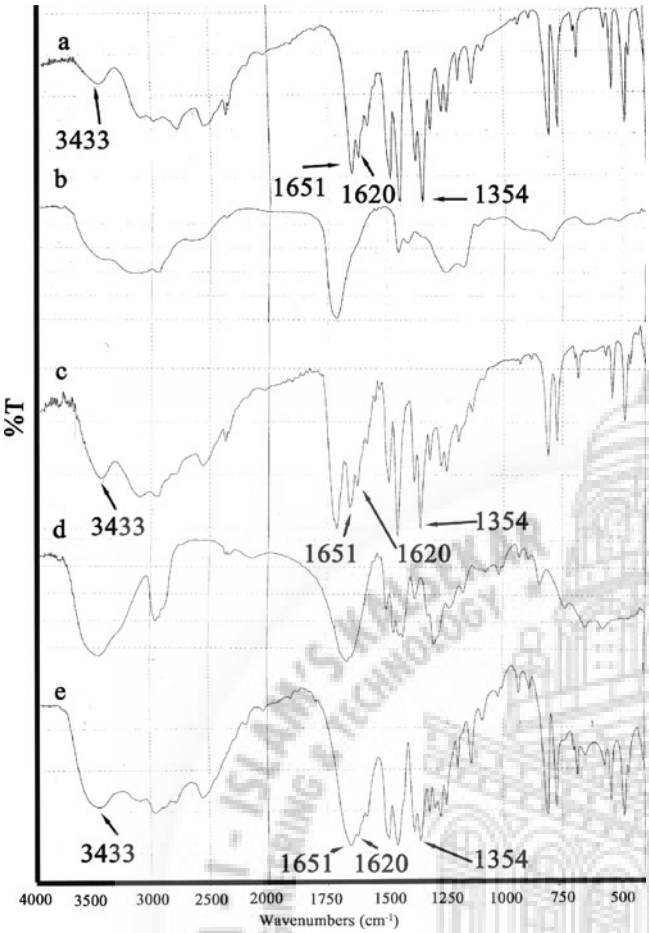

Fig. 2. FT-IR spectra of *a* mesalazine; *b* Carbopol®; *c* mesalazine–carbopol coground mixture (1: 1 *w*/*w*); *d* PVP; *e* mesalazine–PVP coground mixture (1: 1 *w*/*w*)

Experimental Design

A three-factor, three-level Box–Behnken design was employed for the optimization procedure using Design-Expert® 7.1.5 software (Stat-Ease, Inc., USA). The investigated factors (independent variables) were Carbopol® content (X_1), Eudragit® RS content (X_2), and croscarmellose sodium content (X_3). The levels for these three factors were determined from sufficient preliminary trials. The cumulative percentages of drug released at 6, 10, and 14 h (Y_1, Y_2, and Y_3, respectively) were selected as dependent variables as shown in Table I. A zero-order release profile of mesalazine over 16 h was suggested as a targeted release profile which was based on a theoretical release of about 8.3% of the drug per hour after a lag time of 4 h and was deduced from mesalazine release profile of the once daily marketed product.

Preparation of Mesalazine Core Tablets

Accurately weighed quantity of mesalazine, Carbopol®, and Eudragit® RS were mixed for 20 min using a glass mortar and pestle. The mixture was then granulated using a binder solution of PVP (5% *w*/*w*) in isopropyl alcohol. The wet mass was passed through 16# sieve and the resulted granules were dried in a tray drier for 30 min at 50°C. The dried granules were mixed with the required amounts of croscarmellose sodium, 2% *w*/*w* of talc, and 1% *w*/*w* magnesium stearate. Amounts of the resulting granules equivalent to 1,200 mg of mesalazine were compressed with a single-punch tablet press machine (Royal Artist, Bombay, India), using an oblong punch and die set (21×9 mm). Table II depicts the composition of the prepared tablets.

Coating of the Prepared Tablets

Twenty-five grams of Eudragit® S 100 was dissolved in 350 g of 95% ethanol under high-speed stirring until a clear solution was obtained. Two and half grams of triethyl citrate as a plasticizer and 1.25 g talc as a glidant were added (14,15). Then the mixture was stirred for 24 h to ensure sufficient plasticization of the polymer and to get homogeneous solution (16). Coating of tablets was performed by immersion (17) in the coating solution followed by solvent evaporation using hot air electric hand dryer (16). The process was repeated until the target weight gain of 5% (*w*/*w*) was achieved. This ratio was selected based on the results from the preliminary trials.

Characterization of Core Tablets

The prepared tablets were evaluated regarding hardness, friability, and drug content. The hardness of 10 tablets was measured using Monsanto (standard type) tablet hardness tester. Friability was determined by taking 10 tablets in digital tablet friability tester (Model DFI-1, Veego, Bombay, India) for 4 min at 25 rpm. For estimating drug content, 10 tablets were crushed and powdered. The aliquot of powder equivalent to 10 mg of drug was weighed and dissolved in 50 ml freshly prepared phosphate buffer (pH 7.4). The resultant solution was filtered and suitably diluted, then analyzed spectrophotometrically at predetermined λ_{max} of mesalazine (330 nm). From the absorbance value drug content was calculated on average weight basis.

In Vitro Release Studies

The release characteristics of mesalazine from the prepared formulations were determined according to the USP dissolution II paddle method using a dissolution tester (Vision® Classic 6^{TM} Dissolution Tester, Hanson Research Corporation, California, USA) at 37±0.5°C with a rotation speed of 50 rpm. The release profile was studied in a medium of changing pH. The initial condition was 350 ml of 0.1 N HCl (pH 1.2) for 0–2 h. At the end of second hour, 250 ml solution composed of 3.75 g of KH_2PO_4 and 1.2 g of NaOH was added to raise pH of dissolution medium to 4.5 and the total volume of the dissolution medium to 600 ml. At the end of fourth hour, 300 ml phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water) was added to raise pH to 7.4. The study was then continued till the end in 900 ml volume (18). At predetermined time intervals, a 5 ml sample was withdrawn and replaced with fresh dissolution media. Collected samples were filtered through 0.45 μm Millipore filters. After appropriate dilutions, the concentration of mesalazine in samples was spectrophotometrically measured at predetermined $\lambda_{max(s)}$ using a UV spectrophotometer (Jenway UV/Vis. Spectrophotometer, Barloworld Scientific

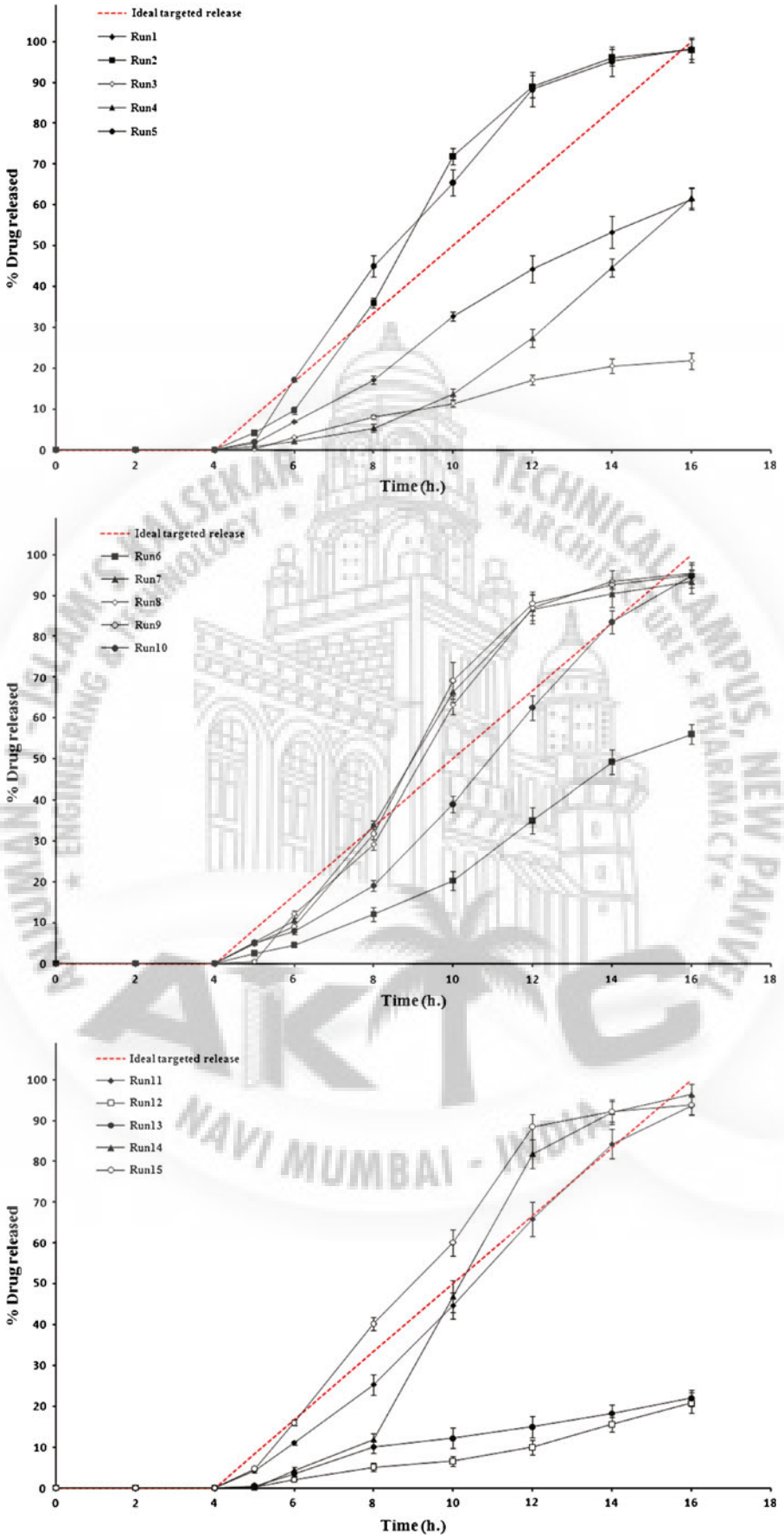

Fig. 3. *In vitro* release profiles of all formulations. Each data point is expressed as mean ± SD (n=3)

Table III. Summary of Results of: Model Analysis, Lack of Fit and R-square Analysis for Measured Responses

Source	Y_1		Y_2		Y_3	
	Sum of squares	P > F	Sum of squares	$P > F$	Sum of squares	$P > F$
Model analysis						
Mean *vs.* total	953.44		25,918.13		69,559.39	
Linear *vs.* mean	203.43	0.0115	3,817.85	0.0609	8,647.99	0.0087
2FI[a] *vs.* linear	18.75	0.7118	455.62	0.8094	1,457.86	0.3927
Quadratic *vs.* 2FI	81.69	0.0487	3,517.22	0.0023	3,032.47	0.0092
Cubic *vs.* quadratic	23.69	0.0667	238.23	0.0899	385.41	0.0628
Residual	1.12		15.44		17.04	
Total	1,282.11		33,962.49		83,100.16	
Lack of fit						
Linear	124.13	0.0395	4,211.07	0.0163	4,875.74	0.0156
2FI	105.38	0.0311	3,755.45	0.0122	3,417.88	0.0148
Quadratic	23.69	0.0667	238.23	0.0899	385.41	0.0628
Cubic	0.000		0.000		0.000	
Pure error	1.12		15.44		17.04	

	R^2	R_a^2	PRESS	R^2	R_a^2	PRESS	R^2	R_a^2	PRESS
R^2 analysis									
Linear	0.619	0.5150	258.03	0.475	0.3313	6,764.99	0.637	0.5401	8,608.39
2FI	0.676	0.4330	524.30	0.531	0.1797	10,468.81	0.746	0.5561	10,430.84
Quadratic	0.925	0.7886	381.63	0.968	0.9117	3,846.43	0.970	0.9168	6,204.88
Cubic	0.997	0.9762	ND[b]	0.998	0.9866	ND	0.999	0.9912	ND

[a] Two-factor interaction
[b] PRESS statistic not defined

Limited, Essex, UK). Cumulative percentage of drug released from the tablets were calculated and plotted as a function of time.

Kinetic Analysis of the Release Data

The mean *in vitro* drug release data were fitted to different kinetic models, namely zero-order kinetics (19), first order (20), Higuchi (21), and Korsmeyer-Peppas (22) using regression analysis to evaluate the kinetics of drug release from the prepared matrices. The model which shows the highest value of coefficient of determination (R^2) was selected as the best model that describes the kinetics of drug release.

Stability Study

According to the ICH guidelines the tablets of the optimized formula were exposed to 6 months accelerated stability study at 40°C/75% RH (23). At the end of 1, 3, and 6 months, the tablets were subjected to visual observation to detect any physical changes and evaluated regarding drug content and *in vitro* release.

Table IV. Standardized Main Effects of the Factors on the Responses

	Y_1			Y_2			Y_3		
	Coefficient estimate	P value	SME[a]	Coefficient estimate	P value	SME	Coefficient estimate	P value	SME
b_0	9.72	0.0240	7.56	69.17	0.0030	16.82	93.03	0.0026	17.96
b_1	−1.32	0.1535	−1.68	0.42	0.8738	0.17	10.64	0.0202	3.35
b_2	−4.33	0.0027	−5.50	−18.85	0.0007	−7.48	−25.19	0.0005	−7.94
b_3	2.21	0.0375	2.81	11.04	0.0071	4.38	18.25	0.0022	5.75
b_{12}	1.65	0.1975	1.49	5.62	0.1754	1.58	8.78	0.1076	1.96
b_{13}	−1.00	0.4109	−0.90	1.32	0.7266	0.37	2.52	0.5984	0.56
b_{23}	0.98	0.4214	0.88	8.98	0.0531	2.52	16.76	0.0135	3.74
b_{11}	−4.14	0.0160	−3.57	−27.55	0.0007	−7.43	−25.85	0.0026	−5.54
b_{22}	1.78	0.1854	1.53	−10.38	0.0380	−2.80	−7.52	0.1683	−1.61
b_{33}	−0.91	0.4669	−0.79	−13.82	0.0136	−3.73	−13.38	0.0352	−2.87

[a] Standardized main effects (SME) were calculated by dividing the main effect by the standard error of the main effect

RESULTS AND DISCUSSION

Compatibility of Mesalazine with Different Excipients

Figure 1 shows the DSC thermograms of mesalazine alone and as physical mixtures with different pharmaceutical excipients. The DSC thermogram of mesalazine exhibited a sharp endothermic peak at 281°C indicating the melting point of the drug (24). There is no observed change in the endothermic peak of mesalazine in cases of drug mixtures with Eudragit® RS, croscarmellose sodium, or Eudragit® S. This result could suggest the absence of interaction between the drug with all the aforementioned excipients.

In the DSC thermogram of the physical mixture of mesalazine with Carbopol®, a broadening and decreased intensity of the endothermic peak of mesalazine was observed. This result could suggest interaction between mesalazine and Carbopol® (25). This interaction could be attributed to hydrogen bond formation between mesalazine and Carbopol® (26,27). However, this interaction between the drug and Carbopol could contribute to reduction of the dissolution rate, which could be considered as an advantage in formulation of a controlled-release delivery system (28,29).

In the case of the physical binary mixture of mesalazine and PVP, the typical melting peak for mesalazine was observed, but broadening and shift of the endothermic peak temperature to a lower temperature (from 281°C to 277°C) were observed. Similar results have been reported between nateglinide (30), glipizide (31), ibuproxan (32), and ibuprofen (33) with PVP in a physical mixture. This shift could be attributed to some solid–solid interaction, although it does not necessarily indicate any incompatibility (33,34). It was reported that minor changes in the melting endotherm of drug could be due to mixing of drug and excipient, which lowers the purity of each component in the mixture and may not necessarily indicate potential incompatibility (30,31).

Accordingly, for a better understanding of the changes in the binary mixtures, physical mixtures of mesalazine with Carbopol® and PVP were subjected to FT-IR studies and their spectra were compared to FT-IR spectrum of mesalazine. Figure 2 shows the infrared spectra of mesalazine, the used excipients, and the drug-excipients physical mixtures. The infrared spectrum of pure mesalazine exhibited the characteristic bands corresponding to the functional groups of the drug at 3,433 cm^{-1} (due to the mutual overlapping of NH and OH stretching), 1,651 cm^{-1} (corresponds to the C=O stretch), 1,620 cm^{-1} (corresponds to NH bending), and 1,354 cm^{-1} (corresponds to CN stretching). The bands in a range of 2,000–3,000 cm^{-1} correspond to the stretching vibrations of the hydrogen bonds in the mesalazine molecule (35). It was remarked that all the spectra of the mixtures exhibited the characteristic bands of the drug. This indicates that there is no change in the drug structure and the absence of chemical interaction between mesalazine and these excipients. The compatibility between mesalazine and the selected excipients will be further investigated by carrying out stability studies on the optimized formulation.

Characterization of Core Tablets

All the prepared tablets were found to be of good quality with acceptable physical characteristics. The hardness was found to vary between 10 and 11 kg. Friability in all the formulations was less than 0.9%. Drug content varied with ±5% of the theoretical value (1,200 mg) for all formulations.

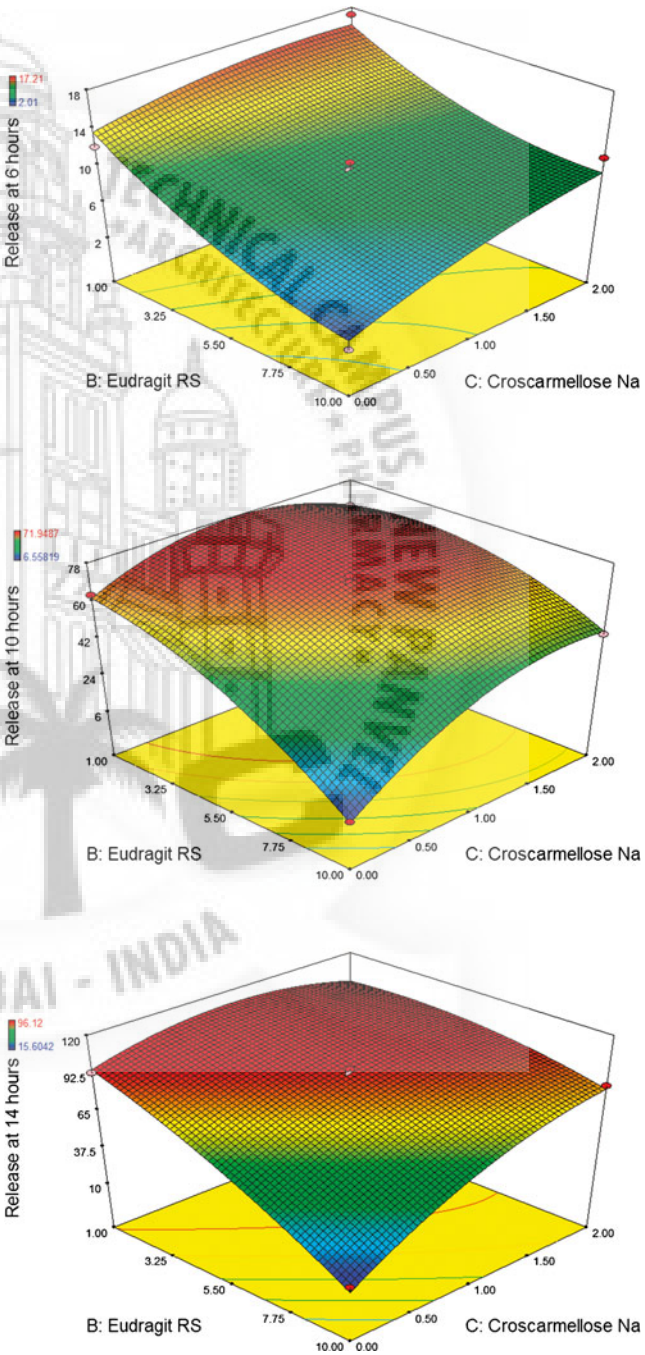

Fig. 4. Effect of the contents of Eudragit® RS (X_1) and croscarmellose Na (X_2) on responses using response surface and contour plots at 4% Carbopol® content

Preparation of Mesalazine Tablets

Because of the high loading of mesalazine (1,200 mg/tablet), we aimed to develop a formulation containing polymers and other excipients at amounts as little as possible, as well as releasing its content in a extended release profile over the specified length of time, and preferably with a zero-order kinetic. Hydrophobic insoluble polymer (36) is a good choice to address all of these requirements. Eudragit® RS is composed of poly (ethylacrylate-methylmethacrylate-trimethylammonioethyl methacrylate chloride) copolymers. Eudragit® RS is a hydrophobic, water-insoluble polymer, and pH-independent sustained drug release profile is exhibited by drug delivery systems prepared using it (37). It has been widely applied in modified release formulations (38–41). In the production of matrix tablets Eudragit® RS has the advantages of excellent compression properties, being suitable for producing tablets using all common process technologies, good binding properties, thermostability, thermoplastic properties, and plastic properties. The plastic properties of Eudragit® RS produce stable characteristics across a range of relevant production parameters such as compression force (42). Such property give rise to similar dissolution profiles for tablets produced at different compression forces.

Carbopol® is a hydrophilic polyacrylic acid polymer which has gel-forming and bioadhesive properties. Due to the chemical nature of Carbopol® polymers, swelling of the polymer occurs in the pH range 5–9, as a result of ionization of the carboxylic acid groups that lead to electronic repulsion of the polymer (43). Such pH-dependent swelling behavior of Carbopol® suggests that it is a good choice ingredient to be included in colon-targeting delivery systems.

A superdisintegrant, croscarmellose sodium, was incorporated extragranularly to assist the breakdown of tablets into smaller granules or fragments and thus, ensure more uniform distribution of mesalazine throughout the colon.

Wet granulation technique, although more time consuming than direct compression (44), was employed in this study because of the high load of the drug which has poor flowability and compressibility as observed in the preliminary trials.

Eudragit S is methylacrylic acid–methylmethacrylate copolymers, which tends to dissolve at pH higher than 7. This makes it a suitable coating material for the colonic drug delivery (45). After application of Eudragit® S coating, all the evaluated formulations released less than 1% of mesalazine in the first two stages of the release studies.

Determination of the Regression Model and Statistical Evaluation

Box–Behnken design is suitable for investigating the quadratic response surfaces and for constructing a second-order polynomial model. Consequently, statistical optimization of the pharmaceutical dosage form could be performed using a small number of experiments runs (15 runs) (46). The experiment runs with independent variables and the observed responses for the 15 formulations are shown in Fig. 3 and Table II. The selection of the best fitting mathematical model involving the individual main effects and interaction factors was based on the comparison of some statistical parameters including the multiple correlation coefficient (R^2), adjusted multiple correlation coefficient (R_a^2), and the predicted residual sum of squares (PRESS), provided by the Design-Expert software. As shown in Table III, the quadratic model was chosen because it had the smallest value of PRESS. Predicted residual sum of squares indicates how well the model fits the data. The smaller the PRESS statistic is, the better the model fits to the data points (47). Additionally, the quadratic model showed a statistically insignificant lack of fit ($P>0.05$). Analysis of variance was applied to estimate the significance of the model at the 5% significance level. The nonlinear computer-generated quadratic model is given as Eq. 1:

$$Y = b_o + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

Where Y is the measured response associated with each factor level combination; b_o is an intercept; b_1 to b_{33} are the estimated regression coefficients computed from the observed experimental values of Y; and X_1, X_2, and X_3 are the coded levels of independent variables. The terms X_iX_i and X_i^2 (i=1, 2, or 3) represent the interaction and quadratic terms, respectively (48).

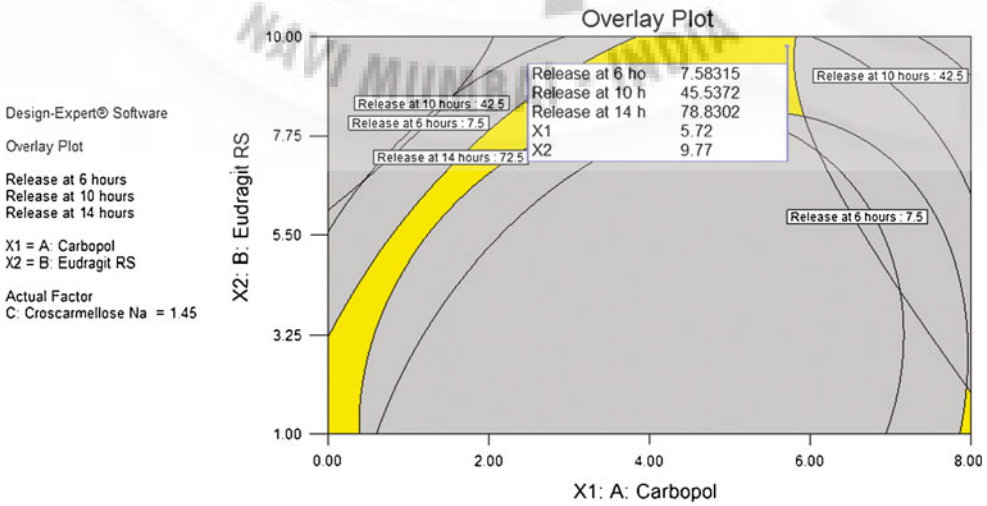

Fig. 5. Overlay plot for optimized variables

Table V. Comparative Levels of Predicted and Observed Responses for the Optimized Formulation

Responses (predicted, %)	Observed (%)	Predicted error[a] (%)
Y_1 (7.58)	8	5.54
Y_2 (45.54)	46.2	1.45
Y_3 (78.83)	82.3	4.40

[a] Predicted error (%)=(observed value−predicted value)/predicted value×100%

The coefficient estimate and standardized main effects (SME) values in the form of a polynomial equation for the responses are listed in Table IV. SME values were calculated by dividing the main effects by the standard error of the main effects (49).

From Table IV, it could be inferred that Eudragit® RS (X_2) and croscarmellose sodium (X_3) were significant in controlling the release of mesalazine throughout the dissolution time ($p \leq 0.05$). In addition, Eudragit® RS content (X_2) showed the largest SME (−5.50, −7.48, and −7.94 at Y_1, Y_2, and Y_3, respectively) indicating that Eudragit® RS content (X_2) was the main influential factor on drug release from the tested tablets in the whole stages of mesalazine *in vitro* release studies.

Figure 4 depicts the contour and three-dimensional response surface plots which show the effects of the independent variables on each response. Analysis of Fig. 4 shows that on increasing Eudragit® RS form 1% to 10% a decrease in drug release was observed at Y_1, Y_2, and Y_3. Such finding was as expected and is in agreement with the findings of many previous reports (41,50,51). These results stem from the fact that Eudragit® RS is insoluble in aqueous media and acts as a shield preventing the penetration of the dissolution medium into the tablets and mesalazine from dissolution (52).

As shown in Table IV, the effect of Carbopol® became only significant (P=0.02) at 14 h (Y_3). Also, the coefficient of b_1 is 10.64 (bearing positive sign) for Y_3 indicating that increasing Carbopol® content augments mesalazine release. This could be attributed to the fact that Carbopol® needs water to swell (43). Availability of water needed for Carbopol® to swell could be retarded by the coherent structure and hydrophobic nature of mesalazine–Eudragit® RS matrices (53).

Eudragit® RS matrix tablets could be thought as a coherent system in which the drug is dispersed. This structure is anticipated to be weakened by incorporating the water swellable polymer, Carbopol®, which swell in water up to 1,000 times its original volume (and 10 times its original diameter) to form a gel when exposed to a pH environment above 4.0 to 6.0 (54). Swelling is suggested to decrease the strength of the matrix and assist the drug leaking out. It is worthy to mention that in Carbopol®-containing tablets, a faster gradual detachment of smaller granules from the core was observed to take place with time during dissolution studies. A consequent increase in drug release is suspected due to the greater surface area available for the dissolution media. However, such effect was expected to be opposed by the formation of a viscous gel layer on the surface of the granules which is postulated to hinder drug release (55). The net effect of Carbopol® depends on which effect is predominant. At the low concentrations used in this study, Carbopol® enhanced mesalazine release from the detached granules. Such finding met that reported by Haney and Dash (56).

Although the correlation between tablet disintegration and drug dissolution is not always observable (57), analysis of Fig. 4 demonstrates that increasing croscarmellose sodium from 0% to 2% led to an increase in mesalazine release at Y_1, Y_2, and Y_3. This result could be attributed to the detachment of granules that was aided by the inclusion of the super-disintegrant croscarmellose sodium into the tablets; hence, the release rate was increased by increasing the surface exposed to the dissolution medium (57,58).

Optimization of Drug Release and Validation of Optimized Formulation

After generating the polynomial equations relating the dependent and independent variables, the release profile was optimized for the responses Y_1, Y_2, and Y_3. The desirable range of these responses was restricted to the values listed in Table I. The optimum values of the variables were obtained by graphical and numerical analyses using the Design-Expert software and based on the criterion of desirability (59,60). Figure 5 represents an overlay plot showing the optimized parameters suggested by the software to get the responses in the required range. The optimized formulation was achieved with 5.72% Carbopol®, 9.77% Eudragit® RS, and 1.45% croscarmellose sodium. To check the validity of the optimization procedure, a new batch of mesalazine tablets with the predicted levels of formulation factors was prepared. Table V illustrates the predicted and observed responses for the optimum formulation. The observed values of Y_1, Y_2, and Y_3 were in a very close agreement to the predicted ones. By this the validity of the optimization procedure was proven. Figure 6 demonstrated that the optimized formulation prepared according to computer-determined levels exhibited a release profile which was close to that of the ideal targeted release profile. Additionally, these dissolution profiles were compared using two fit factors, difference factor (f_1) and similarity factor (f_2). The calculated values of f_1 and f_2 were 8.47 and 67.70, respectively. Such values indicate that the release profiles of the optimized formulation and that of the ideal targeted release profile were similar.

Three kinetic models were applied to study the kinetics of mesalazine release from all the prepared formulations as well as from the optimized formula. Drug release kinetic parameters are presented in Table VI. As shown in Table VI, zero-order kinetic model gave the highest value of the coefficient of determination (R^2) for optimized formula (0.9974), indicating that zero-order kinetic model would be the most suitable model for describing the release of mesalazine.

The *in vitro* release profiles were further studied in terms of three time-based parameters; $t_{20\%}$, $t_{50\%}$, and $t_{80\%}$ values (time required for 20%, 50%, and 80% of drug release, respectively). It was suggested that a $t_{20\%}$ >6 h ensures that less than 20% of drug could be released during the initial gastrointestinal transit while $t_{50\%}$ of 10–11 h and $t_{80\%}$ <14 ensure that 50% of the drug could be released in the ascending and transverse colon and drug release could be completed in 14–16 h during the expected residence time of the dosage form in colon (61). The optimized formula exhibited $t_{20\%}$, $t_{50\%}$, and $t_{80\%}$ values of 7.12, 10.47, and

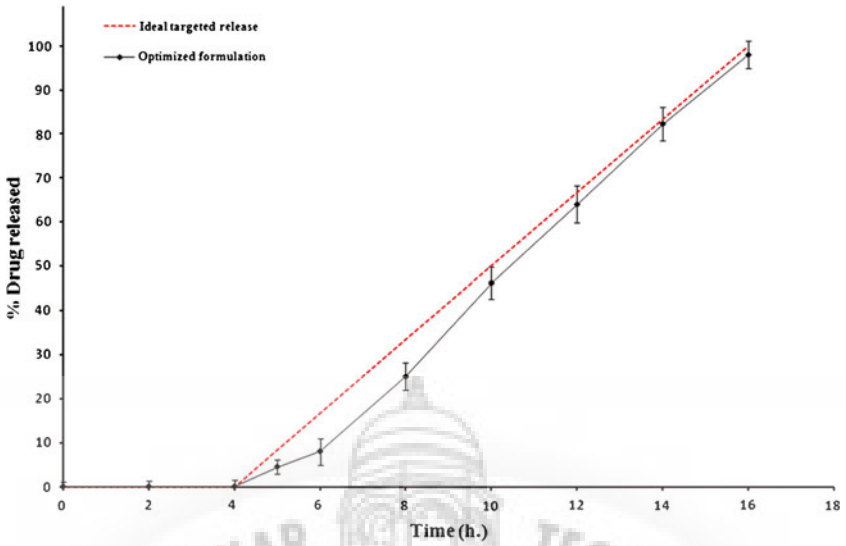

Fig. 6. *In vitro* release profiles of mesalazine for optimized formula and ideal targeted release profiles. Each data point is expressed as mean ± SD (n=3)

13.82 h, respectively, that were within the suggested range regarding these parameters (Table VI).

Stability Study

Neither physical changes nor significant changes in drug content of tablets from the optimized formula had been detected on storage and drug content of the tested tablets was found to be 98.77±0.46%. This result comes in agreement with previous report in which the decrease in the content of mesalazine did not exceed 1% in solid dosage forms even placed in stressed conditions for a period up to 2 years (62). The pair-wise procedures indicated statistically insignificant difference in the *in vitro* drug release profiles from the fresh and stored tablets of the optimized formula. Such results suggest the compatibility between mesalazine and the used excipients and the high stability of mesalazine in the optimized enteric coated tablets.

CONCLUSION

The optimized hydrophilic–hydrophobic, high-loading mesalazine matrix tablets demonstrated a controlled drug release manner potentially suitable for once daily administration. The optimized formulation, containing 5.72% Carbopol®, 9.77% Eudragit® RS, and 1.45% croscarmellose

Table VI. Release Rate Kinetics for the Box–Behnken and Optimized Formulations

Run	R^2				Mechanism	$t_{20\%}$[e]	$t_{50\%}$[e]	$t_{80\%}$[e]
	Zero order[a]	1st order[b]	Diffusion[c]	Peppas[d]				
Run1	0.9809	0.9992	0.9965	0.9743	1st order	8.02	13.23	23.38
Run2	0.9755	0.9187	0.9592	0.9932	Peppas	7.18	8.95	10.02
Run3	0.9746	0.9788	0.9853	0.9844	Diffusion	14.40	45.72	97.96
Run4	0.9974	0.9747	0.9900	0.9960	Zero order	9.74	15.16	20.57
Run5	0.9961	0.9390	0.9966	0.9910	Diffusion	5.99	8.51	12.05
Run6	0.9855	0.9834	0.9806	0.9894	Peppas	9.50	15.04	19.79
Run7	0.9928	0.9543	0.9861	0.9921	Zero order	6.22	9.56	12.91
Run8	0.9856	0.9229	0.9618	0.9921	Peppas	8.12	10.44	12.10
Run9	0.9845	0.9537	0.9776	0.9892	Peppas	6.99	9.97	12.51
Run10	0.9991	0.9499	0.9929	0.9965	Zero order	7.36	10.76	14.17
Run11	0.9961	0.9282	0.9715	0.9989	Peppas	7.30	10.76	13.77
Run12	0.9576	0.9485	0.9016	0.9813	Peppas	14.97	22.72	28.62
Run13	0.9878	0.9811	0.9923	0.9805	Diffusion	15.03	51.33	112.94
Run14	1.0000	0.9590	0.9977	0.9798	Zero order	7.42	10.38	13.34
Run15	0.9956	0.8970	0.9798	0.9970	Peppas	6.68	9.67	12.33
Optimized formulation	0.9974	0.9346	0.9917	0.9889	Zero order	7.12	10.47	13.82

M_t/M_∞ the fraction of drug released up to time t, k the kinetic constant, C constant
[a] Zero order: $M_t/M_\infty = kt + C$
[b] First order: $M_t/M_\infty = 1 - e^{-kt}$
[c] Higuchi: $M_t/M_\infty = k(t)^{0.5} + C$
[d] Korsmeyer-Peppas: $M_t/M_\infty = kt^n + C$
[e] $t_{20\%}$, $t_{50\%}$, and $t_{80\%}$ are the times required for 20%, 50%, and 80% of drug release, respectively

sodium in addition to other excipients, was fabricated utilizing the simple wet granulation technique and produced a zero-order drug release profile over a period of 16 h after a lag time of 4 h. This release profile was similar to that of the ideal target release model deduced from the dissolution profile of a marketed once daily tablet of mesalazine.

REFERENCES

1. Lichtenstein GR, Kamm MA. Review article: 5-aminosalicylate formulations for the treatment of ulcerative colitis-methods of comparing release rates and delivery of 5-aminosalicylate to the colonic mucosa. Aliment Pharmacol Ther. 2008;28:663–73.
2. Dhaneshwar SS, Chail M, Patil M, Naqvi S, Vadnerkar G. Colon-specific mutual amide prodrugs of 4-aminosalicylic acid for their mitigating effect on experimental colitis in rats. Eur J Med Chem. 2009;44:131–42.
3. Mladenovska K, Raicki RS, Janevik EI, Ristoski T, Pavlova MJ, Kavrakovski Z, *et al*. Colon-specific delivery of 5-aminosalicylic acid from chitosan-Ca-alginate microparticles. Int J Pharm. 2007;342:124–36.
4. Sweetman SC. Martindale, The Complete Drug Reference. 36 ed: Pharmaceutical Press; 2009.
5. Kedia P, Cohen RD. Once-daily MMX mesalamine for the treatment of mild-to-moderate ulcerative colitis. Ther Clin Risk Manag. 2007;3:919–27.
6. Tenjarla S, Romasanta V, Zeijdner E, Villa R, Moro L. Release of 5-aminosalicylate from an MMX mesalamine tablet during transit through a simulated gastrointestinal tract system. Adv Ther. 2007;24:826–40.
7. Michael AK, William JS, Miguel G, Stefan S, Lechoslaw J, Todd B, *et al*. Once-daily, high-concentration MMX Mesalamine in active ulcerative colitis. Gastroenterology. 2007;132:66–75.
8. Hu MY, Peppercorn MA. MMX mesalamine: a novel high-dose, once-daily 5-aminosalicylate formulation for the treatment of ulcerative colitis. Expert Opin Pharmacother. 2008;9:1049–58.
9. Lakatos PL, Lakatos L. Once daily 5-aminosalicylic acid for the treatment of ulcerative colitis; are we there yet? Pharmacol Res. 2008;58:190–5.
10. Cada D, Baker D, Levien T. Formulary drug reviews—mesalamine. Hospital Pharmacy. 2007;42:543–52.
11. Karrout Y, Neut C, Wils D, Siepmann F, Deremaux L, Flament MP, *et al*. Novel polymeric film coatings for colon targeting: drug release from coated pellets. Eur J Pharm Sci. 2009;37:427–33.
12. Akhgari A, Afrasiabi Garekani H, Sadeghi F, Azimaie M. Statistical optimization of indomethacin pellets coated with pH-dependent methacrylic polymers for possible colonic drug delivery. Int J Pharm. 2005;305:22–30.
13. Chaudhary H, Kohli K, Amin S, Rathee P, Kumar V. Optimization and formulation design of gels of Diclofenac and Curcumin for transdermal drug delivery by Box-Behnken statistical design. J Parm Sci. 2011;100:580–93.
14. Ibekwe VC, Fadda HM, Parsons GE, Basit AW. A comparative *in vitro* assessment of the drug release performance of pH-responsive polymers for ileo-colonic delivery. Int J Pharm. 2006;308:52–60.
15. Akhgari A, Sadeghi F, Garekani HA. Combination of time-dependent and pH-dependent polymethacrylates as a single coating formulation for colonic delivery of indomethacin pellets. Int J Pharm. 2006;320:137–42.
16. Piao ZZ, Lee MK, Lee BJ. Colonic release and reduced intestinal tissue damage of coated tablets containing naproxen inclusion complex. Int J Pharm. 2008;350:205–11.
17. Alvarez-Fuentes J, Fernandez-Arevalo M, Gonzalez-Rodriguez ML, Cirri M, Mura P. Development of enteric-coated timed-release matrix tablets for colon targeting. J Drug Target. 2004;12:607–12.
18. Asghar LF, Chandran S. Design and evaluation of matrices of Eudragit with polycarbophil and carbopol for colon-specific delivery. J Drug Target. 2008;16:741–57.
19. Harland RS, Dubernet C, Benoît J-P, Peppas NA. A model of dissolution-controlled, diffusional drug release from non-swellable polymeric microspheres. J Control Release. 1988;7:207–15.
20. Vueba ML, Batista de Carvalho LA, Veiga F, Sousa JJ, Pina ME. Influence of cellulose ether polymers on ketoprofen release from hydrophilic matrix tablets. Eur J Pharm Biopharm. 2004;58:51–9.
21. Higuchi T. Mechanism of sustained-action medication. theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci. 1963;52:1145–9.
22. Ritger PL, Peppas NA. A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. J Control Release. 1987;5:37–42.
23. Stability testing of new drug substances and products Q1A (R2), International Conference on Harmonization. 2003.
24. Dash AK, Brittain HG. Mesalamine. In: Harry GB, editor. Analytical profiles of drug substances and excipients: Academic Press; 1998. p. 209–242.
25. Lin S-Y, Chen K-S, Lin Y-Y. pH of preparations affecting the on-off drug penetration behavior through the thermo-responsive liquid crystal-embedded membrane. J Control Release. 1998;55:13–20.
26. Barreiro-Iglesias R, Alvarez-Lorenzo C, Concheiro A. Thermal and FTIR characterization of films obtained from carbopol/surfactant aqueous solutions. J Therm Anal Calorim. 2002;68:479–88.
27. Ozawa M, Hasegawa K, Yonezawa Y, Sunada H. Preparation of solid dispersion for Ethenzamide—Carbopol and Theophylline—Carbopol systems using a twin screw extruder. Chem Pharm Bull (Tokyo). 2002;50:802–7.
28. French DL, Mauger JW. Evaluation of the physicochemical properties and dissolution characteristics of mesalamine: relevance to controlled intestinal drug delivery. Pharm Res. 1993;10:1285–90.
29. Elkheshen SA. Interaction of verapamil hydrochloride with Carbopol 934P and its effect on the release rate of the drug and the water uptake of the polymer matrix. Drug Dev Ind Pharm. 2001;27:925–34.
30. Pani N, Nath L, Acharya S, Bhuniya B. Application of DSC, IST, and FTIR study in the compatibility testing of nateglinide with different pharmaceutical excipients. J Therm Anal Calorim. 2011:1–8.
31. Verma RK, Garg S. Selection of excipients for extended release formulations of glipizide through drug-excipient compatibility testing. J Pharm Biomed Anal. 2005;38:633–44.
32. Mura P, Faucci MT, Manderioli A, Bramanti G, Ceccarelli L. Compatibility study between ibuproxam and pharmaceutical excipients using differential scanning calorimetry, hot-stage microscopy and scanning electron microscopy. J Pharm Biomed Anal. 1998;18:151–63.
33. Novoa GAG, Heinämäki J, Mirza S, Antikainen O, Iraizoz Colarte A, Suzarte Paz A, *et al*. Physical solid-state properties and dissolution of sustained-release matrices of polyvinylacetate. Eur J Pharm Biopharm. 2005;59:343–50.
34. Sekizaki H, Danjo K, Eguchi H, Yonesawa Y, Sunada H, Otsuka A. Solid-state interaction of ibuprofen with polyvinylpyrrolidone. Chem Pharm Bull (Tokyo). 1995;43:988–93.
35. Mladenovska K, Cruaud O, Richomme P, Belamie E, Raicki RS, Venier-Julienne MC, *et al*. 5-ASA loaded chitosan-Ca-alginate microparticles: preparation and physicochemical characterization. Int J Pharm. 2007;345:59–69.
36. Genc L, Bilac H, Guler E. Studies on controlled release dimenhydrinate from matrix tablet formulations. Pharm Acta Helv. 1999;74:43–9.
37. Fujimori J, Yoshihashi Y, Yonemochi E, Terada K. Application of Eudragit RS to thermo-sensitive drug delivery systems: II. Effect of temperature on drug permeability through membrane consisting of Eudragit RS/PEG 400 blend polymers. J Control Release. 2005;102:49–57.
38. Socha M, Sapin A, Damge C, Maincent P. Influence of polymers ratio on insulin-loaded nanoparticles based on poly-epsilon-caprolactone and Eudragit RS for oral administration. Drug Deliv. 2009;16:430–6.

39. Maghsoodi M, Esfahani M. Preparation of microparticles of naproxen with Eudragit RS and Talc by spherical crystallization technique. Pharm Dev Technol. 2009;14:442–50.
40. Kim KS, Park SJ. Characterization and release behaviors of porous PCL/Eudragit RS microcapsules containing tulobuterol. Colloids Surf B Biointerfaces. 2010;76:404–9.
41. Apu AS, Pathan AH, Shrestha D, Kibria G, Jalil RU. Investigation of *in vitro* release kinetics of carbamazepine from Eudragit (R) RS PO and RL PO matrix tablets. Tropical Journal of Pharm Res. 2009;8:145–52.
42. McGinity JW, Cameron CG, Cuff GW. Controlled-release theophylline tablet formulations containing acrylic resins. I. Dissolution properties of tablets. Drug Dev Ind Pharm. 1983;9:57–68.
43. Khan GM, Zhu JB. Studies on drug release kinetics from ibuprofen-carbomer hydrophilic matrix tablets: influence of co-excipients on release rate of the drug. J Control Release. 1999;57:197–203.
44. Augsburger LL, Zellhofer MJ. Tablet Formulation. Encyclopedia of Pharmaceutical Technology: Third Edition. 2006:3641–3652.
45. Chan WA, Boswell CD, Zhang Z. Comparison of the release profiles of a water soluble drug carried by Eudragit-coated capsules in different *in-vitro* dissolution liquids. Powder Technology. 2001;119:26–32.
46. Kramar A, Turk S, Vrecer F. Statistical optimisation of diclofenac sustained release pellets coated with polymethacrylic films. Int J Pharm. 2003;256:43–52.
47. Kim MS, Kim JS, You YH, Park HJ, Lee S, Park JS, *et al*. Development and optimization of a novel oral controlled delivery system for tamsulosin hydrochloride using response surface methodology. Int J Pharm. 2007;341:97–104.
48. Gannu R, Palem CR, Yamsani VV, Yamsani SK, Yamsani MR. Enhanced bioavailability of lacidipine via microemulsion based transdermal gels: formulation optimization, ex vivo and *in vivo* characterization. Int J Pharm. 2010;388:231–41.
49. Chopra S, Patil GV, Motwani SK. Release modulating hydrophilic matrix systems of losartan potassium: optimization of formulation using statistical experimental design. Eur J Pharm Biopharm. 2007;66:73–82.
50. Tsai T, San Y-P, Ho H-O, Wu J-S, Sheu M-T. Film-forming polymer-granulated excipients as the matrix materials for controlled release dosage forms. J Control Release. 1998;51:289–99.
51. Sadeghi F, Afrasiabi Garekani H, Goli F. Tableting of Eudragit RS and propranolol hydrochloride solid dispersion: effect of particle size, compaction force, and plasticizer addition on drug release. Drug Dev Ind Pharm. 2004;30:759–66.
52. Aceves JM, Cruz R, Hernandez E. Preparation and characterization of Furosemide-Eudragit controlled release systems. Int J Pharm. 2000;195:45–53.
53. Grassi M, Grassi G. Mathematical modelling and controlled drug delivery: matrix systems. Curr Drug Deliv. 2005;2:97–116.
54. Shah RB, Nutan M, Reddy IK, Khan MA. A dual controlled gastrointestinal therapeutic system of salmon calcitonin. II. Screening of process and formulation Variables. Clin Res Regul Aff. 2004;21:231–8.
55. French DL, Himmelstein KJ, Mauger JW. Physicochemical aspects of controlled release of substituted benzoic and naphthoic acids from Carbopol® gels. J Control Release. 1995;37:281–9.
56. Haney PW, Dash AK. Simple liquid chromatographic method for the analysis of 5-aminosalicylic acid and its degradation product. J Chromatogr A. 1997;765:233–9.
57. Johnson JR, Wang LH, Gordon MS, Chowhan ZT. Effect of formulation solubility and hygroscopicity on disintegrant efficiency in tablets prepared by wet granulation, in terms of dissolution. J Pharm Sci. 1991;80:469–71.
58. Gorman EA, Rhodes CT, Rudnic EM. An evaluation of croscarmellose as a tablet disintegrant in direct compression systems. Drug Dev Ind Pharm. 1982;8:397–410.
59. Liu Y, Zhang P, Feng N, Zhang X, Wu S, Zhao J. Optimization and *in situ* intestinal absorption of self-microemulsifying drug delivery system of oridonin. Int J Pharm. 2009;365:136–42.
60. Myers RH, Montgomery DC. Response Surface Methodology. 1995.
61. Chandran S, Sanjay KS, Ali Asghar LF. Microspheres with pH modulated release: design and characterization of formulation variables for colonic delivery. J Microencapsul. 2009;26:420–31.
62. Jensen J, Cornetta C, Olsenc CE, Tjørnelunda J, Hansen SH. Identification of major degradation products of 5-aminosalicylic acid formed in aqueous solutions and in pharmaceuticals. Int J Pharm. 1992;88:177–87.

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Research paper

Evaluation of alginate based mesalazine tablets for intestinal drug delivery

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Abstract

The aim of this study was to develop the alginate based mesalazine tablets for intestinal delivery. Sodium alginate is a biocompatible, natural polymer with pH-sensitive gel-forming ability.

Matrix tablets were prepared with two types of sodium alginate of different amounts. The in vitro release characteristics of mesalazine from alginate tablets were compared with those of the commercial product (Salofalk®). X-ray imaging was used to monitor the tablets throughout the gastrointestinal system.

Although alginate tablets gave a faster release in an acidic medium compared with the commercial product (Salofalk®), the cumulative amount of released drug of the optimum formulation was found to be almost the same as that of the commercial product at the end of 4 h. The alginate type and amount in the matrices played an important role in basic media. The release of the optimum formulation containing low viscosity alginate was found to be almost identical to that of the commercial product in acidic and basic media.

Tablets were visualized to determine whether they were located in the terminal ileum or cecum for 3–6 h. Mesalazine-alginate matrix tablet formulations can deliver the drug to the small and large intestine. Thus, the alginate matrix system may be a promising system for the treatment of Crohn's disease involving both the ileum and large intestine.

Keywords: Mesalazine; Alginate; Intestinal drug delivery; X-ray imaging

1. Introduction

Oral administration is the most convenient and preferred means of drug delivery into systemic circulation [1]. For a number of drugs this approach is generally adequate. In some situations it would be highly beneficial to target a drug to a particular site within the gastrointestinal tract, either to maximize therapeutic response or to reduce side effects caused by drug delivery to an inopportune region of the gut. In recent years there has been a significant increase in available strategies for providing site-specific delivery in the gastrointestinal tract [2].

The natural pH environment of the gastrointestinal tract varies from acidic to slightly alkaline. pH-sensitive hydrogels may be an alternative for site specific drug delivery. In the design of oral delivery of peptide or protein drugs, pH-sensitive hydrogels have attracted increasing attention [3]. Swelling of such hydrogels in the stomach is minimal, and thus the drug release is also minimal. The extent of swelling increases as hydrogels pass down the intestinal tract due to the increase in pH. A variety of synthetic or natural polymers with acidic or basic pendent groups have been employed to fabricate pH-sensitive hydrogels. Among them, alginate is one of the more commonly used [4].

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Alginates, which are naturally occurring substances found in brown seaweed and algae, have received much attention for use in pharmaceutical dosage forms, particularly as a vehicle for controlled drug delivery. The formation of a matrix upon hydration causes a gelatinous layer which can act as a drug diffusion barrier [5,6]. Alginate is a family of polysaccharides composed of α-L-guluronic acid and β-D-mannuronic acid residues, arranged in homopolymeric blocks of each type and in heteropolymeric blocks. The alginate monomer composition is reported to have a major impact on the drug release properties of the different formulation systems [7].

Liew et al. [8] used 17 grades of sodium alginate with different properties to screen the factors influencing drug release. It was reported that alginate particle size, viscosity and concentration affect not only the rate of drug release, but also the release mechanism [8].

Mesalazine, or 5-aminosalicylic acid (5-ASA), has been used for several years in the treatment of inflammatory bowel disease. When pure mesalazine is administered directly in the proximal part of the small bowel or orally as a conventional tablet, it is rapidly and almost completely absorbed, with little drug reaching the distal small intestine and colon [9]. Therefore the premature absorption of mesalazine can be prevented by the preparation of enteric coated tablets or colon-specific dosage forms. Orally administered delayed-release mesalazine acts locally from within the lumen of the inflamed bowel and is partly absorbed into systemic circulation. To prevent proximal small-intestinal absorption, and allow mesalazine to reach the inflamed small bowel and/or colon, a variety of mesalazine delivery systems have been developed [9,10].

5-ASA can be effective in treating Crohn's disease and ulcerative colitis if the drug can be delivered topically onto the inflamed intestinal lining. Most patients with Crohn's disease involving both the ileum (distal small intestine) and large intestine must take 5-ASA orally [11,12]. There are several formulations of mesalazine. One of them is the Salofalk® tablet which has an outer coating with a semi-permeable membrane of ethylcellulose and an inner coating of acrylic resin (Eudragit L). This pH-sensitive polymer is resistant to gastric conditions but soluble above pH 6.0 in the intestine. In this formulation mesalazine is designed to be released in highly dispersed form in the distal small bowel and the colon [13].

The aims of the present study were to develop a site specific matrix tablet of mesalazine with sodium alginate and to investigate the in vitro release characteristics of the tablets and to compare them with those of the commercial product (Salofalk®). The developed dosage form was also monitored in vivo in healthy volunteers. The transit of drug delivery systems throughout the gastrointestinal tract was monitored in vivo either in animals using various techniques [14,15] or in humans using γ-scintigraphy [16,17] or X-ray studies [18–20]. X-ray imaging was used in the present study. To our knowledge no in vivo X-ray study has been performed with alginate tablets.

2. Materials and methods

2.1. Materials

The mesalazine was a kind gift of the Ali Raif Drug Co., Turkey. Two different grades of sodium alginate, namely Protanal LF 240 D and Protanal LF 120 M, were kindly supplied by FMC Biopolymer (Switzerland). Viscosity measurements of 1% w/v aqueous dispersion of the polymers were carried out using a Brookfield viscometer (Brookfield Model LVTD, USA) at 25 °C. The viscosities of the alginates were 1600 cPs and 720 cPs for protonal LF 240 D and LF 120 M, respectively. Other materials, namely microcrystalline cellulose (Avicel PH 102) (FMC Biopolymer, Switzerland), magnesium stearate (Riedel Mannouen, Germany), silicon dioxide (Aerosil 200) (Werksboschemigung, Germany) and barium sulphate (Opti-Up, Lafayette Pharmaceuticals, USA), were of pharmacopeial quality (US/NF). The commercially available mesalazine product, Salofalk® (S®) (Batch No. 99F 10E), was kindly supplied by the Ali Raif Drug Co. (Turkey).

2.2. Preparation of mesalazine matrix tablets

Due to the poor flowability of the drug powder, matrix tablets of mesalazine were prepared using the slugging method.

Mesalazine, alginate and Avicel PH 102 were passed through a #45 (0.350 mm) mesh screen separately and blended for 20 min. The mixture was compacted in the Erweka tablet machine (Korsh-Erweka GmbH, Germany), using a 20 mm flat-faced punch. Slugged tablets were broken and passed through a #18 (1 mm) mesh screen. Then Aerosil 200 and magnesium stearate were added and mixed for an additional 5 min. Tablets were compressed, using the Erweka tablet machine with a 12 mm flat-faced punch. Each tablet (average weight of 660 mg) contained 250 mg of mesalazine. The compositions of the matrix tablets are given in Table 1. Barium sulphate was added to the final formulation (Tablet C) for in vivo studies.

2.3. Physical characteristics of the tablets

The tablets were characterized immediately after preparation. Twenty tablets were tested for weight (AB 104, Mettler Toledo, Switzerland), thickness (Vernier Caliper, portable dial hand micrometer, Russia), diametrical crushing force (CGS, Hardness tester HDT 1V-3, Germany) and friability (USP 27/Roche friability tester). The mean values were calculated with confidence intervals (CI).

The disintegration time of the tablets was determined using the compendial USP method with the disintegration apparatus (Aymes, Turkey). Six tablets were evaluated from each formulation. The apparatus was operated using simulated gastric fluid for 1 h. Then the dissolution medium was replaced with simulated intestinal fluid.

Table 1
Formulation of the tablets

Code	Mesalazine (mg)	Alginate (LF 240 D) (mg)	Alginate (LF 120 M) (mg)	Avicel PH102 (mg)	Aerosil 200 (mg)	Magnesium stearate (mg)	Barium sulphate (mg)
A1	250	350	–	48	5	6.5	–
A2	250	150	–	248	5	6.5	–
A3	250	75	–	323	5	6.5	–
B1	250	–	350	48	5	6.5	–
B2	250	–	250	148	5	6.5	–
B3	250	–	150	248	5	6.5	–
C	250	–	150	148	5	6.5	100

The drug content of the tablets was measured spectrophotometrically. For this purpose 10 tablets were individually weighed, and then each of them was dissolved at pH 7.4 in 150 mL buffer solution. Samples were assayed spectrophotometrically (Beckman DU-600, ABD) at wavelengths of 298 nm (pH = 1.2, 4,5) or 330 nm (pH = 6.8, 7.4). The spectrophotometric assay method was fully validated according to USP 27. The same experiments were carried out with the commercial product Salofalk®.

The results are shown in Table 2.

2.4. Drug release studies

Drug release from the tablet formulations was assessed using the flow-through dissolution apparatus at a flow rate of 8 ml/min, fitted with 22 mm dissolution cells (USP Aparatus IV, Sotax AG, Switzerland). Six tablets from each formulation were tested. The tablets were tested for drug release for 2 h in 0.1 M HCl, based on the assumption that the average gastric emptying time is about 2 h [21,22]. The following dissolution media were used: 2 h in pH 4.5, 2 h in pH 6.8 phosphate buffer and lastly, 2 h at pH 7.4 at 37 °C considering the pH of the GI tract [23–25].

The flow rate of 8 mL/min was chosen to keep the sink conditions during the dissolution test in all dissolution media. Mean data values are presented with their deviation (means ± SD). Following the drug release test for release comparison, analysis of variance (ANOVA) was used for all data analysis.

2.5. In vivo studies

X-ray imaging was used to monitor the tablets throughout the gastrointestinal system. Eight healthy volunteers, six female and two male, with a mean age of 29 years (range 22–40) and 50–80 kg body weight, participated in in vivo studies. They were non-alcoholics, non-smokers and had not taken any drugs. The purpose of the study had been fully explained, and all volunteers gave their written consent. Each subject orally ingested barium sulphate containing alginate matrix tablets with 200 ml of water, after an overnight fast. Abdominal radiographs were taken at fixed time intervals, and the tablets were visualized using X-ray imaging to establish whether they had reached the large intestine or not over 6 h. Volunteers were served with food after 2 h (breakfast) and 4 h (lunch) after the administration of the tablet.

In the present study, X-ray imaging was used on the tablets, in order to monitor the alginate matrix tablets throughout the gastrointestinal system and to test them in vivo. Meanwhile the fluoroscopy technique was also applied to subjects to ascertain where the tablets localized through the GI system. The position of the tablets in the body was monitored at different points in time.

The Ethics Committee of the Faculty of Medicine, Gazi University, in accordance with internationally accepted principles, had approved the experimental protocol (2001/5). Each volunteer received about 0.1 rem of radiation during the taking of the gastrointestinal X-ray radiograph. Normally, in a routine abdominal investigation

Table 2
The physical characteristics of the tablets

Code	Weight average (g) ± SD ($n = 20$)	Diameter average (mm) ± SD ($n = 20$)	Thickness average (mm) ± SD ($n = 20$)	Strength average (N) ± SD ($n = 20$)	Friability (%) ($n = 10$)	Disintegration time average (h) ± SD ($n = 6$)	Mesalazine content average ± SD ($n = 10$)
A1	0.648 ± 0.016	12.1 ± 0.0	4.40 ± 0.06	128.1 ± 0.8	0.38	3.30 ± 0.18	253 ± 5
A2	0.643 ± 0.058	12.1 ± 0.2	4.48 ± 0.09	132.9 ± 1.1	0.33	2.50 ± 0.33	248 ± 6
A3	0.661 ± 0.038	12.2 ± 0.2	4.53 ± 0.04	135.6 ± 0.7	0.36	2.15 ± 0.72	249 ± 3
B1	0.668 ± 0.047	12.1 ± 0.0	4.38 ± 0.03	123.5 ± 1.1	0.39	3.10 ± 0.81	245 ± 1
B2	0.670 ± 0.018	12.1 ± 0.1	4.43 ± 0.08	128.9 ± 1.5	0.32	2.35 ± 0.93	251 ± 0
B3	0.661 ± 0.003	12.0 ± 0.0	4.41 ± 0.03	130.6 ± 0.8	0.31	2.15 ± 0.14	250 ± 3
C	0.664 ± 0.081	12.0 ± 0.4	4.39 ± 0.06	133.9 ± 0.9	0.41	2.20 ± 0.22	250 ± 1
S®	0.515 ± 0.005	11.4 ± 0.0	5.64 ± 0.03	–	–	6.17 ± 0.27	256 ± 8

with barium sulphate, a patient receives 0.7 rem of radiation [26]. Therefore, the total radiation dose (about 0.5 rem) received by each volunteer was found not to be higher than that of standard abdominal radiography.

3. Results and discussion

All the tablet formulations were evaluated from the point of the view of the physical properties of the tablets (Table 2) and their in vitro releases. The tablet strengths were almost identical for all of the formulations, and the crushing forces were found to be in the range of 123.5–135.6 N (Table 2). The effect of alginate type and amount on the disintegration process was important as it starts to swell immediately on contact with water. The disintegration times of the tablets varied between 2.15 ± 0.72 to 3.30 ± 0.18 and 2.15 ± 0.14 to 3.10 ± 0.81 min for the formulations prepared with high and low viscosity alginate, respectively. As expected the increase in the amount of alginate delayed the disintegration time for both types of alginate (Table 2).

The mean drug content of all the mesalazine tablets was found to be in the range of 245–253 mg. This indicates that the tablets passed the content uniformity test, as they contained 98.1–102% of mesalazine.

The release profiles of the commercial product (Salofalk®) were investigated using the paddle or flow through dissolution methods [27,28]. Rudolf et al. [27] carried out the in vitro release of several mesalazine preparations and compared their multi-unit dosage forms. In that study the paddle method was used and dissolution studies were performed in different media such as pH 1.2, 4.5, 6.8 and 7.4. In our experiments flow-through cell apparatus was used since it was thought that in vivo GI transit conditions may be best imitated by in vitro flow-through cell apparatus using different but sequential pH media.

The in vitro release profiles of the tablets are shown in Figs. 1 and 2. As can be seen from Figs. 1 and 2 the amount of alginate was found to affect the drug release significantly between 4 and 8 h at pH 6.8 and 7.4 ($P < 0.05$), whereas there was no statistically significant effect for the first 4 h for the two different types of alginate ($P > 0.05$). Mesalazine has good solubility at both acidic and neutral pHs (10.2, 8.12, and 9.37 mg/mL at pH 1.2, 6.8 and 7.4, respectively) [29]; this would ensure that drug release is primarily dependent on the properties of the matrix and not on drug solubility.

When we compared the alginate types from the perspective of in vitro release profiles, no significant difference was found between the release of drug from the tablets prepared with two different types of alginate for the first 4 h at pH 1.2 and 4.5. The comparison of in vitro release profiles of the formulation with those of the commercial product (Salofalk®) showed that the matrix tablets released 8.4–11.9% of the drug during the first 2 h at pH 1.2, whereas no mesalazine release was found from Salofalk® tablets at pH 1.2 (Fig. 1). However drug release increased

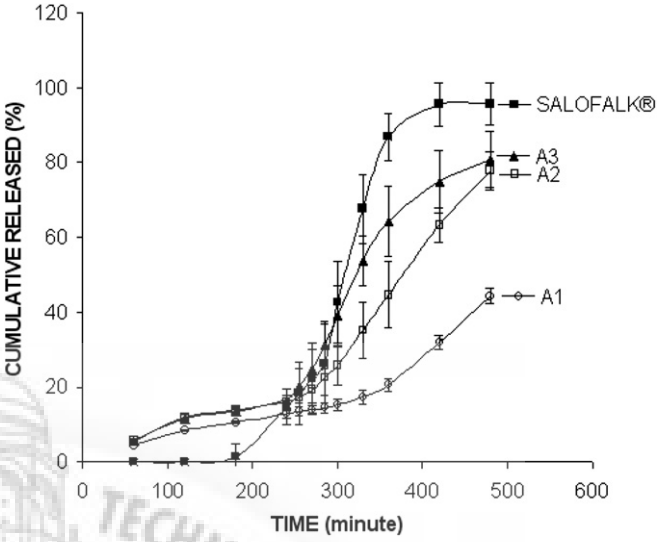

Fig. 1. Dissolution profiles of mesalazine from the Alginate-LF 240 D matrix tablets and commercial tablet Salofalk®. {A1 (350 mg Alginate-LF 240 D), A2 (150 mg Alginate-LF 240 D), A3 (75 mg Alginate-LF 240 D).}

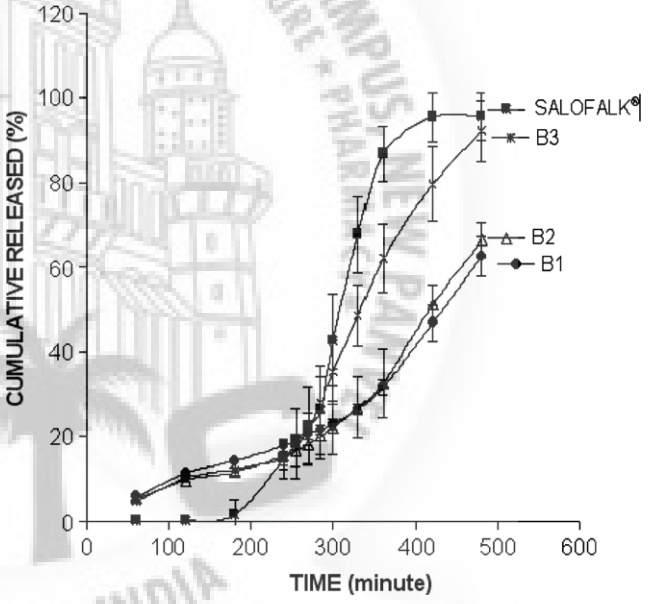

Fig. 2. Dissolution profiles of mesalazine from Alginate-LF 120 M tablets and commercial SALOFALK® tablet. {B1 (350 mg Alginate-LF 120 M), B2 (250 mg Alginate-LF 120 M), B3 (150 mg Alginate-LF 120 M).}

at pH 4.5, and it was found that 12.8–16.7% and 14.7% of the drug were released from the A1–A3 tablets and Salofalk® tablets, respectively, over 4 h (Fig. 1).

It was observed that the alginate type and amount in the matrices played an important role in basic media. The drug release decreased when the amount of high viscosity alginate increased in the matrices of A1, A2 and A3 formulation in basic media (Fig. 1). A1 and A3 formulations released 20% of drug within 6 and 4 h, respectively. Cumulative release of drug from A3 formulation was approximately 80%, whereas, 44% drug had been released from A1 formulation at 8 h. The increasing amount of alginate

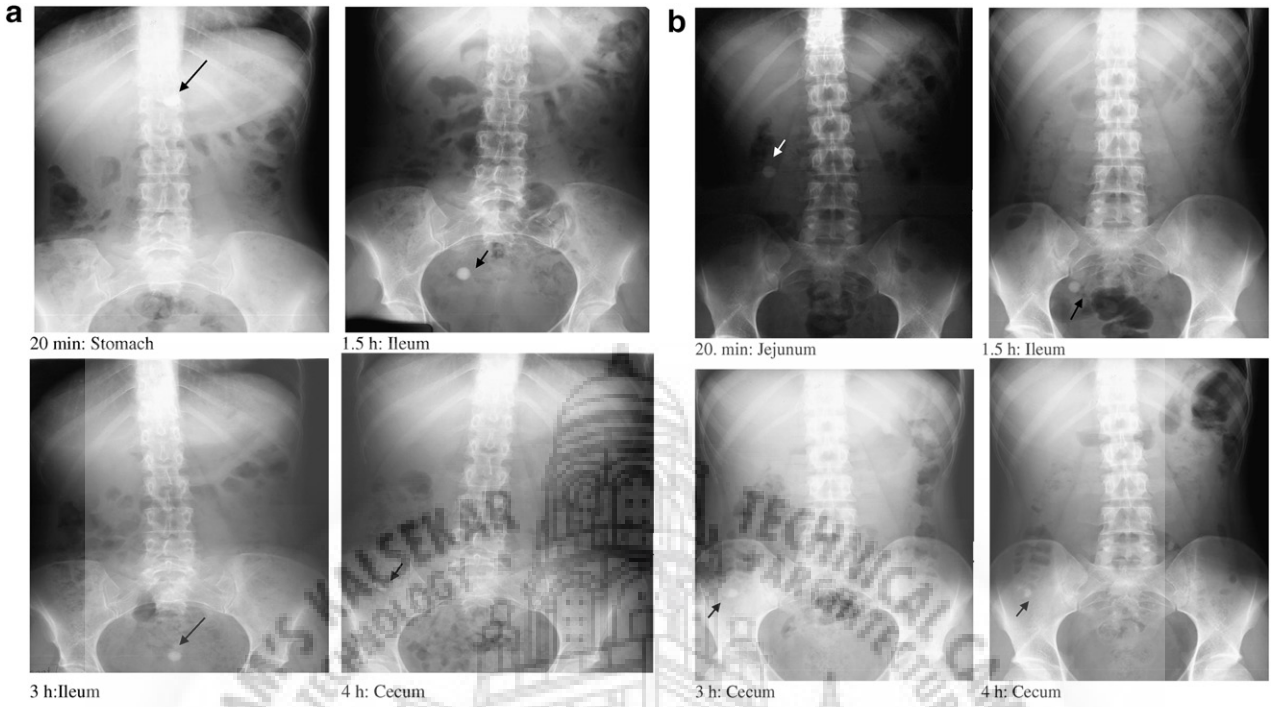

Fig. 3. The localization of the tablet in the gastrointestinal tract in subject 1 (a). The localization of the tablet in the gastrointestinal tract in subject 2 (b).

results in the increase in the viscosity of the gel layer, which retards the drug diffusion from the tablet [8].

The release profiles of B1 and B2 formulations were found to be almost identical in basic media. It was observed that the release rates of B1 and B2 formulations were found to be slow compared with B3 formulation (Fig. 2).

Formulation B3 released 20% and 40% of drug approximately at 4.5 and 5 h similar to Salofalk® tablets. The 47% and 80% release were obtained in 7 h for B1 and B3 formulations, respectively. On the other hand, 80% of drug had been released at 7 h from B3 formulation and at 6 h from Salofalk® tablets (Fig. 2). Salofalk® tablets released mesalazine more rapidly due to the increase in pH because of enteric coating. Efentakis and Koutlis [30] also reported that the viscosity of sodium alginate affected the release rate of furosemide from hard gelatin capsules. Low viscosity formulations exhibited greater erosion, while high viscosity formulations exhibited less erosion and drug release was completed in 8 h.

Although Salofalk® tablets are acid resistant enteric coated tablets, almost the same amount of drug was released with B3 and Salofalk® tablets (Fig. 2). Therefore, tablet B3 was chosen for further in vivo studies, and formulation C was prepared with the addition of barium sulphate as a marker for monitoring the tablets through the GI system.

From the abdominal radiographs, taken at different points of time, it was seen that after 20 min the tablets remained in the stomach in five subjects, whereas the tablets had reached the upper intestinal region in the other three subjects. The X-ray image of tablets throughout the gastrointestinal systems is shown in Fig. 3 for two subjects (subjects 1 and 2).

The position of the tablets at different time intervals is shown in Table 3. The tablet formulation reached the ileum after approximately 1.5 h in four subjects (subjects 1–4).

Table 3
The position of the tablets throughout the gastrointestinal tract in the subjects at certain points in time

Subjects	20 min	1.5 h	3 h	4 h	5 h	6 h
1	Stomach	Ileum	Ileum	Cecum	ND[a]	ND
2	Jejunum	Ileum	Cecum	Cecum	ND	ND
3	Stomach	Ileum	Cecum	ND	ND	ND
4	Jejunum	Terminal ileum	Transverse colon	ND	ND	ND
5	Stomach	Jejunum	Ileum	Ileum	Disentegrated	ND
6	Stomach	Stomach	Ileum	Ileum	Terminal ileum	Cecum
7	Duodenum	Jejunum	Jejunum	Disentegrated	ND	ND
8	Stomach	Jejunum	Terminal ileum	Disentegrated	ND	ND

[a] ND, not detected.

However, the tablets were monitored in the jejunum in the other three subjects (subjects 5, 7 and 8) at 1.5 h. Krishnaiah et al. [16] reported that the mean gastric emptying time was found to be 1.08 ± 0.11 h and the mean small intestinal transit time was 1.75 ± 0.25 h, while evaluating guar gum as a matrix tablet for colonic drug delivery using gamma scintigraphy.

The tablets were seen in the cecum, transverse colon and terminal ileum at 3 h in subjects 2, 3, 4 and 8. However, the tablet was monitored in the cecum at 4 h in subject 1. For four subjects (3, 4, 7 and 8) the intensity of the tablet image decreased at 4 h, due to possible disintegration of the tablet, and finally the tablet was not detected at 5 and 6 h, except in the case of subject 6, because of complete disintegration.

It was reported that the average small intestinal transit time and cecal arrival time were 3.11 and 4.6 h, respectively [31].

In our previous study, X-ray imaging showed that the colonic arrival time of the guar gum-mesalazine matrix tablets was 3–8 h for six volunteers, whereas it took 24 h for two volunteers [29]. It was reported that the tablets were visualized in the ileum for seven subjects, and the tablets reached the colon in five of them.

Our in vivo experiments showed that the tablets reached the small intestine in 3 h in the majority of healthy volunteers and this finding agrees with that observed by the above studies. The in vitro release test experiments of the tablets showed that $10.1 \pm 0.3\%$ of the active compound was released in the first 2 h. These results indicate that alginate matrix tablets reached the upper part of the large intestine after releasing a small amount of mesalazine.

It was observed that the transit time of the tablets throughout the gastrointestinal tract was variable. Billa et al. [31] also reported wide intersubject variations in the gastric emptying values.

It was concluded that mesalazine-alginate matrix tablet formulations can deliver the drug to the small and large intestine. Thus, the alginate matrix tablets may be a promising site specific delivery system for the treatment of Crohn's disease involving both the ileum and large intestine.

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References

[1] S.-J. Hwang, H. Park, K. Park, Gastric retentive drug-delivery systems, Cric. Rev. Ther. Drug Carr. Systems 5 (1998) 243–284.
[2] I. Wilding, Site-specific drug delivery in the gastrointestinal tract, Cric. Rev. Ther. Drug Carr. Systems 17 (2000) 557–620.
[3] M. Morishita, A.M. Lowman, K. Takayama, T. Nagai, N.A. Peppas, Elucidation of the mechanism of incorporation of insulin in controlled release systems based on complexation polymers, J. Control. Rel. 81 (2002) 25–32.
[4] Y.-H. Lin, H.-F. Liang, C.-K. Chung, M.-C. Chen, H.-W. Sung, Physical crosslinked alginate/N, O-carboxymethyl chitosan hydrogels with calcium for oral delivery of protein drugs, Biomaterials 26 (2005) 2105–2113.
[5] Ø. Holte, E. Onsøyen, R. Myrvold, J. Karlsen, Sustained release of water-soluble drug from directly compressed alginate tablets, Eur. J. Pharm. Sci. 20 (2003) 403–407.
[6] S.K. Bajpai, S. Shubhra, Investigation of swelling/degradation behaviour of alginate beads crosslinked with Ca^{2+} and Ba^{2+} ions, Reac. Func. Poly. 59 (2004) 129–140.
[7] S. Takka, F. Acartürk, Calcium alginate microparticles for oral administration: I-Effect of sodium alginate type on drug release and drug entrapment efficiency, J. Microencap. 16 (1999) 275–290.
[8] C.V. Liew, L.W. Chan, A.L. Ching, P.W.S. Heng, Evaluation of sodium alginate as drug release modifier in matrix tablets, Int. J. Pharm. 309 (2006) 25–37.
[9] A. Prakash, A. Markham, Oral delayed-release mesalazine, Drugs 57 (1999) 383–408.
[10] Altamash I. Qureshi, Russell D. Cohen, Mesalamine delivery systems: do they really make much difference? Adv. Drug Del. Rev. 57 (2005) 281–302.
[11] K. Lauritsen, L.S. Laursen, J. Rask-Madsen, Clinical pharmacokinetics of drugs used in the treatment of gastrointestinal disease (Part II), Clin. Pharmacokinet. 19 (1990) 94–125.
[12] S.N. Rasmussen, K. Lauritsen, U. Tage-Jensen, O.H. Nielsen, P. Bytzer, O. Jacobsen, K. Ladefoged, M. Vilien, V. Binder, J. Rask-Madsen, S. Bondesen, S.H. Hansen, E.F. Hvidberg, 5-aminosalicylic acid in the treatment of Crohn's disease, Scan. J. Gastroenterol. 22 (1987) 877–883.
[13] F. Martin, Oral 5-aminosalicylic acid preparations in treatment of inflammatory bowel disease an uptake, Dig. Dis. Sci. 32 (1987) 57S–63S.
[14] G. Van Den Mooter, C. Samyn, R. Kinget, In vivo evaluation of a colon-specific drug delivery system: an absorption study of theophylline from capsules coated with azo polymers in rats, Pharm. Res. 12 (1995) 244–247.
[15] N. Gardner, W. Haresing, R. Spiller, N. Farraj, J. Wiseman, H. Norbury, L. Illum, Development and validation of a pig model for colon-specific drug delivery, J. Pharm. Pharmacol. 48 (1996) 689–693.
[16] Y.S.R. Krishnaiah, S. Satyanarayana, Y.V.R. Prasad, S.N. Rao, Gamma scintigraphic studies on guar gum matrix tablets for colonic drug delivery in healthy human volunteers, J. Control. Rel. 55 (1998) 245–252.
[17] M. Sakkinen, J. Marvola, H. Kanerva, K. Lindevall, A. Ahonen, M. Marvola, Are chitosan formulations mucoadhesive in the human small intestine? An evaluation based on gamma scintigraphy, Int. J. Pharm. 307 (2006) 285–291.
[18] M.J. Dew, R.E.J. Ryder, N. Evans, B.K. Evans, J. Rhodes, Colonic release of 5-aminosalicylic acid from an oral preparation in active ulcerative colitis, Br. J. Clin. Pharmacol. 6 (1983) 85–187.
[19] V. Iannuccelli, G. Coppi, R. Sansone, G. Ferolla, Air compartment multiple-unit system for prolonged gastric residence. Part II. In vivo evaluation, Int. J. Pharm. 174 (1998) 55–62.
[20] J. Chen, W.E. Blevins, H. Park, K. Park, Gastric retention properties of superporous hydrogel composites, J. Control. Rel. 64 (2000) 39–51.
[21] H.P. Osterwald, Pharmaceutic Development: Mesalazine, Scand. J. Gastroenterol. 25 (1990) 43–46.
[22] K.P. Steed, G. Hooper, N. Monti, M.S. Benedetti, G. Fornasini, I.R. Wilding, The use of pharmacoscintigraphy to focus the development strategy for a novel 5-ASA colon targeting system ("TIME CLOCK (R)" system), J. Control. Rel. 49 (1997) 115–122.
[23] P.J. Watts, L. Illum, Colonic drug delivery, Drug Dev. Ind. Pharm. 23 (1997) 893–913.
[24] V.S. Mastiholimath, P.M. Dandagi, S. Samata Jain, A.P. Gadad, A.R. Kulkarni, Time and pH dependent colon specific, pulsatile delivery of theophylline for nocturnal asthma, Int. J. Pharm. 328 (2007) 49–56.

[25] A.W. Basit, Advances in colonic drug delivery, Drugs 65 (2005) 1991–2007.
[26] Radiation Protecting 118 Referral Guidelines for Imaging, Office for Official Publications of the European Commission Directorate-General for the Environment, Luxembourg http://europaeuint/comm/energy/nuclear/radioprotection/publication/doc/118_enpdf.2000.
[27] M.W. Rudolph, S. Klein, T.E. Beckert, H.-U. Petereit, J.B. Dressman, A new 5-aminosalicylic acid multi-unit dosage form for the therapy of ulcerative colitis, Eur. J. Pharm. Biopharm. 51 (2001) 183–190.
[28] L.M.L. Stolk, R. Rietbroek, E.H. Wilting, J.J. Tukker, Dissolution profiles of mesalazine formulation in vitro, Pharm. Weekblad. Sci. Ed. 12 (1990) 200–204.
[29] F. Tuğcu-Demiröz, F. Acartürk, S. Takka, Ö.L. Konuş-Boyunağa, In vitro and in vivo evaluation of mesalazine-guar gum matrix tablets for colonic drug delivery, J. Drug. Target. 12 (2004) 105–112.
[30] M. Efentakis, A. Koutlis, Release of furosemide from multiple-unit and single-unit preparations containing different viscosity grades of sodium alginate, Pharm. Dev. Tech. 6 (2001) 91–98.
[31] N. Billa, K. Yuen, M.A.A. Khader, A. Omar, Gamma-scintigraphic study of the gastrointestinal transit and in vivo dissolution of a controlled release diclofenac sodium formulation in xanthan gum matrices, Int. J. Pharm. 201 (2000) 109–120.

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REVIEW

Long-term efficacy and safety of once-daily mesalazine granules for the treatment of active ulcerative colitis

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Abstract: In 1977, 5-aminosalicylic acid (5-ASA) was discovered as a therapeutically active moiety of sulfasalazine (SASP) and was launched for topical and oral therapy of ulcerative colitis (UC) in 1984. As a first-step, delivery systems had to be developed to protect 5-ASA against absorption in the upper gastrointestinal tract, resulting in different and competing strategies (azo compounds, controlled release, and pH-dependent release). In a second step, at the beginning of the new century, coinciding with the expiration of patent protection for the first 5-ASA formulations, two component composite release mechanisms (pH-dependent and controlled release) were developed. Furthermore, the drug was formulated as granules instead of tablets, allowing higher unit strengths compared with tablets. Neither Salofalk Granu-Stix®, nor MMX 5-ASA, nor Pentasa® granules have initially been developed for once-daily (OD) dosing. A review of the achievements of 20 years of 5-ASA development has demonstrated that 5-ASA has equal efficacy compared with SASP at best, that there are no measurable differences in efficacy between various 5-ASA preparations, and that in a group of patients tolerating SASP, adverse event profiles of SASP and 5-ASA did not differ significantly, with SASP being the far cheaper substance. Therefore, drug adherence came into focus as a new goal for improving UC therapy. Although adherence is a complex and multifactorial construct, a simple dosing schedule may contribute to higher drug adherence and better efficacy of treatment. Simultaneously, the US 5-ASA market, estimated to be worth US$1.4 billion, is expected to grow continuously. Naturally, this very competitive market is not only driven by scientific progress but also by commercial interests. Thus, patents for minor changes to the formulation may serve as protection against drug companies trying to launch generic versions. Randomized controlled trials performed on OD dosing in induction of remission have demonstrated that OD administration of 5-ASA is as effective as conventional dosing in mild to moderate active UC. The three 5-ASA products MMX, Salofalk®, and Pentasa® employed in those studies so far have not shown differences in efficacy between OD and conventional dosing. No differences regarding safety outcomes have been detected between OD and conventional dosing, including incidence of adverse events, serious adverse events, or withdrawal from treatment due to an adverse event. Although the majority of patients prefer OD dosing to conventional dosing, it was not possible to detect differences in adherence between OD and multiple dose regimens in the clinical trial setting. Well-designed and controlled large-scale community-based studies are necessary to further investigate and prove the point of improved long-term adherence and treatment efficacy in OD dosing.

Keywords: dosing, adherence, mesalamine, 5-aminosalicylic acid

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Introduction

Ulcerative colitis (UC) is a chronic inflammatory disorder involving the colonic mucosa. Most frequently, the mucosal inflammation involves the rectum, but it may

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extend proximally, resulting in procto-sigmoiditis, left-sided colitis, or pancolitis. In addition, patients may suffer from extraintestinal manifestations of UC, including affections of the skin, eyes, joints, or the liver in the form of primary sclerosing cholangitis. In addition, there is an increased risk for colorectal cancer, with longstanding inflammation. UC most commonly affects teenagers and young adults, but may occur in any age group.[1] The prevalence in the US adult population is 238 per 100,000, and the worldwide incidence varies from 0.5 to 24.5 per 100,000 person-years.[2,3] The clinical presentation includes bloody diarrhea, rectal urgency, tenesmus, and abdominal cramping. UC follows a relapsing and remitting course necessitating therapy for induction of remission as well as maintenance of remission. 5-aminosalicylic acid (5-ASA) (the terms 5-ASA, mesalazine, and mesalamine are synonymous) was introduced into UC therapy 30 years ago and remains the backbone of treatment in both indications. Thus, for induction of remission, current national and international guidelines recommend oral 5-ASA alone or in combination with topical application in the management of active mild to moderate left-sided or extensive UC.[4–6]

Lately, 5-ASA formulations developed more than 10 years ago for a multiple daily dosing schedule have been marketed for once-daily (OD) dosing. Adherence issues have been cited as the main reason for this shift. According to this hypothesis, a more inconvenient drug regimen can interfere with the everyday life of a patient, reduce quality of life, and thus have a negative impact on adherence to the drugs, resulting in a poorer long-term prognosis.

In this review, we trace the evolution of 5-ASA formulations, examine the rationale and motivation for the introduction of OD dosing in UC therapy, and sum up efficacy and safety of oral 5-ASA in the treatment of active UC administered OD following this new therapeutic trend.

The path to OD dosing of oral 5-ASA

Sulfasalazine

The development of modern treatment of UC started with the introduction of sulfasalazine (SASP) by the Swedish physician Nanna Svartz in 1942. Serving as Professor of Internal Medicine at the Karolinska Institute from 1937 to 1957, she was the first woman ever to be appointed professor at a Swedish university.[7] Svartz synthesized and described SASP as anti-inflammatory principle in rheumatic arthritis and UC. SASP contains 5-ASA bound to sulfapyridine via a diazobond. This bond is cleaved by bacterial azoreductases in the colon to release the two components and thus deliver 5-ASA to the intended site of action.[1,8]

Identification of 5-ASA as the therapeutically active component

Indeed, about 35 years ago, 5-ASA was found to be the therapeutically active moiety, while sulfapyridine is thought to function as the inactive carrier molecule.[9–11] Sulfapyridine is absorbed into the systemic circulation and is believed to be mostly responsible for the adverse effects associated with SASP.[12,13] Oral administration of unbound or uncoated 5-ASA results in rapid and nearly complete absorption by the proximal small bowel and conversion to the inactive metabolite N-acetyl-5-ASA, thus preventing delivery of therapeutically sufficient concentrations to the colon where it supposedly acts locally on the mucosa.[1,8,14,15] Therefore, key factors governing the development of 5-ASA formulations are minimizing systemic absorption of 5-ASA from the small intestine and maximizing delivery of the active drug to the site of inflammation in the colon.[16,17]

Mechanism of action

A number of different but not mutually exclusive mechanisms of action have been proposed for the polypotent 5-ASA, including inhibition of the activity of the nuclear factor-kappa B (NF-κB) pathway.[18–22] Lately evidence is accumulating that the anti-inflammatory effects of 5-ASA are mediated, at least in part, by peroxisome proliferator-activated receptor gamma (PPARγ).[1,23,24] PPARγ is a nuclear receptor that modulates the inflammatory response of monocytes and macrophages by inhibiting the production of nitric oxide (iNOS) and macrophage-derived cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6.[1] Novel PPARγ modulators having similarities to 5-ASA have been developed, one of them (GED-0507-34-Levo) is evaluated in Phase 2 clinical trials with promising results.[24]

Pharmacokinetics

The pharmakokinetic profiles of oral mesalazine formulations and mesalazine pro-drugs have been extensively reviewed.[16] Another paper reviews whether pharmacological methods for assessing 5-ASA release and colonic distribution from oral formulations are useful for guiding clinical decisions. The strengths and weaknesses of in-vitro gastrointestinal models, gamma-scintigraphy, plasma pharmacokinetic studies, and mucosal biopsies are discussed. The latter provide direct evidence of colonic distribution and may predict clinical efficacy, but must be interpreted cautiously because of

considerable inter-subject variability and other confounding factors. The paper concludes that limitations of individual measurement techniques mean that randomized clinical studies in UC patients remain the best guide for dosing and treatment regimen decisions.[25]

Topical 5-ASA formulations

The requirements of minimizing systemic absorption of 5-ASA from the small intestine and maximizing delivery of the active drug to the site of inflammation in the colon are ideally met by topical 5-ASA formulations like suppositories, foam, or enemas.[17,26] Topical 5-ASA formulations result in 200-fold higher mucosal concentrations than those achieved by oral administration. Data from clinical trials show that topical 5-ASA therapy is superior to oral therapy in distal UC and is an important part of the therapy in more extensive forms of UC up to pancolitis and is therefore recommended in current international guidelines.[4,6,27,28] Topical use of 5-ASA was first described in 1981.[26] Dr Falk Pharma's (Freiburg, Germany) 250 mg Salofalk® suppositories launched in March 1984 were the first pure 5-ASA preparation worldwide. By the early 1990s, topical 5-ASA was considered firmly established in UC therapy.[29] Since about 80% of UC patients suffer from proctitis or left-sided colitis,[30] it is all the more surprising that between 1992 and 2009 prescriptions for oral 5-ASA increased nearly six-fold, while those for topical 5-ASA remained almost constant at a low 10% share of the entire 5-ASA market.[27] Reasons for this disparity cited are patient discomfort or inconvenience caused by topical therapy, the opinion held by many practitioners that patients with active colitis have difficulties retaining rectal suspensions, and more aggressive marketing of oral 5-ASA preparations with patented release mechanisms claiming to enhance distal colon 5-ASA release.[17,27,31]

First-generation oral 5-ASA formulations

Only a few months after the suppositories, Dr Falk Pharma introduced also in 1984 the first pure oral 5-ASA preparation (Salofalk®), that was protected against absorption in the upper gastrointestinal tract by a pH-sensitive acrylic coating (Eudragit L) that delays 5-ASA release until luminal conditions approach pH 7, the pH present in the terminal ileum and cecum.[8,16,17,27] Other formulations using this strategy include Claversal® (1987; Merkle, Blaubeuren, Germany) and Asacol® (1988; Medeva Pharma Suisse, Bulle, Switzerland), Pentasa® (1986; Ferring; Saint-Prex, Switzerland) is a microsphere formulation that consists of 5-ASA microgranules enclosed within a semipermeable membrane of ethylcellulose, which is designed for controlled drug release that begins in the duodenum and continues into the affected regions of the lower bowel.

Another strategy of protecting 5-ASA from early absorption is incorporation of 5-ASA into a prodrug, in which 5-ASA is covalently bound to an active carrier molecule. Examples for this prodrug strategy other than SASP are olsalazine/Dipentum® (1990; UCB Pharma, Anderlecht, Belgium). which consists of two 5-ASA molecules linked by a diazo bond or balsalazide/Colazal®/Colazide® (1998 Salix Pharmaceuticals Inc., Raleigh, NC, USA; Almirall, Barcelona, Spain), which is composed of a 5-ASA molecule azo-bonded to a benzoic acid derivative. Like SASP, these compounds are poorly absorbed in the upper digestive tract but are readily metabolized by bacterial diazoreductases of the intestinal flora in the lower bowel.[8,16,17,27]

It should be mentioned that for some of these 5-ASA products invention, production, and distribution lie in different hands, and those may differ between continents or even between countries in Europe. Importantly, a formulation in one country may not be identical to a formulation with the same name produced in a different country. For example, for Asacol®, differences with respect to the pH at which 5-ASA is released, the overall dissolution profile, efficacy and safety have been described between tablets produced in the US and within Europe.[32,33]

The unit strength for the controlled-release 5-ASA products range between 250 and 500 mg, with daily target doses between 1.6 and 4.8 g for active UC and 0.75 and 4.8 g for maintenance, resulting in a number of units to swallow per day of up to 16. In the case of the azo compounds, unit strengths between 250 and 750 mg were available with daily target doses of 2.00–6.75 g for active UC and 1.00–6.75 g for maintenance, resulting in up to nine pills per day (16). The first 5-ASA product offered in a unit strength of 1,000 mg was Pentasa® in 1999.[34,35]

Second-generation oral 5-ASA formulations

To offer the 1,000 mg unit strength, the Pentasa® microgranules were no longer packaged into a tablet, which would have been difficult to swallow because of its size, but loosely filled into a sachet. Arguments for this product alteration were the necessity of administration of higher daily 5-ASA doses as a result of meta-analyses at that time finding a dose–response relationship and the resulting inconvenience of taking large amounts of tablets. The compliance issue was also mentioned.[34,35] Lately, data were presented that patients indeed prefer granules over tablets.[36] Interestingly, the paper

dealing with the pharmacology of the new preparation is also discussing the use of systemic bioequivalence data in the regulatory assessment of marketing authorities for generic or copy products in locally acting preparations.[34] Indeed, at the beginning of the new century, the available oral 5-ASA products were reaching the end of their formulation patent protection, and 5-ASA copies were expected to compete with the proprietary products.[33] For example, in 2003, Lagap Pharmaceuticals Ltd, now Sandoz Ltd, launched with Ipocol a copy of Asacol® with 400 mg 5-ASA in a Eudragit S coating, although with different characteristics.[37] Therefore, modifications of 5-ASA products in the early 2000s were probably in part also driven by patent issues.

In 2005, Claversal®, similarly to Pentasa®, was also offered as a multi-unit 1,500 mg micropellet sachet in addition to the single-unit tablets.[38,39]

While Pentasa® and Claversal® were now basically just marketed as a multi-unit instead of a single-unit drug at a higher dose, Dr Falk Pharma developed its Salofalk® one step further. Salofalk Granu-Stix® launched in 2001 was now to release 5-ASA in a first-step pH-dependent by depolymerization of the acrylic coating and in a second step from a matrix core, that is designed to provide a continuous release of 5-ASA even in the distal colon. In vitro dissolution and plasma concentration data suggested a slower and more prolonged release of 5-ASA from the pellets compared with the tablets. An in vivo pharmacologic and scintigraphic study demonstrated that the novel 5-ASA pellets and Salofalk® tablets release active 5-ASA in the same target region and pass through the gastrointestinal tract under fasting conditions in healthy volunteers in a comparable time.[40] Salofalk Granu-Stix® were marketed as 500 and 1,000 mg sachets. They were, however, not specifically developed for OD dosing. In a dose-finding study in UC, the drug regimen was three times a day (TID).[41]

A similar two-component drug-release mechanism was employed by Italy's pharmaceutical company Giuliani SpA in developing its multi-matrix (MMX) 5-ASA. The molecule is incorporated into a lipophilic matrix, which is itself dispersed within a hydrophilic matrix, to delay and prolong dissolution. Similar to other 5-ASA products a gastroresistent polymer film leads to pH-dependent dissolution of the tablet at pH 7 in the terminal ileum. The hydrophilic matrix is then exposed to intestinal fluids and swells, resulting in the formation of a viscous gel mass supposedly leading to a slow gradual release of mesalazine throughout the length of the colon.[42] The pharmacoscintigraphic study to evaluate its in vivo properties came to the conclusion that 5-ASA was mainly and selectively delivered to the colonic lumen, with a continuous release along the whole colon, and a lower systemic absorption was observed compared with other mesalazine sustained-release formulations.[43] The MMX 5-ASA tablet has a unit strength of 1,200 mg, nevertheless, similar to the other 5-ASA products, it was not primarily developed for OD dosing. The schedule in the pharmacological study was twice a day (BID); in the first clinical study even TID.[44] MMX 5-ASA was launched in 2007 by Shire (Basingstone, UK) as Lialda® and Mezavant® in the US and European markets, respectively.

Drawing the balance after 20 years of 5-ASA development

5-ASA preparations were intended to avoid the adverse effects of SASP while maintaining its therapeutic benefits. After more than 20 years of development of multiple competing oral 5-ASA formulations, Sutherland and MacDonald[8,45] measured the progress been made in their Cochrane systematic review on oral 5-ASA in induction and maintenance of remission of UC, published in 2006. The result was sobering in three regards:

- Efficacy of 5-ASA versus SASP
  For maintenance of remission, SASP was found to have a higher degree of therapeutic effectiveness compared with 5-ASA, with an odds ratio of 1.29 (95% confidence interval [CI] 1.05–1.57). For induction of remission studies, investigating complete global or clinical remission found no difference between SASP and 5-ASA; only for endoscopic improvement was there a trend toward the superiority of 5-ASA over SASP, which did not reach statistical significance.[8,45]
- Adverse events of 5-ASA versus SASP
  Regarding adverse events in the maintenance situation SASP and 5-ASA had similar profiles, with odds ratios of 1.16 (95% CI 0.62–2.16) and 1.31 (95% CI 0.86–1.99), respectively. The NNH (number needed to harm) values were determined to be 171 and 78, respectively. However, it is mentioned that there may have been a bias in favor of SASP, since many trials comparing 5-ASA and SASP involved patients who were known to have tolerated SASP in the past. This might have minimized SASP-related adverse events in these trials.[45] The meta-analysis examining induction of remission trials found a significantly higher proportion of withdrawals associated with SASP.[8]
- Differences in efficacy between various 5-ASA preparations

Both reviews arrive at the conclusion that there is little evidence to suggest that there are differences in the efficacy of various oral 5-ASA drugs. This conclusion was also bolstered by more recent studies comparing a pH-dependent (Asacol®) and a time-dependent (Pentasa®) 5-ASA formulation[46] as well as Asacol® with MMX.[47]

Incidentally, the updated versions of the pair of Cochrane systematic reviews in 2012 corroborated those results further.[48,49]

The authors drew the following conclusions for practice and research:

- In the light of the fact "that the newer 5-ASA preparations have yet to be proven to be more clinically beneficial than SASP" and that they are three to four times more expensive than SASP, they "should be reserved for SASP-intolerant individuals, men concerned about fertility, and other patients within special populations that may, in the future, be shown to gain unique therapeutic benefits from alternative 5-ASA delivery systems."[8,45] A share of 25% for SASP among 5-ASA prescriptions in the US testifies to the continued value of SASP in daily practice.[27]
- "There is little evidence to suggest that there is a difference in efficacy of the oral 5-ASA drugs. Given, that the differences in efficacy are likely to be marginal, further trials comparing the efficacy of various 5-ASA agents do not appear to be justified. Future trials should look at enhancing patient adherence with medication rather than comparing the efficacy of various 5-ASA agents. Adherence to therapy is important for treatment success and may be an important predictor of relapse."[8,45]

Thus a lack of substantial progress in efficacy and safety contributed to a shift of focus in 5-ASA drug development or rather marketing to the topic of adherence. Two important issues regarding this field are the dosing schedule (ie, multiple daily dosing versus OD dosing, as well as total dose).

Adherence and OD dosing

In chronic diseases like UC, therapy often must continue on an indefinite basis; this is especially true for the maintenance situation. This can result in significant levels of medication non-adherence.[50–52]

Prevalence of medication non-adherence in inflammatory bowel disease

While clinical trials in the inflammatory bowel disease (IBD) field report patient adherence rates between 70% and 95%, in normal clinical practice non-adherence rates being defined as taking less than 80% of prescribed medication, range between 40% and 72%.[53–56]

The impact of non-adherence on clinical recurrence

A cohort study of 99 UC patients in remission demonstrated a considerable impact of non-adherence on clinical recurrence.[57] Patients who were non-adherent to their prescribed 5-ASA therapy had a greater than fivefold increased risk of clinical relapse. Moreover, adherent patients were shown to have an 89% chance of maintaining remission, compared with only 39% in non-adherent patients.[57] Other consequences of non-adherence to 5-ASA therapy are an increased risk of developing colon cancer and increased health care costs.[51,52,56]

Reasons for non-adherence

Adherence is a complex and multifactorial issue, in which a wide variety of factors play a role (eg, poor physician–patient relationship, lack of insight into illness, perceptions and beliefs about the illness, treatment of asymptomatic disease, and forgetfulness). Therefore, dosing regimen is just one of many factors potentially influencing drug adherence.[51,52,56] Nevertheless, the dosing schedule as a potential avenue to improve therapeutic outcome in UC has been addressed already, some 30 years ago. Van Hees and van Tongeren[58] measured urine levels of acetylated SASP as a marker for adherence and found considerably lower urine levels months after hospital discharge in 41.2% of patients. They suggested investigating whether SASP can be given BID in maintenance therapy instead of TID or QID (four times daily), citing studies from the late 1960s and early 1970s, which had demonstrated much higher adherence for BID schedules.[58]

OD dosing

A meta-analysis by Claxton et al[59] published in 2001 examined the relationship between number of daily doses and rate of adherence. It included studies where adherence was measured only by electronic monitoring and excluded studies based on patient self-report, blood-level monitoring, prescription refills, or pill count data. A total of 76 studies from several disease areas were identified. The result suggested that less frequent dosing is related to higher adherence. However, significant differences were only demonstrated between OD and three-times daily, or four-times daily dosing. No significant difference was found between OD and BID regimens.[59]

First steps

The pioneers of OD dosing of 5-ASA in UC were Hussain et al,[60] who performed a pharmacokinetic study published in 2001, and Kane et al,[50] who undertook a first pilot feasibility study of OD versus conventional dosing for maintenance in UC, published in 2003.

In the pharmacokinetic study 12 healthy volunteers each received either 1.2 or 2.4 g of Eudragit S-coated 5-ASA (Asacol®) either as three doses of 400 and 800 mg, respectively, or as an OD dose for 7 days. Peak and trough serum levels and serum area under the curve values were similar with both regimens. Furthermore, urinary, fecal, and most importantly, rectal tissue concentrations were similar following single or divided dosing. The authors suggested clinical trials examining efficacy and toxicity of OD dosing in patients with UC.[60]

In the clinical pilot study, 22 patients with UC in remission were randomized to OD 5-ASA versus conventional dosing (BID or TID), with the exact 5-ASA formulation and dose not further specified to assess adherence rates with both regimens. At 6 months nine patients (75%) in the OD group versus seven (70%) in the conventional dosing group were adherent. However, the amount of medication taken approached significance (90% versus 76%, P=0.07), and all patients in the OD group reported being either "very satisfied" or "satisfied" with their regimen. The performance of larger trials was suggested.[50]

In 2007, a further pharmacokinetic study compared an OD dosing regimen of 4 g Pentasa® with the current twice-daily dosage of 2 × 2 g/day. Similar to the previous study of Hussein et al,[60] concentrations of 5-ASA and its metabolites were similar in plasma, urine, feces, and rectal tissue following single or divided daily dosing in 30 healthy volunteers.[61]

OD dosing and safety considerations

It was also in 2007 when a host of studies started to be published exploring the effect of OD dosing versus conventional dosing on efficacy, safety, and adherence on a broader basis. Interestingly, safety concerns of employing OD dosing, which could potentially result in a higher peak dosis in the patient, are barely discussed in the literature. After all, the practice of divided dosing stemmed from the desire to reduce the toxicity and side effects that were originally associated with SASP therapy.[62,63] It may be speculated that it was reasoned that the high OD 5-ASA doses would be well tolerated because the pharmacokinetic study of Hussain et al[60] had not found relevant differences between OD and conventional dosing, and newer 5-ASA agents are generally tolerated better than SASP.

Dose-response relationship

Another important aspect closely related to safety and efficacy is the issue of dose–response relationship. Safety might depend on the total dose necessary to gain efficacy. In this respect, it was observed early on that the clear dose–response relationship established for SASP between 1 and 18 g could not be reproduced for 5-ASA compounds. Related to dose-dependent side effects of SASP, commonly 4 g daily for active disease and 2 g for maintenance treatment, providing 1,600 and 800 mg 5-ASA, respectively, are used as a compromise between efficacy and safety.[64] While studies have shown 5-ASA compounds to be as effective as 3–4 g SASP, none has been superior, even with doses of 4.8 g 5-ASA, equivalent to 12 g SASP, suggesting there is more to the action of SASP than delivering 5-ASA to its site of action.[64] The meta-analysis by Sutherland and MacDonald[8,45] states no dose–response trend for the maintenance situation, while a dose–response trend was observed for induction therapy.

The ECCO (European Crohn's and Colitis Organisation) guideline from 2012 states that for maintenance of remission, there is a minimum effective dose of oral 5-ASA of 1.2 g per day. In the comment, it is explained that a dose–response for maintenance of remission with mesalazine at doses greater than 0.8 g/day has not been established. It is possible that higher doses of maintenance oral mesalazine are required in some patients, perhaps in those that require high doses of oral 5-ASA to induce remission or those with frequently relapsing disease, but at present, there is no robust evidence to support this.[4,48,65] For treatment of left-sided or extensive active colitis of mild to moderate severity, use of oral 5-ASA at a dose >2 g is recommended. An advantage for doses beyond 2 g was suggested regarding response and mucosal healing but not remission, in the Assessing the Safety and Clinical Efficacy of a New Dose of 5-ASA (ASCEND) II study.[66,67] The ASCEND III study suggested a benefit of the higher dose strategy for induction of remission (43% versus 35%; P=0.04),[68] while a study with MMX did not demonstrate a benefit of 4.8 g/day compared to 2.4 g/day at 8 weeks.[69]

In summary, patient adherence is an important issue in the attempt to improve therapy of UC. Simplifying the dosing schedule and adapting it to the needs of the patient can be one element of improving drug adherence. A missing clear dose–response relationship limiting the maximal doses, pharmacokinetic studies demonstrating comparable results between OD and conventional dosing and overall

good tolerability of 5-ASA compounds allowed clinicians and drug companies to follow the avenue of OD dosing in the therapy of UC.

Market

Innovation – or pseudo-innovation in some cases – in drug development is not only driven by scientific progress, but naturally also by commercial interests. 5-ASA has been in clinical use now for 30 years; patents on the substance have long expired. The last significant changes to the formulation were made almost 15 years ago, and those patents are expiring as well. The market is more and more challenged from companies specializing in generics. A market with a considerable volume is to be protected.

Market volume

According to numbers from 2009, the US 5-ASA market was estimated to be worth US$1.4 billion, with continuing growth being expected.[1] While the Shire plc[70] annual report, 2010 claims that 88% of all UC patients receive treatment with 5-ASA, a German health insurer covering 8.6 million people gives a lower number, with 49%.[71] Those insured with Barmer GEK had 5-ASA prescriptions worth €14.2 million in 2012, a little down from 2011, with €14.5 million.[72] The fraction of patients receiving 5-ASA for the treatment of Crohn's disease ranges from 16% to 31%, depending on the source.[71,73]

The 5-ASA market is highly competitive, with several companies offering their products to physicians and patients. Tables 1 and 2 show the market share of different products in the US and the European markets for the year 2010 and 2010/2011, respectively. The market is especially under pressure, since mesalamine and balsalazide products are generally protected by formulation patents only, and generic drug companies are looking to make a push into the 5-ASA market.

Table 1 Market shares of oral 5-aminosalicylic acid products in the 2010 United States market[70]

Product	Manufacturer	Licensed to	Market share, %
Asacol®	Medeva Pharma Suisse	Warner-Chilcott	42.4
Lialda®	Shire		20
Pentasa®	Ferring	Shire	14
Asacol HD®	Warner-Chilcott		9
Balsalazide	Generic		6.9
Apriso®	Dr Falk	Salix	6
Colazal®	Salix		0.9
Dipentum®	UCB	Alaven Pharm LLC	0.6

Competition through expiring patents

Salix's patent on its balsalazide-containing product Colazal® expired in 2007. The company fought since 2005 against approval of generic versions of Colazal® and was denied by the US Food and Drug Administration (FDA) in 2007. Salix subsequently announced the launch of an authorized generic version by Watson Pharmaceuticals; the FDA approved subsequently also three other generic versions of Colazal®, which had a 6.9% and 7.5% share of the US oral market in December 2010 and December 2011, respectively.[70] In an attempt to offset the loss of patent protection of Colazal®, Salix acquired rights to develop Dr Falk Pharma's mesalamine granules and received approval from the FDA in October 2008 to market the granules packaged in 375 mg capsules as Apriso® in the US. Apriso® was supposed to be patent-protected until 2018. In September 2012, Salix Pharmaceuticals and Dr Falk Pharma filed a lawsuit against the Indian drug company Lupin Ltd for patent infringement. Lupin seeks approval to market a generic version of Apriso®.

The patent for Asacol® expired in July 2013. In 2009, Proctor and Gamble launched (Cincinnati, OH, USA) launched an 800 mg mesalamine tablet as Asacol HD® in addition to its 400 mg tablet, which has been marketed since 1992. The packages of Asacol® and Asacol HD® carried the notice, that both are not bioequivalent to each other. In the same year, Proctor and Gamble sold the Asacol® franchise to Warner-Chilcott (Rockaway, NJ, USA). The sales of Asacol®, which was the market leader, with a 52% share of the US oral 5-ASA market in 2008, were slowly eroding over the years (Table 3, column 9). Meanwhile the production of Asacol® was discontinued in the US market by Warner and Chilcott and substituted by the launch of Delzicol® in March 2013. Delzicol® offers 400 mg of mesalamine as an easier to swallow capsule instead of the tablet. Patents are supposed to protect Delzicol® until 2020; however, it was not granted a 3-year new drug product exclusivity, limiting the protection in the marketplace. Investors and shareholders are consoled that generic competition for the company's mesalamine-based UC franchise (Asacol®, Asacol HD®, and Delzicol®) remains highly unlikely over the next few years, given the challenging pathway to approval.[74]

Competition through new products

Even more competition in the 5-ASA market arrived with the "new kid on the block" in the form of Shire's products Lialda® for the US market and Mezavant® for the European market. Lialda® was launched in March 2007, and Mezavant®

Table 2 Market shares of oral 5-aminosalicylic products in the 2010 and 2011 European market[70]

Product	Manufacturer	Market share, %									
		UK		Germany		Spain		France		EU5 (UK, Germany, Spain, France, Italy)	
		2010	2011	2010	2011	2010	2011	2010	2011	2010	2011
Asacol®	Warner-Chilcott	56	45.8							21	19
Salofalk®	Dr Falk			56	53.2						
Mezavant®	Shire			17							
Pentasa®	Ferring	25	27.1		18	46	35.2	78			29.6
Claversal®	Recordati			15		41	29.3				
Fivasa®	Norgine BV							19			

started in the UK in November 2007. Lialda's® share of the US oral mesalamine market jumped from 3.9% in 2007 to 20% in 2009, the combined worldwide revenue for Lialda® and Mezavant® reached in 2012, with US$400 million, eight times the level of 2007 (Table 3, columns 1 and 4). Meanwhile the market share of Pentasa®, which is also marketed by Shire in the US, dropped continuously (Table 3, column 6). The revenue for Pentasa® still grew continuously, despite lower prescription demand due to the impact of price increases (Table 3, column 7).[70] As mentioned above, the US market leader Asacol® also lost market share in the years following the launch of Lialda®. A decrease in prescriptions was offset in net sales (US$793 million for 2012) by higher selling prices and a decrease in sales-related deductions.[75] In May 2013, Watson Pharmaceuticals (now Actavis plc; Dublin, Ireland) sought approval from the FDA to market a generic version of Lialda®. The Southern District Court of Florida, however, upheld the validity of the patent covering Lialda® until it expires in 2020.

OD dosing: randomized controlled trials

From 2007 on, a series of randomized studies were published dealing with the comparison of OD and conventional dosing for maintenance and induction of remission. The critical questions to be answered are as follows: What is the efficacy of OD dosing compared with conventional dosing? In the case that OD dosing is more efficient, is this because of better adherence or because of other reasons? Does OD dosing lead to more adverse events compared with conventional dosing?

OD dosing in maintenance of remission

Ford and coauthors published a meta-analysis on OD dosing versus a conventional dosing schedule of 5-ASA in maintenance of remission in 2011.[63] They identified seven eligible randomized controlled trials (RCTs) comprising a total of 2,745 patients.[47,65,76–80] Five RCTs compared OD with BID dosing, and two compared OD with TID. The drugs used in the studies were Asacol® (4×), MMX (2×), Pentasa® (1×), and Salofalk® (1×). Duration of treatment and follow-up was 12 months in all studies.

Relapse rates were not significantly different between OD and conventional dosing schedules for 5-ASA, with 423 (31.4%) versus 461 (33.0%) patients relapsing, respectively (relative risk [RR] of relapse 0.94; 95% CI 0.82–1.08).[63]

Four trials with 994 patients could be analyzed for noncompliance. Definitions of compliance in these trials

Table 3 Market share and revenue reached by Shire's products Lialda® and Mezavant® in comparison with Pentasa® and Asacol®[70]

Year	1	2	3	4 (=2 + 3)	5	6	7	8 (=1 + 6)	9
	Lialda® US oral mesalamine market share, %	Lialda® US oral mesalamine revenue, million USD	Mezavant® Europe oral mesalamine revenue, million USD	Lialda® + Mezavant® worldwide revenue, million USD	Lialda® total US UC market share, %	Pentasa® US oral mesalamine market share, %	Pentasa® US market revenue, million USD	Shire US oral mesalamine market share (Lialda® + Pentasa®), %	Asacol® US oral mesalamine market share, %
2007	3.9	50.3	0.2	50.5		17.2	176.4	21.1	
2008	11.7	134.8	5.6	140.4	18%	16.7	185.5	28.4	52.3
2009	20.0			235.9		12.0	214.8	32.0	42.4
2010				293.4			235.9	34.5	37.4
2011				372.1			251.1	35.8	
2012				399.9			265.8		

Abbreviation: UC, ulcerative colitis; USD, US dollars.

included taking between 75% and 90% or more of the study medication, according to self-report. There was no significant difference in compliance, as 43 (8.8%) and 52 (10.3%) patients in the OD and the conventional dosing group were classed as being noncompliant (RR of noncompliance =0.87 (95% CI 0.46–1.66). The failure to demonstrate better adherence in the OD arm was explained with the very high adherence rate of about 90%. This high rate is thought to mirror the high motivation of patients participating in RCTs and unlikely to be representative of patients seen in the real world.[63] The result was especially disappointing, since poor adherence was thought to be particularly problematic in quiescent disease.

Finally, five trials comprising 1,356 patients provided data concerning total adverse events, but there were insufficient data regarding individual adverse events. Again, there was no statistically significant difference between adverse events in the OD arm (332; 50%) and the conventional dosing arm (320; 46.2%) resulting in an RR for experiencing any adverse event in the OD group =1.08 (95% CI 0.97–1.20).[63]

In summary, patients with UC in remission can be switched to an OD dosing schedule without compromising efficacy and safety. Outside of clinical trials, OD dosing may lead to enhanced drug adherence.

OD dosing in induction of remission

Up to this point, there were four RCTs on the comparison of OD dosing with conventional dosing in induction of remission,[69,81–83] three of which were summarized in the meta-analysis by Feagan and MacDonald.[84] The study by Flourie et al[83] published in 2013 was not included. The characteristics of those studies are summarized in Table 4.

The study of Lichtenstein et al[81] compared MMX 5-ASA 2.4 g BID, 4.8 g MMX 5-ASA OD or placebo for 8 weeks in patients with mild to moderately active UC. The primary endpoint was the percentage of patients in clinical and endoscopic remission at week 8 (UC-Disease Activity Index (DAI) score ≤1). This endpoint was achieved by 34.1% and 29.2% of patients receiving MMX 5-ASA 2.4 g BID and MMX 4.8 g/day OD, respectively, versus 12.9% receiving placebo ($P<0.01$). There was no significant difference between the MMX 5-ASA groups.

According to the double-blind, double-dummy design of this study performed between 2003 and 2005, each patient received medication BID. Therefore, the hypothesis of better adherence with OD dosing was not addressed. Adherence was similar in all the treatment groups. Ninety percent of patients in the safety population took between 80% and 120% of the study medication.

Both doses of MMX 5-ASA (2.4 g BID and 4.8 g OD) were well tolerated in this study, with a safety profile similar to other 5-ASA formulations. There was no evidence of a dose–response relationship for any safety parameter, and no clinically significant differences in safety were observed between placebo and either dose of MMX 5-ASA.[81]

The study by Kamm et al[69] was conducted in 2003 and 2004. Patients with active, mild-to-moderate UC received MMX 5-ASA 2.4 g OD or 4.8 g OD, Asacol® 0.8 g TID, or placebo for 8 weeks. The primary endpoint was the proportion of patients in clinical and endoscopic remission

Table 4 Characteristics of randomized controlled trials of once-daily dosing versus conventional dosing in induction of remission in ulcerative colitis patients

Study	Year	Number of patients	Country, number of centers	Intervention	Duration of therapy	Methodology
Lichtenstein et al[81]	2007	280	Eight countries, 52 centers	4.8 mg MMX OD (n=94) 2.4 mg MMX BID (n=93) Placebo (n=93)	8 weeks	Double-blind Double-dummy
Kamm et al[69]	2007	341	Ten countries, 49 centers	2.4 mg MMX OD (n=84) 4.8 mg MMX OD (n=86) 800 mg Asacol® TID (n=86) Placebo (n=86)	8 weeks	Double-blind Double-dummy
Kruis et al[82]	2009	380	13 countries, 54 centers	3 g Salofalk® OD (n=191) 1 g Salofalk® TID (n=189)	8 weeks	Double-blind Double-dummy Non-inferiority
Flourie et al[83]	2013	206	Four countries, 44 centers	4 g Pentasa® OD (n=102) + 1 g 5-ASA enema 2 g Pentasa® BID (n=104) + 1 g 5-ASA enema	8 weeks Enema: 4 weeks	Single-blind Non-inferiority

Note: Copyright © 2012. John Wiley & Sons Ltd. Adapted from Feagan BG, MacDonald JK. Once daily oral mesalamine compared to conventional dosing for induction and maintenance of remission in ulcerative colitis: a systematic review and meta-analysis. *Inflamm Bowel Dis*. 2012;18:1785–1794.[84]
Abbreviations: MMX, multi-matrix; OD, once daily; BID, twice a day; TID, three times a day.

(UC-DAI ≤1). In the intention-to-treat (ITT) population, a statistically significantly greater proportion of patients receiving MMX 5-ASA 2.4 g OD (40.5%; *P*=0.010) or 4.8 g OD (41.2%; *P*=0.007) achieved clinical and endoscopic remission compared with placebo (22.1%). The proportion of patients receiving Asacol® 0.8 g TID who achieved clinical and endoscopic remission was not statistically significantly greater than placebo (32.6%; *P*=0.124). In the subgroup analysis of the ITT population, including patients with mild or moderate disease at baseline and patients with left-sided or extensive disease, remission rates (clinical and endoscopic) were not statistically significantly greater for either dose of MMX mesalamine or Asacol® than for placebo. This was felt to be due to the limited patient numbers.

Similar to the study by Lichtenstein et al,[81] every patient received medication three times a day because of the double-blind, double-dummy study design. Therefore, adherence according to the dosing scheme could not be evaluated. More than 92% of patients in each treatment group took between 80% and 120% of the study medication.

There were no notable differences between the treatment groups with respect to the frequency of treatment-emergent adverse events, and there was no evidence of a dose–response relationship with MMX 5-ASA for any safety parameter. Hepatobiliary, renal, and urinary adverse events were very infrequent.[69]

In the study by Kruis et al,[82] 380 patients with confirmed diagnosis of UC (either established or first attack) with a clinical activity index >4 and endoscopic index ≥4 at baseline were randomized and treated with either 3 g OD or 1 g TID of Salofalk® 5-ASA granules. The primary endpoint was the percentage of patients achieving clinical remission defined as a clinical activity index ≤4 at the end of the study. In the ITT population, clinical remission was achieved by 151/191 patients (79.1%) in the OD group and 143/189 patients (75.7%) in the TID group, demonstrating non-inferiority between the OD and TID group, with a highly significant *P*-value of 0.0001.[82]

Endoscopic remission using the endoscopic index was obtained in 135/191 patients (71%) in the OD group and 132/189 (70%) in the TID group at the end of the study (ITT). There was no statistically significant difference between the groups.

In the subgroup analysis within the OD group, significantly more patients with mild as compared with moderate disease achieved clinical remission (85% versus 69%; *P*=0.0067). Disease localization also had an impact on the remission rates achieved. Whereas no significant difference in proximal disease (ie, left-sided, subtotal, and pancolitis) was observed between the OD and TID groups, there was a significant difference in distal disease between the groups (86% versus 73%; *P*=0.0298) as well as within the OD group itself between distal and proximal disease (86% versus 72%; *P*=0.0247). A pooled analysis also suggested higher efficacy in distal disease for 5-ASA granules administered OD in comparison with 5-ASA tablets TID,[85] although the conclusion was questioned by others due to the heterogeneity of the pooled studies.[86]

In a post hoc analysis, the efficacy data from the study by Kruis et al[82] were recalculated using a more stringent definition of remission used in the MMX 5-ASA trials. In this analysis of the ITT population, 70/191 patients (37%) in the OD group and 73/189 (39%) in the TID group achieved remission. These numbers are nearly identical to those reported in a pooled analysis[87] of the two trials discussed above,[69,81] with remission rates of 64/172 (37%) for 2.4 g MMX 5-ASA OD and 61/174 (35%) for 4.8 g MMX 5-ASA OD.

Again the double-blind, double-dummy design precluded an analysis of medication adherence depending on the schedule. However, asked which dosing schedule they prefer, the vast majority of patients 313/380 (82%) favored an OD dosing regimen; only 6/380 patients (2%) preferred the TID schedule, and 55/380 (14%) had no preference.[82]

Treatment with the study medication was well tolerated, and there was no difference in the occurrence of adverse events between the two dosing regimens. The majority of adverse events were mild or moderate in intensity, and no unexpected side effects occurred. Special emphasis had been put on potential adverse effects on renal function. Urinary function tests using sensitive early markers of renal disease (α1-microglobulin, β-N-acetyl-D-glucosaminidase (β-NAG), and cystatin C) showed no impairment of renal function, and indicated that an oral OD dose of 3 g mesalazine, which may be associated with higher peak plasma levels as compared with a 1 g TID regimen, is at least as safe as a 1 g TID dose with regard to potential tubulo-toxicity.[82]

The meta-analysis of Feagan and MacDonald[84] pooled the three trials discussed above with 738 patients.[69,81,82] Of the patients in the OD dosing group, 42% (155/370) failed to enter remission compared with 44.3% (163/368) of patients in the conventional dosing group. The pooled RR was 0.95 (95% CI 0.82–1.10), demonstrating no statistically significant difference between OD dosing and conventional dosing (*P*=0.49). Furthermore, none of the subgroup comparisons by formulation showed any differences in efficacy between OD dosing and conventional dosing.[84]

Finally, the study by Flourie et al[83] randomized 206 patients with mild-to-moderately active UC to 8 weeks of 4 g Pentasa® OD or 2 g BID. Patients additionally received a 1 g 5-ASA enema per day for 4 weeks. The primary endpoint was the percentage of patients in clinical and endoscopic remission after 8 weeks (defined as UC-DAI score ≤1). Although recruiting fell short of the goal, the primary endpoint was reached, and non-inferiority of OD versus BID dosing was demonstrated with 52.1% of patients in the ITT OD group and 41.8% of patients in the BD group in clinical and endoscopic remission at week 8.[83]

As this study was only investigator-blinded, an evaluation of acceptability and compliance for the two dosing schedules could be done. Acceptability using a visual analog scale was numerically but not statistically higher at week 8 for the OD arm compared with the BID arm (OD 73.2%±22.3% versus BID 66.3%±29.4%; P=0.10). Compliance was high, with median compliance rates of 100% in both treatment arms.

There were no differences in adverse events, laboratory results, or vital signs between the two study groups.[83]

In summary, available RCTs demonstrated consistently, the following:[84]

- 5-ASA administration OD is as effective as conventional dosing for induction therapy in mild to moderate active UC.
- Subgroup analysis by drug formulation showed no differences in efficacy between OD and conventional dosing for induction of remission. 5-ASA formulations employed for OD dosing are MMX, Salofalk®, and Pentasa®.
- No differences regarding safety outcomes were detected between OD and conventional dosing, including incidence of adverse events, serious adverse events, or withdrawal from treatment due to an adverse event.
- OD dosing did not lead to superior efficacy, although three out of four studies showed a trend in this sense. Due to the study design, dosing-dependent adherence could not be evaluated in three of four studies. In the study by Flourie et al,[83] there was no difference in adherence between the OD and the BID group. In the study by Kruis et al[82] that measured patient preference, the majority preferred OD dosing to conventional dosing; in the study by Flourie et al,[83] the preference for OD dosing was not statistically significant. Overall, it is felt that it may be difficult to detect differences in adherence between OD and multiple-dose regimens in the clinical trial setting because of the adherence rates beyond 90%. To examine this issue further, large-scale community-based studies are suggested, although they should be most promising in the maintenance of remission situation.

To this end, several Shire-sponsored studies have been published suggesting an advantage of OD MMX 5-ASA over other 5-ASA formulations regarding adherence and persistency.[88–90] However, these studies harbor several limitations regarding the collection and interpretation of the data. Furthermore, 5-ASA formulations approved for OD dosing other than MMX were not considered. Overall, there remain doubts about the size of the contribution OD dosing can make to a better drug adherence.[79,84,91]

Safety

In 1991, Hayllar and Bjarnason[64] argued for a continued role of SASP in the treatment of UC, also warning against 5-ASA toxicity due to the altered pharmacokinetics in comparison to SASP, which may lead to nephrotoxicity among others. In 2002, Ransford and Langman[92] stirred up the scientific and clinical community with their comparison of reported serious adverse advents between SASP and 5-ASA. With a total of 4.7 million prescriptions evaluated for SASP compared with 2.8 million for 5-ASA, interstitial nephritis was only described for 5-ASA, with 11.1 reports per million prescriptions, and pancreatitis was reported seven times as frequently for 5-ASA (7.5 per million prescriptions) compared with SASP (1.1 per million prescriptions). In contrast, there were more serious adverse events reported for SASP regarding blood dyscrasias and hepatic disorders than for 5-ASA.[92] The authors came to the conclusion that there is no evidence to indicate a safety advantage of 5-ASA over SASP in the treatment of inflammatory bowel disease and that advice on renal monitoring in patients who receive 5-ASA may need reinforcing. The study was soundly criticized by others on several accounts.[93]

Meanwhile, numerous studies and systematic reviews demonstrate that 5-ASA has an adverse event profile and is frequency similar to placebo with intolerance occurring in up to 15%. No differences between different 5-ASA formulations can be detected. Diarrhea (3%), headache (2%), nausea (2%), rash (1%) and thrombocytopenia (<1%) are reported.[4,22,48,94,95]

In particular, meta-analyses suggest that there is no difference in safety between OD and conventionally dosed mesalamine. No differences have been observed for safety outcomes including the overall incidence of adverse events or withdrawal from treatment due to an adverse event. Adverse events reported in the studies assessing OD dosing are mild to moderate in intensity and include gastrointestinal symp-

toms (eg, flatulence, abdominal pain, nausea, and diarrhea), headache, and worsening UC.[48,63,84]

Nephrotoxicity

Among idiosyncratic reactions attributed to 5-ASA nephrotoxicity remains the most debated.[96,97] A continued issue remains the potential nephrotoxicity of 5-ASA and the measures to be taken to avoid an affection of the kidney function.

Gisbert et al[98] summarized the current knowledge about the potential relationship between 5-ASA treatment and nephrotoxicity. They found that renal impairment in IBD patients may be partly attributable to the underlying disease, although users of 5-ASA may have an increased risk of renal disease. Epidemiological studies evaluating nephrotoxicity in IBD patients treated with 5-ASA suggest the incidence to be less than 0.5%. 5-ASA treatment-related nephrotoxicity is reported most often within the first 12 months, but also delayed presentation after several years has been observed. Nephrotoxicity is unlikely to be detected by urinalysis (eg, leukocyturia and low-grade proteinuria), therefore emphasizing the importance of monitoring serum creatinine in patients with IBD treated with 5-ASA. The low overall incidence of renal disease during 5-ASA treatment reported in the literature, and the absence of a clear relationship between 5-ASA dose and the risk of nephrotoxicity, suggest that the renal reactions may be idiosyncratic rather than dose-related in nature. 5-ASA-associated nephrotoxicity most frequently takes the form of an indolent, severe, chronic, and progressive interstitial nephritis. The nephrotoxicity potential of mesalazine and SASP seems to be similar; potential differences in the relative risk with different oral preparations of 5-ASA are probably too small to influence the choice of agent. Although data in the literature about the safety of 5-ASA compounds in patients with IBD and chronic renal failure are lacking, there needs to be more attention and scrutiny for those patients.

In a patient with IBD in whom no other cause can be readily identified for renal impairment, 5-ASA should be discontinued. If withdrawal of 5-ASA treatment does not result in a fall in serum creatinine, then the patient should be referred for renal biopsy, as only this will determine whether interstitial nephritis or glomerulonephritis associated with IBD is the cause of the persistent impaired renal function. Although the data are ambiguous, a trial of high-dose steroid (60 mg/day or 1 mg day/kg for up to 3 months) has been suggested in patients whose renal function does not respond to drug withdrawal alone.

It has been calculated that approximately 10% of the patients with 5-ASA nephrotoxicity will develop end-stage renal disease, emphasizing the need for timely recognition of renal impairment and prompt discontinuation of 5-ASA treatment of affected patients. Thus, many authors agree on performing a monitoring of renal function by serum creatinine measurements, although the optimal monitoring schedule remains to be established, and there is presently no evidence that such screening or monitoring improves patient outcomes.[4,22,98]

A recent study with the largest collection of patients (n=156) with suspected 5-ASA-related nephrotoxicity in IBD patients emphasizes some of the points made above.[99] The adverse effect was seen with all aminosalicylates (mesalazine, balsalazide, olsalazine, and SASP). The first abnormal blood test occurred in 22.4% of patients within the first 12 months after introduction of 5-ASA. After drug withdrawal, 81.1% had a recovery of renal function, 17 patients required renal replacement therapy, including 15 with kidney transplantation. The study includes genome-wide association analysis and subsequent sequencing to identify clinically useful predictive genetic markers so that these drugs can be either avoided or monitoring intensified in high-risk patients.[99]

Disclosure

The authors report no conflicts of interest in this work.

References

1. Ham M, Moss AC. Mesalamine in the treatment and maintenance of remission of ulcerative colitis. *Expert Rev Clin Pharmacol*. 2012;5:113–123.
2. Loftus EV. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology*. 2004;126:1504–1517.
3. Lakatos PL. Recent trends in the epidemiology of inflammatory bowel diseases: up or down? *World J Gastroenterol*. 2006;12:6102–6108.
4. Dignass A, Lindsay JO, Sturm A, et al. Second European evidence-based consensus on the diagnosis and management of ulcerative colitis: current management. *J Crohns Colitis*. 2012;6:991–1030.
5. Dignass A, Preiss JC, Aust DE, et al. [Updated German guideline on diagnosis and treatment of ulcerative colitis, 2011]. *Z Gastroenterol*. 2011;49:1276–1341. German.
6. Kornbluth A, Sachar DB. Ulcerative colitis practice guidelines in adults. American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol*. 2010;105:501–523.
7. Svartz N. Salazopyrin, a new sulfanilamide preparation: A. Therapeutic results in rheumatic polyarthritis. B. Therapeutic results in ulcerative colitis. C. Toxic manifestations in treatment with sulfanilamide preparation. *Acta Med Scand*. 1942;11:557–590.
8. Sutherland L, MacDonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev*. 2006;2:CD000543.
9. Azad Kahn AK, Piris J, Truelove SC. An experiment to determine the active therapeutic moiety of sulphasalazine. *Lancet*. 1977;310:892–895.
10. van Hees PA, Bakker JH, van Tongeren JH. Effect of sulphapyridine, 5-aminosalicyclic acid, and placebo in patients with idiopathic proctitis: a study to determine the active therapeutic moiety of sulphasalazine. *Gut*. 1980;21:632–635.

11. Klotz U, Maier K, Fischer C, et al. Therapeutic efficacy of sulfasalazine and its metabolites in patients with ulcerative colitis and Crohn's disease. *N Engl Med J*. 1980;303:1499–1502.
12. Nielsen OH. Sulfasalzine intolerance. A retrospective survey of the reasons for discontinuing treatment with sulfasalazine in patients with chronic inflammatory bowel disease. *Scand J Gastroenterol*. 1982;17:389–393.
13. Peppercorn MA. Sulfasalazine. Pharmacology, clinical use, toxicity, and related new drug development. *Ann Intern Med*. 1984;101:377–386.
14. Nielsen OH, Bondesen S. Kinetics of 5-aminosalicylic acid after jejunal instillation in man. *Br J Clin Pharmacol*. 1983;16:738–740.
15. Myers B, Evans DN, Rhodes J, et al. Metabolism and urinary excretion of 5-aminosalicylic acid in healthy volunteers when given intravenously or released for absorption at different sites in the gastrointestinal tract. *Gut*. 1987;28:196–200.
16. Sandborn WJ, Hanauer SB. Systematic review: the pharmacokinetic profiles of oral mesalazine formulations and mesalazine pro-drugs used in the management of ulcerative colitis. *Aliment Pharmacol Ther*. 2003;17:29–42.
17. Sandborn WJ. Oral 5-ASA therapy in ulcerative colitis: what are the implications of the new formulations? *J Clin Gastroenterol*. 2008;42:338–344.
18. Egan LJ, Mays DC, Huntoon CJ, et al. Inhibition of interleukin-1-stimulated NF-kappaB RelA/p65 phosphorylation by mesalamine is accompanied by decreased transcriptional activity. *J Biol Chem*. 1999;274:26448–26453.
19. Liptay S, Bachem M, Hacker G, et al. Inhibition of nuclear factor κB and induction of apoptosis in T-lymphocytes by sulfasalazine. *Br J Pharmacol*. 1999;128:1361–1369.
20. Bantel H, Berg C, Vieth M et al. Mesalazine inhibits activation of transcription factor NF-kappaB in inflamed mucosa of patients with ulcerative colitis. *Am J Gastroenterol*. 2000;95:3452–3457.
21. Desreumaux P, Ghosh S. Review article: mode of action and delivery of 5-aminosalicylic acid – new evidence. *Aliment Pharm Ther*. 2006; 24(Suppl 1):2–9.
22. Klotz U. The pharmacological profile and clinical use of mesalazine (5-aminosalicylic acid). *Arzneimittelforschung*. 2012;62:53–58.
23. Desreumaux P. Understanding the mechanism of 5-ASA in treating colonic inflammation. *Gastroenterol Hepatol*. 2008;4:319–320.
24. Bertin B, Dubuquoy L, Colombel JF, Desreumaux P. PPAR-gamma in ulcerative colitis: a novel target for intervention. *Curr Drug Targets*. 2013;14:1501–1507.
25. Lichtenstein GR, Kamm MA. Review article: 5-aminosalicylate formulations for the treatment of ulcerative colitis – methods of comparing release rates and delivery of 5-aminosalicylate to the colonic mucosa. *Aliment Pharmacol Ther*. 2008;28:663–673.
26. Campieri M, Lanfranchi GA, Bazzocchi G, et al. Treatment of ulcerative colitis with high-dose 5-aminosalicylic acid enemas. *Lancet*. 1981;2(8241):270–271.
27. Harris MS, Lichtenstein GR. Review article: delivery and efficacy of topical 5-aminosalicylic acid (mesalazine) therapy in the treatment of ulcerative colitis. *Aliment Pharmacol Ther*. 2011;33:996–1009.
28. Ford AC, Khan KJ, Achkar JP, et al. Efficacy of oral vs topical, or combined oral and topical 5-aminosalicylates, in ulcerative colitis: systematic review and meta-analysis. *Am J Gastroenterol*. 2012;107: 167–176.
29. Campieri M, Corbelli C, Gionchetti P, et al. Spread and distribution of 5-ASA colonic foam and 5-ASA enema in patients with ulcerative colitis. *Dig Dis Sci*. 1992;37:1890–1897.
30. Ghosh S, Shand A, Ferguson A. Ulcerative colitis. *BMJ*. 2000;320: 1119–1123.
31. Seibold F, Fournier N, Beglinger C, et al. Swiss IBD cohort study group. Topical therapy is underused in patients with ulcerative colitis. *J Crohns Colitis*. 2014;8:56–63.
32. Farrell RJ, Peppercorn MA. Equimolar doses of balsalazide and mesalamine: are we comparing apples and oranges? *Am J Gastroenterol*. 2002;97:1283–1285.
33. Forbes A, Cartwright A, Marchant S, McIntyre P, Newton M. Review article: Oral, modified-release mesalazine formulations – proprietary versus generic. *Aliment Pharmacol Ther*. 2003;17:1207–1214.
34. Wilding IR, Kenyon CJ, Hooper G. Gastroinestinal spread of oral prolonged-release mesalazine microgranules (Pentasa) dosed as either tablets or sachet. *Aliment Pharmacol Ther*. 2000;14:163–169.
35. Farup PG, Hinterleitner TA, Lukas M, et al. Mesalazine 4 g daily given as prolonged-release granules twice daily and four times daily is at least as effective as prolonged-release tablets four times daily in patients with ulcerative colitis. *Inflamm Bowel Dis*. 2001;7:237–242.
36. Kruis W, Klugmann T, Duffelmeyer C, et al. Detailed analysis of factors determining patients adherence to therapy in ulcerative colitis. *UEG J*. 2013;1(Suppl 1):A224 (P342).
37. Forbes A, Al-Damluji A, Ashworth S, et al. Multicentre randomized-controlled clinical trial of Ipocol, a new enteric-coated form of mesalazine, in comparison with Asacol in the treatment of ulcerative colitis. *Aliment Pharmacol Ther*. 2005;21:1099–1104.
38. Wilding IR, Behrens C, Tardif SJ, et al. Combined scintigraphic and pharmacokinetic investigation of enteric-coated mesalazine micropellets in healthy subjects. *Aliment Pharmacol Ther*. 2003;17:1153–1162.
39. Raedler A, Behrens C, Bias P. Mesalazine (5-aminosalicylic acid) micropellets show similar efficacy and tolerability to mesalazine tablets in patients with ulcerative colitis – results from a randomized-controlled trial. *Aliment Pharmacol Ther*. 2004;20:1353–1363.
40. Brunner M, Greinwald R, Kletter K, et al. Gastrointestinal transit and release of 5-aminosalicylic acid from 153Sm-labelled mesalazine pellets vs tablets in male healthy volunteers. *Aliment Pharmacol Ther*. 2003;17:1163–1169.
41. Kruis W, Bar-Meir S, Feher J, et al. The optimal dose of 5-aminosalicylic acid in active ulcerative colitis: a dose-finding study with newly developed mesalamine. *Clin Gastroenterol Hepatol*. 2003;1:36–43.
42. Lakatos PL. Use of new once-daily 5-aminosalicylic acid preparations in the treatment of ulcerative colitis: is there anything new under the sun? *World J Gastroenerol*. 2009;15:1799–1804.
43. Brunner M, Assandri R, Kletter K, et al. Gastrointestinal transit and 5-ASA release from a new mesalazine extended-release formulation. *Aliment Pharmacol Ther*. 2003;17:395–402.
44. Prantera C, Viscido A, Biancone L, et al. A new oral delivery system for 5-ASA: preliminary clinical findings for MMX. *Inflamm Bowel Dis*. 2005;11:421–427.
45. Sutherland L, MacDonald JK. Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev*. 2006;2:CD000544.
46. Ito H, Iida M, Matsumoto T, et al. Direct comparison of two different mesalamine formulations for the induction of remission in patients with ulcerative colitis: a double-blind, randomized study. *Inflamm Bowel Dis*. 2010;16:1567–1574.
47. Prantera C, Kohn A, Campieri M, et al. Clinical trial: ulcerative colitis maintenance treatment with 5-ASA: a 1-year, randomized multicentre study comparing MMX with Asacol. *Aliment Pharmacol Ther*. 2009;30:908–918.
48. Feagan BG, Macdonald JK. Oral 5-aminosalicylic acid for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev*. 2012;10:CD000544.
49. Feagan BG, Macdonald JK. Oral 5-aminosalicylic acid for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev*. 2012;10:CD000543.
50. Kane S, Huo D, Magnanti K. A pilot feasibility study on once daily versus conventional dosing mesalamine for maintenance of ulcerative colitis. *Clin Gastroenterol Hepatol*. 2003;1:170–173.
51. Robinson A. Review article: improving adherence to medication in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2008;27(Suppl 1):9–14.
52. Hawthorne AB, Rubin G, Ghosh S. Review article: medication non-adherence in ulcerative colitis – strategies to improve adherence with mesalazine and other maintenance therapies. *Aliment Pharmacol Ther*. 2008;27:1157–1166.

53. Kane SV, Cohen RD, Aikens JE, et al. Prevalence of non-adherence with maintenance mesalamine in quiescent ulcerative colitis. *Am J Gastroenterol*. 2001;96:2929–2933.
54. Shale MJ, Riley SA. Studies of compliance with delayed-release mesalazine therapy in patients with inflammatory bowel disease. *Aliment Pharmacol Ther*. 2003;18:191–198.
55. Lakatos PL. Prevalence, predictors, and clinical consequences of medical adherence in IBD: how to improve it? *World J Gastroenterol*. 2009;15:4234–4239.
56. Kane SV, Robinson A. Review article: understanding adherence to medication in ulcerative colitis – innovative thinking und evolving concepts. *Aliment Pharmacol Ther*. 2010;32:1051–1058.
57. Kane S, Huo D, Aikens J, et al. Medication non-adherence and outcomes of patients with quiescent ulcerative colitis. *Am J Med*. 2003;114:39–43.
58. van Hees PAM, van Tongeren JHM. Compliance to therapy in patients on a maintenance dose of sulfasalazine. *J Clin Gastroenterol*. 1982;4:333–336.
59. Claxton AJ, Cramer J, Pierce C. A systematic review of the associations between dose regimens and medication compliance. *Clin Ther*. 2001;23:1296–1310.
60. Hussain FN, Ajjan RA, Kapur K, Moustafa M, Riley SA. Once versus divided daily dosing with delayed-release mesalazine: a study of tissue drug concentrations and standard pharmacokinetic parameters. *Aliment Pharmacol Ther*. 2001;15:53–62.
61. Gandia P, Idier I, Houin G. Is once-daily mesalazine equivalent to the currently used twice-daily regimen? A study performed in 30 healthy volunteers. *J Clin Pharmacol*. 2007;47:334–342.
62. Das KM, Eastwood MA, McManus JP, et al. Adverse reactions during salicylazosulfapyridine therapy and the relation with drug metabolism and acetylator phenotype. *N Engl Med J*. 1973;289:491–495.
63. Ford AC, Khan KJ, Sandborn WJ, et al. Once daily dosing vs conventional dosing schedule of mesalamine and relapse of quiescent ulcerative colitis: systematic review and meta-analysis. *Am J Gastroenterol*. 2011;106:2070–2077.
64. Hayllar J, Bjarnason I. Sulphasalazine in ulcerative colitis: in memoriam? *Gut*. 1991;32:462–463.
65. Kruis W, Jonaitis L, Pokrotnieks J, et al. Randomised clinical trial: a comparative dose-finding study of three arms of dual release mesalazine for maintaining remission in ulcerative colitis. *Aliment Pharmacol Ther*. 2011;33:313–322.
66. Hanauer SB, Sandborn WJ, Kornbluth A, et al. Delayed-release oral mesalamine at 4.8 g/day (800 mg tablet) for the treatment of moderately active ulcerative colitis: the ASCEND II trial. *Am J Gastroenterol*. 2005;100:2478–2485.
67. Lichtenstein GR, Ramsey D, Rubin DT. Randomised clinical trial: delayed-release oral mesalazine 4.8 g/day vs 2.4 g/day in endoscopic mucosal healing – ASCEND I and II combined analysis. *Aliment Pharmacol Ther*. 2011;33:672–678.
68. Sandborn WJ, Regula J, Feagan BG, et al. Delayed-release oral mesalamine 4.8 g/day (800-mg tablet) is effective for patients with moderately active ulcerative colitis. *Gastroenterology*. 2009;137:1934–1943.
69. Kamm MA, Sandborn WJ, Gassull M, et al. Once-daily, high-concentration MMX mesalamine in active ulcerative colitis. *Gastroenterology*. 2007;132:66–75.
70. Shire plc. Annual Reports from 2008 to 2012. Available from: http://www.shire.com/shireplc/en/investors/reports. Accessed August 4, 2013.
71. Stallmach A, Häuser W, L'hoest H, et al. Die chronisch entzündlichen Darmerkrankungen Morbus Crohn und Colitis ulcerosa: Herausforderungen an die Versorgung. [The inflammatory bowel diseases Crohns disease and ulcerative colitis: Challenges for health care]. In: Repschläger U, Schulte C, Osterkamp N, editors. Barmer GEK Gesundheitswesen aktuell 2012. [Barmer GEK public health care up to date 2012]. Düsseldorf, Germany: 37 Grad GmbH; 286–309. German.
72. Glaeske G, Schicktanz C. Schriftenreihe zur Gesundheitsanalyse, Band 14. [Monograph series for analysis of health. Volume 14]. Barmer GEK Arzneimittelreport 2012. Available from: https://presse.barmer-gek.de/barmer/web/Portale/Presseportal/Subportal/Infothek/Studien-und-Reports/Arzneimittelreport/Einstieg-Arzneimittelreport.html. Accessed July 30, 2013. German.
73. Blumenstein I, Tacke W, Filmann N, et al. [Integrated management of patients with chronic inflammatory bowel disease in the Rhine-Main region: results of the first integrated health-care project IBD in Germany]. *Z Gastroenterol*. 2013;51:613–618. German.
74. Istock Analyst [homepage on the Internet]. Warner Chilcott Plc: numerous sources of potential upside. February 20, 2013. Available from: http://www.istockanalyst.com/finance/story/6300463/warner-chilcott-plc-numerous-sources-of-potentialupside. Accessed August 11, 2013.
75. Warner Chilcott plc. Form 10-K for December 31, 2012. Available from: https://tickerpot.com/symbol/wcrx/1323854/topic/asacol. Accessed August 11, 2013.
76. Kane S, Holderman W, Jacques P, Miodek T. Once daily versus conventional dosing of pH-dependent mesalamine long-term to maintain quiescent ulcerative colitis: Preliminary results from a randomized trial. *Patient Prefer Adherence*. 2008;2:253–258.
77. Kamm MA, Lichtenstein GR, Sandborn WJ, et al. Randomised trial of once- or twice-daily MMX mesalazine for maintenance of remission in ulcerative colitis. *Gut*. 2008;57:893–902.
78. Dignass AU, Bokemeyer B, Adamek H, et al. Mesalamine once daily is more effective than twice daily in patients with quiescent ulcerative colitis. *Clin Gastroenterol Hepatol*. 2009;7:762–769.
79. Sandborn WJ, Korzenik J, Lashner B, et al. Once-daily dosing of delayed-release oral mesalamine (400-mg tablet) is as effective as twice-daily dosing for maintenance of remission of ulcerative colitis. *Gastroenterology*. 2010;138:1286–1296.
80. Hawthorne AB, Stenson R, Gillespie D, et al. One-year investigator-blind randomized multicenter trial comparing Asacol 2.4 g once daily with 800 mg three times daily for maintenance of remission in ulcerative colitis. *Inflamm Bowel Dis*. 2012;18:1885–1893.
81. Lichtenstein GR, Kamm MA, Boddu P, et al. Effect of once- or twice-daily MMX mesalamine (SPD476) for the induction of remission of mild to moderate active ulcerative colitis. *Clin Gastroenterol Hepatol*. 2007;5:95–102.
82. Kruis W, Kiudelis G, Racz I, et al. Once daily versus three times daily mesalazine granules in active ulcerative colitis: a double-blind, double-dummy, randomised, non-inferiority trial. *Gut*. 2009;58:233–240.
83. Flourie B, Hagege H, Tucat G, et al. Randomised clinical trial: once- vs twice-daily prolonged-release mesalazine for active ulcerative colitis. *Aliment Pharmacol Ther*. 2013;37:767–775.
84. Feagan BG, MacDonald JK. Once daily oral mesalamine compared to conventional dosing for induction and maintenance of remission in ulcerative colitis: a systematic review and meta-analysis. *Inflamm Bowel Dis*. 2012;18:1785–1794.
85. Leifeld L, Pfützer R, Morgenstern J, et al. Mesalazine granules are superior to Eudragit-L-coated mesalazine tablets for induction of remission in distal ulcerative colitis – a pooled analysis. *Aliment Pharmacol Ther*. 2011;34:1115–1122.
86. Brooks AJ, Lobo AJ. Are mesalazine granules superior to Eudragit-L-coated mesalazine tablets for induction of remission in distal ulcerative colitis? *Aliment Pharmacol Ther*. 2012;35:193–194.
87. Sandborn WJ, Kamm MA, Lichtenstein GR, et al. MMX Multi Matrix System mesalazine for the induction of remission in patients with mild-to-moderate ulcerative colitis: a combined analysis of two randomized, double-blind, placebo-controlled trials. *Aliment Pharmacol Ther*. 2007;26:205–215.
88. Kane SV, Sumner M, Solomon D, Jenkins M. Twelve-month persistency with oral 5-aminosalicylic acid therapy for ulcerative colitis: results from a large pharmacy prescriptions database. *Dig Dis Sci*. 2011;56:3463–3470.

89. Yen L, Wu J, Hodgkins PL, Cohen RD, Nichol MB. Medication use patterns and predictors of nonpersistence and nonadherence with oral 5-aminosalicylic acid therapy in patients with ulcerative colitis. *J Manag Care Pharm*. 2012;18:701–712.
90. Lachaine J, Yen L, Beauchemin C, Hodgkins P. Medication adherence and persistence in the treatment of Canadian ulcerative colitis patients: analyses with the RAMQ database. *BMC Gastroenterol*. 2013;13:23.
91. Jackson CA, Clatworthy J, Robinson A, Horne R. Factors associated with non-adherence to oral medication for inflammatory bowel disease: a systematic review. *Am J Gastroenterol*. 2010;105:525–539.
92. Ransford RAJ, Langman MJS. Sulphasalazine and mesalazine: serious adverse reactions re-evaluated on the basis of suspected adverse reaction reports to the Committee on Safety of Medicines. *Gut*. 2002;51:536–539.
93. D'Haens G, van Bodegraven AA. Mesalazine is safe for the treatment of IBD. *Gut*. 2004;53:155.
94. Loftus EV Jr, Kane SV, Bjorkman D. Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. *Aliment Pharmacol Ther*. 2004;19:179–189.
95. Feagan BG, Chande N, MacDonald JK. Are there any differences in the efficacy and safety of different formulations of oral 5-ASA used for induction and maintenance of remission in ulcerative colitis? Evidence from cochrane reviews. *Inflamm Bowel Dis*. 2013;19:2031–2040.
96. Schroeder KW. Review: is mesalamine safe? *Gastroenterol Hepatol*. 2007;3:878–879.
97. van Staa TP, Travis S, Leufkens HG, Logan RF. 5-aminosalicylic acids and the risk of renal disease: a large British epidemiologic study. *Gastroenterology*. 2004;126:1733–1739.
98. Gisbert JP, González-Lama Y, Maté J. 5-Aminosalicylates and renal function in inflammatory bowel disease: a systematic review. *Inflamm Bowel Dis*. 2007;13:629–638.
99. So K, Bewshea BW, Heap GA, et al. 5-Aminosalicylate (5-ASA) induced nephrotoxicity in inflammatory bowel disease. *Gastroenterology*. 2013;144(Suppl 1):112(A638).

AP&T Alimentary Pharmacology and Therapeutics

Mesalazine granules are superior to Eudragit-L-coated mesalazine tablets for induction of remission in distal ulcerative colitis – a pooled analysis

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SUMMARY

Background
Different oral formulations of 'mesalazine (mesalamine)' may have different efficacy in distal ulcerative colitis.

Aim
To evaluate the efficacy of mesalazine granules (Salofalk granules) vs. mesalazine tablets (Salofalk tablets) as induction therapy in patients with distinct extensions of ulcerative colitis.

Methods
A pooled analysis of 705 patients from four prospective, randomised, double-blind phase III trials was performed. The efficacy of 8 weeks' induction with 3 g/day mesalazine granules [3 g once daily (o.d.) or 1 g three times daily (t.d.s)] vs. 3 g/day mesalazine tablets (1 g t.d.s.) was compared in terms of clinical remission (CR: CAI ≤ 4) and endoscopic remission (ER: EI ≤ 3) (both according to Rachmilewitz) in subgroups with pancolitis, left-sided colitis, or proctosigmoiditis.

Results
Mesalazine granules were equipotent to mesalazine tablets in pancolitis regarding CR (72% vs. 71%, $P = 0.909$) and ER (58% vs. 49%, $P = 0.338$). In left-sided colitis, both mesalazine formulations were equipotent regarding CR (66% vs. 67%; $P = 0.843$) but mesalazine granules were superior regarding ER (56% vs. 37%; $P = 0.025$). In proctosigmoiditis, mesalazine granules were significantly more effective than mesalazine tablets regarding CR (78% vs. 55% $P < 0.001$) and ER (67% vs. 43% $P < 0.001$). Furthermore, o.d. application of mesalazine granules was more effective than t.d.s. dosing in left-sided colitis (CR 73% vs. 62%, $P = 0.181$; ER 71% vs. 48% $P = 0.005$) and proctosigmoiditis (CR 86% vs. 73%, $P = 0.020$; ER 75% vs. 61%, $P = 0.021$), but not in pancolitis.

Conclusion
This pooled analysis supports the hypothesis that mesalazine granules are superior to mesalazine tablets in induction of remission in distal colitis and should be taken once daily.

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INTRODUCTION

Mesalazine (mesalamine) [5-aminosalicylate (5-ASA)] has demonstrated therapeutic efficacy for induction and maintenance of remission in ulcerative colitis (UC). Efficacy and a favourable safety profile make mesalazine the gold standard for induction of remission in mild-to-moderately active UC. Because of its topical mode of action, mesalazine must reach the inflamed mucosa from the luminal side of the bowel. This can be achieved via oral administration of delayed-release formulations which deliver 5-ASA at the appropriate location in the bowel, or by rectal application of mesalazine in the form of enemas, foams, or suppositories. Topical rectal therapy is highly effective for distal UC. Furthermore, increasing intraluminal mesalazine concentrations in the left-sided bowel by adding topical application of mesalazine to oral administration enhances the induction of remission not only in left-sided but also in extended mild-to-moderately active UC,[1] and is recommended by the European Crohn's and Colitis Organisation (ECCO) consensus.[2] However, long-term rectal therapy is considered difficult by some patients, leading to inconvenience and non-adherence.

Manifestation of UC within the colon can range from limited inflammation confined to the rectum up to extensive disease throughout the entire colon, with important implications for the treatment of individual patients. UC often involves only the distal colon, with less frequent involvement of the complete colon. It is therefore highly relevant to compare the effects of different pharmaceutical formulations of mesalazine in the treatment of varying manifestations of UC to support individualised therapy of patients. Patients with proctosigmoiditis are of particular interest, since the effectiveness of conventional oral formulations of mesalazine can sometimes be disappointing in this setting,[3–5] leading to a requirement for application of rectal formulations.

Preparations of oral mesalazine have been introduced which aim to deliver 5-ASA to more distal parts of the colon, too, and an analysis of their effects in subgroups of patients with proctosigmoiditis is merited. Herein, we compare the effect of conventional Eudragit-L-coated mesalazine tablets (Salofalk tablets) with mesalazine granules that have a delayed and extended release profile (Salofalk granules) in a pooled analysis of four recently published studies (SAG-2,[6] SAG-15,[7] SAG-26,[8] and SAT-14[9]). Conventional mesalazine tablets have an acid-resistant enteric coating (Eudragit-L), allowing pH-controlled release of mesalazine starting at a pH of approximately ≥6.0 from the terminal ileum and the right-sided colon onwards. The mesalazine granules combine this pH-controlled release with an extended-release mechanism mediated by an inner polymer matrix core in which mesalazine is embedded. While mesalazine tablets (Salofalk tablets) lead to high luminal concentrations of the active drug in the terminal ileum and right colon, the delayed and extended release profile of mesalazine granules (Salofalk granules) ensures a continuous release of mesalazine throughout the entire colon.[10]

MATERIALS AND METHODS

We included all prospective, randomised, double-blind phase III trials on induction therapy of mild-to-moderately active UC with mesalazine granules (Salofalk granules, Dr. Falk Pharma GmbH, Freiburg, Germany) or mesalazine tablets (Salofalk tablets, Dr. Falk Pharma GmbH). Patients with mild-to-moderately active UC participating in two trials of mesalazine granules with a delayed and extended release profile (Salofalk granules) (SAG-2,[6] SAG-26[8]) and one trial with Eudragit-L-coated mesalazine tablets (Salofalk tablets) (SAT-14[9]) and one trial with mesalazine granules or mesalazine tablets (SAG-15[7]), were included in this pooled subgroup efficacy analysis. The analysis included all patients from the intention-to-treat population who were randomised, had received at least one dose of study medication, had active UC at baseline, and for whom the extension of the disease at baseline was known. Patients were to be treated with mesalazine 3.0 g/day for 8 weeks.

Study population, inclusion and exclusion criteria and assessments were very similar in each of the four studies, permitting pooling of data for the subgroup analysis. Patients with either a first attack or established UC were eligible. Activity of colitis was assessed based on the Clinical Activity Index (CAI) according to Rachmilewitz.[11] The total score consists of the following parameters: stool frequency, blood in stools, abdominal pain/cramps, general well-being, temperature caused by colitis, extra-intestinal manifestations and laboratory data (erythrocyte sedimentation rate and haemoglobin). Patients with a CAI score above 4[8] or between 6 and 12[6, 7, 9] were enrolled in the studies, corresponding to mild-to-moderately active UC. The Endoscopic Index (EI) according to Rachmilewitz[11] was recorded for each patient. Diagnosis of UC was confirmed by colonoscopy and histological examination of mucosal tissue samples. Microbiological examination of stools ruled out any bacteria causing infectious colitis. Exclusion criteria were immunosuppressive drugs within at least 1 month prior to baseline and mesalazine >500 mg/day[7] or

>2 g/day.[6, 8, 9] All concomitant treatments for UC were stopped at baseline.

The primary endpoint in each of the studies was clinical remission, defined as CAI ≤ 4 at the end of the study. Endoscopic remission was defined as EI ≤ 3 at study end.

In SAG-15, the initial treatment was 0.5 g mesalazine three times daily (t.d.s.) (1.5 g/day). In the event of an inadequate response to this regimen, the daily dose could be increased to 3 g mesalazine. The patients treated with 3 g mesalazine in this trial might thus represent a subgroup with more severe colitis compared to the other trials. Therefore, pooled data from the other three trials were also analysed separately, excluding patients from SAG-15.

Aim of the study

The aim of the study was to evaluate the efficacy of mesalazine granules with a delayed and extended release profile vs. Eudragit-L-coated mesalazine tablets as induction therapy in subgroups of patients with distinct extensions of UC (pancolitis, left-sided colitis and proctosigmoiditis).

Statistical methods

The statistical analyses were carried out using the SAS statistical software (version 9.2, SAS Institute Inc., Cary, NC, USA).

Within each subgroup of patients with distinct extensions of UC, the proportions of patients with clinical remission and with endoscopic remission were tabulated for each formulation and application frequency. For patients without a valid endpoint (clinical remission and endoscopic remission, respectively), nonresponse was assumed.

Two-sided chi-squared tests were performed to evaluate differences between the granule and tablet formulations and between the application frequencies once daily (o.d.) and t.d.s. (granules only). *P* values were calculated to compare the overall incidences of different adverse event categories between formulations and application frequencies using the two-sided chi-squared test. *P* values to compare incidences of individual adverse events were calculated using Fisher's exact test.

As all analyses were exploratory, no adjustment for multiplicity was performed.

Ethical considerations

The studies were performed in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki and were approved in each participating country by a central Independent Ethics Committee and/or local independent Ethics Committees. Written informed consent was obtained from each patient.

RESULTS

A total of 705 patients were included in the analysis. From SAG-2, 107 patients were included, who were treated with mesalazine granules 1 g t.d.s. From SAG-15, 96 patients were included, 44 of whom were treated with mesalazine granules 1 g t.d.s., while 52 were treated with mesalazine tablets 1 g t.d.s. From SAG-26, 380 patients were included, 191 of whom were treated with 3 g mesalazine granules o.d. and 189 of whom were treated with mesalazine granules 1 g t.d.s. Lastly, from SAT-14, 122 patients were included who were treated with mesalazine tablets 1 g t.d.s. In total, 191 of these patients were treated with mesalazine granules 3 g o.d., 340 patients were treated with mesalazine granules 1 g t.d.s., and 174 patients were treated with mesalazine tablets 1 g t.d.s. for 8 weeks. Three hundred and fifty patients suffered from proctosigmoiditis, 204 from left-sided colitis and 151 from pancolitis, according to baseline endoscopic status. Baseline characteristics of the patients are summarised in Table 1.

In pancolitis, no difference was observed between the different galenic formulations, either between mesalazine granules vs. mesalazine tablets or between mesalazine granules o.d. vs. t.d.s. schedule. Clinical remission was achieved with mesalazine granules in 72% of patients in the o.d. group and 73% in the t.d.s. group ($P = 0.916$) (overall t.d.s. + o.d.: 72%) compared to 71% clinical remission with mesalazine tablets ($P = 0.909$) (Figures 1 and 3). The results for clinical remission were confirmed by endoscopic index data in patients with pancolitis. Endoscopic remission was achieved with mesalazine granules in 59% of patients in the o.d. group and 57% in the t.d.s. group ($P = 0.850$) (overall t.d.s. + o.d.: 58%) compared to 49% endoscopic remission with mesalazine tablets ($P = 0.338$) (Figures 2 and 4).

The equipotency of different galenical formulations of mesalazine was also observed for clinical remission in left-sided colitis, for which there was no difference between 3 g o.d. vs. 1 g t.d.s. mesalazine granules (73% vs. 62%; $P = 0.181$; Figure 3). Furthermore, mesalazine granules were not superior to mesalazine tablets in left-sided colitis with respect to clinical remission (66% vs. 67%; $P = 0.843$; Figure 1). However, a significant difference was observed for endoscopic remission between 3 g o.d. vs. 1 g t.d.s. mesalazine granules (71% vs. 48%; $P = 0.005$; Figure 4) and between mesalazine granules (o.d. + t.d.s.) compared to mesalazine tablets (56% vs. 37%; $P = 0.025$; Figure 2).

Table 1 | Baseline characteristics

		Mesalazine granules 3 g			Mesalazine tablets 3 g (1 g t.d.s.)
		Total	3 g o.d.	1 g t.d.s.	
Total number of patients	*n* (%)	531 (75.3)	191 (27.1)	340 (48.2)	174 (24.7)
Age	Mean (s.d.)	42.3 (13.6)	41.8 (14.0)	42.6 (13.4)	41.4 (13.3)
Gender					
Female	*n* (%)	269 (50.7)	97 (50.8)	172 (50.6)	92 (52.9)
Male	*n* (%)	262 (49.3)	94 (49.2)	168 (49.4)	82 (47.1)
Body mass index (kg/m^2)	Mean (s.d.)	24.9 (4.4)	24.8 (4.6)	24.9 (4.3)	25.2 (4.3)
White	*n* (%)	528 (99.4)	189 (99)	339 (99.7)	171 (98.3)
Smoker: smoker/ex-smoker/ nonsmoker	%	9.2/21.7/69.1	9.4/20.9/69.6	9.1/22.1/68.8	8/22.4/69.5
Diagnosis: established/new	%	79.1/20.9	73.8/26.2	82.1/17.9	80.5/19.5
Course (in patients with established diagnosis) continuous/recurrent	%	4.3/95.7 *n* = 420	3.5/96.5 *n* = 141	4.7/95.3 *n* = 279	10.7/89.3 *n* = 140
Time (years) since diagnosis	Median (min/max)	2.9 (0/37)	1.8 (0/36)	3.6 (0/37)	3.5 (0/40)
Number of previous episodes	Mean (s.d.)	5.0 (5.3)	4.3 (5.2)	5.3 (5.4)	6.8 (8.9)
Duration (days) of acute episodes (in relapsing disease)	Median (min/max)	31 (2/644) *n* = 402	27 (2/428) *n* = 136	33.5 (2/644) *n* = 266	37 (3/739) *n* = 125
CAI	Mean (s.d.)	8.1 (2.1)	8.1 (2.2)	8.1 (2.0)	8.2 (1.6)
EI	Mean (s.d.)	7.6 (1.9)	7.5 (1.9)	7.6 (1.9)	8.0 (1.9)
Disease severity CAI 5-8/>8	%	63.8/36.2	63.4/36.6	64.1/35.9	65.5/34.5
Disease localisation					
Proctosigmoiditis	*n* (%)	257 (48.4)	97 (50.8)	160 (47.1)	93 (53.4)
Left-sided colitis	*n* (%)	158 (29.8)	55 (28.8)	103 (30.3)	46 (26.4)
Pancolitis	*n* (%)	116 (21.8)	39 (20.4)	77 (22.6)	35 (20.1)

CAI, clinical activity index; EI, endoscopic index.

In contrast, for patients with proctosigmoiditis, significant differences were found in all analysed parameters including induction of clinical and endoscopic remission by mesalazine granules compared to mesalazine tablets, and for o.d. vs. t.d.s. administration of mesalazine granules. Eight weeks of therapy with mesalazine granules led to 78% clinical remission, compared to 55% in the mesalazine tablets group ($P < 0.001$) (Figure 1). Endoscopic remission was also achieved more frequently with mesalazine granules (67%) than with mesalazine tablets (43%, $P < 0.001$) (Figure 2). Within the mesalazine granules group, there was a significant advantage for o.d. administration of 3 g compared to 1 g t.d.s. in terms of clinical remission (86% vs. 73%, $P = 0.020$; Figure 3), and endoscopic remission (75% vs. 61% $P = 0.021$) (Figure 4).

Similar results were obtained after excluding the 96 patients from SAG-15. In pancolitis, 74% achieved clinical remission with mesalazine granules (o.d. 72%, t.d.s. 75%; $P = 0.745$) compared to 71% with mesalazine tablets ($P = 0.779$). In left-sided colitis, 69% achieved clinical remission by mesalazine granules (o.d. 73%, t.d.s. 67%; $P = 0.450$) compared to 81% with mesalazine tablets ($P = 0.193$). In proctosigmoiditis, 79% achieved clinical remission with mesalazine granules (o.d. 86%, t.d.s. 75%; $P = 0.051$) compared to 54% by mesalazine tablets ($P < 0.001$). Endoscopic remission was achieved in 60% of pancolitis patients with mesalazine granules (o.d. 59%, t.d.s. 61%; $P = 0.871$) and in 50% with mesalazine tablets ($P = 0.368$). In left-sided colitis, endoscopic remission was achieved in 60% by mesalazine granules (o.d. 71%, t.d.s. 52%; $P = 0.029$) and in 37% by mesalazine tablets

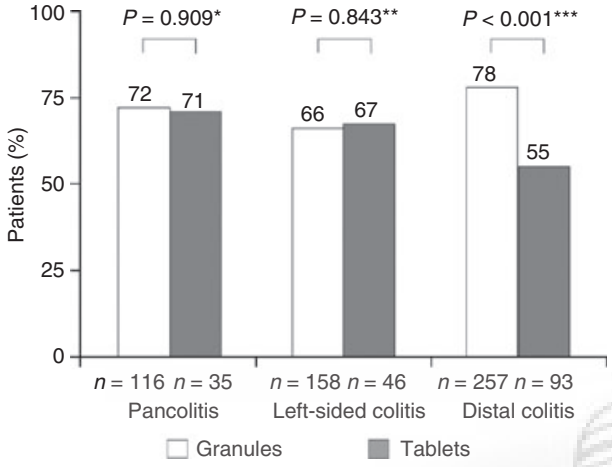

Figure 1 | Clinical remission (CAI ≤ 4) after 8 weeks induction therapy with mesalazine granules (3 g 5-ASA o.d. or 1 g 5-ASA t.d.s.) vs. mesalazine tablets (1 g 5-ASA t.d.s.) in patients with mild to moderately active pancolitis, left-sided colitis or proctosigmoiditis. *Δ1%, 95% CI (−16.0%; 17.9%), **Δ−1%, 95% CI (−14.0%; 17.1%), ***Δ23%, 95% CI (12.3%; 33.7%).

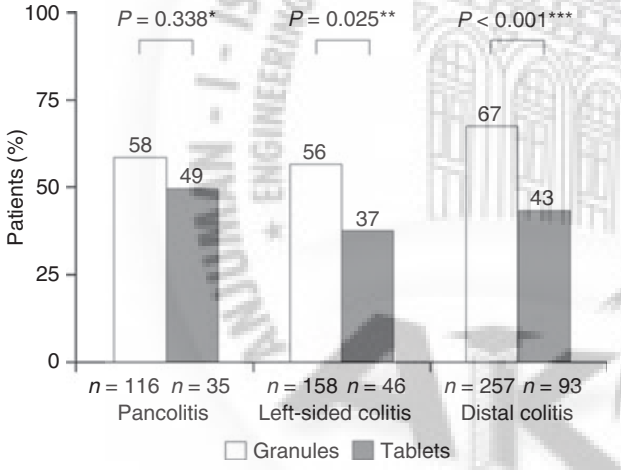

Figure 2 | Endoscopic remission (EI ≤ 3) after 8 weeks induction therapy with mesalazine granules (3 g 5-ASA o.d. or 1 g 5-ASA t.d.s.) vs. mesalazine tablets (1 g 5-ASA t.d.s.) in patients with mild to moderately active pancolitis, left-sided colitis or proctosigmoiditis. *Δ9%, 95% CI (−9.7%; 28.0%), **Δ19%, 95% CI (2.8%; 34.7%), ***Δ24%, 95% CI (11.9%; 35.1%).

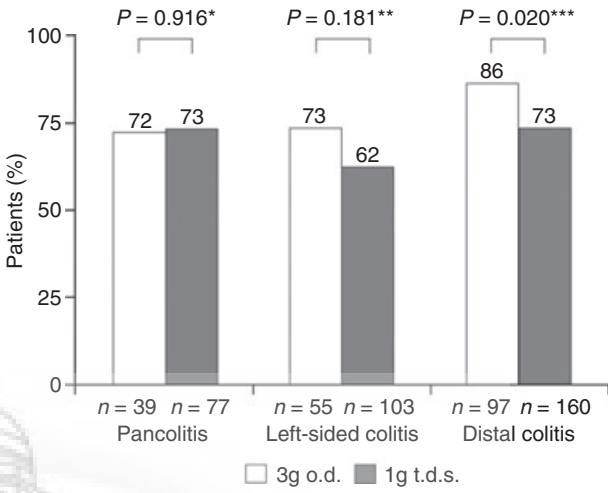

Figure 3 | Clinical remission (CAI ≤ 4) after 8 weeks induction therapy with 3 g 5-ASA o.d. vs. 1 g 5-ASA t.d.s. administered as granules in patients with mild to moderately active pancolitis, left-sided colitis or proctosigmoiditis. *Δ−1%, 95% CI (−16.3%; 18.2%), **Δ11%, 95% CI (−4.9%; 26.1%), ***Δ13%, 95% CI (2.0%; 22.9%). o.d., once daily; t.d.s., three times daily.

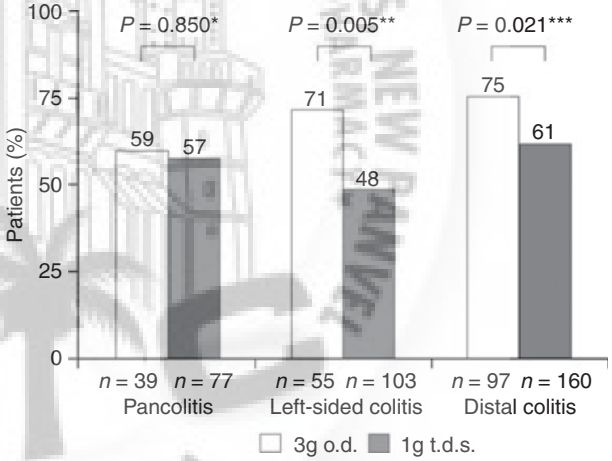

Figure 4 | Endoscopic remission (EI ≤ 3) after 8 weeks induction therapy with 3 g 5-ASA o.d. vs. 1 g 5-ASA t.d.s. administered as mesalazine granules in patients with mild to moderately active pancolitis, left-sided colitis or proctosigmoiditis. *Δ2%, 95% CI (−17.2%; 20.8%), **Δ23%, 95% CI (7.9%; 38.7%), ***Δ14%, 95% CI (2.6%; 25.4%). o.d., once daily; t.d.s., three times daily.

(P = 0.030), while in proctosigmoiditis endoscopic remission was achieved in 68% in mesalazine granules (o.d. 75%, t.d.s. 64%; P = 0.062) compared to 39% by mesalazine tablets (P < 0.001).

Adverse events and adverse drug reactions are summarised in Table 2. Fewer patients in the mesalazine granules group experienced adverse events. No unexpected serious adverse events occurred in either group, and there were no between-group differences in serious adverse events.

Table 2 | Adverse events and adverse drug reactions

		Mesalazine granules 3 g			Mesalazine tablets 3 g (1 g t.d.s.)	
		Total	3 g o.d.	1 g t.d.s.		
Number of adverse events	*n*	294	78	216	186	
Number of patients with adverse event	*n* (%)	197 (37.1)*	55 (28.8)†	142 (41.8)†	90 (51.7)*	$P = 0.001$* $P = 0.003$†
Intensity of adverse events mild/moderate/severe	% of patients	66/27/7	68/22/10	65/28/6	53/38/9	
Number of discontinuation due to adverse events	*n*	2	0	2	5	
Most frequent adverse events						
Headache	*n* (%)	51 (9.6)	9 (4.7)	42 (12.4)	34 (19.5)	
Abdominal pain	*n* (%)	12 (2.3)	3 (1.6)	9 (2.7)	5 (2.9)	
Aggravation of ulcerative colitis	*n* (%)	28 (5.3)	8 (4.2)	20 (5.9)	11 (6.3)	
Influenza-like symptoms	*n* (%)	11 (2.1)	4 (2.1)	7 (2.1)	3 (1.7)	
Nasopharyngitis	*n* (%)	17 (3.2)	6 (3.1)	11 (3.2)	4 (2.3)	
Nausea	*n* (%)	1 (0.2)	0 (0)	1 (0.3)	6 (3.5)	
Adverse events of special interest						
Allergic reactions	*n* (%)	1 (0.2)	0 (0)	1 (0.3)	2 (1.1)	
Nephritis	*n* (%)	1 (0.2)	0 (0)	1 (0.3)	0 (0)	
Number of patients with serious adverse events	*n* (%)	11 (2.1)*	4 (2.1)†	7 (2.1)†	4 (2.3)*	$P = 0.857$* $P = 0.978$†
Individual serious adverse events						
Deterioration of ulcerative colitis	*n*	7	4	3	3	
Viral upper respiratory tract infection	*n*	1	1	0	0	
Loose wire pacemaker	*n*	1	0	1	0	
Acute hearing loss	*n*	1	0	1	0	
Scheduled operation (not performed)	*n*	1	0	1	0	
Febrile state after colonoscopy with abdominal pain	*n*	0	0	0	1	
Measles	*n*	1	0	1	0	
Number of adverse drug reactions	*n*	35	8	27	45	
Number of patients with adverse drug reaction	*n* (%)	31 (5.8)*	6 (3.1)†	25 (7.4)†	25 (14.4)*	$P < 0.001$* $P = 0.047$†

A patient could experience more than one adverse event or adverse drug reaction.

* Granules vs. tablets.

† o.d. vs. t.d.s.

DISCUSSION

The results of this pooled subgroup analysis support the hypothesis that mesalazine granules with a delayed and extended release profile offer higher therapeutic efficacy than conventional Eudragit-L-coated mesalazine tablets for the induction of remission in patients with UC confined to the rectum and sigmoid. In pancolitis and left-sided mild-to-moderately active UC, mesalazine granules appear equipotent to mesalazine tablets. Favourable effects of mesalazine granules in distal colitis are plausible and consistent with the galenical properties of this formulation, since the extended release system allows more 5-

ASA to reach the distal parts of the colon. Moreover, the o.d. regimen was significantly more effective in patients with distal colitis than the t.d.s. dosing schedule, leading to 86% clinical remission after 8 weeks of therapy.

Topical application of 5-ASA is established as a highly effective therapy in mild-to-moderately active distal UC. It has shown superiority over placebo, topical corticosteroids and conventional galenical formulations of oral 5-ASA.[3–5, 12] Furthermore, the combination of rectal and oral 5-ASA is superior to oral application alone.[1] The efficacy of rectal 5-ASA is the result of up to a 100-fold increase in mucosal concentrations of 5-ASA compared to oral 5-ASA.[12] Nevertheless, the somewhat uncomfortable application, occasional difficulties in retaining rectal preparations in active UC and the tendency of mesalazine foams to cause anal irritation[12] make topical mesalazine less attractive for patients than oral mesalazine. Thus, oral mesalazine with extended release to the distal colon would be a valuable option in the treatment of distal UC. Mesalazine granules (Salofalk granules) with a delayed and extended release profile which provide high clinical remission rates in distal UC could be such an alternative.

The MMX (Multi Matrix System)-mesalazine provides another 5-ASA delivery system that also leads to extended colonic release and allows once daily administration. In two phase III studies and one combined analysis of all 517 patients, 8 weeks' induction treatment with 2.4–4.8 g MMX mesalazine in mild-to-moderately active UC achieved clinical remission rates of 21.2–41.2%.[13–15] The lower rates of remission in these studies compared to mesalazine granules (Salofalk granules) may be explained by a different outcome definition. When we used the same criteria for remission in a trial of Salofalk granules[8] as were used in the MMX trial (i.e. remission defined as total modified DAI score of 1 or less, with scores of zero for rectal bleeding and stool frequency, a combined PGA and sigmoidoscopy score of 1 or less, no friability, and at least a 1-point reduction from baseline in the sigmoidoscopy score), remission fell to 37% with Salofalk granules – a result similar to that obtained with MMX-mesalazine.

A preliminary study has suggested similar rates for induction of remission with 5-ASA enemas and MMX-mesalazine for patients with left-sided ulcerative colitis,[16] which is consistent with our findings that with Salofalk granules, which similar to MMX-mesalazine have also a delayed and extended release profile, one could achieve a better clinical outcome in the distal colon as compared to conventional mesalazine tablets.

Advanced therapeutic effects of o.d. mesalazine granules are of particular importance in distal colitis. Adherence to treatment is much higher when a drug can be taken o.d.,[17–22] and it is well known that long-term compliance is low in 5-ASA therapy for UC. In prospective, community-based studies, adherence rates are particularly poor among patients in symptomatic remission, with 60% of patients failing to adhere to a prescribed dose regimen and taking less than 70% of their prescribed medication.[18–20, 22, 23] Patients who fail to adhere to the prescribed 5-ASA regimen have a significantly higher chance of relapse[20] and an increased risk of developing colorectal carcinoma.[24, 25] Therefore, the high therapeutic efficacy of o.d. mesalazine granules may influence treatment decision-making in distal UC.

Overall, both mesalazine granules and mesalazine tablets proved to be safe and well tolerated drugs.

Based on this pooled analysis the hypothesis that mesalazine granules offer substantial therapeutic benefits in patients with distal UC should be confirmed in prospective controlled trials.

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REFERENCES

1. Marteau P, Probert CS, Lindgren S, *et al.* Combined oral and enema treatment with Pentasa (mesalazine) is superior to oral therapy alone in patients with extensive mild/moderate active ulcerative colitis: a randomised, double blind, placebo controlled study. *Gut* 2005; **54**: 960–5.

2. Travis SPL, Stange EF, Lémann M, *et al.* European evidence-based Consensus on the management of ulcerative colitis:

current management. *J Crohn Colitis* 2008; **2**: 24–62.
3. Gionchetti P, Rizzello F, Venturi A, *et al.* Comparison of oral with rectal mesalazine in the treatment of ulcerative proctitis. *Dis Colon Rectum* 1998; **41**: 93–7.
4. Kam L, Cohen H, Dooley C, *et al.* A comparison of mesalamine suspension enema and oral sulfasalazine for treatment of active distal ulcerative colitis in adults. *Am J Gastroenterol* 1996; **91**: 1338–42.
5. Safdi M, DeMicco M, Sninsky C, *et al.* A double-blind comparison of oral versus rectal mesalamine versus combination therapy in the treatment of distal ulcerative colitis. *Am J Gastroenterol* 1997; **92**: 1867–71.
6. Kruis W, Bar-Meir S, Feher J, *et al.* The optimal dose of 5-aminosalicylic acid in active ulcerative colitis: a dose-finding study with newly developed mesalamine. *Clin Gastroenterol Hepatol* 2003; **1**: 36–43.
7. Marakhouski Y, Fixa B, Holoman J, *et al.* A double-blind dose-escalating trial comparing novel mesalazine pellets with mesalazine tablets in active ulcerative colitis. *Aliment Pharmacol Ther* 2005; **21**: 133–40.
8. Kruis W, Kiudelis G, Racz I, *et al.* Once daily versus three times daily mesalazine granules in active ulcerative colitis: a double-blind, double-dummy, randomised non-inferiority trial. *Gut* 2009; **58**: 233–40.
9. Gibson PR, Fixa B, Pekarkova B, *et al.* Comparison of the efficacy and safety of Eudragit-L-coated mesalazine tablets with ethylcellulose-coated mesalazine tablets in patients with mild to moderately active ulcerative colitis. *Aliment Pharmacol Ther* 2006; **23**: 1017–26.
10. Brunner M, Greinwald R, Kletter K, *et al.* Gastrointestinal transit and release of 5-aminosalicylic acid from (153)Sm-labelled mesalazine pellets vs. tablets in male healthy volunteers. *Aliment Pharmacol Ther* 2003; **17**: 1163–9.
11. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: a randomised trial. *BMJ* 1989; **298**: 82–6.
12. Bergman R, Parkes M. Systematic review: the use of mesalazine in inflammatory bowel disease. *Aliment Pharmacol Ther* 2006; **23**: 841–55.
13. Kamm MA, Sandborn WJ, Gassull M, *et al.* Once-daily, high-concentration MMX mesalamine in active ulcerative colitis. *Gastroenterology* 2007; **132**: 66–75.
14. Lichtenstein GR, Kamm MA, Boddu P, *et al.* Effect of once- or twice-daily MMX mesalamine (SPD476) for the induction of remission of mild to moderately active ulcerative colitis. *Clin Gastroenterol Hepatol* 2007; **5**: 95–102.
15. Sandborn WJ, Kamm MA, Lichtenstein GR, *et al.* MMX Multi Matrix™ mesalazine for the induction of remission in patients with mild-to-moderate ulcerative colitis: a combined analysis of two randomized, double-blind, placebo-controlled trials. *Aliment Pharmacol Ther* 2007; **26**: 205–15.
16. Prantera C, Viscido A, Biancone L, *et al.* A new oral delivery system for 5-ASA: preliminary clinical findings for MMX. *Inflamm Bowel Dis* 2005; **11**: 421–7.
17. Desreumaux P, Ghosh S. Review article: mode of action and delivery of 5-aminosalicylic acid – new evidence. *Aliment Pharmacol Ther* 2006; **24**: 2–9.
18. Kane S, Huo D, Aikens J, *et al.* Medication nonadherence and the outcomes of patients with quiescent ulcerative colitis. *Am J Med* 2003; **114**: 39–43.
19. Kane SV, Cohen RD, Aikens JE, *et al.* Prevalence of nonadherence with maintenance mesalamine in quiescent ulcerative colitis. *Am J Gastroenterol* 2001; **96**: 2929–33.
20. Kane SV. Systematic review: adherence issues in the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 2006; **23**: 577–85.
21. Sewitch MJ, Abrahamowicz M, Barkun A, *et al.* Patient nonadherence to medication in inflammatory bowel disease. *Am J Gastroenterol* 2003; **98**: 1535–44.
22. Shale MJ, Riley SA. Studies of compliance with delayed-release mesalazine therapy in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2003; **18**: 191–8.
23. Levy RL, Feld AD. Increasing patient adherence to gastroenterology treatment and prevention regimens. *Am J Gastroenterol* 1999; **94**: 1733–42.
24. Moody GA, Jayanthi V, Probert CSJ, *et al.* Long-term therapy with sulphasalazine protects against colorectal cancer in ulcerative colitis: a retrospective study of colorectal cancer risk and compliance with treatment in Leicestershire. *Eur J Gastroenterol Hepatol* 1996; **8**: 1179–83.
25. van Staa TP, Card T, Logan RF, *et al.* 5-Aminosalicylate use and colorectal cancer risk in inflammatory bowel disease: a large epidemiological study. *Gut* 2005; **54**: 1573–8.

ORIGINAL ARTICLE

INTESTINAL RESEARCH

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Randomized, crossover questionnaire survey of acceptabilities of controlled-release mesalazine tablets and granules in ulcerative colitis patients

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Background/Aims: Oral mesalazine is an important treatment for ulcerative colitis (UC), and non-adherence to mesalazine increases the risk of relapse. Controlled-release (CR) mesalazine has 2 formulations: tablets and granules. The relative acceptabilities of these formulations may influence patient adherence; however, they have not been compared to date. This study aimed to evaluate the acceptabilities of the 2 formulations of CR mesalazine in relation to patient adherence using a crossover questionnaire survey. **Methods:** UC patients were randomly assigned to 2 groups in a 1:1 ratio. Patients in each group took either 4 g of CR mesalazine tablets or granules for 6 to 9 weeks, and then switched to 4 g of the other formulation for a further 6 to 9 weeks. The acceptability and efficacy were evaluated by questionnaires, and adherence was assessed using a visual analog scale. The difference in acceptabilities between the 2 formulations and its impact on adherence were assessed. **Results:** A total of 49 patients were prospectively enrolled and 33 patients were included in the analysis. Significantly more patients found the tablets to be less acceptable than the granules (76% vs. 33%, *P*=0.0005). The granules were preferable to the tablets when the 2 formulations were compared directly (73% vs. 21%, *P*=0.004), for their portability, size, and numbers of pills. The adherence rate was slightly better among patients taking the granules (94% vs. 91%) during the observation period, but the difference was not significant (*P*=0.139). **Conclusion:** CR mesalazine granules are more acceptable than tablets, and may therefore be a better option for long-term medication. (**Intest Res 2019;17:87-93**)

Key Words: Colitis, ulcerative; Mesalamine; Medication adherence; Patient acceptance of health care; Drug compounding

INTRODUCTION

Ulcerative colitis (UC) is a life-long disorder of the colon, characterized by a relapsing–remitting course.[1] The optimal goal of medical treatment in UC patients is to induce and maintain long-term remission. Oral 5-aminosalicylate (5-ASA) plays an important role in both induction and maintenance therapies, and numerous studies have revealed that non-adherence to mesalazine is associated with an increased risk of clinical relapse.[2,3] Improving adherence to mesalazine is therefore an important goal in daily clinical practice.

Ethylcellulose-coated controlled-release (CR) mesalazine (PENTASA®) is one of the major forms of oral 5-ASA.[4,5] CR mesalazine is available as 2 different formulations, tablets and granules, with no apparent difference in efficacy between them for UC, because both have the same mechanism of mesalazine release.[6] However, the acceptabilities of the formulations may differ. In addition to the general differences between tablets and granules, CR mesalazine granules include a signifi-

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cantly lower percentage of additives compared with the tablets, while the numbers of tablets or sachets that need to be taken differ. It is therefore possible that these differences may influence patient adherence;[7,8] however, no study has yet compared these 2 formulations in terms of acceptability and adherence.

This study aimed to evaluate the acceptabilities of the 2 formulations of CR mesalazine in relation to adherence among UC patients, using a crossover questionnaire survey.

METHODS

1. Patients

Outpatients diagnosed with UC at Kitasato University Kitasato Institute Hospital or Kyorin University Hospital and who were eligible for CR mesalazine were recruited from January to December 2016 in Kitasato University Kitasato Institute Hospital, and from April to August 2017 in Kyorin University Hospital. There was no age limit as long as the patients could assess the acceptability of the medications and answer the questionnaires unaided.

2. Formulations of CR Mesalazine

This crossover study compared PENTASA® tablets and granules. PENTASA® tablets (250 mg, 500 mg, and 1 g) are approved in over 100 countries, and PENTASA® granules (250 mg, 500 mg, 1 g, 2 g, and 4 g) are approved in over 80 countries worldwide. A single tablet contains about 33% additives, and a total weight of 6 g is therefore needed to deliver 4 g mesalazine, compared with only 4.24 g of the granules. PENTASA® tablets (500 mg/tablet) and granules (2 g/sachet) were used in this study.

3. Study Design

The outline of the study is shown in Fig. 1. Enrolled patients were randomly assigned to group 1 or group 2 in a 1:1 ratio. Patients in group 1 took CR mesalazine tablets and patients in group 2 took the granules for 6–9 (±3) weeks, and each group then switched to the other formulation for a further 6–9 (±3) weeks. Patients who were administered 2 g twice daily were further evaluated to compare the acceptability, adherence, and efficacy of the formulations. The endpoints of the study were the acceptability, preference, adherence, efficacy, and safety of the 2 formulations. Acceptability and preference were assessed based on the answers to the questionnaires, efficacy was assessed based on the changes of the partial Mayo score

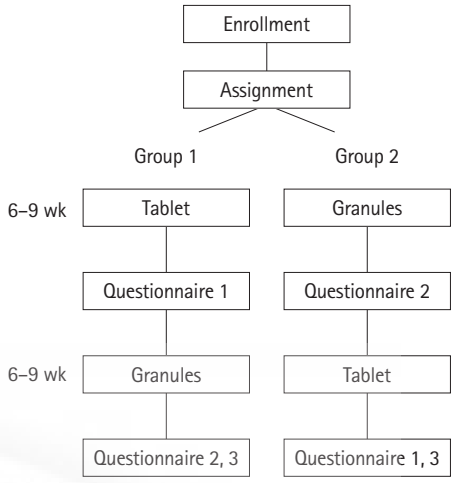

Fig. 1. Study design. Questionnaire 1, acceptability of tablets; questionnaire 2, acceptability of granules; questionnaire 3, comparison of tablets and granules.

before and after taking each formulation and based on the answers to the questionnaires. The differences between the 2 formulations were evaluated. Adherence rate (%) was assessed by a visual analog scale,[9] with an adherence rate of ≥80% defined as high adherence, and a rate of <80% defined as low adherence, as reported previously.[2,3] The average adherence rate, numbers of patients with high and low adherence, and adherence rate in each patient were compared between the 2 formulations. Safety was evaluated by assessing adverse events during the study period. The information on the enrolled patients was obtained from medical records.

4. Questionnaires

The English versions of the questionnaires are shown in Fig. 2 (the original was written in Japanese).

5. Statistical Analysis

All numerical values are shown as the median and range, or average±SD. Continuous variables were compared by *t*-tests, and proportions of categorical variables were compared by chi-square and Fisher exact tests. A *P*-value ≤0.05 was considered statistically significant. Statistical analyses were performed using EZR version 1.33 (Saitama Medical Center, Jichi Medical University, Saitama, Japan).

6. Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki and with good clinical practice. The study protocol was approved by the Institutional Review Boards of Kitasato

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University Kitasato Institute Hospital and Kyorin University School of Medicine. Written informed consent was obtained from all participating patients.

RESULTS

1. Patients

A total of 49 patients were prospectively enrolled. Twelve patients were excluded, including 11 who did not attend within the scheduled period and 1 who chose to withdraw from the study. Thirty-seven patients therefore completed the study. To ensure an accurate comparison of the acceptabilities and efficacies of the 2 formulations, 4 patients were excluded from the final analysis (33 patients) because they did not receive doses of 4 g/day, almost all patients were in remission (partial Mayo score, 1.0 ± 1.4) (Table 1). There was no significant difference in any patient characteristics or questionnaire responses between the 2 groups, and we therefore assessed the acceptabilities of the 2 formulations without distinguishing between the groups.

2. Formulation Acceptabilities

The results of questionnaires 1 and 2 (Fig. 2) regarding the difficulties in taking each formulation are shown in Fig. 3. Significantly more patients considered the tablets difficult to take compared with the granules (P=0.0005). When the 2 formulations were compared directly (questionnaire 3) (Fig. 2C), patients considered the granules to be significantly more acceptable than the tablets (P=0.004) (Fig. 4A), largely due to the reduced volume of medication in the granule formulation (Fig. 4B).

3. Medication Adherence

The adherence rate in patients taking the tablets was 91% ± 11% compared with 94% ± 8% in patients taking the granules (Fig. 5). There was no significant difference between the adherence rates for the 2 formulations (P=0.139), although adherence to the granules tended to be higher. Thirty percent of patients showed better adherence to the granules compared with the tablets, while 12% showed better adherence to the tablets (P=0.180). Eighteen percent of patients missed taking tablets because of their acceptability, compared with only 3% who

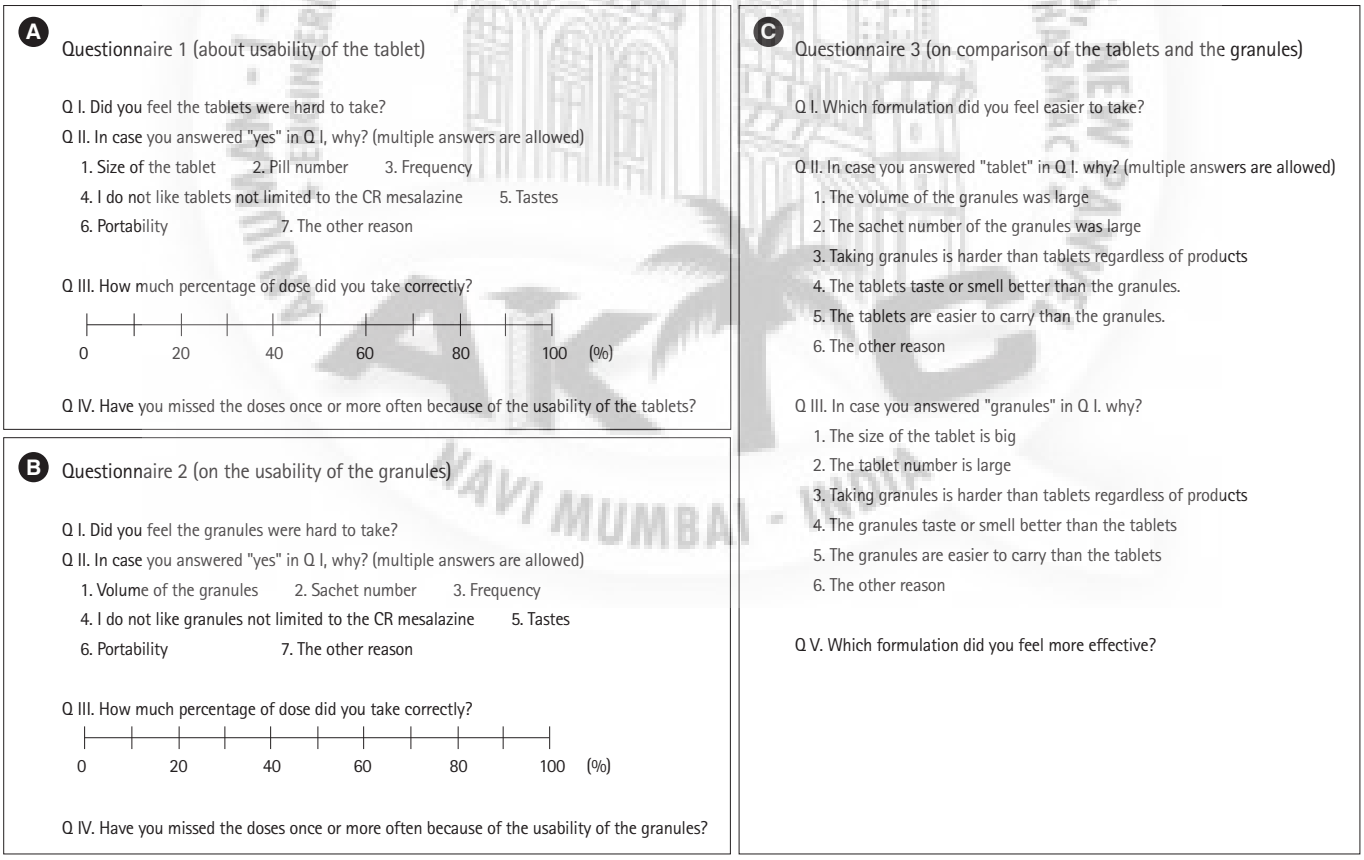

Fig. 2. (A) Questionnaire 1: acceptability of tablets. (B) Questionnaire 2: acceptability of granules. (C) Questionnaire 3: comparison of tablets and granules. All the original questionnaires were written in Japanese. CR, controlled-release.

Table 1. Patient Characteristics

Characteristics	All (n=33)	Group 1 (n=18)	Group 2 (n=15)	*P*-value[a]
Age (yr)	41±13	40±13	43±13	0.675
Sex (male/female)	15/18	10/8	5/10	0.296
Disease duration (yr)	9±9	7±6	12±11	0.174
Period of tablet (day)	53±10	55±6	52±13	0.342
Period of granules (day)	52±10	52±10	52±9	0.801
Concomitant medications				
Steroids	0	0	0	-
Thiopurines	11	7	4	0.458
Anti-TNF-α antibodies	6	3	3	0.804
Topical medications	5	3	2	0.790
Steroid-dependent	6	3	3	0.804
Steroid-refractory	3	2	1	0.698
Partial Mayo score at enrollment	1.0±1.4	1.2±1.6	0.8±1.1	0.405

Values are presented as mean±SD or number.
[a]Group 1 vs. group 2.

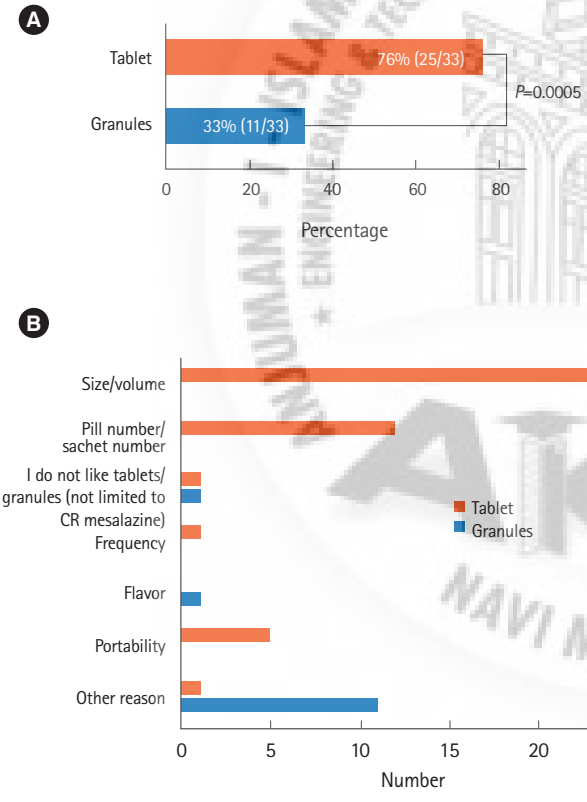

Fig. 3. Answers to the questions: (A) "Did you find the tablets/granules difficult to take?" (questionnaires 1 and 2, Q. I; n=33, chi-square test, *P*=0.0005), and (B) the reasons for the answers (multiple answers allowed). CR, controlled-release.

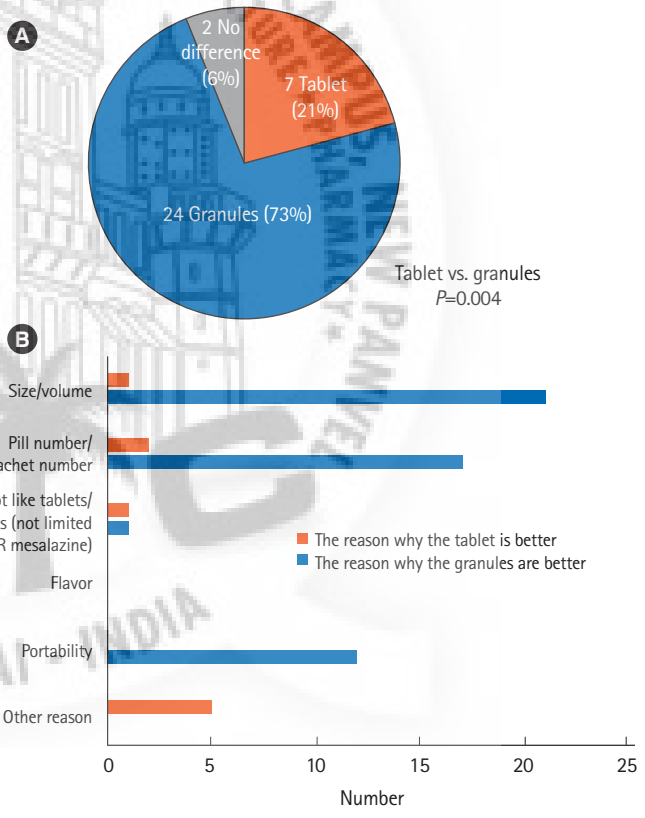

Fig. 4. Answers to the question: (A) "Which formulation did you find easier to take?" (questionnaire 3, Q. I; n=33, chi-square test, *P*=0.004), and (B) the reasons for the answers (multiple answers allowed). CR, controlled-release.

missed taking the granules (*P*=0.0456). Eighteen percent also felt that the tablets were more likely to be missed than the granules, whereas no patient felt that the granules were more likely to be missed. Six percent of patients showed high adherence

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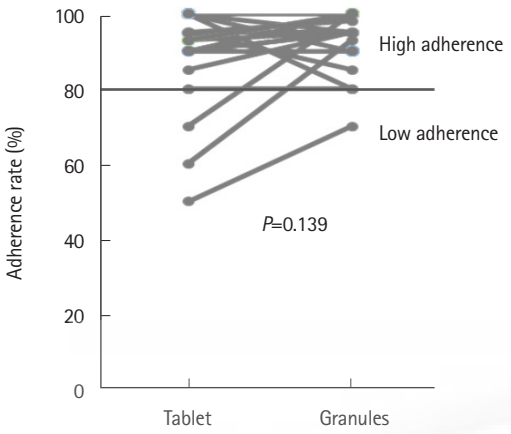

Fig. 5. Patient adherence rates to tablets and granules (n=33, paired *t*-test, *P*=0.139). High adherence, ≥80%; low adherence, <80%.

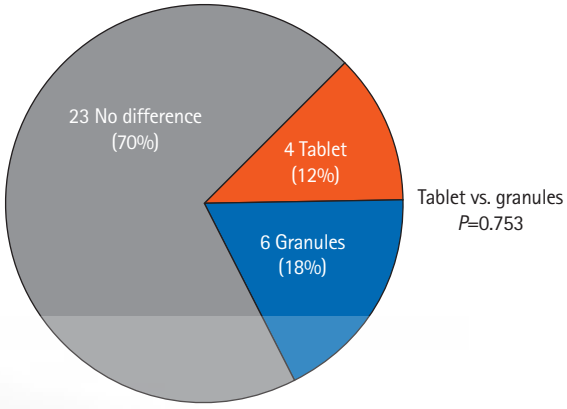

Fig. 7. Answers to the question "Which formulation did you feel was more effective?" (questionnaire 3, Q. V; n=33, chi-square test, *P*=0.753).

Fig. 6. Change of partial Mayo score before and after taking each formulation (no significant change was observed through this study. (A) Tablet (n=33, *P*=0.478) and (B) granules (n=33, *P*=0.838).

to the granules but low adherence to the tablets.

4. Efficacy

Before and after taking each formulation, no significant change of partial Mayo score was observed in both the 2 formulations (Fig. 6). The responses regarding the patients' perceptions of formulation efficacy are shown in Fig. 7. A total of 70% of patients noticed no difference between the 2 formulations, while 18% and 12% considered the granules and tablets to be more effective, respectively. There was no significant difference between the 2 formulations in terms of efficacy.

5. Adverse Events

Most patients experienced no adverse events related to CR mesalazine during the study period. One patient who had taken CR mesalazine tablets before enrollment experienced deterioration of abdominal symptoms during the granule period and improved after switching to the tablets; the partial Mayo score of the patient was 0 at enrollment, increased to 3 after taking the granules and decreased to 2 after taking the tablets.

DISCUSSION

To the best of our knowledge, this was the first study to compare the acceptability, adherence, and efficacy of 2 different formulations of CR mesalazine directly, using a crossover design to avoid potential bias. We demonstrated that CR mesalazine granules were more acceptable than tablets, and patient adherence tended to be slightly better for granules than for tablets, although there was no statistically significant difference.

The questionnaire responses revealed that many patients found the size and number of the tablets unpleasant. It is necessary to take 8 or 16 (500 or 250 mg, respectively) CR mesalazine tablets to take in 4 g of mesalazine. The situation is similar for other mesalazine formulations, such as pH-dependent mesalazine (Asacol®) (e.g., 10 tablets of 400 mg to administer 4 g mesalazine). Furthermore, the relatively large volume of CR mesalazine tablets, due to the large additive content, was also a major reason for the patient dissatisfaction. CR mesalazine granules were therefore preferable because of both their formulation and the reduced volume required.

The acceptability of the granules was significantly superior to that of the tablets, with approximately three-quarters of patients considering the granules to be preferable to the tablets by the end of this crossover study. Interestingly, the granule

formulation was preferred not only because of the reduced pill burden in terms of volume and number, but also because of its portability. Medication portability may be an important factor for patients who need to take their medications outside their homes during their daily life, and patients may hesitate or forget to carry medications with them because of poor portability. Granules might thus be a good option for patients who take their medications outside their home.

Although the acceptabilities of the 2 formulations differed, their average adherence rates were not significantly different. The frequency of taking medications has been identified as one of the most important factors affecting adherence in patients with various diseases, including IBD.[10-12] In this study, the frequency of intake was the same for both formulations, which may help to explain why the average adherence rates were similar. However, the study design may have led to differences in adherence between the formulations being underestimated. It is difficult to assess real-world adherence in prospective studies, because patients may pay more attention to the protocol and/or overestimate their adherence under trial settings. In fact, the adherence rates in this study were very high (granules 95%, tablet 91%) compared with the previous reports.[2,3] In addition, we did not assess long-term adherence in this short-term study, and it is possible that long-term adherence to the granules might be superior to that of the tablets in a real-world situation, because of significantly better acceptance.

The short-term nature of this study also limited the efficacy evaluation, and a longer observation period may be needed to assess the difference in efficacy in terms of maintaining remission. Adherence guidelines produced by the National Collaborating Centre for Primary Care suggest that although there is no convincing evidence that changes in drug formulation improve adherence, the number, taste, smell, size, and shape of the pills might nonetheless affect medication adherence.[13] Kane et al.[2] also reported that 30% of UC patients did not take their medication because of the large number of pills, and a lower adherence rate was associated with a higher risk of future clinical relapse. These findings suggest that adherence declines with lower acceptability of the medication in some patients, possibly leading to a flare-up. Indeed, 6% of patients in the current study showed high adherence to the granules but low adherence to the tablets, and all said that they had missed a dose at least once because of the poor acceptability of the tablets. Interestingly, a lower pill burden was also reported to be associated with better adherence and virological suppression in patients with human immunodeficiency virus infection, which also requires good adherence to the daily medication, and may be associated with treatment fatigue after long-term treatment.[14] UC treatment has similar characteristics from the aspect of long-term maintenance, and the obvious difference in acceptability in the present study may thus have an important impact on the long-term treatment outcomes in patients with UC.

In conclusion, CR mesalazine granules are a highly acceptable formulation of 5-ASA, and may be associated with better long-term outcomes than tablets as a result of improved patient adherence to the medication.

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CONFLICT OF INTEREST

TK received lecture fees from Mitsubishi-Tanabe Pharma, Eisai, Kyorin Pharmaceutical, Abbvie, Janssen, JIMRO, Ajinomoto Pharma, EA Pharma, Astellas, Mochida Pharmaceutical, Asahi Kasei Medical, Takeda Pharmaceutical, Gilead Sciences, Celltrion, Nippon Kayaku, and Alfresa Pharma, advisory/consultancy fees from Janssen, Pfizer, Kyorin Pharmaceutical, Mochida Pharmaceutical, Takeda Pharmaceutical, Eli Lilly, Ferring Pharmaceuticals, Nippon Kayaku, Thermo Scientific, and Covidien, and research grants from EA Pharma, Thermo Scientific, and Alfresa Pharma. KY received lecture fees from Eisai, Mitsubishi-Tanabe Pharma, EA Pharma, Abbvie, and Pfizer and advisory fees from Janssen, EA Pharma, and Mochida Pharmaceutical. RO received lecture fees from ZERIA and Pfizer Pharmaceutical Co., Ltd. MN received consulting fees from Medtronic Co., Ltd., Takeda Pharmaceutical, Mochida Pharmaceutical, and ZERIA Pharmaceutical. ES received lecture fees from Abbvie, Ajinomoto Pharma, and EA Pharma, chairmanship etc. fees from Abbvie, EA Pharma, and Mitsubishi-Tanabe Pharma, and research grants from EA Pharma, Abbvie, JIMRO, and ZERIA Pharma. TaH received lecture fees from Mitsubishi-Tanabe Pharma, EA Pharma, Kyorin Pharmaceutical, Abbvie, Janssen, JIMRO, Mochida Pharmaceutical, Takeda Pharmaceutical, Gilead Sciences, and Nippon Kayaku, advisory/consultancy fees from Janssen, Pfizer, Kyorin Pharmaceutical, Mochida Pharmaceutical, Takeda Pharmaceutical, Eli Lilly, Nichi-Iko Pharmaceutical Co., Ltd., and Celgene, and research grants from Mitsubishi-Tanabe Pharma, EA Phar-

ma, Kyorin Pharmaceutical, Abbvie, JIMRO, Mochida Pharmaceutical, Takeda Pharmaceutical, Nippon Kayaku, Daiichi Sankyo Co. Ltd., Astellas Pharma Inc., Mylan EPD, and Boston Scientific. ToH received lecture fees from Abbvie Inc., Kyorin Pharmaceutical Co., Ltd., Eisai Co., Ltd., Mitsubishi Tanabe Pharma Co., Ltd., EA Pharma Co., Ltd., JIMRO Co., Ltd., and ZERIA Pharmaceutical Co., Ltd. No funding was received for this work from any of the organizations.

AUTHOR CONTRIBUTION

Conceptualization: Yagisawa K, Kobayashi T. Methodology: Yagisawa K, Kobayashi T. Formal analysis: Yagisawa K, Kobayashi T. Project administration: Kobayashi T. Visualization: Yagisawa K. Writing-original draft: Yagisawa K, Kobayashi T. Writing-review and editing: Ozaki R, Hisamatsu T, and Hibi T. Approval of final manuscript: all authors.

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REFERENCES

1. Magro F, Gionchetti P, Eliakim R, et al. Third European evidence-based consensus on diagnosis and management of ulcerative colitis. Part 1: definitions, diagnosis, extra-intestinal manifestations, pregnancy, cancer surveillance, surgery, and ileo-anal pouch disorders. J Crohns Colitis 2017;11:649-670.
2. Kane S, Huo D, Aikens J, Hanauer S. Medication nonadherence and the outcomes of patients with quiescent ulcerative colitis. Am J Med 2003;114:39-43.
3. Kawakami A, Tanaka M, Nishigaki M, et al. Relationship between non-adherence to aminosalicylate medication and the risk of clinical relapse among Japanese patients with ulcerative colitis in clinical remission: a prospective cohort study. J Gastroenterol 2013;48:1006-1015.
4. Hanauer S, Schwartz J, Robinson M, et al. Mesalamine capsules for treatment of active ulcerative colitis: results of a controlled trial. Pentasa Study Group. Am J Gastroenterol 1993;88:1188-1197.
5. Flourié B, Hagège H, Tucat G, et al. Randomised clinical trial: once- vs. twice-daily prolonged-release mesalazine for active ulcerative colitis. Aliment Pharmacol Ther 2013;37:767-775.
6. Wilding IR, Kenyon CJ, Hooper G. Gastrointestinal spread of oral prolonged-release mesalazine microgranules (Pentasa) dosed as either tablets or sachet. Aliment Pharmacol Ther 2000;14:163-169.
7. Devlen J, Beusterien K, Yen L, Ahmed A, Cheifetz AS, Moss AC. Barriers to mesalamine adherence in patients with inflammatory bowel disease: a qualitative analysis. J Manag Care Spec Pharm 2014;20:309-314.
8. Kawakami A, Tanaka M, Ochiai R, et al. Difficulties in taking aminosalicylates for patients with ulcerative colitis. Gastroenterol Nurs 2012;35:24-31.
9. Severs M, Zuithoff PN, Mangen MJ, et al. Assessing self-reported medication adherence in inflammatory bowel disease: a comparison of tools. Inflamm Bowel Dis 2016;22:2158-2164.
10. Ford AC, Khan KJ, Sandborn WJ, Kane SV, Moayyedi P. Once-daily dosing vs. conventional dosing schedule of mesalamine and relapse of quiescent ulcerative colitis: systematic review and meta-analysis. Am J Gastroenterol 2011;106:2070-2077.
11. McLaughlin T, Hogue SL, Stang PE. Once-daily bupropion associated with improved patient adherence compared with twice-daily bupropion in treatment of depression. Am J Ther 2007;14:221-225.
12. Amara W, Antoniou S. Benefits of once-daily dosing with non-vitamin K antagonist oral anticoagulants in patients with atrial fibrillation. Eur Heart J Suppl 2016;18(Suppl D):D1-D6.
13. National Collaborating Centre for Primary Care (UK). Medicines adherence: involving patients in decisions about prescribed medicines and supporting adherence. London: Royal College of General Practitioners (UK); 2009.
14. Nachega JB, Parienti JJ, Uthman OA, et al. Lower pill burden and once-daily antiretroviral treatment regimens for HIV infection: a meta-analysis of randomized controlled trials. Clin Infect Dis 2014;58:1297-1307.

Aliment Pharmacol Ther 1999; 13: 381–388.

A new mesalazine gel enema in the treatment of left-sided ulcerative colitis: a randomized controlled multicentre trial

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SUMMARY

Background: A new mesalazine rectal gel preparation (without propellant gas) has been recently developed to improve topical treatment in distal ulcerative colitis.

Aim: To evaluate the efficacy, safety and patient tolerability of mesalazine gel enema compared with mesalazine foam enema in the treatment of patients with acute left-sided ulcerative colitis.

Methods: In a randomized multicentre investigator-blind parallel group trial, 103 patients with mild to moderate left-sided colitis or proctosigmoiditis were randomly allocated to mesalazine 2 g gel enema ($n = 50$ evaluable patients) and mesalazine 2 g foam enema ($n = 53$ evaluable patients) for 4 weeks. Clinical symptoms, endoscopic and histological findings were assessed at entry, 2 and 4 weeks. Patients' evaluation of treatment tolerability and acceptability was assessed at 2 and 4 weeks.

Results: After 4 weeks of treatment, clinical remission was achieved by 76% of mesalazine gel enema-treated patients and 69% of patients treated with mesalazine foam enema ($P = 0.608$). Endoscopic remission rates at week 4 were 51 and 52% for the mesalazine gel and foam enemas, respectively ($P = 0.925$). Histological remission was achieved by 30% of patients in both groups. Patients reported that the new mesalazine gel preparation was significantly better tolerated than the foam enema.

Patients in the foam group had significantly more difficulty in retention (25% vs. 6%, $P < 0.05$), abdominal bloating (50% vs. 26%, $P < 0.005$) and discomfort during administration (48% vs. 26%, $P < 0.05$).

Conclusion: The new mesalazine gel enema is efficacious and significantly better tolerated than the mesalazine foam enema.

INTRODUCTION

In recent years the rectal administration of mesalazine has become an established treatment for distal ulcerative colitis. This approach has been designed in order to ensure the delivery of large amounts of mesalazine to the distal colon, with low systemic absorption and a low incidence of side-effects.[1–3] Mesalazine solution, or foam enemas and suppositories are commercially available and widely used for the treatment of patients with proctosigmoiditis or distal ulcerative colitis and proctitis, respectively.[4–10]

Data on retrograde spread in the colon show that suppositories have a limited spread in the rectum and distal sigmoid colon,[11] while enema solutions have a greater, although highly variable, range capacity.[12, 13]

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In addition, solution enemas can be difficult to administer and have reduced patient compliance due to problems with retention.[10]

The foam enemas that are commercially available in Italy (Asacol foam, Giuliani & Bracco, Milan, Italy) offer a greater and more rapid capacity for retrograde distribution because of the generation of large volumes (120–200 mL) within the colon due to the addition of hydrocarbon propellants in the formulation.[14]

In order to further improve both tolerance and patient compliance to topical therapy, a new 60 mL single-dose high-viscosity mesalazine suspension (a thixotropic suspension) has recently been developed (Enterasin gel, Crinos S.p.A, Como, Italy).

The preparation is contained in a canister fitted with a valve. The spray system consists in a two-chamber device: an inner chamber (a flexible polyethylene-coated aluminium bag) containing the mesalazine suspension, and an outer chamber (an air-tight can) filled with pressurized nitrogen acting as a propellant. After activating the valve, the pressurized nitrogen squeezes the inner bag containing the suspension, which is released into the colon via a disposable rectal applicator.

The novelty of this device lies in the fact that the propellant gas is retained inside the can and is not delivered into the colon. In addition, the peculiar characteristics of the suspension are such that they permit an easy and complete release of the mesalazine suspension from the canister, as well as a better adhesion to the colonic mucosa. Furthermore, spreading is achieved more slowly and gradually compared to foam enemas.

The available data on retrograde spread in the colon show that the ready-to-use mesalazine gel enema displays a good spreading capacity, reaching the splenic flexure, with homogeneous distribution in the colon.[15]

This study was designed to compare the efficacy and safety of mesalazine gel enema with a commercially available mesalazine foam enema over a 4-week period of treatment of active proctosigmoiditis or colitis extending to the splenic flexure, and further to evaluate patient acceptance of both treatments.

METHODS

The study was a randomized, investigator-blind, parallel group trial conducted in patients attending out-patient clinics in nine Italian centres from October 1995 through to October 1996. The protocol was approved by the Ethics Committee or Internal Review Board of each participating centre and all patients gave informed, written consent.

Patients

Eligibility criteria included patients of either sex and aged 18–70 years, with clinical and endoscopically confirmed active mild to moderate proctosigmoiditis or ulcerative colitis extending to the splenic flexure. Patients were admitted into the study either in a state of clinical and endoscopic relapse or with first attacks of the disease and with negative stool cultures. At entry, they were required to have a minimum score of 3 on the 12-point Disease Activity Index (DAI).[4]

Patients were excluded if they had relapsed during treatment with rectal corticosteroid or rectal mesalazine preparations, if they had used oral corticosteroids or immunosuppressive drugs in the previous 3 months, they had Crohn's colitis, hypersensitivity to aminosalicylates, impaired liver and renal function, pregnancy or lactation.

Patients who were taking oral maintenance treatment with sulphasalazine or mesalazine at entry were allowed to continue, using the same dose throughout the study.

Treatment

All patients were randomly assigned to receive at bedtime, over a 4-week period, either 2 g mesalazine gel enema (Enterasin gel, Crinos S.p.A, Como, Italy) given rectally in one single application (total volume 60 mL) or 2 g mesalazine foam enema (Asacol foam, Giuliani & Bracco, Milan, Italy), given rectally as a single application (total volume ≈ 120 mL).

The drugs were packaged at a central location, and labelled and randomised in blocks of four according to a randomization list generated by a computer.[16]

Both treatments were presented as blank cylindrical aerosol cans with disposable applicators; however, the mesalazine foam can was half the size of the mesalazine gel cans. To preserve investigator blindness the endoscopist and the histopathologist were both blind to the type of treatment.

Trial assessments

At baseline, patients were examined clinically, endoscopically and histologically in order to confirm

diagnosis. The patients' demography, medical history, and concomitant medication were recorded. Once they had been randomised to either treatment, patients were asked to keep a daily record of symptoms (stool consistency and frequency, rectal bleeding, mucus and pus in stool, urgency, tenesmus, abdominal pain), the time of retention of gel enema or foam, as well as possible adverse events. These data were collected at each visit.

Clinical assessments of therapy were made at baseline, after 2 weeks (14 ± 3 days) and 4 weeks (28 ± 3 days) according to the 12-point Disease Activity Index (DAI)—measuring stool frequency, rectal bleeding, endoscopic findings, and physician's overall assessment of disease severity (Table 1).

Signs and symptoms such as mucus and pus in stools, abdominal pain, tenesmus and urgency were also recorded.

The endoscopic appearance of the colonic mucosa was assessed by the same physician in each centre, according to the criteria of Baron *et al.*[17] (Table 1).

Clinical and endoscopic remission were defined as a score of zero in the clinical and endoscopic portion of the DAI, respectively; an improvement in clinical and endoscopic activity was defined as a decrease in the severity of symptoms and mucosal inflammation (by at least one grade), respectively.

Table 1. Disease Activity Index (DAI)

Stool frequency
0 = Normal number of stools for this patient
1 = 1–2 stools/day greater than normal
2 = 3–4 stools/day greater than normal
3 = 5 or more stools/day greater than normal
Rectal bleeding
0 = No blood seen in stool
1 = Streaks of blood with stools less than half the time
2 = Obvious blood with stools most of the time
3 = Blood alone passed
Mucosal appearance
0 = Normal mucosa or inactive disease
1 = Mild inflammatory changes (erythema, decreased vascular pattern; mild friability)
2 = Moderate inflammatory changes (marked erythema; absent vascular pattern; friability, erosions)
3 = Severe inflammatory changes (spontaneous bleeding, and ulcerations)
Physician's overall assessment of disease severity
0 = Normal
1 = Mild disease
2 = Moderate disease
3 = Severe disease
Maximum total score = 12

Histological disease activity was also assessed at study entry, and after 2 and 4 weeks, according to the criteria of Truelove & Richard.[18] Two biopsy specimens were taken 10 cm from the anal margin on the anterior rectal wall. The histological disease activity index score was determined by a single pathologists (G.B.) who was blinded to patient identification, clinical status and treatment, and was graded as follows: 0 = normal; 1 = chronic inflammatory cell infiltrate in lamina propria; 2 = mild crypt injury with acute cell infiltrate, some crypt abscesses; 3 = marked crypt destruction with crypt abscesses and ulcerations. Histological remission and histological improvement were defined as a histological disease score of zero or one, and a decrease in the histological disease index of one or two points, respectively.

At weeks 2 and 4 a Physicians Global Assessment (PGA) scale was used to assess changes in the disease state of each patient. This scale ranged from 1 to 6 and was determined by the physician's overall clinical assessment, based on patient symptoms, endoscope, and histological findings, as well as the patient's general well-being: 6 = much worse, 5 = minimally worse, 4 = no change, 3 = minimally improved, 2 = much improved, 1 = very much improved.

Safety was assessed by recording adverse events either observed by the investigator or reported by the patient at each follow-up visit.

At weeks 2 and 4, the patients were asked to express their opinion regarding the acceptability and tolerability of the formulations according to a questionnaire which assessed the following: difficulty of retention, discomfort during enema delivery, rectal pain, abdominal pain and abdominal bloating during enema administration, leakage. A two-point scale was adopted (0 = no problems at all, 1 = presence of problems).

Statistical methods

The trial was designed to have an 80% power, with significance set at the 5% level. Using the end-point of patients' tolerability and acceptance of therapy, it was calculated that at least a total of 90 patients would be required in order to show that the mesalazine foam was 30% less well tolerated than the mesalazine gel.

A total of 103 patients were enrolled: 96 were included in the efficacy analysis, and 102 in the tolerability and acceptability analysis, according to a per protocol analysis.

The homogeneity of the groups was tested using a χ^2-test and Wilcoxon's rank sum test for qualitative variables, and Student's *t*-test for independent samples for the quantitative variable parameters.

Treatment efficacy and tolerability was verified by using a χ^2-test corrected for continuity. Scores from the DAI were analysed by nonparametric methods using ranks (Wilcoxon's rank sum test). Two-tailed tests of significance were applied throughout.

The 95% confidence limits for difference in rates between the treatment groups were also calculated.[19]

The analysis was performed using SAS statistical software and CIA (Confidence Interval Analysis) computer programs.[20]

RESULTS

One hundred and three patients entered the study; 50 received mesalazine gel and 53 mesalazine foam. Seven patients (one in the mesalazine gel group and six in the mesalazine foam group) were excluded from the efficacy analysis; four because of incorrect entry criteria, two discontinued treatment after only a few days and failed to keep further appointments and one was noncompliant (Table 2).

A further 11 patients (eight in the mesalazine gel group and three in the mesalazine foam group) were excluded from the histological analysis because of histology in remission at entry. However, this patient group was included in the clinical and endoscopic analysis because they had an initial DAI score in the range from 3 to 8: one patient in the mesalazine gel group had a DAI score of 3, four patients (three in the mesalazine gel group and one in the mesalazine foam group) had a DAI of 4, two patients (each in the mesalazine gel and foam group) had a DAI score of 5, two patients in the mesalazine gel group had a DAI score of 6, and two patients, one in the mesalazine foam group and one in the gel group, had DAI scores of 7 and 8, respectively.

Ninety-six patients (49 in the mesalazine gel group and 47 in the foam group) were included in the clinical and endoscopic analysis population and 85 (41 in the mesalazine gel group and 44 in the foam) in the histological assessment.

One hundred and two patients (50 in the mesalazine gel group and 52 in the foam group) were included in the tolerability/acceptability evaluation.

Five patients in the mesalazine foam group withdrew during the trial: four because of lack of improvement, one because of poor compliance.

Characteristics of the two treatment groups are presented in Table 3 and were comparable with regard

Table 2. Data sets analysed

	No. of patients	
	5-ASA gel	5-ASA foam
Randomized	50	53
Non-compliance	0	1
Tolerability analysis	50	52
Protocol violation (entry)	1	3
Lost to follow-up	0	2
Clinical and endoscopic analysis	49	47
Histology in remission (entry)	8	3
Histological analysis	41	44

Table 3. Baseline entry characteristics

	5-ASA gel (n = 50)	5-ASA foam (n = 53)
Sex (%)		
Male	36 (72)	30 (57)
Female	14 (28)	23 (43)
Age (years) mean (s.d.)	42.2 (12.7)	37.4 (12.4)
Duration of disease (years)		
Mean (s.d.)	5.9 (5.0)	5.8 (5.5)
Range	0.3–20	0.25–31
Extent of disease (%)		
Proctosigmoiditis	38 (76)	36 (68)
Left-sided colitis	12 (24)	17 (32)
Concomitant oral 5-ASA/SSZ (%)	40 (80)	36 (68)
Initial DAI score		
Mean (s.d.)	6.12 (1.88)	6.19 (1.55)
Range	3–11	4–10
Endoscopy score		
Grade 1	9	6
Grade 2	39	41
Grade 3	2	5
Not available	—	1
Histology score		
Grade 1	9	6
Grade 2	19	22
Grade 3	22	24
Not available	—	1

Table 4. Mean values (s.d.) of DAI score at baseline and weeks 2 and 4

	Baseline	Week 2	Week 4
5-ASA gel	6.12 (1.88)	2.36 (2.32)*	1.44 (2.18)*
95% CI	5.57–6.66	1.69–3.03	0.82–2.07
5-ASA foam	6.19 (1.55)	2.82 (2.24)*	1.57 (2.29)*
95% CI	5.73–6.64	2.16–3.49	0.85–2.28

* $P < 0.001$ in comparison to baseline

to extent of disease, grade of endoscopic or histological score, and number of patients with oral maintenance therapy.

Efficacy assessments

Both treatments significantly reduced the mean total DAI scores from baseline (Table 4). After 2 and 4 weeks, the mean DAI score declined by 3.76 and 4.68, respectively for patients receiving mesalazine gel, and by 3.37 and 4.62 in the mesalazine foam group. There were no significant differences between treatments ($P = 0.22$ and $P = 0.92$, respectively), but both treatments significantly decreased the scores ($P < 0.001$ at 2 and 4 weeks).

Table 5 shows the clinical, endoscopic and histological rate of remission, improvement and failure at 2 and 4 weeks of treatment.

Both treatments produced a significant improvement from baseline in all symptoms. In the group treated with gel, 17 of 49 (35%) were in clinical remission after 2 weeks compared with 19 of 47 (40%) treated with foam. Four patients in the foam group discontinued the study at week 2 following inadequate response. After 4 weeks, 37 of 49 (76%) in the gel group and 29 of 42 (69%) in the foam group were in remission. No statistical differences were observed between treatments.

The endoscopic appearances showed a significant improvement after both treatments. In the group treated with the gel, 14 of 49 (29%) were in endoscopic remission after 2 weeks, compared with seven of 47 (15%) in the foam group ($P = 0.120$). After 4 weeks, 25 of 49 (51%) in the gel group and 22 of 42 (52%) in the foam group were in remission, with no statistical difference between treatments.

In addition, there was no statistically significant difference between treatment groups in terms of histological response.

Physicians Global Assessment scores also indicated progressive improvement in both groups (Figure 1). At week 2 there was a slightly greater frequency of 'very much' improvement in the mesalazine gel group than in the foam group. However, the difference between treatments in PGA scores was not significant at any time.

Table 5. Clinical, endoscopic and histological results

	Week 2			Week 4		
	5-ASA gel	5-ASA foam	*P*-value	5-ASA gel	5-ASA foam	*P*-value
Clinical symptoms						
Remission	17 (35%)	19 (40%)		37 (76%)	29 (69%)	
Improvement	30 (61%)	23 (49%)	0.320	9 (18%)	8 (19%)	0.608
Failure	2 (4%)	5 (11%)		3 (6%)	5 (12%)	
Endoscopy						
Remission	14 (29%)	7 (15%)		25 (51%)	22 (52%)	
Improvement	25 (51%)	23 (49%)	0.120	18 (37%)	14 (34%)	0.925
Failure	10 (20%)	17 (36%)		6 (12%)	6 (14%)	
No. of patients	49	47		49	42	
Histology						
Remission	8 (20%)	9 (21%)		12 (30%)	12 (30%)	
Improvement	17 (41%)	16 (37%)	0.931	18 (45%)	20 (50%)	0.756
Failure	16 (39%)	18 (42%)		10 (25%)	8 (20%)	
No. of patients	41	43		40	40	

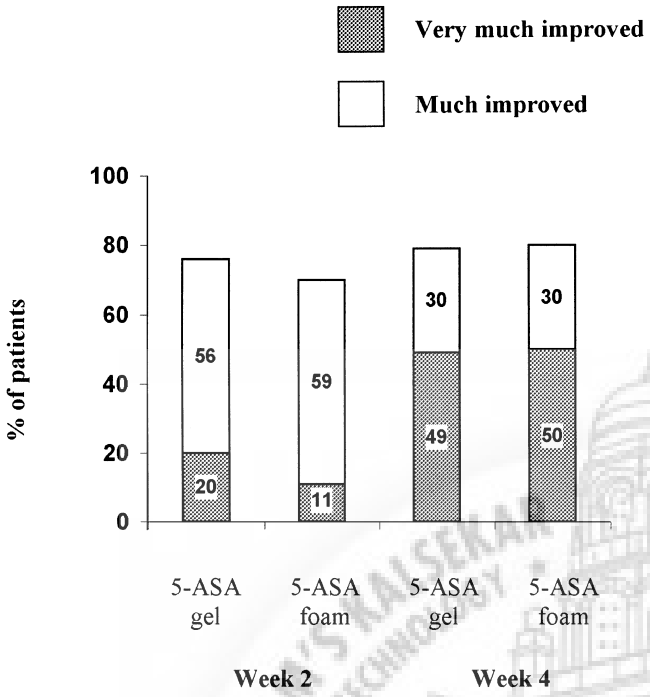

Figure 1. Physician's Global Assessment scoring.

Safety assessments

Adverse events. Two patients receiving mesalazine gel enemas and one patient taking foam enemas developed, respectively, self limiting renal colic, insomnia and skin eruption which were not thought to be related to the trial medications.

Patient evaluation of therapy. The analysis of the data collected showed that the mesalazine gel enema was significantly better tolerated than the mesalazine foam (Table 6).

The most common problems reported by patients during mesalazine foam treatment administration were: difficulty in retention, abdominal bloating and discomfort during administration.

After 2 weeks, the mesalazine foam patients had significantly more difficulty in retention, abdominal bloating and discomfort during administration (37, 56 and 58%, respectively), compared to the mesalazine gel group (4, 18 and 18%, respectively) ($P < 0.001$). After 4 weeks, the percentage of patients with difficulty in retention, abdominal bloating and discomfort during administration was 25, 50 and 48% in mesalazine foam group and 6, 26 and 26%, respectively, in the gel group ($P < 0.005$).

DISCUSSION

The present study was designed to evaluate the efficacy and patient acceptability and tolerability of a new 5-ASA gel preparation, in comparison to that of 5-ASA foams in patients with left-sided ulcerative colitis. Although the enemas were not identical in appearance, they were only labelled with a trial number, and every attempt was made to ensure the investigator blinding. Endosopic and histological assessments were carried out without the knowledge of the patient group.

After 4 weeks there were significant improvements in symptoms as well as in endoscopic and histological grades, that were of a similar degree in both treatment groups. Data regarding the patients' acceptability and tolerability showed that the new 5-ASA gel enema was significantly better tolerated by the patients because it was easier to retain and caused significantly less discomfort, abdominal pain and abdominal bloating.

Significant differences between the two groups in the results from the tolerability questionnaire were found at both 2 and 4 weeks of treatment. However, at 2 weeks a significantly greater proportion of patients reported no problems at all in using 5-ASA gel compared with the foam, suggesting that the new formulation is better

Table 6. Patient evaluation of tolerability and acceptability of therapy

	Week 2			Week 4		
	5-ASA gel (n = 50)	5-ASA foam (n = 52)	Difference (95%)	5-ASA gel (n = 50)	5-ASA foam (n = 44)	Difference (95%)
Difficulty in retention	2 (4%)**	19 (37%)	33% (19–47)	3 (6%)*	11 (25%)	19% (5–33)
Abdominal bloating	9 (18%)**	29 (56%)	38% (21–55)	13 (26%)*	22(50%)	24% (5–43)
Discomfort during administration	9 (18%)**	30 (58%)	40% (23–57)	13 (26%)*	21 (48%)	22% (3–41)

*$P < 0.05$, ** $P < 0.001$, in comparison with 5-ASA foam (χ^2; Yates correction).

accepted in the initial phases of the disease when activity is more pronounced.

The better tolerability of the 5-ASA gel is most likely to be linked to its innovative formulation (a thixotropic suspension) and release system, which does not deliver the propellant gas into the colon, and permits an easy and complete release of the active ingredient, together with a better adhesion and homogeneous distribution to the colonic mucosa.

The topical treatment of distal colitis makes it possible to administer a high dosage of the active drug directly to the inflamed mucosa, as well as achieving a low level of systemic absorption. Rectal formulations of 5-ASA represent the first choice treatment for distal colitis, being significantly superior both to placebo and topical corticosteroids, as has been confirmed by two recent meta-analyses.[7, 8]

Mesalazine suppositories are thought to be the best treatment for patients with proctitis,[9, 21] while liquid enemas and foams, thanks to their retrograde spread, are suitable for more extensive disease.[12, 13] Mesalazine foam enemas have been shown to be superior to prednisolone foam enemas,[22] and have a more uniform distribution as well as a greater persistence than the liquid enema in the descending and sigmoid colon.[15] Moreover, when mesalazine foam enemas were given in equal doses, they gave a faster remission compared with mesalazine liquid enemas, and patient evaluation of the therapy showed that the foam was more comfortable, more practical, easier to retain, and interfered less with daily living.[10]

The extent of spread of the mesalazine gel enema used in this study has been investigated and was found to reach repeatably into the splenic flexure, with a homogeneous distribution into the left colon. In addition, the systemic absorption of the new gel enema was found to be similar to that of other mesalazine topical preparations on the market.[15]

We conclude that the new mesalazine gel enema is a highly efficacious and safe preparation and that it is better tolerated than the mesalazine foam enema. This technological advance should help with patients compliance to topical treatment.

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REFERENCES

1 Campieri M, Lanfranchi GA, Bazzocchi G, *et al.* Treatment of ulcerative colitis with high dose 5-aminosalicylic acid enemas. Lancet 1981; ii: 270–1.
2 Campieri M, Lanfranchi GA, Boschi S, *et al.* Topical administration of 5-aminosalicylic acid enemas in patients with ulcerative colitis. Studies on rectal absorption and excretion. Gut 1985; 26: 400–5.
3 Thomson ABR. Review article: new developments in the use of 5-aminosalicylic acid in patients with inflammatory bowel disease. Aliment Pharmacol Ther 1991; 5: 449–70.
4 Sutherland LR, Martin F, Greer S, *et al.* 5-Aminosalicylic acid enema in the treatment of distal ulcerative colitis, proctosigmoiditis, and proctitis. Gastroenterology 1987; 92: 1894–8 .
5 Campieri M, Gionchetti P, Belluzzi A, *et al.* Optimum dosage of 5-aminosalicylic acid as rectal enemas in patients with active ulcerative colitis. Gut 1991; 32: 929–31.
6 Biddle WL, Miner PB. Long-term use of mesalamine enemas to induce remission of ulcerative colitis. Gastroenterology 1990; 99: 113–18
7 Marshall JK, Irvine ES. Rectal aminosalicylate therapy for distal ulcerative colitis: a meta-analysis. Aliment Pharmacol Ther 1995; 9: 293–300.
8 Marshall JK, Irvine EJ. Rectal corticosteroids versus alternative treatments in ulcerative colitis: a meta-analysis. Gut 1997; 40: 775–81.
9 Campieri M, De Franchis R, Bianchi Porro G, Ranzi T, Brunetti G, Barbara L. Mesalazine (5-aminosalicylic) suppositories in the treatment of ulcerative proctitis or distal proctosigmoiditis. A randomized controlled trial. Scand J Gastroenterol 1990; 25: 663–8.
10 Campieri M, Paoluzi P, D'Albasio G, Brunetti G, Pera A, Barbara L. Better quality of therapy with 5-ASA colonic foam in active ulcerative colitis. A multicenter comparative trial with 5-ASA enema. Dig Dis Sci 1993; 38(10): 1843–50.
11 Williams CN, Haber G, Aquino GA. Double blind, placebo controlled evaluation of 5-ASA suppositories in active distal colitis and measurement of extent spread using ^{99m}Tc labelled 5-ASA suppositories. Dig Dis Sci 1990; 32(Suppl.): 71S–5S.
12 Wood E, Wilson CG, Hardy JG. The spreading of foam and solution enemas. Int J Pharm 1985; 25: 191–7.
13 Campieri M, Lanfranchi GA, Brignola C, *et al.* Retrograde spread of 5-aminosalicylic acid enemas in patients with ulcerative colitis. Dis Colon Rectum 1986; 29: 108–10.
14 Campieri M, Corbelli C, Gionchetti P, *et al.* Colonic spread and distribution of 5-ASA colonic foam and 5-ASA enema in patients with ulcerative colitis. Dig Dis Sci 1992; 37: 1890–7.
15 Gionchetti P, Venturi A, Rizzello F, *et al.* Retrograde colonic spread of a new mesalazine rectal enema in patients with distal ulcerative colitis. Aliment Pharmacol Ther 1997; 11: 679–84.
16 Tiplady B. A basic program for constructing a dispensing list for a randomized clinical trial. Br J Clin Pharmacol 1981; 11: 617–18.

17 Baron JH, Connel AM, Lennard Jones JE. Variation between observers in describing mucosal appearances in proctocolitis. Br Med J 1964; 1: 89–92.
18 Truelove SC, Richard WCD. Biopsy studies in ulcerative colitis. Br Med J 1956; 1: 1315–18.
19 Gardner MJ, Gardner SB, Winter PD. (eds) Confidence interval analysis (CIA). Microcomputer Program Manual. Br Med J 1991.
20 Fleiss JL. Statistical Methods for Rates and Proportions. New York: John Wiley & Sons, 1981.
21 Campieri M, Gionchetti P, Belluzzi A, *et al.* 5-aminosalicylic acid as enemas or suppositories in distal ulcerative colitis? J Clin Gastroenterol 1988; 10(4): 406–9.
22 Lee FI, Jewell DP, Mani V, *et al.* A randomized trial comparing mesalazine and prednisolone foam enemas in patients with acute distal ulcerative colitis. Gut 1996; 38: 229–33.

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Water-in-Water Emulsion as a New Approach to Produce Mesalamine-Loaded Xylan-Based Microparticles

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Featured Application: Controlled Release of Drugs.

Abstract: The water-in-water emulsion method has been reported as a technique able to prepare microparticles without using harmful solvents. However, there are few reports showing the encapsulation of small molecules into microparticles produced within this technique. The probable reason relays on the rapid diffusion of these molecules from the discontinuous phase to the continuous phase. In the present study, xylan microparticles containing mesalamine were produced and the doubled crosslinking approach, used to promote higher encapsulation rates, was disclosed. To achieve this goal, a 2^3 full factorial design was carried out. The results revealed that all formulations presented spherical-shaped microparticles. However, at specific conditions, only few formulations reached up to 50% of drug loading. In addition, the new xylan-based microparticles formulation retained almost 40% of its drug content after 12 h of a dissolution assay likely due to the degree of crosslinking. Thus, the doubled crosslinking approach used was effective on the encapsulation of mesalamine and may pave the way to successfully produce other polysaccharide-based carriers for clinical use.

Keywords: xylan; water-in-water emulsion; mesalamine; mathematical modeling

1. Introduction

The preparation of microparticles based on hydrophilic polymers, such as polysaccharides derivatives, using techniques based on emulsion templates, majorly requires the use of organic solvents in order to form the hydrophilic and hydrophobic phases in which the internal hydrophilic phase contains the polymer [1–3]. In addition, together with the use of crosslinkers such as glutaraldehyde and terephthaloyl chloride, the microparticles produced by this way may present an important degree of toxicity [1,4].

Xylan, which is the second most abundant polysaccharide of the biosphere, is mainly presented in hardwood and perennial plants such as grasses, cereals, and herbs [5]. The use of xylan for biomedical applications is based on its important biocompatibility and selective degradation in the gastrointestinal

tract, which occurs in the colon through enzymatic hydrolysis by the microbiota. Our group has lately exploited this feature in order to develop colon-specific drug delivery systems [6–8].

As previously mentioned, the preparation of polysaccharide-based microparticles, including xylan-based microparticles (XBM), mostly requires the use of organic/harmful solvents such as cyclohexane, and crosslinkers, such as terephthaloyl chloride, during their preparation [1,9,10]. On this behalf, the water-in-water emulsion approach can produce microparticles by the mixture of two immiscible aqueous polymeric solutions. Once these solutions are mixed, there is a formation of a system with a positive Gibbs free energy (ΔG) (Equation (1)).

$$\Delta G = \Delta H_{\text{mix}} - \text{T}\,\Delta S_{\text{mix}} \quad (1)$$

This phenomenon happens due to the high molecular weight of the polyethylene glycol (PEG), which limited the translational motion of the polymeric chains. This results in a smaller value of ΔS_{mix} than ΔH_{mix}. Hence, it results in a positive ΔG, and, therefore, leads to phase separation [11]. Similarly to the traditional oil-in-water and water-in-oil emulsions, these two polymeric phases, once homogenized, may form two different phases, which include a continuous and a discontinuous one. Thus, once the two phases are obtained and a crosslinker is added to the dispersion, particles can be obtained [12,13].

The reports about microparticles prepared by the water-in-water emulsion technique hardly ever describe the encapsulation of active molecules [14–16]. Nonetheless, when tried, the encapsulated molecules are mostly biomolecules, such as proteins and peptides [17]. A probable explanation for such a phenomenon would be related to the free mobility of the small hydrophilic molecules into the dispersion. Once both phases are aqueous, the encapsulation efficiency is hindered [14].

The preparation of microparticles loaded with small active molecules through the water-in-water emulsion technique has been a pharmaceutical technology-based challenge. In this sense, in the present work, xylan microparticles loaded with mesalamine (5-ASA), which is a low molecular weight molecule, were prepared through a water-in-water emulsification process using different crosslinking approaches, designed through a 2^3 full factorial design with a central point.

2. Materials and Methods

2.1. Materials

Xylan from beech wood, 5-ASA, and trisodium trimetaphosphate (STMP) were purchased from Sigma-Aldrich® (São Paulo, Brazil). Polyethylene glycol (PEG, MW = 20,000 Da) came from Merck® (São Paulo, Brazil). Potassium chloride was purchased from QEEL® (São Paulo, Brazil). Sodium chloride, sodium hydroxide, calcium chloride, and sodium phosphate dibasic anhydrous were purchased from Vetec® Chemical (Rio de Janeiro, Brazil). Anhydrous monobasic potassium phosphate was from ISOFAR® (Rio de Janeiro, Brazil) and ethanol 98%$_{(v/v)}$ from Atriom® (São Paulo, Brazil). Water (conductivity ≤ 3.5 µS/cm), obtained from deionization and followed by a reverse osmosis process, was used to produce the microparticles and prepare the dissolution medium. All chemicals were of an analytical grade and used as received.

2.2. Phase Diagram of Xylan and PEG

To evaluate the concentrations of xylan and PEG required to achieve the formation of water-in-water emulsions, a phase diagram was obtained from acquired data in different experiments. First, a stock solution containing 35%$_{(w/v)}$ of xylan and another with 32%$_{(w/v)}$ of PEG were prepared. Afterward, successive dilutions were made using 1N NaOH and water, to reach a concentration range from 0.1%$_{(w/v)}$ to 32%$_{(w/v)}$ for xylan and from 0.5%$_{(w/v)}$ to 29%$_{(w/v)}$ for PEG, respectively. These solutions were brought together in a 1:4$_{(v/v)}$ ratio and the phase separation was evaluated by the naked eye [16].

2.3. Production of the 5-ASA-Loaded XBM

The 5-ASA-loaded XBM was prepared from an emulsion obtained by the previously mentioned phase diagram. The microparticles were produced following the flow chart presented in Figure 1. First, a solution containing 4%$_{(w/v)}$ of xylan, 1%$_{(w/v)}$ of STMP, and 0.05%$_{(w/v)}$ of 5-ASA was prepared. Simultaneously, a PEG solution containing 32%$_{(w/v)}$, with or without $CaCl_2$, was also prepared. In the sequence, the xylan solution was slowly poured into the PEG solution to reach a proportion of 1:4$_{(v/v)}$, respectively. The dispersion was kept under constant magnetic stirring for 5 min and, then, incubated at 45 °C for 6 h [10]. Afterward, to cease the reaction and lead to microparticles precipitation, ethanol 98%$_{(v/v)}$ was added to the mixture. Then, washing steps were carried out by centrifugation (2136 g for 5 min) with ethanol 98%$_{(v/v)}$ and deionized water. Lastly, the 5-ASA-loaded XBM were dried at room temperature and stored at 25 °C.

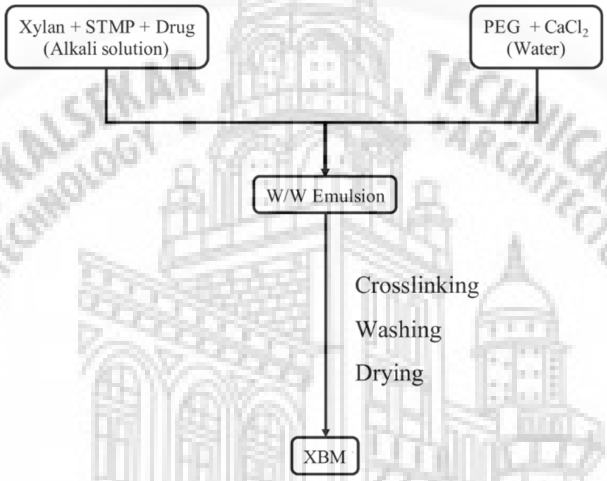

Figure 1. Flow chart for the production of the xylan-based microparticles (XBM) and XBM-Ca. STMP = Sodium trimethaphosphate. PEG = Polyethylene glycol. MW = 20,000. $CaCl_2$ = Calcium chloride.

2.4. Full Factorial Design

In order to improve the drug encapsulation into the XBM, a 2^3 full factorial design with a center point was built (Table 1). The concentrations of xylan, $CaCl_2$, and STMP were tested according to Table 1, while 5.0 mg of 5-ASA was added in all formulations (XBM-Ca-F1–XBM-Ca-F9). Afterward, a mathematical modeling of the data was carried out in order to determine the parameters that had higher influence over the encapsulation efficiency.

Table 1. Experimental results of average diameter size and EE% of XBM and all XBM-Ca prepared based on the 2^3 experimental design.

Samples	Xylan (%)	STMP (%)	$CaCl_2$ (%)	Average Size μm ± (SD *)	EE (%) ± (SD)
XBM	4.0	0.5	-	10.7 ± 2.0	3.6 ± 2.5
XBM-Ca-F1	5.0	0.5	0.5	4.6 ± 0.1	29.1 ± 2.3
XBM-Ca-F2	5.0	1.0	0.5	4.6 ± 0.1	16.4 ± 6.2
XBM-Ca-F3	2.0	0.5	0.5	6.1 ± 0.4	20.9 ± 3.6
XBM-Ca-F4	5.0	1.0	2.0	5.1 ± 1.1	35.3 ± 9.3
XBM-Ca-F5	2.0	1.0	0.5	6.1 ± 0.4	32.6 ± 5.5
XBM-Ca-F6	3.5	0.75	1.25	6.3 ± 0.2	32.6 ± 0.8
XBM-Ca-F7	5.0	0.5	2.0	5.6 ± 0.2	35.4 ± 12.3
XBM-Ca-F8	2.0	0.5	2.0	4.5 ± 0.1	29.8 ± 8.8
XBM-Ca-F9	2.0	1.0	2.0	4.8 ± 0.1	49.6 ± 0.5

STMP = trisodium trimetaphosphate. $CaCl_2$ = calcium chloride. XBM = xylan-based microparticles. XBM-Ca = XBM also crosslinked with calcium chloride. EE% = encapsulation efficiency. F = Formulation. The PEG (polyethylene glycol) concentration remained constant in all formulations (32%$_{(w/v)}$). * This standard deviation is from the average size obtained during the analysis of 1500 particles (3 times 500), and not from the particle size distribution.

2.5. Morphologic Analyses and Particle Size Distribution

The morphology and particle size distribution were evaluated by optical microscopy, using two optical microscopes, Model TM 800 (Taimin®, João Pessoa, Brazil) and Model 020507.010 (Leica®, Bellevue, WA USA) at a magnification of 10χ. In order to enhance the contrast of the particles during the analyses, several droplets of methylene blue solution $0.01\%_{(w/v)}$ were used to re-suspend the microparticles. For the measurement of the particle size distribution, the powder of the microparticles was placed onto glass slides followed by the counting of 1500 particles (3 × 500). The particle size of each formulation was assessed, according to the Feret's diameter [8]. Then, the acquired data were plotted using the Prism® (Version 5.03, GraphPad®, San Diego, CA, USA).

2.6. Drug Loading Efficiency (%)

The amount of 5-ASA encapsulated into the microparticles was evaluated through an UV/Vis Spectrophotometry technique (Biochrom® Libra® S32 UV/Vis, Cambridge, UK), at λ = 322 nm, with the following parameters: $y = 27.87x - 0.0303$, $R^2 = 0.999$, and relative standard deviation below 0.05% for all data points, in which it was demonstrated that the microparticles shell did not interfere. The microparticles content was released, using 1N NaOH and an ultrasound probe (13 mm probe, Vibra-cell® 75041, Fischer Bioblock Scientific, Aalst, Belgium). In addition, 3 mg of the microparticles powder was immersed into 3 mL of 1N NaOH solution. Then, the mixture was kept at 37 °C under orbital stirring at 100 rpm, overnight. Afterward, the suspension was sonicated with an amplitude of 40% and 20 KHz for 1 min and centrifuged at 2136 g for 5 min. The amount of 5-ASA loaded was determined in the supernatant, and measured at λ = 322 nm, which followed the previously validated spectrophotometric method. The drug loading efficiency (EE%) was calculated by the following equation.

$$\text{Encapsulation efficiency (\%)} = (\text{quantified drug content/theoretical drug content}) \times 100 \quad (2)$$

2.7. Attenuated Total Reflectance Infrared (ATR-IR) Spectroscopy Analysis

The interaction between the xylan, the 5-ASA, and the STMP during the crosslinking process was evaluated by ATR-IR spectroscopy (Spectrum 65, Waltham, MA, USA). The ATR-IR spectroscopy measurements were performed using the samples on the solid state. The samples were placed on the crystal area and the pressure arm was positioned over the crystal/sample area. Each sample was subjected to four scans at 1 cm^{-1} resolution at room temperature using acetone to clean the crystal between the samples. The runs were carried out from the range of 4000 to 500 cm^{-1}. Once the analyses were performed, the graphs were plotted using OriginPro® (Version 2015, Northampton, MA, USA).

2.8. X-ray Diffraction (XRD)

XRD analysis was performed for the 5-ASA and xylan alone, and the formulation XBM-Ca-F9 with and without the 5-ASA. X-ray scattering angle measurements were performed with a copper anode radiation Kα (λ = 0.15418 nm, 40 kV, 20 mA) attached to the diffractometer (Bruker, model D8 Advance, Karlsruhe, Germany). A scan rate of 2°/minute across the range of 20° to 80° 2θ was used to determine each spectrum.

2.9. In Vitro Drug Release

Initially, 10 mg of XBM-Ca-F9 was added in 30 mL of phosphate buffered saline pH = 7.4 at 37 °C. Subsequently, the recipients were sealed and maintained under orbital stirring of 100 rpm, and at specific times (0.5, 1, 2, 3, 4, 5, 6, and 12 h), aliquots of 5 mL were withdrawn from the recipients and centrifuged at 2136 g for 5 min. Five mL of fresh media were added in order to maintain the sink condition. Thereafter, the amount of 5-ASA in the supernatant was determined through UV/Vis Spectrophotometry (Biochrom® Libra® S32 UV/Vis, Cambridge, UK). The amount of 5-ASA released

was calculated and expressed as the accumulative percentage of the drug released versus time through the Prism® (Version 5.03, GraphPad®, San Diego, CA, USA). After 12 h, the amount of 5-ASA remaining into the microparticles was analyzed following the procedure described in Section 2.6, after incubation at 37 °C for 2 h.

2.10. Mathematical Modeling of the In Vitro 5-ASA Release

The mathematical modeling of the data from the drug release assay was performed using the Add-in *DDsolver* for Microsoft® Excel [18] in order to describe the mechanism by which the 5-ASA was released from the microparticles. The choice of the model that best fitted the experimental data was based on the adjusted coefficient of determination (adjusted-R^2), which corresponds to the adjustment of the theoretical models to the experimentally obtained data. Additionally, the Root Average Square Error (RMSE) was used to evaluate the difference between the values obtained experimentally and those predicted by the model. Therefore, the model that best describes the experimental data was the one showing the largest adjusted-R^2 and the lower RMSE [18].

2.11. Statistical Analysis

The experimental design was performed in duplicate. Results were presented as average ± standard deviation (SD) of two independent analyses. The analysis of variance (ANOVA) was performed using the STATISTICA® (Version 10, StatSoft, Palo Alto, CA, USA). P-values lower than 0.05 were assumed to be statistically significant.

3. Results and Discussion

3.1. Phase Diagram of Xylan and PEG

Water-in-water emulsions can be obtained by mixing two aqueous solutions of hydrophilic polymers. A biphasic system only is obtained when a certain polymer concentration is reached. These emulsions recently have been granted important attention due to the great potential applications in food and cosmetics preparations. In this sense, the achievement of a monophasic dispersion is mandatory [19]. The water-in-water emulsification process allows obtaining microparticles with narrow size distribution loaded with an active compound by avoiding the use of organic solvents and surfactant agents [11]. Stenekes and Hennink, still in the 1990s, showed that the average size of the particles prepared using the water-in-water emulsion method depends on the volume ratio of the discontinuous/continuous phase, the viscosity of the solutions, and the molecular weight of the polymers [20].

On the other hand, the microencapsulation into xylan-based microparticles of low molecular weight molecules, such as 5-ASA, has been a pharmaceutical technology challenge due to the restricted microparticles' physicochemical stability and the use of harmful solvents to produce them [10]. In this regard, water-in-water emulsion systems can solve all drawbacks to produce such xylan-based microparticles. The phase diagram is the more common approach used to develop water-in-water emulsions [11]. Using such an approach, it is not only possible to define the region of the phase separation, but also to control the size of the internal phase domains, which will be further used as a template to produce the microparticles.

In this regard, the phase diagram prepared with PEG and xylan solutions was obtained and the results are available in Figure 2. The obtained data revealed the potential templates for the preparation of XBM. The region that shows the different and stable combinations between PEG and xylan are identified by the points above the tie-line (solid line, Figure 2). Located at this region, the emulsion constituted by PEG and xylan, at the concentration of 32%$_{(w/v)}$ and 4%$_{(w/v)}$, respectively, was further used due to the clear formation of droplets at the micro-scale range (data not shown).

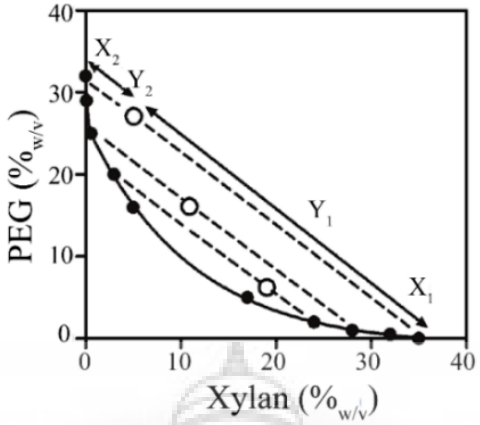

Figure 2. Phase diagram of xylan and PEG solutions. The solid line (–) represents the binodal line and the dashed lines (- - -) or the tie-lines. Below the binodal lines, the systems have just one phase, and, above it, the open circle (○) represents the point at which the phase separation occurs. X_1 stands for the xylan dispersion at $40\%_{(w/v)}$, X_2 is the PEG enriched solution $32\%_{(w/v)}$, and Y_1 and Y_2 represent the volume ratio of the two phases for the formation of the emulsions.

3.2. *Production and Characterization of XBM*

The production of the XBM using the template chosen through the phase diagram was successfully achieved by the addition of STMP, as a cross-linker, and the incubation of the mixture at 45 °C for 6 h. From the macroscopic point of view, after stirring the two solutions, the emulsion presented a yellowish color and a homogeneous appearance. Once 5-ASA was added, samples also remained homogeneous, but reddish in tone. It is noteworthy that all formulations did not show phase separation after the incubation period. In addition, the XBM formulations had about a 35% yield, while the formulations containing $CaCl_2$ showed about a 60% yield. Thus, this parameter was the first evidence of the rapid formation of the microparticles pulling together xylan chains and hindering their diffusion to the continuous phase. Microscopically, the XBM formulation exhibited non-aggregated spherical microparticles (Figure 3A), with a homogeneous particle population distribution, and an average diameter of 10.7 ± 2.0 μm (Figure 3C).

The encapsulation efficiency of the 5-ASA was below 4% for the XBM formulation (Table 1). The probable reason for such a low encapsulation rate might be due to the diffusion of the 5-ASA to the continuous phase. This phenomenon might be the consequence of the use of the 1N NaOH on the production of the xylan solution and a long time to achieve the crosslinking process. The alkaline solution increases the pH of the emulsion to approximately 12 and allows the 5-ASA to be soluble on both phases of the emulsion and the crosslinking process lasts for around 6 h. In fact, the 5-ASA is largely soluble into alkaline solutions while its solubility in water is 1.41 mg/mL at 37 °C [21,22].

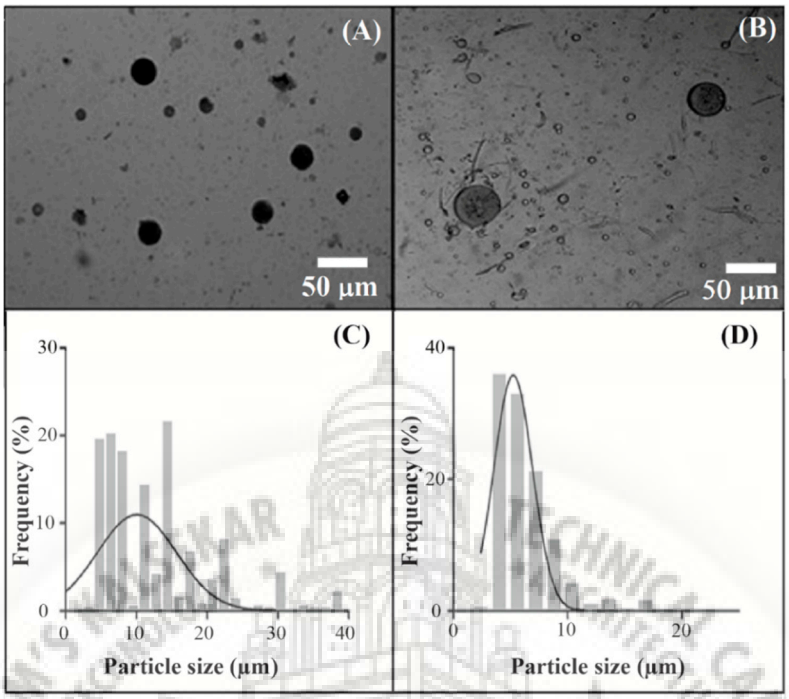

Figure 3. Optical microscopy (**A**,**B**) and particle size distribution (**C**,**D**) of XBM (**A**,**C**, respectively) and XBM-Ca-F9 (**B**,**D**, respectively), at a magnification of 10χ with their respective Gaussian distribution (bold line, **C**,**D**).

3.3. *Influence of the $CaCl_2$ in the 5-ASA Loading*

In order to increase the drug encapsulation into the XBM and improve its hardness, the addition of $CaCl_2$ during the microparticles production was used as a strategy to promote a faster crosslinking within the xylan chains. This approach could prevent the diffusion of the 5-ASA within the continuous phase, while hardening the xylan droplet. Simultaneously, the covalent chemical crosslinking between STMP and xylan would happen during the incubation period. In addition, the $CaCl_2$ might perform a role as a crosslinker, on the sites that would not be occupied by the STMP, which may increase the number of interaction points along the polymeric chains (Figure 4). Consequently, the crosslinking and compactness of the produced microparticles would also increase, which can be observed by a reduction on the average size on the formulations containing $CaCl_2$ (XBM-Ca-F1 to XBM-Ca-F9) compared to the ones without $CaCl_2$ (XBM) (Table 1) [23].

Similarly, based on XBM, the optical microscopy of the formulation XBM-Ca-F9 (Figure 3B) shows not only a spherical shape and absence of aggregates, but also a uniform distribution particle size (Figure 3D). In addition, the $CaCl_2$ promoted a reduction of approximately 50% on the average diameter of the formulation, from 10.70 (for XBM) to 4.77 µm (for XBM-Ca-F9). As can be seen in the formulations XBM-Ca-F4 and XBM-Ca-F6 (Table 1), the increase of $CaCl_2$ to $2\%_{(w/v)}$ promoted a decrease in the average particle size.

The factorial design analysis showed that only the main effect of $CaCl_2$ and the xylan-STMP interaction were statistically significant to increase the 5-ASA encapsulation into XBM (p-value < 0.05). As shown in Figure 5, the increment of the $CaCl_2$ improved the encapsulation of 5-ASA into the microparticles. This phenomenon was related to the faster crosslinking process of the Ca^{2+}, mainly on the surface of the droplet, when compared to the STMP alone. The Ca^{2+} could be able to keep the drug entrapped while the network between xylan an STMP was formed [24]. On the other hand, the interaction between xylan and STMP has a negative impact on the 5-ASA encapsulation. It is likely that the amount of STMP used for the crosslinking reactions was insufficient to tightly link the xylan chains.

Previous experiments showed that the ideal condition to produce XBM was the use of 1%$_{(w/v)}$ of STMP. At high polymer concentrations, the formation of microparticles was hindered (data not shown).

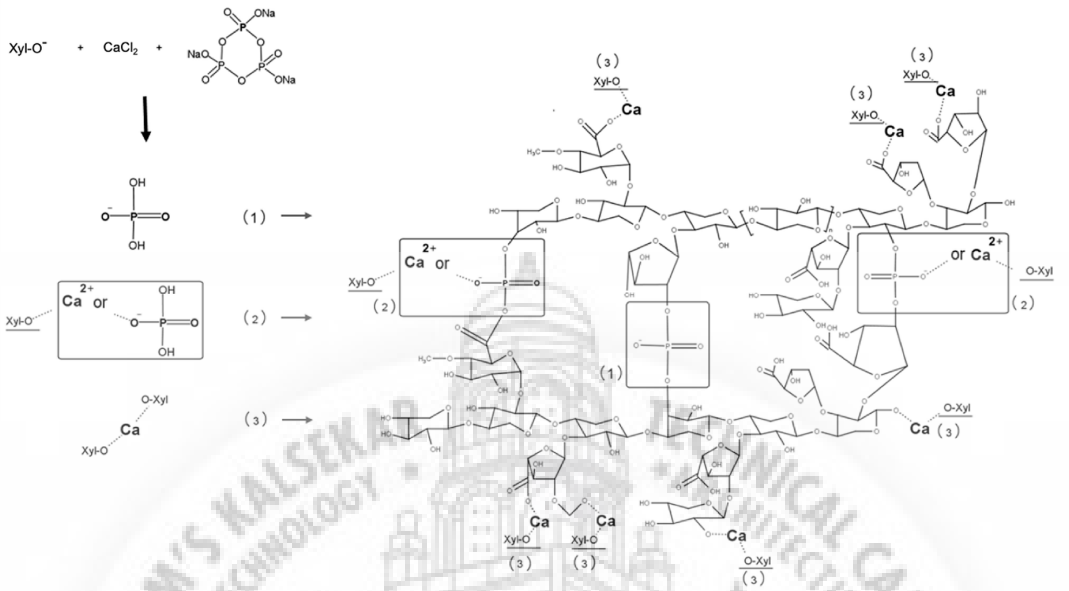

Figure 4. Scheme of the crosslinking reaction between xylan (Xyl), $CaCl_2$, and STMP. Reaction (1) represents only the chemical bond between STMP-Xyl. Reaction (2) represents the process of chemical crosslinking between Ca^{2+}-Xyl-STMP, respectively, and Reaction (3) represents the ionic interaction between Ca^{2+}-Xyl.

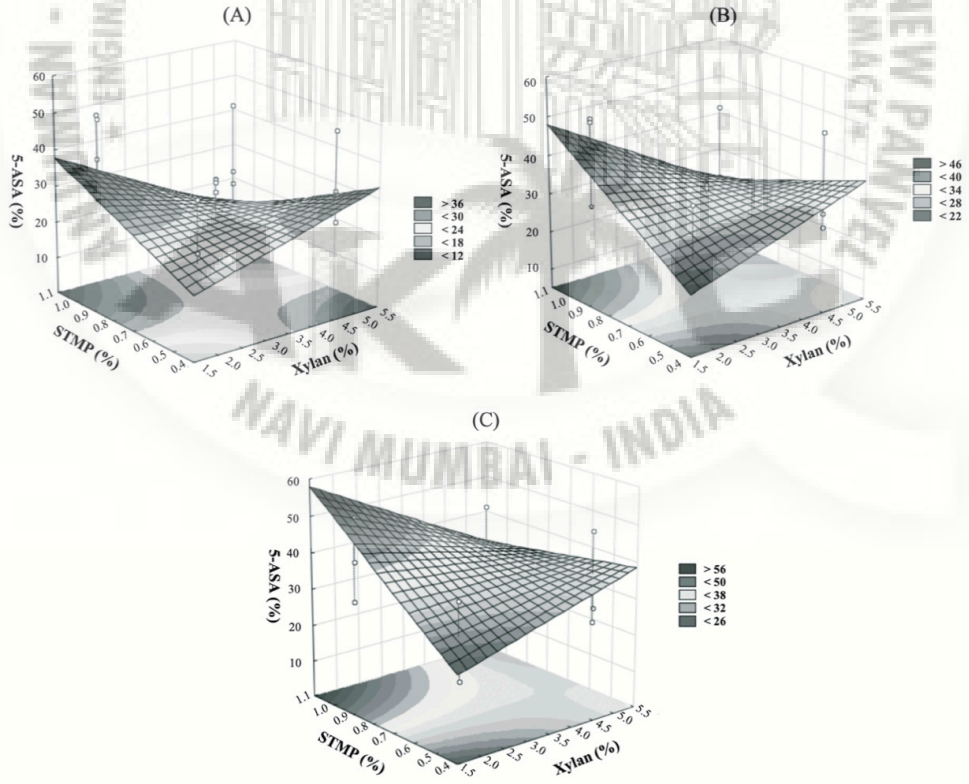

Figure 5. Response surface plot. Correlation of the encapsulation of 5-ASA with xylan-STMP and $CaCl_2$ concentrations into the formulation. The STMP and xylan concentration were maintained (x-axis), while the concentration of $CaCl_2$ was increased. In (**A**) it was used 0.5%$_{(w/v)}$ of $CaCl_2$, in (**B**) 1.25%$_{(w/v)}$ and in (C) 2%$_{(w/v)}$.

Additionally, the encapsulation of 5-ASA was enhanced at the lower level of xylan and at the higher level of STMP (Figure 5A–C). The particles formed within these concentrations likely have a smaller pore size due to the high crosslinking degree, which, consequently, improves the encapsulation of 5-ASA [25].

From the mathematical modeling of the factorial design experimental data, it was possible to retrieve a predictive model with an adjusted-R^2 = 0.4661 and a predictive-R^2 = 0.1253. This large difference might be justified by the standard deviation obtained from some formulations, such as XBM-Ca-F4, XBM-Ca-F7, and XBM-Ca-F8. However, all these formulations showed a high xylan-STMP mass ratio, 5:1, 10:1, and 4:1, respectively, while the optimal formulation (XBM-Ca-F9) had a xylan-STMP mass ratio of 2:1 (Table 1, Figure 5). This data also corroborates to the ones from the literature, in which it could be observed that the crosslinking reaction was important not only to the encapsulation of the drug into the microparticles, but also to the control of their release to the media (buffers and physiological fluids) [23,24]. Recently, xylan-based microspheres were prepared by an emulsion-template technique, and the crosslinking concentration was also an important parameter not only for the formation of the microspheres, but also for their swelling capacity [10].

3.4. Chemical Characterization of XBM and XBM-Ca by ATR-FTIR

To better understand the chemical interactions between the polymeric matrix and the 5-ASA, the ATR-IR was performed. Figure 6A shows the ATR-IR spectra of xylan (I), XBM (II), and PEG (III), while Figure 6B depicted the ATR-IR spectra of XBM-Ca-F9 without 5-ASA (I), XBM-Ca-F9-loaded with 5-ASA (II), and pure 5-ASA (III).

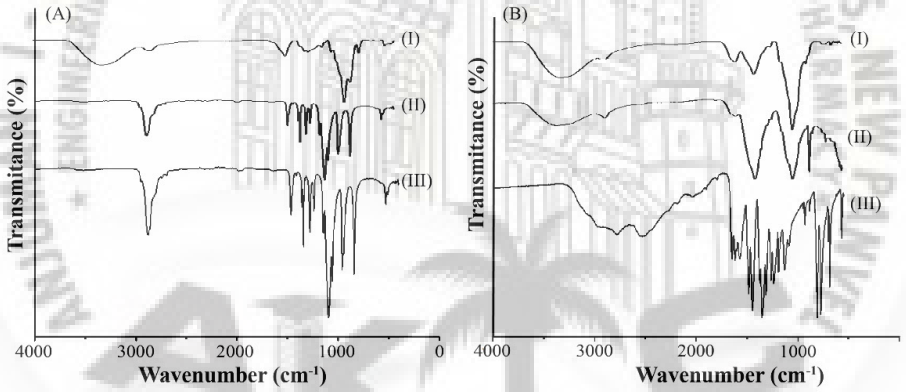

Figure 6. ATR-IR spectra (**A**) of xylan (I), XBM (II), and PEG (III) and (**B**) formulation XBM-Ca-F9 without drug (I), XBM-Ca-F9 loaded with 5-ASA (II), and 5-ASA alone (III).

The xylan infrared spectrum was like other spectra observed elsewhere (Figure 6A (I)) [26,27]. Furthermore, it was observed that the spectrum of XBM and PEG (Figure 6A (II and III, respectively)) had similar peaks, likely due to the shielding effect presented by the PEG in the formulation. In fact, after the drying process, it could be possible that the PEG shielding effect form a film and prevent ATR-IR from analyzing XBM. On the other hand, in formulations containing $CaCl_2$ (Figure 6B (I and II)), this phenomenon was not observed. It could be speculated that, likely, the $CaCl_2$ play an important role at the interface of the microparticles, which hinders the shielding effect of PEG.

The ATR-IR results also revealed that the bond P=O formed during the crosslinking process between the STMP and the polysaccharides was not observed. This phenomenon was equally observed for the empty and loaded XBM-Ca-F9 and might reveal a low degree of crosslinking, which was suggested by Li and co-workers (2012) [15]. The presence of a sharp peak at the 848 cm^{-1} region was observed for the XBM-Ca-F9 loaded with 5-ASA (Figure 6B (II)), while it was not seen on the empty XBM-Ca-F9 (Figure 6B (I)). This peak is commonly attributed to the C-H bonds of aromatic groups, such as the aromatic ring presented at the 5-ASA structure and was also observed on its spectrum

in Figure 6B (III) [22]. In addition, the increase on the intensity of the peak near 1500 cm^{-1} may be associated with the enhancement of drug 5-ASA loading, once these peaks are associated to the C-C and C=C stretching mode, and also to the N-H bond [22].

3.5. XRD Analysis

As reported in the literature, xylan XRD analysis (Figure 7 (II)) revealed the profile of an amorphous polymer [26]. Likewise, the diffractogram of the unloaded XBM-Ca-F9 (Figure 7 (III)) also revealed an amorphous structure. This result is very different from the previous reports of crosslinked xylan microparticles prepared with terephthaloyl chloride. However, this is commonly seen in STMP crosslinking products [28]. On the other hand, the diffractogram of 5-ASA alone (Figure 7 (IV)) showed very pronounced peaks at 22.5°, 30.6°, and 40.6°, which was also identified in XBM-Ca-F9 loaded with 5-ASA (Figure 7 (I)) XRD analysis. The presence of the peaks from 5-ASA on the XBM-Ca-F9 diffractogram reveals the association of the 5-ASA with the xylan through physical interactions. Once no new bound was identified in the ATR-IR analysis, there was only an enhancement of the peaks at the region between 1400 to 1600 cm^{-1}, as previously discussed.

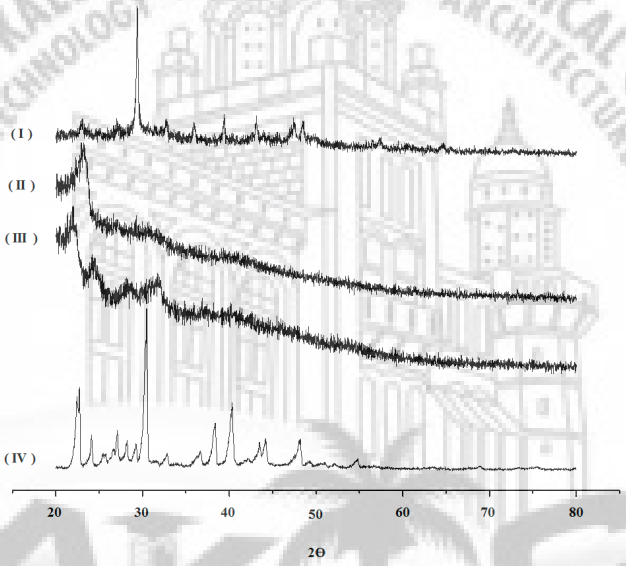

Figure 7. X-ray diffractograms of pure 5-ASA (IV), Formulation XBM-Ca-F9 without drug (III), xylan (II), and Formulation XBM-Ca-F9 loaded with 5-ASA (I).

3.6. In Vitro Drug Release and Mathematical Modeling

From the developed formulations shown in Table 1, the formulation XBM-Ca-F9 presented the highest EE% and, therefore, was used as a model in the study of drug release. According to Figure 8, approximately half of the initial loading of 5-ASA was released into the dissolution medium in approximately 4 h. In fact, despite the initial release, the amount released after 12 h of the experiment reached 60%. This result indicates that the formulation XBM-Ca-F9 might be able to reach the large intestine with approximately 40% of its initial loading in 5-ASA, which was later confirmed (36.4% ± 1.1%) by the extraction of the 5-ASA from the matrix after 12 h.

This initial release observed in the beginning of the experiment may happen due to the hydration and relaxation of the xylan chains that allow the drug diffusion [29]. However, the ability to hold 40% of its initial load after 4 h might be enough for the local treatment of the inflammation. In fact, previous studies with successful pharmacological response carried out with microgranules containing 5-ASA (Pentasa®) showed that the delivery systems reached the colon region in approximately 4 to 6 h [30]. The retention of the 5-ASA for such a long period into the XBM-Ca-F9 matrix leads to the hypothesis of the ability to deliver the 5-ASA in the colon region by the XBM-Ca-F9 formulation, since it was for

xylan conjugates with 5-fluoracil (Sauraj and co-workers (2017)). In this work, the authors performed a dissolution assay using the materials and fluids from rats' gastrointestinal tract [31].

In order to understand the mechanism on the initial release of 5-ASA from XBM-Ca-F9, some mathematical models were applied to the data. Initially, a first attempt was made to fit the data to a concentration independent model (e.g., linear model). However, the drug was not released in a constant rate. Thus, models that considered a non-linear release were tested. The Higuchi, Korsmeyer-Peppas, and Peppas-Shalin models were, then, tried. The Higuchi model showed a poor adjustment to the data, likely due to the non Fickian diffusion of the drug through the polymeric matrix, which will be later discussed. On the other hand, the Korsmeyer-Peppas and the Peppas-Shalin model, which consider the Fickian diffusion and the relaxation of the polymeric chains, were able to best fit the data. Therefore, the Peppas-Sahlin model with t-lag was the one with best fitting parameters, once it considers a lag time for the beginning of the drug release (See Table S1) [29,32]. Thus, according to the predictive model, the main mechanism of the 5-ASA release from the Formulation XBM-Ca-F9 was the relaxation of the xylan chains. In addition, it was seen that the contribution of the Fickian diffusion decreased, while the contribution of the relaxation increased over the time.

The release profile observed for the XBM-Ca-F9 was quite different from the xylan-based particles previously produced by our group. The crosslinked microcapsules prepared by Silva et al. (2013) kept constantly the release of the drug into the buffer media, while the spray-dried formulations completely release the drug once in contact to the medium [9]. Thus, to the best of our knowledge, the xylan-based particles developed had the highest encapsulation efficiency and the best drug release profile for physical encapsulation of 5-ASA.

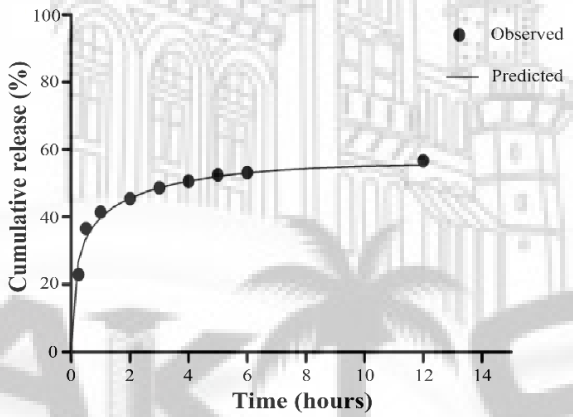

Figure 8. Cumulative drug release profile of the microparticles prepared by the water-in-water emulsion method (Formulation XBM-Ca-F9. Average ± SD. n = 3).

4. Conclusions

The encapsulation of low molecular weight drug using the water-in-water emulsions technique can be achieved using the ionic gelation technique, which is a method to quickly harden the polymeric matrix. Additionally, the addition of $CaCl_2$ did not hinder the assembling of the xylan polymeric chains. Furthermore, the $CaCl_2$ apparently sharpens the particles' size distribution, and all the microparticles produced were spherical and non-aggregated, which reveals the feasibility of the methodology. The kinetic release of the 5-ASA was linked to the relaxation of the xylan chains. Even though there was a 50% release, seen in the first 4 h, there was a high amount of the 5-ASA that moved into the microparticles after 12 h (36.4% ± 1.1%). To the best of our knowledge, this was the first time that a low molecular weight drug was successfully encapsulated into microparticles produced by this technique, which broadens the application of this technology. This approach can also be used to enhance the therapeutic efficiency of other small molecules, such as budesonide, metronidazole, and dicyclomine.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/9/17/3519/s1. Table S1: Mathematical modeling of the experimental data, their equations and statistical parameters.

Author Contributions: B.S.S. performed the experiments, analyzed and discussed the results, and wrote the manuscript. H.R.M. performed the experiments, analyzed and discussed the results, and wrote the manuscript. F.A.J. performed the experiments, analyzed the results, and discussed the results. S.C.C.U. performed the experiments, and analyzed the results. K.C.H.S. performed the experiments. D.C.F.S. contributed to the discussion of the data and edited and revised the manuscript. E.S.T.E. supervised the research work, received the funding, contributed to the discussion of the data, and edited and revised the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Marcelino, H.R.; da Silva, A.E.; Gomes, M.C.S.; Oliveira, E.E.; Nagashima, T., Jr.; Pinheiro, G.S.; Silva, A.E.; Timoteo, A.R.S.; Agnez-Lima, L.F.; Ayala, A.P.; et al. Leads from Physical, Chemical, and Thermal Characterization on Cytotoxic Effects of Xylan-Based Microparticles. *Polymers (Basel)* **2015**, *7*, 2304–2315. [CrossRef]
2. Ferreira, I.S.; Bettencourt, A.; Betrisey, B.; Goncalves, L.M.D.; Trampuz, A.; Almeida, A.J. Improvement of the antibacterial activity of daptomycin-loaded polymeric microparticles by Eudragit RL 100: An assessment by isothermal microcalorimetry. *Int. J. Pharm.* **2015**, *485*, 171–182. [CrossRef]
3. Liu, M.; Zhong, X.; Yang, Z. Chitosan functionalized nanocochleates for enhanced oral absorption of cyclosporine A. *Sci. Rep.* **2017**, *7*, 41322. [CrossRef]
4. Furst, W.; Banerjee, A. Release of Glutaraldehyde From an Albumin-Glutaraldehyde Tissue Adhesive Causes Significant in vitro and in vivo Toxicity. *Ann. Thorac. Surg.* **2005**, *79*, 1522–1529. [CrossRef]
5. Nicolai, T.; Murray, B. Particle stabilized water in water emulsions. *Food Hydrocoll.* **2017**, *68*, 157–163. [CrossRef]
6. Frith, W.J. Mixed biopolymer aqueous solutions—Phase behaviour and rheology. *Adv. Colloid Interface Sci.* **2010**, *161*, 48–60. [CrossRef]
7. Naidu, D.S.; Hlangothi, S.P.; John, M.J. Bio-based products from xylan: A review. *Carbohydr. Polym.* **2018**, *179*, 28–41. [CrossRef]
8. Nagashima, T., Jr.; Oliveira, E.E.; Silva, A.E.; Marcelino, H.R.; Gomes, M.C.S.; Aguiar, L.M.; Araújo, I.B.; Soares, L.A.L.; Oliveira, A.G.; Egito, E.S.T. Influence of the lipophilic external phase composition on the preparation and characterization of xylan microcapsules—A Technical Note. *AAPS Pharm. Sci. Tech.* **2008**, *9*, 814–817. [CrossRef]
9. Silva, A.E.; Oliveira, E.E.; Gomes, M.C.S.; Marcelino, H.R.; Silva, K.C.; Souza, B.S.; Nagashima, T., Jr.; Ayala, A.P.; Oliveira, A.G.; Egito, E.S.T. Producing xylan/Eudragit(R) S100-based microparticles by chemical and physico-mechanical approaches as carriers for 5-aminosalicylic acid. *J. Microencapsul.* **2013**, *30*, 787–795. [CrossRef]
10. Urtiga, S.C.C.; Gabi, C.A.A.L.; Eleamen, G.R.A.; Souza, B.S.; Pessoa, H.L.F.; Marcelino, H.R.; Mendonça, E.A.M.; Egito, E.S.T.; Oliveira, E.E. Preparation and characterization of safe microparticles based on xylan. *Drug Dev. Ind. Pharm.* **2017**, *43*, 1601–1609. [CrossRef]
11. Esquena, J. Water-in-water (W/W) emulsions. *Curr. Opin. Colloid Interface Sci.* **2016**, *25*, 109–119. [CrossRef]
12. Franssen, O.; Hennink, W.E. A novel preparation method for polymeric microparticles without the use of organic solvents. *Int. J. Pharm.* **1998**, *168*, 1–7. [CrossRef]
13. Shum, H.C.; Varnell, J.; Weitz, D.A. Microfluidic fabrication of water-in-water (w/w) jets and emulsions. *Biomicrofluidics* **2012**, *6*, 12808–128089.
14. Franssen, O.; Stenekes, R.J.H.; Hennink, W.E. Controlled release of a model protein from enzymatically degrading dextran microspheres. *J. Control. Release* **1999**, *59*, 219–228. [CrossRef]

15. Li, B.-Z.; Wang, L.-J.; Li, D.; Adhikari, B.; Mao, Z.-H. Preparation and characterization of crosslinked starch microspheres using a two-stage water-in-water emulsion method. *Carbohydr. Polym.* **2012**, *88*, 912–916. [CrossRef]
16. Freitas, R.A.; Nicolai, T.; Chassenieux, C.; Benyahia, L. Stabilization of water-in-water emulsions by polysaccharide-coated protein particles. *Langmuir* **2016**, *32*, 1227–1232. [CrossRef]
17. Hasan, A.S.; Socha, M.; Lamprecht, A.; Ghazouani, F.E.; Sapin, A.; Hoffman, M.; Maincent, P.; Ubrich, N. Effect of the microencapsulation of nanoparticles on the reduction of burst release. *Int. J. Pharm.* **2007**, *344*, 53–61. [CrossRef]
18. Zhang, Y.; Huo, M.; Zhou, J.; Zou, A.; Li, W.; Yao, C.; Xie, S. DDSolver: An add-in program for modeling and comparison of drug dissolution profiles. *AAPS J.* **2010**, *12*, 263–271. [CrossRef]
19. Zhang, J.; Hwang, J.; Antonietti, M.; Schmidt, B.V.K.J. Water-in-water pickering emulsion stabilized by polydopamine particles and cross-linking. *Biomacromolecules* **2019**, *20*, 204–211. [CrossRef]
20. Stenekes, R.J.H.; Hennink, W.E. Equilibrium water content of microspheres based on cross-linked dextran. *Int. J. Pharm.* **1999**, *189*, 131–135. [CrossRef]
21. Ham, M.; Moss, A.C. Mesalamine in the treatment and maintenance of remission of ulcerative colitis. *Expert Rev. Clin. Pharmacol.* **2012**, *5*, 113–123. [CrossRef]
22. Dash, A.K.; Brittain, H.G. Mesalamine. In *Analytical Profiles of Drug Substances and Excipients*; Academic Press: Cambridge, MA, USA, 1998; Volume 25, pp. 209–242.
23. Koetting, M.C.; Guido, J.F.; Gupta, M.; Zhang, A.; Peppas, N.A. pH-responsive and enzymatically-responsive hydrogel microparticles for the oral delivery of therapeutic proteins: Effects of protein size, crosslinking density, and hydrogel degradation on protein delivery. *J. Control. Release* **2016**, *221*, 18–25. [CrossRef]
24. Nguyen, M.-H.; Tran, T.-T.; Hadinoto, K. Controlling the burst release of amorphous drug-polysaccharide nanoparticle complex via crosslinking of the polysaccharide chains. *Eur. J. Pharm. Biopharm.* **2016**, *104*, 156–163. [CrossRef]
25. Li, B.-Z.; Wang, L.-J.; Li, D.; Chiu, Y.L.; Zhang, Z.-J.; Shi, J.; Chen, X.D.; Mao, Z.-H. Physical properties and loading capacity of starch-based microparticles crosslinked with trisodium trimetaphosphate. *J. Food Eng.* **2009**, *92*, 255–260. [CrossRef]
26. Oliveira, E.E.; Silva, A.E.; Nagashima, T., Jr.; Gomes, M.C.S.; Aguiar, L.M.; Marcelino, H.R.; Araujo, I.B.; Bayer, M.P.; Ricardo, N.M.P.S.; Oliveira, A.G.; et al. Xylan from corn cobs, a promising polymer for drug delivery: Production and characterization. *Bioresour. Technol.* **2010**, *101*, 5402–5406. [CrossRef]
27. Gao, C.; Ren, J.; Zhao, C.; Kong, W.; Dai, Q.; Chen, Q.; Liu, C.; Sun, R. Xylan-based temperature/pH sensitive hydrogels for drug controlled release. *Carbohydr. Polym.* **2016**, *151*, 189–197. [CrossRef]
28. Li, B.-Z.; Wang, L.-J.; Li, D.; Bhandari, B.; Li, S.-J.; Lan, Y.; Chen, X.D.; Mao, Z.-H. Fabrication of starch-based microparticles by an emulsification-crosslinking method. *J. Food Eng.* **2009**, *92*, 250–254. [CrossRef]
29. Brazel, C.S.; Peppas, N.A. Mechanisms of solute and drug transport in relaxing, swellable, hydrophilic glassy polymers. *Polymer (Guildf)* **1999**, *40*, 3383–3398. [CrossRef]
30. Wilding, I.R.; Kenyon, C.J.; Hooper, G. Gastrointestinal spread of oral prolonged-release mesalazine microgranules (Pentasa) dosed as either tablets or sachet. *Aliment. Pharmacol. Ther.* **2000**, *14*, 163–169. [CrossRef]
31. Kumar, S.U.; Gopinath, P.; Negi, Y.S. Synthesis and bio-evaluation of xylan-5-fluorouracil-1-acetic acid conjugates as prodrugs for colon cancer treatment. *Carbohydr. Polym.* **2017**, *157*, 1442–1450. [CrossRef]
32. Peppas, N.A.; Sahlin, J.J. A simple equation for the description of solute release. III. Coupling of diffusion and relaxation. *Int. J. Pharm.* **1989**, *57*, 169–172. [CrossRef]

Mesalamine Rectal Suspension, USP Enema

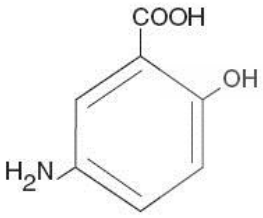
4g/unit (60 mL)

Rx Only

DESCRIPTION

The active ingredient in Mesalamine Rectal Suspension, USP Enema, a disposable (60 mL) unit, is mesalamine, also known as 5-aminosalicylic acid (5-ASA). Chemically, mesalamine is 5-amino-2-hydroxybenzoic acid.

The empirical formula is $C_7H_7NO_3$, representing a molecular weight of 153.14. The structural formula is:



Each rectal suspension enema unit contains 4 grams of mesalamine. In addition to mesalamine the preparation contains the inactive ingredients carbomer 934P, edetate disodium, potassium acetate, potassium metabisulfite, purified water and xanthan gum. Sodium benzoate is added as a preservative. The disposable unit consists of an applicator tip protected by a polyethylene cover and lubricated with USP white petrolatum. The unit has a one-way valve to prevent back flow of the dispensed product.

CLINICAL PHARMACOLOGY

Each Mesalamine Rectal Suspension Enema delivers up to 4 g of mesalamine to the left side of the colon.

The mechanism of action of mesalamine (and sulfasalazine) is not fully understood, but appears to be a topical anti-inflammatory effect on colonic epithelial cells. Mucosal production of arachidonic acid (AA) metabolites, both through the cyclooxygenase pathways, i.e., prostanoids, and through the lipoxygenase pathways, i.e., leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs) is increased in patients with ulcerative colitis, and it is possible that mesalamine diminishes inflammation by blocking cyclooxygenase and inhibiting prostaglandin (PG) production in the colon.

Preclinical Toxicology -

Preclinical studies have shown the kidney to be the major target organ for mesalamine toxicity. Adverse renal function changes were observed in rats after a single 600 mg/kg oral dose, but not after a 200 mg/kg dose. Gross kidney lesions, including papillary necrosis, were observed after a single oral >900 mg/kg dose, and after I.V. doses of > 214 mg/kg. Mice responded similarly. In a 13-week oral (gavage) dose study in rats, the high dose of 640 mg/kg/day mesalamine caused deaths, probably due to renal failure, and dose-related renal lesions (papillary necrosis and/or multifocal tubular injury) were seen in most rats given the high dose (males and females) as well as in males receiving lower doses 160 mg/kg/day. Renal lesions were not observed in the 160 mg/kg/day female rats. Minimal tubular epithelial damage was seen in the 40 mg/kg/day males and was reversible. In a six-month oral study in dogs, the no-observable dose level of mesalamine was 40 mg/kg/day and doses of 80 mg/kg/day and higher caused renal pathology similar to that described for the rat. In a combined 52-week toxicity and 127-week carcinogenicity study in rats, degeneration in kidneys was observed at doses of 100 mg/kg/day and above admixed with diet for 52 weeks, and at 127 weeks increased incidence of kidney degeneration and hyalinization of basement membranes and Bowman's capsule were seen at 100 mg/kg/day and above. In the 12-month eye toxicity study in dogs, Keratoconjunctivitis Sicca (KCS) occurred at oral doses of 40 mg/kg/day and above. The oral preclinical studies were done with a highly bioavailable suspension where absorption throughout the gastrointestinal tract occurred. The human dose of 4 grams represents approximately 80 mg/kg but when mesalamine is given rectally as a suspension, absorption is poor and limited to the distal colon (see **Pharmacokinetics**). Overt renal toxicity has not been observed (see **ADVERSE REACTIONS** and **PRECAUTIONS**), but the potential must be considered.

Pharmacokinetics -

Mesalamine administered rectally as Mesalamine Rectal Suspension Enema is poorly absorbed from the colon and is excreted principally in the feces during subsequent bowel movements. The extent of absorption is dependent upon the retention time of the drug product, and there is considerable individual variation. At steady state, approximately 10 to 30% of the daily 4-gram dose can be recovered in cumulative 24-hour urine collections. Other than the kidney, the organ distribution and other bioavailability characteristics of absorbed mesalamine in man are not known. It is known that the compound undergoes acetylation but whether this process takes place at colonic or systemic sites has not been elucidated.

Whatever the metabolic site, most of the absorbed mesalamine is excreted in the urine as the N-acetyl-5-ASA metabolite. The poor colonic absorption of rectally administered mesalamine is substantiated by the low serum concentration of 5-ASA and N-acetyl-5-ASA seen in ulcerative colitis patients after dosage with mesalamine. Under clinical conditions patients demonstrated plasma levels 10 to 12 hours post mesalamine administration of 2 µg/mL, about two-thirds of which was the N-acetyl metabolite. While the elimination half-life of mesalamine is short (0.5 to 1.5 h), the acetylated metabolite exhibits a half-life of 5 to 10 hours [U. Klotz, **Clin. Pharmacokin.** 10:285-302 (1985)]. In addition, steady state plasma levels demonstrated a lack of accumulation of either free or metabolized drug during repeated daily administrations.

Efficacy -

In a placebo-controlled, international, multicenter trial of 153 patients with active distal ulcerative colitis, proctosigmoiditis or proctitis, mesalamine rectal suspension enema reduced the overall disease activity index (DAI) and individual components as follows:

EFFECT OF TREATMENT ON SEVERITY OF DISEASE DATA FROM U.S.-CANADA TRIAL COMBINED RESULTS OF EIGHT CENTERS

		Activity Indices, mean				
		N	Baseline	Day 22	End Point	Change Baseline to End Point *
Overall DAI	Mesalamine Rectal Suspension Enema	76	7.42	4.05†	3.37‡	-55.07%‡
	Placebo	77	7.40	6.03	5.83	-21.58%
Stool Frequency	Mesalamine Rectal Suspension Enema		1.58	1.11§	1.01†	-0.57§
	Placebo		1.92	1.47	1.50	-0.41
Rectal Bleeding	Mesalamine Rectal Suspension Enema		1.82	0.59‡	0.51‡	-1.30‡
	Placebo		1.73	1.21	1.11	-0.61
Mucosal Inflammation	Mesalamine Rectal Suspension Enema		2.17	1.22†	0.96‡	-1.21†
	Placebo		2.18	1.74	1.61	-0.56
Physician's Assessment of Disease Severity	Mesalamine Rectal Suspension Enema		1.86	1.13‡	0.88‡	-0.97‡
	Placebo		1.87	1.62	1.55	-0.30

Each parameter has a 4-point scale with a numerical rating:
0 = normal, 1 = mild, 2 = moderate, 3 = severe. The four parameters are added together to produce a maximum overall DAI of 12.
*Percent change for overall DAI only (calculated by taking the average of the change for each individual patient).
†Significant Mesalamine Rectal Suspension, USP /placebo difference. p<0.01
‡Significant Mesalamine Rectal Suspension, USP /placebo difference. p<0.001
§Significant Mesalamine Rectal Suspension, USP /placebo difference. p<0.05

Differences between mesalamine rectal suspension enema and placebo were also statistically different in subgroups of patients on concurrent sulfasalazine and in those having an upper disease boundary between 5 and 20 or 20 and 40 cm. Significant differences between mesalamine rectal suspension enema and placebo were not achieved in those subgroups of patients on concurrent prednisone or with an upper disease boundary between 40 and 50 cm.

INDICATIONS AND USAGE

Mesalamine Rectal Suspension Enema is indicated for the treatment of active mild to moderate distal ulcerative colitis, proctosigmoiditis or proctitis in adults.

CONTRAINDICATIONS

Mesalamine Rectal Suspension Enema is contraindicated in patients with known or suspected hypersensitivity to salicylates, aminosalicylates, sulfites or any other component of this medication.

WARNINGS

Hypersensitivity Reactions

Sulfite-Related Reactions

Mesalamine Rectal Suspension Enema contains potassium metabisulfite, a sulfite that may cause allergic-type reactions including anaphylactic symptoms and life-threatening or less severe asthmatic episodes in certain susceptible people. The overall prevalence of sulfite sensitivity in the general population is unknown but probably low. Sulfite sensitivity is seen more frequently in asthmatic or in atopic nonasthmatic persons.

Epinephrine is the preferred treatment for serious allergic or emergency situations even though epinephrine injection contains sodium or potassium metabisulfite with the above-mentioned potential liabilities. The alternatives to using epinephrine in a life-threatening situation may not be satisfactory. The presence of a sulfite(s) in epinephrine injection should not deter the administration of the drug for treatment of serious allergic or other emergency situations.

Sulfasalazine-Associated Reactions

Hypersensitivity reactions have been reported in patients taking sulfasalazine. Some patients may have a similar reaction to Mesalamine Rectal Suspension Enema or to other compounds that contain or are converted to mesalamine.

As with sulfasalazine, mesalamine-induced hypersensitivity reactions may present as internal organ involvement, including myocarditis, pericarditis, nephritis, hepatitis, pneumonitis and hematologic abnormalities. Evaluate patients immediately if signs or symptoms of a hypersensitivity reaction are present. Discontinue Mesalamine Rectal Suspension Enema if an alternative etiology for the signs and symptoms cannot be established.

Renal Impairment

Renal impairment, including minimal change disease, acute and chronic interstitial nephritis, and renal failure have been reported in patients given products that contain mesalamine or are converted to mesalamine. In animal studies, the kidney was the principal organ of mesalamine toxicity.

Evaluate the risks and benefits of using Mesalamine Rectal Suspension Enema in patients with known renal impairment or a history of renal disease or taking concomitant nephrotoxic drugs. Mesalamine is known to be substantially excreted by the kidney, and the risk of adverse reactions may be greater in patients with impaired renal function. Evaluate renal function in all patients prior to initiation and periodically while on Mesalamine Rectal Suspension Enema therapy.

Mesalamine-Induced Acute Intolerance Syndrome

Mesalamine has been associated with an acute intolerance syndrome that may be difficult to distinguish from a flare of inflammatory bowel disease. Although the exact frequency of occurrence cannot be ascertained, it has occurred in 3% of patients in controlled clinical trials of mesalamine or sulfasalazine. Symptoms include cramping, acute abdominal pain and bloody diarrhea, sometimes fever, headache, and rash. Monitor patients for worsening of these symptoms while on treatment. If acute intolerance syndrome is suspected, promptly discontinue treatment with Mesalamine Rectal Suspension Enema.

PRECAUTIONS

Hepatic Failure

There have been reports of hepatic failure in patients with pre-existing liver disease who have been administered other products containing mesalamine. Evaluate the risks and benefits of using Mesalamine Rectal Suspension Enema in patients with known liver impairment.

Photosensitivity

Patients with pre-existing skin conditions such as atopic dermatitis and atopic eczema have reported more severe photosensitivity reactions. Advise patients to avoid sun exposure, wear protective clothing, and use a broad-spectrum sunscreen when outdoors.

Nephrolithiasis

Cases of nephrolithiasis have been reported with the use of mesalamine, including stones with 100% mesalamine content. Mesalamine-containing stones are radiotransparent and undetectable by standard radiography or computed tomography (CT). Ensure adequate hydration during treatment.

Interference with Laboratory Tests

Use of mesalamine may lead to spuriously elevated test results when measuring urinary normetanephrine by liquid chromatography with electrochemical detection because of the similarity in the chromatograms of normetanephrine and mesalamine's main metabolite, N-acetyl-5-aminosalicylic acid (N-Ac-5-ASA). Consider an alternative, selective assay for normetanephrine.

Drug Interactions

Nephrotoxic Agents, Including Non-Steroidal Anti-Inflammatory Drugs

The concurrent use of mesalamine with known nephrotoxic agents, including non-steroidal anti-inflammatory drugs (NSAIDs), may increase the risk of nephrotoxicity. Monitor patients taking nephrotoxic drugs for changes in renal function and mesalamine-related adverse reactions.

Azathioprine or 6-Mercaptopurine

The concurrent use of mesalamine with azathioprine or 6-mercaptopurine and/or any other drugs known to cause myelotoxicity may increase the risk for blood disorders, bone marrow failure, and associated complications. If concomitant use of Mesalamine Rectal Suspension Enema and azathioprine or 6-mercaptopurine cannot be avoided, monitor blood tests, including complete blood cell counts and platelet counts.

Information for Patients -

See patient information enclosed.

Carcinogenesis, Mutagenesis, Impairment of Fertility -

Mesalamine caused no increase in the incidence of neoplastic lesions over controls in a 2-year study of Wistar rats fed up to 320 mg/kg/day of mesalamine admixed with diet. Mesalamine is not mutagenic to Salmonella typhimurium tester strains TA98, TA100, TA1535, TA1537, TA1538. There were no reverse mutations in an assay using E. coli strain WP2UVRA. There were no effects in an *in vivo* mouse micronucleus assay at 600 mg/kg and in an *in vivo* sister chromatid exchange at doses up to 610 mg/kg. No effects on fertility were observed in rats receiving up to 320 mg/kg/day. The oligospermia and infertility in men associated with sulfasalazine has very rarely been reported among patients treated with mesalamine.

Pregnancy -

Teratologic studies have been performed in rats and rabbits at oral doses up to five and eight times respectively, the maximum recommended human dose, and have revealed no evidence of harm to the embryo or the fetus. There are, however, no adequate and well-controlled studies in pregnant women for either sulfasalazine or 5-ASA. Because animal reproduction studies are not always predictive of human response, 5-ASA should be used during pregnancy only if clearly needed.

Nursing Mothers -

It is not known whether mesalamine or its metabolite(s) are excreted in human milk. As a general rule, nursing should not be undertaken while a patient is on a drug since many drugs are excreted in human milk.

Pediatric Use -

Safety and effectiveness in pediatric patients have not been established.

Geriatric Use -

Clinical trials of mesalamine rectal suspension enema did not include sufficient numbers of patients aged 65 years and over to determine whether they respond differently from younger patients. Reports from uncontrolled clinical studies and postmarketing reporting systems suggested a higher incidence of blood dyscrasias (i.e., agranulocytosis, neutropenia and pancytopenia) in patients receiving mesalamine-containing products such as mesalamine rectal suspension enema who were 65 years or older compared to younger patients.

Consider monitor complete blood cell counts and platelet counts in elderly patients during treatment with mesalamine rectal suspension enema. In general, consider the greater frequency of decreased hepatic, renal, or cardiac function, and of concurrent disease or other drug therapy in elderly patients when prescribing mesalamine rectal suspension enema.

To report SUSPECTED ADVERSE REACTIONS, contact Perrigo at 1-866-634-9120 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

ADVERSE REACTIONS

Clinical Adverse Experience -

Mesalamine Rectal Suspension Enema is usually well tolerated. Most adverse effects have been mild and transient.

ADVERSE REACTIONS OCCURRING IN MORE THAN 0.1 % OF MESALAMINE RECTAL SUSPENSION ENEMA TREATED PATIENTS (COMPARISON TO PLACEBO)

	MESALAMINE RECTAL SUSPENSION ENEMA		PLACEBO	
SYMPTOM	N=815 N	%	N=128 N	%
Abdominal Pain/Cramps/Discomfort	66	8.10	10	7.81
Headache	53	6.50	16	12.50
Gas/Flatulence	50	6.13	5	3.91
Nausea	47	5.77	12	9.38
Flu	43	5.28	1	0.78
Tired/Weak/Malaise/Fatigue	28	3.44	8	6.25
Fever	26	3.19	0	0.00
Rash/Spots	23	2.82	4	3.12
Cold/Sore Throat	19	2.33	9	7.03
Diarrhea	17	2.09	5	3.91
Leg/joint Pain	17	2.09	1	0.78
Dizziness	15	1.84	3	2.34
Bloating	12	1.47	2	1.56
Back Pain	11	1.35	1	0.78
Pain on Insertion of Enema Tip	11	1.35	1	0.78
Hemorrhoids	11	1.35	0	0.00
Itching	10	1.23	1	0.78
Rectal Pain	10	1.23	0	0.00
Constipation	8	0.98	4	3.12
Hair Loss	7	0.86	0	0.00
Peripheral Edema	5	0.61	11	8.59
UTI/Urinary Burning	5	0.61	4	3.12
Rectal Pain/Soreness/Burning	5	0.61	3	2.34
Asthenia	1	0.12	4	3.12
Insomnia	1	0.12	3	2.34

In addition, the following adverse events have been identified during post-approval use of products which contain (or are metabolized to) mesalamine in clinical practice: nephrotoxicity, pancreatitis, fibrosing alveolitis, elevated liver enzymes, nephrogenic diabetes insipidus, intracranial hypertension and nephrolithiasis. Cases of pancreatitis and fibrosing alveolitis have been reported as manifestations of inflammatory bowel disease as well. Published case reports and/or spontaneous post marketing surveillance have described rare instances of aplastic anemia, agranulocytosis, thrombocytopenia, eosinophilia, pancytopenia, neutropenia, oligospermia, and infertility in men. Anemia, leukocytosis and thrombocytosis can be part of the clinical presentation of inflammatory bowel disease.

Hair Loss

Mild hair loss characterized by "more hair in the comb" but no withdrawal from clinical trials has been observed in seven of 815 mesalamine patients but none of the placebo-treated patients. In the literature there are at least six additional patients with mild hair loss who received either mesalamine or sulfasalazine. Retreatment is not always associated with repeated hair loss.

OVERDOSAGE

Mesalamine absorption from the colon is limited; however, Mesalamine Rectal Suspension Enema is an aminosalicylate, and symptoms of salicylate toxicity include nausea, vomiting and abdominal pain, tachypnea, hyperpnea, tinnitus, and neurologic symptoms (headache, dizziness, confusion, seizures). Severe salicylate intoxication may lead to electrolyte and blood pH imbalance and potentially to other organ (e.g., renal and liver) involvement. There is no specific antidote for mesalamine overdose. Correct fluid and electrolyte imbalance by the administration of appropriate intravenous therapy and maintain adequate renal function.

DOSAGE AND ADMINISTRATION

The recommended adult dosage of Mesalamine Rectal Suspension Enema in 60 mL units is one rectal instillation (4 grams) once a day, preferably at bedtime, and retained for approximately eight hours. While the effect of Mesalamine Rectal Suspension Enema may be seen within 3 to 21 days, the usual course of therapy would be from 3 to 6 weeks depending on symptoms and sigmoidoscopic findings. Studies available to date have not assessed if Mesalamine Rectal Suspension Enema will modify relapse rates after the 6-week short-term treatment. Mesalamine Rectal Suspension Enema is for rectal use only.

Drink an adequate amount of fluids during treatment.

Patients should be instructed to shake the bottle well to make sure the suspension is homogeneous. The patient should remove the protective sheath from the applicator tip. Holding the bottle at the neck will not cause any of the medication to be discharged. The position most often used is obtained by lying on the left side (to facilitate migration into the sigmoid colon); with the lower leg extended and the upper right leg flexed forward for balance. An alternative is the knee-chest position. The applicator tip should be gently inserted in the rectum pointing toward the umbilicus. A steady squeezing of the bottle will discharge most of the preparation. The preparation should be taken at bedtime with the objective of retaining it all night. Patient instructions are included with every seven units.

HOW SUPPLIED

Mesalamine Rectal Suspension, USP Enema for rectal administration is an off-white to tan colored suspension. Each disposable enema bottle contains 4.0 grams of mesalamine in 60 mL aqueous suspension. Enema bottles are supplied in boxed, foil-wrapped trays as follows:

Carton of 7 Bottles - NDC 45802-**098**-51

Carton of 28 Bottles - NDC 45802-**098**-28

Combo Kit with 7 Bottles and Wipes - NDC 45802-**923**-41

Combo Kit with 28 Bottles and Wipes - NDC 45802-**929**-49

Mesalamine Rectal Suspension Enemas are for rectal use only.

KEEP OUT OF REACH OF CHILDREN

Patient instructions are included.

Storage

Store at 20-25°C (68-77°F). [See USP Controlled Room Temperature]. Once the foil-wrapped unit of seven bottles is opened, all enemas should be used promptly as directed by your physician.

Contents of enemas removed from the foil pouch may darken with time. Slight darkening will not affect potency, however, enemas with dark brown contents should be discarded.

NOTE: Mesalamine Rectal Suspension Enema will cause staining of direct contact surfaces, including but not limited to fabrics, flooring, painted surfaces, marble, granite, vinyl, and enamel. Take care in choosing a suitable location for administration of this product.

For more information call Perrigo at 1-866-634-9120.

Rx Only

Made in Israel

Manufactured By Perrigo

Yeruham, Israel

Distributed By Perrigo®

Allegan, MI 49010

www.perrigorx.com

Rev 10-20

2N900 RC J8

PATIENT INSTRUCTIONS

How to Use this Medication

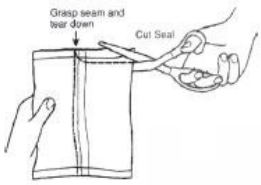
Best results are achieved if the bowel is emptied immediately before the medication is given.

NOTE: Mesalamine Rectal Suspension Enema will cause staining of direct contact surfaces, including but not limited to fabrics, flooring, painted surfaces, marble, granite, vinyl, and enamel. Take care in choosing a suitable location for administration of this product.

1. Remove the Bottles

a. Remove the bottles from the protective foil pouch by tearing or by using scissors as shown, being careful not to squeeze or puncture bottles. Mesalamine Rectal Suspension Enema is an off-white to tan colored suspension. Once the foil-wrapped unit of seven bottles is opened, all enemas should be used promptly as directed by your physician.

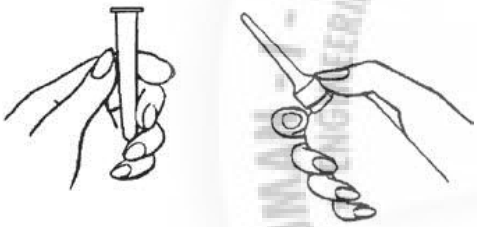
Contents of enemas removed from the foil pouch may darken with time. Slight darkening will not affect potency, however, enemas with dark brown contents should be discarded.



2. Prepare the Medication for Administration

a. Shake the bottle well to make sure that the medication is thoroughly mixed.

b. Remove the protective sheath from the applicator tip. Hold the bottle at the neck so as not to cause any of the medication to be discharged.

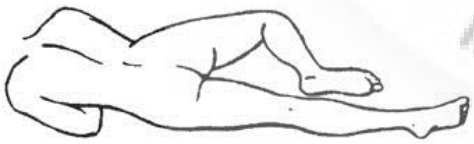


Prepare the Medication for Administration Image

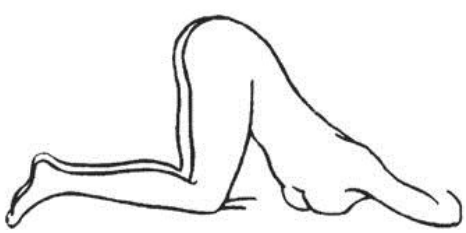
Prepare the Medication for Administration Image

3. Assume the Correct Body Position

a. Best results are obtained by lying on the left side with the left leg extended and the right leg flexed forward for balance.



b. An alternative to lying on the left side is the "knee-chest" position as shown here.



4. Administer the Medication

a. Gently insert the lubricated applicator tip into the rectum to prevent damage to the rectal wall, pointed slightly toward the navel.

b. Grasp the bottle firmly, then tilt slightly so that the nozzle is aimed toward the back, squeeze slowly to instill the medication. Steady hand pressure will discharge most of the medication. After administering, withdraw and discard the bottle.

c. Remain in position for at least 30 minutes to allow thorough distribution of the medication internally. Retain the medication all night, if possible.

Rx Only

Made in Israel

Manufactured By Perrigo

Yeruham, Israel

Distributed By Perrigo®

Allegan, MI 49010

www.perrigorx.com

Rev 10-20

2N900 RC J8

Principal Display Panel

Rx Only

Mesalamine Rectal Suspension, USP Enema

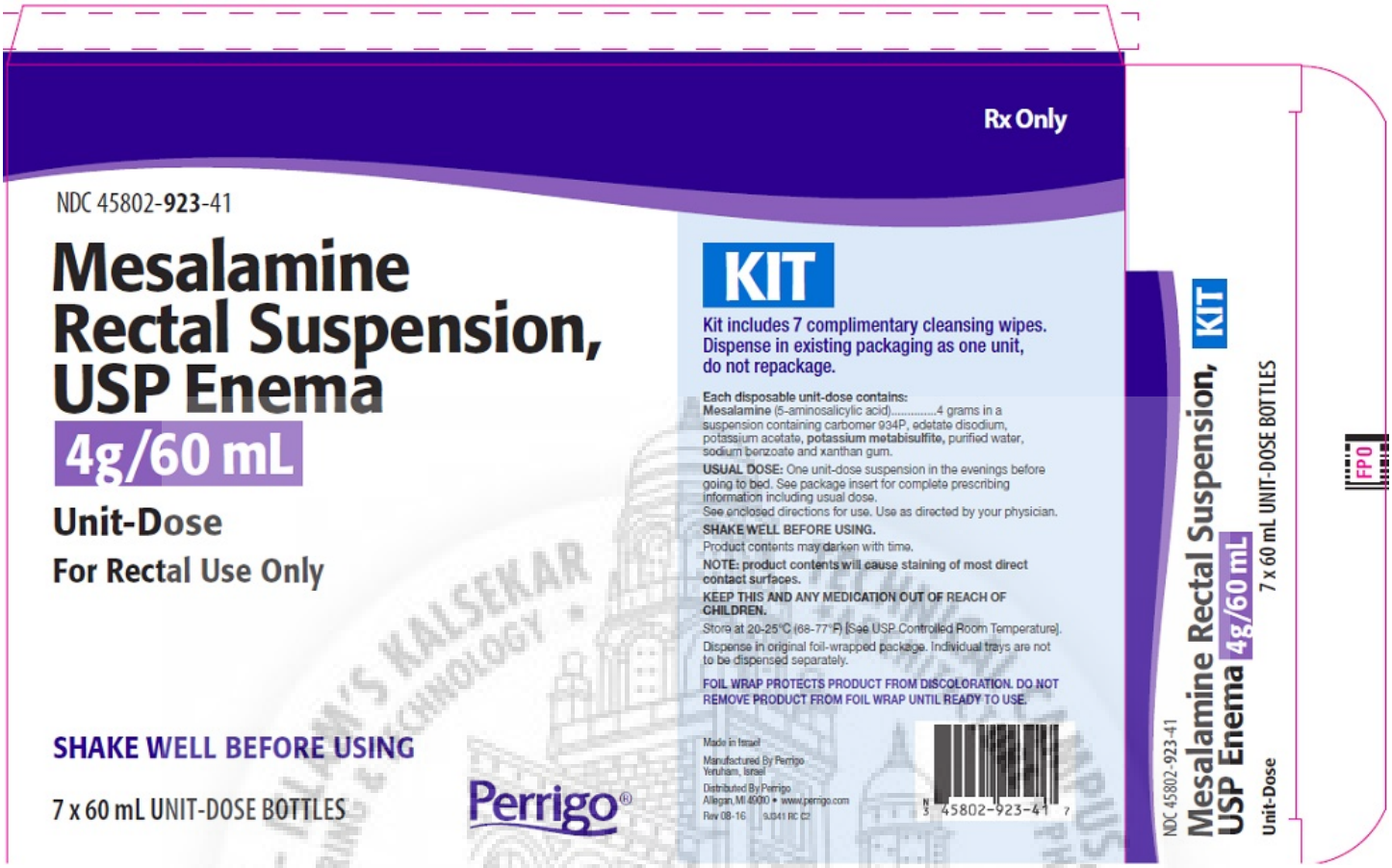
4g/60 mL

Unit-Dose

For Rectal Use Only

SHAKE WELL BEFORE USING

7 X 60 mL UNIT-DOSE BOTTLES



The following image is a placeholder representing the product identifier that is either affixed or imprinted on the drug package label during the packaging operation.

S/N [insert product's serial number]
Lot [insert product's lot number]
Exp [insert product's expiration date]

MESALAMINE
mesalamine kit

Product Information

Product Type	HUMAN PRESCRIPTION DRUG	**Item Code (Source)**	NDC:45802-923

Packaging

#	Item Code	Package Description	Marketing Start Date	Marketing End Date
1	NDC:45802-923-41	1 in 1 CARTON; Type 0: Not a Combination Product	09/01/2009	

Quantity of Parts

Part #	Package Quantity	Total Product Quantity
Part 1	7 BOTTLE	420 mL
Part 2	7 PACKET	7

Part 1 of 2

MESALAMINE

mesalamine enema

Product Information

Item Code (Source)	NDC:45802-098
Route of Administration	RECTAL

Active Ingredient/Active Moiety

Ingredient Name	Basis of Strength	Strength
MESALAMINE (UNII: 4Q81I59GXC) (MESALAMINE - UNII:4Q81I59GXC)	MESALAMINE	4 g in 60 mL

Inactive Ingredients

Ingredient Name	Strength
CARBOMER HOMOPOLYMER TYPE B (ALLYL PENTAERYTHRITOL OR ALLYL SUCROSE CROSSLINKED) (UNII: K6MOM3T5YL)	
EDETATE DISODIUM (UNII: 7FLD91C86K)	
POTASSIUM ACETATE (UNII: M911911U02)	
POTASSIUM METABISULFITE (UNII: 65OE787Q7W)	
WATER (UNII: 059QF0KO0R)	
XANTHAN GUM (UNII: TTV12P4NEE)	
SODIUM BENZOATE (UNII: OJ245FE5EU)	

Product Characteristics

Color	WHITE (off white to tan)	**Score**	
Shape		**Size**	
Flavor		**Imprint Code**	
Contains			

Packaging

#	Item Code	Package Description	Marketing Start Date	Marketing End Date
1	NDC:45802-098-46	60 mL in 1 BOTTLE; Type 0: Not a Combination Product		

Marketing Information

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
ANDA	ANDA076751	09/16/2009	

Part 2 of 2

CLEANSING WIPES

wipes swab

Product Information

Route of Administration	TOPICAL

Inactive Ingredients

Ingredient Name	Strength
BENZOIC ACID (UNII: 8SKN0B0MIM)	
LEVOMENOL (UNII: 24WE03BX2T)	
BUTYLENE GLYCOL (UNII: 3XUS85K0RA)	
BUTYLPARABEN (UNII: 3QPI1U3FV8)	
CARAMEL (UNII: T9D99G2B1R)	
DEHYDROACETIC ACID (UNII: 2KAG279R6R)	
DEXPANTHENOL (UNII: 1O6C93RI7Z)	
DIETHYLENE GLYCOL MONOETHYL ETHER (UNII: A1A1I8X02B)	
ETHYLPARABEN (UNII: 14255EXE39)	
DEXTROSE, UNSPECIFIED FORM (UNII: IY9XDZ35W2)	

ISOBUTYLPARABEN (UNII: 0QQJ25X58G)	
LACTIC ACID, UNSPECIFIED FORM (UNII: 33X04XA5AT)	
METHYLPARABEN (UNII: A2I8C7HI9T)	
PHENOXYETHANOL (UNII: HIE492ZZ3T)	
POTASSIUM SORBATE (UNII: 1VPU26JZZ4)	
PROPYLENE GLYCOL (UNII: 6DC9Q167V3)	
PROPYLPARABEN (UNII: Z8IX2SC1OH)	
SODIUM BENZOATE (UNII: OJ245FE5EU)	
WATER (UNII: 059QF0KO0R)	

Packaging

#	Item Code	Package Description	Marketing Start Date	Marketing End Date
1		1 in 1 PACKET; Type 0: Not a Combination Product		

Marketing Information

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
Unapproved drug other		09/16/2009	

Marketing Information

Marketing Category	Application Number or Monograph Citation	Marketing Start Date	Marketing End Date
ANDA	ANDA076751	09/01/2009	

Labeler - Perrigo New York Inc (078846912)

Revised: 12/2020

Perrigo New York Inc

INFLAMMATORY BOWEL DISEASE

Open

Treatment of Relapsing Mild-to-Moderate Ulcerative Colitis With the Probiotic VSL#3 as Adjunctive to a Standard Pharmaceutical Treatment: A Double-Blind, Randomized, Placebo-Controlled Study

Antonio Tursi, MD[1], Giovanni Brandimarte, MD[2], Alfredo Papa, MD, PhD[3], Andrea Giglio, MD[4], Walter Elisei, MD[2], Gian Marco Giorgetti, MD[5], Giacomo Forti, MD[6], Sergio Morini, MD[7], Cesare Hassan, MD[7], Maria Antonietta Pistoia, MD[8], Maria Ester Modeo, MD[9], Stefano Rodino', MD[4], Teresa D'Amico, MD[4], Ladislava Sebkova, MD[4], Natale Sacca', MD[4], Emilio Di Giulio, MD, PhD[10], Francesco Luzza, MD, PhD[11], Maria Imeneo, MD[11], Tiziana Larussa, MD[11], Salvatore Di Rosa, MD[12], Vito Annese, MD[13], Silvio Danese, MD, PhD[14] and Antonio Gasbarrini, MD, PhD[3]

OBJECTIVES: **VSL#3 is a high-potency probiotic mixture that has been used successfully in the treatment of pouchitis. The primary end point of the study was to assess the effects of supplementation with VSL#3 in patients affected by relapsing ulcerative colitis (UC) who are already under treatment with 5-aminosalicylic acid (ASA) and/or immunosuppressants at stable doses.**

METHODS: **A total of 144 consecutive patients were randomly treated for 8 weeks with VSL#3 at a dose of 3,600 billion CFU/day (71 patients) or with placebo (73 patients).**

RESULTS: **In all, 65 patients in the VSL#3 group and 66 patients in the placebo group completed the study. The decrease in ulcerative colitis disease activity index (UCDAI) scores of 50% or more was higher in the VSL#3 group than in the placebo group (63.1 vs. 40.8; per protocol (PP) $P=0.010$, confidence interval $(CI)_{95\%}$ 0.51–0.74; intention to treat (ITT) $P=0.031$, $CI_{95\%}$ 0.47–0.69). Significant results with VSL#3 were recorded in an improvement of three points or more in the UCDAI score (60.5% vs. 41.4%; PP $P=0.017$, $CI_{95\%}$ 0.51–0.74; ITT $P=0.046$, $CI_{95\%}$ 0.47–0.69) and in rectal bleeding (PP $P=0.014$, $CI_{95\%}$ 0.46–0.70; ITT $P=0.036$, $CI_{95\%}$ 0.41–0.65), whereas stool frequency (PP $P=0.202$, $CI_{95\%}$ 0.39–0.63; ITT $P=0.229$, $CI_{95\%}$ 0.35–0.57), physician's rate of disease activity (PP $P=0.088$, $CI_{95\%}$ 0.34–0.58; ITT $P=0.168$, $CI_{95\%}$ 0.31–0.53), and endoscopic scores (PP $P=0.086$, $CI_{95\%}$ 0.74–0.92; ITT $P=0.366$, $CI_{95\%}$ 0.66–0.86) did not show statistical differences. Remission was higher in the VSL#3 group than in the placebo group (47.7% vs. 32.4%; PP $P=0.069$, $CI_{95\%}$ 0.36–0.60; ITT $P=0.132$, $CI_{95\%}$ 0.33–0.56). Eight patients on VSL#3 (11.2%) and nine patients on placebo (12.3%) reported mild side effects.**

CONCLUSIONS: **VSL#3 supplementation is safe and able to reduce UCDAI scores in patients affected by relapsing mild-to-moderate UC who are under treatment with 5-ASA and/or immunosuppressants. Moreover, VSL#3 improves rectal bleeding and seems to reinduce remission in relapsing UC patients after 8 weeks of treatment, although these parameters do not reach statistical significance.**

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon characterized by bloody diarrhea and abdominal pain. Despite recent advances in the understanding of the genetics, immune and inflammatory mechanisms, as well as potential environmental factors that contribute to the disease, an exact pathogenesis remains elusive. Hence, the treatment is aimed at modifying the pathogenic mechanisms involved, mostly by using anti-inflammatory drugs such as mesalazine, corticosteroids, immunosuppressant agents, or biologics (1).

Recently, modulation of the gut flora has been suggested as an approach to manage UC. The role of microbiome in inflammatory bowel disease is clearly supported by many experimental observations. Gut flora can be modified either by antibiotics or by probiotics. Antibiotics are not good candidates for patients with chronic disorders because of antibiotic resistance, potential side effects, and ecological concerns.

Probiotics have proven to be effective in the management of pouchitis (3,4), and preliminary data are available for the treatment of UC (5,6), but strong data are still lacking in both UC and Crohn's disease. In particular, there is limited evidence that probiotics, in addition to standard therapy, may provide benefits in terms of reduction of disease activity in patients with mild to moderately active UC because of a lack of well-designed, large, randomized, placebo-controlled trials (7).

The present study has been conducted with VSL#3, a product that has proven to be effective for the treatment and prevention of pouchitis (3). The aim of this investigation was to assess whether, by adding VSL#3 to the current standard treatment of patients with mild-to-moderate UC, it would be possible to decrease the ulcerative colitis disease activity index (UCDAI) score by at least 50% and improve some of the symptoms associated with UC. Positive results would encourage a new approach in managing UC patients to avoid or delay step-up therapies with drugs burdened by potentially serious side effects.

METHODS

A multicenter, double-blind, randomized, placebo-controlled, parallel study was conducted in a population of UC patients with relapsing disease of mild-to-moderate severity.

We defined "relapsing mild-to-moderate UC" as a disease showing symptomatic recurrence after at least 6 months of remission (8), with a new increase in UCDAI (see **Table 1**) of at least three points (between three and eight) (9).

The protocol was approved by the Investigational Review Board of each center. All patients gave written informed consent for their participation.

Sample size

The sample size was based on a power of 80% and a statistical significance (α) of 95% ($P=0.05$). This calculation was based on the assumption that a response to treatment at 8 weeks, such as with oral 5-aminosalicylic acid (ASA) preparations, was expected to occur in 71% of patients treated with VSL#3 compared with a 40% expected response for patients treated with placebo. This assumed that the probiotic is as effective as oral 5-aminosalycilic acid. Therefore, 59 patients were required in each group, with an additional 15% for dropouts and 5% for patients failing to undergo final endoscopic assessment; hence a total of 144 patients were planned for the trial.

Table 1. Ulcerative colitis (UC) disease activity index

1. Stool frequency	
Normal	0
1–2 Stools/day > normal	1
3–4 Stools/day > normal	2
>4 Stools/day > normal	3
2. Rectal bleeding	
None	0
Streaks of blood	1
Obvious blood	2
Mostly blood	3
3. Mucosal appearance	
Normal	0
Mild friability	1
Moderate friability	2
Exudation, spontaneous bleeding	3
4. Physician's rating of disease activity	
Normal	0
Mild	1
Moderate	2
Severe	3

The index assesses four variables, which include stool frequency, severity of bleeding, colonic mucosal appearance, and the physician's overall assessment of disease activity.
Each variable is scored from 0–3 so that the total index score ranges from 0–12; 0–2: remission; 3–6: mild; 7–10: moderate; >10: severe UC.

Study procedures

The study procedures were conducted for each patient enrolled in the study.

At the screening visit, each patient's demographic characteristics, medical history, and current medications were recorded. β-Chorionic gonadotropin hormone was also assessed in women of childbearing age and was collected and analyzed to exclude pregnancy.

Eligible patients were randomly assigned to receive either VSL#3 or placebo twice daily for 8 weeks. The study product, VSL#3, was provided in plastic sealed individual dose sachets. Placebo was supplied in identical sachets. Patients were asked to take the contents of the sachets in the morning and evening. Individual disease activity quantified by the patient's UCDAI was calculated. The UCDAI was calculated by the investigator, who added the individual scores of the four parameters (bowel frequency, rectal bleeding, endoscopic score, and physician's rating of severity). At each visit, a detailed physical

examination and history were performed. All adverse events were documented, classified, and graded. Study participants were supplied with diary cards to assess and record their symptoms (stool frequency, bleeding, and abdominal pain) on a daily basis. Participants' compliance was assessed by the investigators, who counted the unused sachets that the patients were requested to bring back at week 8.

Inclusion criteria

Patients had to meet all the inclusion criteria described in **Table 2** to be eligible for participation. Moreover, women who had a negative pregnancy test at the screening visit and agreed to use a valid contraceptive method for the duration of the study, as well as patients not requiring hospitalization and patients willing and able to provide written informed consent, were considered eligible for inclusion in the study.

Exclusion criteria

Patients who met any of the exclusion criteria as described in **Table 3** were not enrolled in this study.

Significant hepatic, renal, endocrine, respiratory, neurological, or cardiovascular diseases, as determined by the investigator, were also considered as exclusion criteria. Other exclusion criteria that were also taken into consideration included the following:

- a history of severe adverse reaction or known hypersensitivity to maltose and/or silicon dioxide;
- patients requiring hospitalization;
- use of any investigational drug and/or participation in any clinical trial within 3 months before entering this study;
- inability to give a valid written informed consent or to properly follow the protocol.

Treatment

Patients meeting the inclusion criteria were randomly assigned to one of the two groups of treatment and received the product for 8 weeks in addition to their standard pharmaceutical therapy (5-ASA and/or immunosuppressant). VSL#3 consists of sachets, each containing 900 billion viable lyophilized bacteria, comprising four strains of lactobacilli (*L. paracasei*, *L. plantarum*, *L. acidophilus*, and *L. delbrueckii* subsp *bulgaricus*), three strains of bifidobacteria (*B. longum*, *B. breve*, and *B. infantis*), and one strain of *Streptococcus thermophilus* (VSL Pharmaceuticals, MD). The daily dose was two sachets twice a day taken orally (3,600 billion bacteria per day). The patient was asked to mix the contents of the sachets in a glass of cold water or in yogurt. Hot beverages were excluded, as an elevated temperature may inactivate the bacteria. The placebo was in the form of identical sachets that did not contain any lyophilized bacteria.

Concomitant treatments

Patients who were taking maintenance oral 5-ASA and/or azathioprine or 6-mercapropurine continued to do so at stable doses. The 5-ASA doses had to be fixed for 4 weeks and azathioprine or 6-mercaptopurine doses were fixed for at least 3 months before study entry, and had to be maintained at the same dose throughout the study. Any change in dosing of oral 5-ASA or in dosing of oral 6-mercaptopurine and azathioprine drugs throughout the 8-week study period was considered as a protocol violation.

Rectally administered medications, steroids, antibiotics, probiotics, and antidiarrheal drugs were not allowed, nor were any fruits, vegetables, milk, or fresh milk by-products.

VSL#3 supplementation had to be interrupted for a minimum of 14 days before inclusion in the study.

Primary end point

The primary end point was the evaluation of the beneficial effects of food supplementation with VSL#3 in relapsing mild-to-moderate UC patients, assessed by a decrease in the UCDAI of 50% or more, from baseline to week 8.

Table 2. Inclusion criteria

Male and female patients aged more than 18 years;
Diagnosis of UC established by previous colonoscopy, with consistent histology and clinical course;
UC involving at least the rectosigmoid region; activity confirmed by colonoscopy at the beginning of the study;
Mild-to-moderate relapsing UC, defined as a UCDAI score ranging from three to eight;
Symptoms (relapsing episodes) for less than 4 weeks before study entry;
A minimum endoscopic score of three on the UCDAI at screening (mucosal appearance);
Use of oral 5-ASA at least 4 weeks before study entry at a stable dose (mesalazine at least 1.6 g/day or balsalazide at least 4.5 g/day) and/or use of azathioprine (at least 1.5 mg/kg/day) or 6-mercaptopurine (at least 1 mg/kg/day) at least 3 months before study entry at a stable dose.

ASA, aminosalicylic acid; UC, ulcerative colitis; UCDAI, ulcerative colitis disease activity index.

Table 3. Exclusion Criteria

Crohn's disease or pouchitis;
A UCDAI score greater than eight (need for emergency surgery or the presence of severe disease);
Use of oral steroids within the last 4 weeks before study entry;
Use of antibiotics within the last 2 weeks before study entry;
Change in dose of oral 5-ASA within the last 4 weeks before study entry and throughout the 8-week study period or a change in dose of oral 6-mercaptopurine and azathioprine drugs within the last 3 months before the study;
Use of rectal 5-ASA or steroids within 1 week before entering the study or throughout the 8-week study period;
Use of probiotic preparations either prescribed or over-the-counter within 2 weeks before study entry;
Use of NSAIDs for 1 week before and throughout the 8-week study period.

ASA, aminosalicylic acid; NSAID, non-steroidal anti-inflammatory drug; UCDAI, ulcerative colitis disease activity index.

Secondary end points

Secondary end points were the possible beneficial effects of VSL#3 on the following:

- activity of relapsing UC;
- remission, considered as UCDAI ≤2, assessed at week 8;
- improvement in endoscopic scores, assessed by the endoscopic subgroup score of the UCDAI at week 8;
- change in objective symptoms (rectal bleeding and stool frequency);
- change in subjective symptoms (physician rating of disease activity);
- lack of beneficial effects, defined by the need for pharmacological treatment or inability to remain on the study regimen until week 8.

Randomization

Each center enrolled patients according to the randomization list. Patients who fulfilled the eligibility criteria specified above were randomly assigned to receive VSL#3 or placebo in a random order, using only one randomization list. The randomization number was strictly given according to the order of the patient's enrollment, assigning each patient the first available number on the randomization list. The randomization number, or the reason for not enrolling the patient, was reported for each patient in the appropriate forms. Randomization was carried out in a double-blind manner in blocks of four patients using 1:1 allocation to the two groups.

Assessment of compliance

The investigators assessed compliance by checking the number of unused sachets that the patients brought back at each visit.

Statistical assessment

Baseline characteristics of patients were compared using Student's *t*-test for independent samples or Pearson's χ^2-test as appropriate. Values of $P \geq 0.05$ were considered statistically significant. Pearson's χ^2-test was used to compare the UCDAI score at each visit with the basal visit score after adjustment of data using the last-observation-carried-forward method. Comparison of stool frequency, rectal bleeding, and mucosal appearance at each time between treatment groups and at each visit vs. the basal value was performed using Pearson's χ^2-test. The 95% confidence interval (CI) was also assessed.

A multivariate analysis was also performed. The general linear model multivariate procedure is based on a general linear model in which factors and covariates are assumed to have linear relationships to the dependent variables. As dependent variables, we chose UCDAI overall response at visit three (increase of 50% or more in the UCDAI score compared with the screening score) and disease extension at visit three (left-sided colitis, distal colitis, pancolitis). Fixed factors categorical predictors were selected as factors in the model (treatment with placebo or VLS#3, and concomitant treatment with or without the combination of 5-ASA and immunosuppressors). The general linear model multivariate procedure assumes that all model factors are fixed, i.e., they are generally thought of as variables, the values of interest of which are all represented in the data file, usually by design.

The statistical analysis of all the data sets pertaining to efficacy (specifically, primary and secondary end points) and safety (specifically, serious adverse events as defined by federal guidelines) has been independently performed by a biostatistician who is not employed by the corporate entity. The corresponding author had full access to all data and takes full responsibility for the veracity of the data and analysis.

RESULTS

Participant flow

A total of 144 patients (71 in the VSL#3 group and 73 in the placebo group) were enrolled. No patient was withdrawn before treatment assignment (see **Figure 1**).

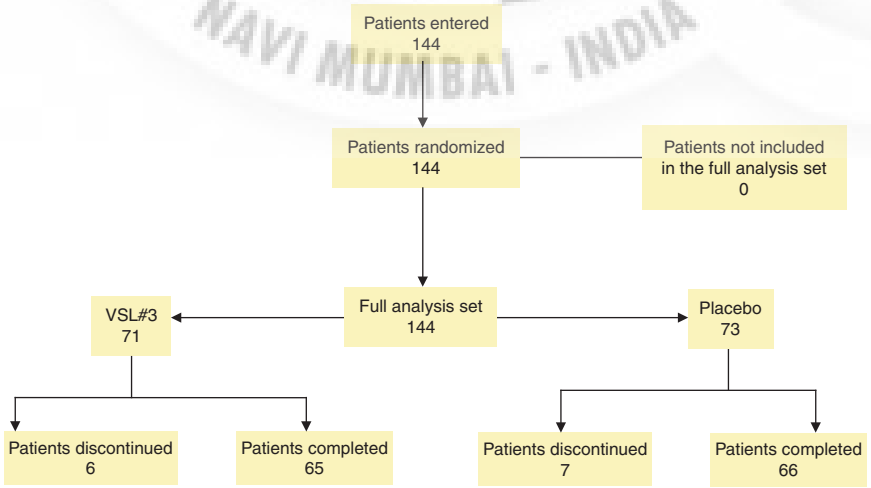

Figure 1. Patient disposition.

Baseline data

The clinical characteristics of patients in the two groups were comparable (**Table 4**). No significant differences were identified in terms of demographic characteristics (mean age, male–female ratio, weight, height, and mean UCDAI).

Clinical response

The main clinical outcomes of the study according to per-protocol (PP) and intention-to-treat (ITT) methods are shown in **Table 5**.

Table 4. Patient demographic and baseline characteristics

Characteristic	VSL#3	Placebo
Gender (male:female)	49:22 (69%)	44:29 (60.3%)
Age in years (mean±s.d.)	47.7±14.1	46.4±14.4
Number of previous relapses (mean±s.d.)	2.24±1.05	2.37±1.04
UCDAI at entry (mean±s.d.)	5.52±1.33	5.42±1.43
Disease extent (number of patients) (%)		
Proctosigmoiditis	36 (50.7%)	38 (52.1%)
Left-sided colitis	24 (33.8%)	21 (28.8%)
Pancolitis	11 (15.5%)	14 (19.1%)
Concomitant medications		
Mesalamine alone (mean/median±s.d.)	65 (91.55%) (2.08/2.4±0.39)	69 (94.52%) (2.08/2.4±0.40)
Balsalazide alone (mean/median±s.d.)	2 (2.82%) (4.5/4.5)	2 (2.74%) (4.5/4.5)
Azathioprine alone (mean/median±s.d.)	1 (1.23%) (1.62/1.5±0.25)	0 (0%)
Methotrexate alone (mean/median±s.d.)	1 (1.23%) (15 mg i.m./week)	0 (0%)
No medications	0 (0%)	0 (0%)
Combinations of drugs		
Mesalamine+azathioprine (mean/median±s.d.)	2 (3.90%) (2.08/2.4±0.39)+ (1.62/1.5±0.25)	2 (2.74%) (2.08/2.4±0.40)+ 1.75/1.75±0.25
Balsalazide+azathioprine	0 (0%)	0 (0%)
Balsalazide+methotrexate	0 (0%)	0 (0%)
Total	71 (100%)	73 (100%)

i.m., intra-muscular; UCDAI, ulcerative colitis disease activity index.

Six patients in the VSL#3 group withdrew during the follow-up, two had protocol violations (these patients took beclomethasone dipropionate and prednisone), two withdrew their informed consent, and three were lost to follow-up. Among the seven patients in the placebo group who withdrew during the follow-up, five patients experienced a worsening of symptoms, one was lost to follow-up, and one withdrew informed consent (see **Table 6**).

Primary end point

Overall, VSL#3 was significantly superior to placebo in reducing the disease activity of mild-to-moderate UC. Significantly more patients in the VSL#3 group experienced an improvement in their UCDAI score of at least 50% at the end of 8 weeks than those who received the placebo (41 (63.1%) vs. 29 (40.8%), respectively; PP $P=0.010$, $CI_{95\%}$ 0.51–0.74; ITT $P=0.031$, $CI_{95\%}$ 0.47–0.69) (see **Figure 2**).

To evaluate a more homogeneous set of patients, we also excluded patients who were under immunosuppressive treatment from the final evaluation. However, no statistical difference was found because VSL#3 was still significantly better in improving UCDAI scores of at least 50% at the end of 8 weeks than placebo (37 (56.1%) vs. 25 (36.2%), respectively; PP $P=0.008$; ITT $P=0.025$).

Secondary end points

Similarly, a significantly higher number of patients in the VSL#3 group had a decrease of three or more points in their UCDAI score from baseline to week 8 than the placebo group (39 (60%) vs. 29 (43.94%), respectively; PP $P=0.017$, $CI_{95\%}$ 0.51–0.74; ITT $P=0.046$, $CI_{95\%}$ 0.47–0.69) (see **Figure 2**).

Regarding the induction of remission, 31 (47.7%) patients in the VLS#3 group and 23 (32.4%) patients in the placebo group experienced remission by the end of 8 weeks; although a Δ value of 15.3% was observed, this difference was not statistically significant (PP $P=0.069$, $CI_{95\%}$ 0.36–0.60; ITT $P=0.132$, $CI_{95\%}$ 0.33–0.56) (see **Figure 2**). None of the parameters assessed in the multivariate analysis was found to have a significant role in influencing remission.

To evaluate a more homogeneous set of patients, we also excluded patients under immunosuppressive treatment from the final evaluation. However, no difference was found because VSL#3 was still better in obtaining remission at the end of 8 weeks than placebo, and the result did not reach statistical significance (28 (42.4%) vs. 20 (29%), respectively; PP $P=0.067$; ITT $P=0.110$).

Table 5. Clinical outcomes

	Per-protocol			Intention-to-treat		
	VSL#3	Placebo	*P* value	VSL#3	Placebo	*P* value
≥50% Improvement in UCDAI (week 8)	41	29	0.010	41	29	0.031
≥3 Decrease in UCDAI score (week 8)	39	29	0.017	39	28	0.046
Remission (week 8)	31	23	0.069	31	23	0.132

UCDAI, ulcerative colitis disease activity index.

Table 6. Reasons for discontinuation of treatment

	VSL#3 number of patients (%)	Placebo number of patients (%)
Lack of efficacy	0 (0.0)	5 (6.8)
Clinical episode	0 (0.0)	0 (0.0)
Abnormal laboratory result	0 (0.0)	0 (0.0)
Death	0 (0.0)	0 (0.0)
Protocol violation	2 (1.4)	0 (0.0)
Lost to follow-up	3 (4.2)	1 (1.4)
Protocol interim criteria not met	0 (0.0)	0 (0.0)
Patient's consent withdrawn	2 (2.8)	1 (1.4)

It is interesting that **Tables 7** and **8** show that none of the patients in the VSL#3 group experienced a worsening of symptoms during the follow-up, whereas several patients in the placebo group showed a worsening of symptoms, and five of them had to be withdrawn from the study.

Patients receiving VSL#3 had a significant reduction in rectal bleeding (PP $P=0.014$, $CI_{95\%}$ 0.46–0.70; ITT $P=0.036$, $CI_{95\%}$ 0.41–0.65). On the other hand, we did not find any significant difference in stool frequency (PP $P=0.202$, $CI_{95\%}$ 0.39–0.63; ITT $P=0.229$, $CI_{95\%}$ 0.35–0.57), physician's rating of disease activity (PP $P=0.088$, $CI_{95\%}$ 0.34–0.58; ITT $P=0.168$, $CI_{95\%}$ 0.31–0.53), or mean endoscopy scores (PP $P=0.086$, $CI_{95\%}$ 0.74–0.92; ITT $P=0.366$, $CI_{95\%}$ 0.66–0.86) (see **Figure 3**).

Safety and tolerability

No major adverse event was reported. Eight patients on VSL#3 (11.2%) reported mild side effects (one patient reported dizziness, one reported a flu-like syndrome, and six initially complained of abdominal bloating and discomfort), whereas nine patients on placebo (12.3%) reported mild side effects (one reported a fever, one had cystitis, three had abdominal bloating, and four patients had an unpleasant taste in their mouth).

DISCUSSION

UC is a chronic inflammatory disease of the colon involving still largely unknown interactions between genetic, environmental, and immunological factors.

UC is characterized by flare-ups of inflammation and periods of remission or quiescence that can be achieved or maintained by drugs having, as a common denominator, anti-inflammatory and/or immunosuppressive properties (5-aminosalicylates, 6-mercaptopurine, azathioprine, and anti-TNFα antibodies). If left without any maintenance drug, about 70% of patients will relapse within 12 months (2), and many patients on maintenance drugs will still require step-up therapy.

After the initial report by Gionchetti *et al.* (3) on pouchitis, followed by other confirmatory clinical studies, it is now accepted that VSL#3, a combination of probiotic bacteria, can place this disease in remission or quiescence in a large number of patients with a J-pouch, as recommended in the guidelines of international gastroenterological associations (10,11).

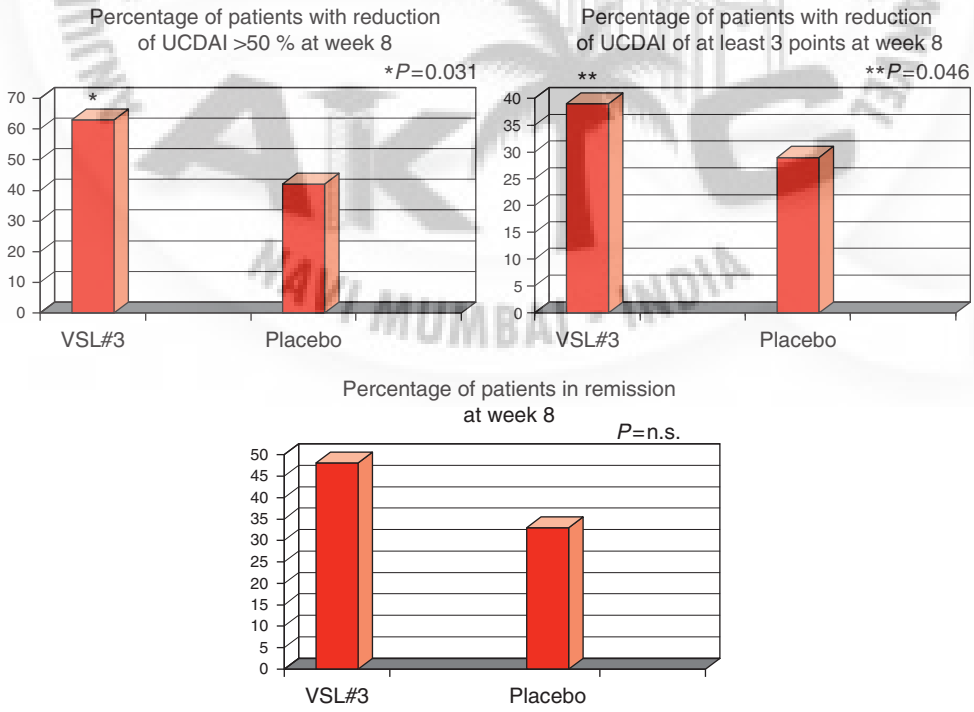

Figure 2. Percentage of patients with reduction of ulcerative colitis disease activity index (UCDAI) > 50% or of at least three points, and patients in remission at week 8 (on intention-to-treat analysis). n.s., not significant.

Table 7. Overall UCDAI response after 8 weeks (per-protocol analysis)

UCDAI after 8 weeks	Treatment					
	VLS#3		Placebo		Overall	
	n	%	*n*	%	*n*	%
None or light (0–2)	31	47.7	23	32.4	54	39.7
Mild (3–5)	27	41.5	34	47.9	61	44.9
Moderate (6–8)	7	10.8	11	15.5	18	13.2
Severe (9–12)	0	0	3	4.2	3	2.2
Overall	65	100	71	100	136	100

UCDAI, ulcerative colitis disease activity index.

Table 8. Overall UCDAI response after 8 weeks (on intention-to-treat analysis)

UCDAI after 8 weeks	Treatment					
	VLS#3		Placebo		Overall	
	n	%	*n*	%	*n*	%
None or light (0–2)	31	43.7	23	31.5	54	37.5
Mild (3–5)	30	42.3	35	47.9	65	45.1
Moderate (6–8)	10	14.1	12	16.4	22	15.3
Severe (9–12)	0	0	3	4.1	3	2.1
Overall	71	100	73	100	144	100

UCDAI, ulcerative colitis disease activity index.

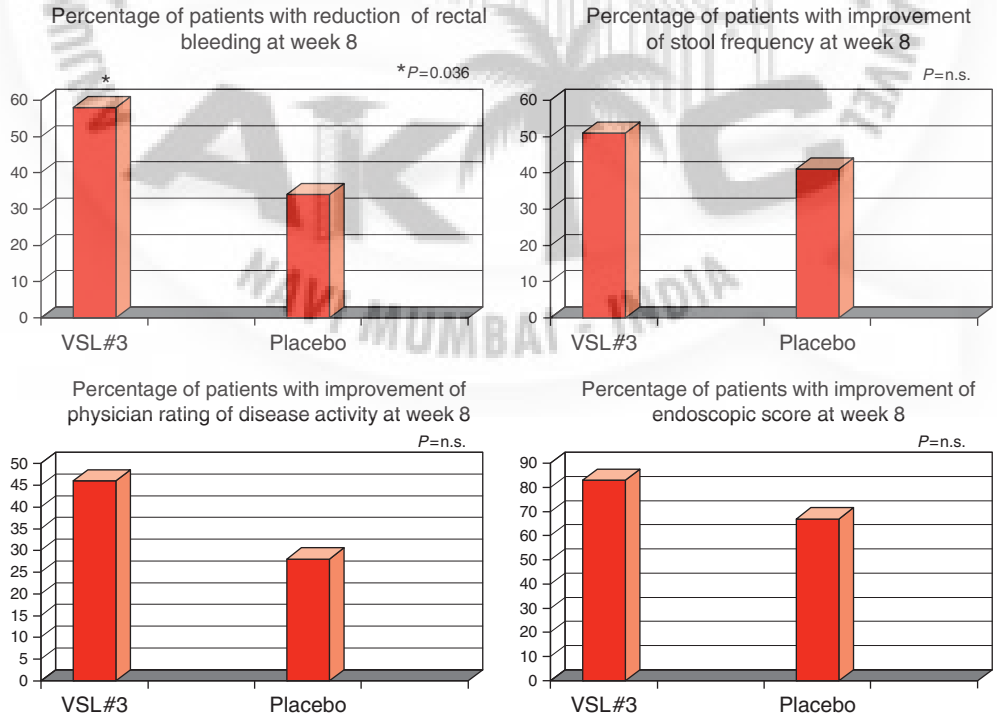

Figure 3. Percentage of patients with improvement in different subgroups of ulcerative colitis disease activity index (UCDAI; rectal bleeding, stool frequency, physician rating of disease activity, and endoscopic score) at week 8 (on intention-to-treat analysis). n.s., not significant.

We report the results of an Italian multicenter study aimed at evaluating the efficacy of the specific probiotic product, VSL#3, for the treatment of mild-to-moderate UC used in conjunction with standard treatment. Our study is a double-blind randomized placebo-controlled trial on adult patients affected by relapsing mild-to-moderate UC, in which VSL#3 or placebo was added to the standard treatment, and aimed to assess the decrease in UCDAI score of 50% or more. For ethical reasons, the "placebo" group was a group in which the patients continued to take their standard treatment (5-ASA and/or immunosuppressant), with the simple addition of a placebo.

Overall, VSL#3 was significantly superior to the placebo in reducing the activity of mild-to-moderate UC (primary end point). A significantly higher proportion of patients in the VSL#3 group experienced an improvement in their UCDAI score of at least 50% at week 8 over those who received placebo (63.1% vs. 40.8%, $P=0.010$). As a secondary end point, 31 individuals (47.7%) in the VLS#3 group and 23 individuals (32.4%) in the placebo group experienced remission by the end of 8 weeks, reaching results that did not show a significant difference (PP $P=0.069$; ITT $P=0.132$). We believe that this might represent a type II error and that a larger study might have had enough power to detect a statistically significant difference. None of the patients in the VSL#3 group experienced any worsening of symptoms during follow-up (**Tables 6** and **7**), whereas five individuals in the placebo group showed a deterioration in their clinical status and had to be withdrawn from the study. No significant difference in stool frequency, physician rating of disease activity, and mean endoscopy scores was detected between the two groups ($P=$ n. s. (not significant)). However, VSL#3 patients had a significant reduction in rectal bleeding compared with the placebo group (PP $P=0.014$; ITT $P=0.036$). Finally, no major adverse event was reported in either group. To confirm the efficacy of VSL#3, we also considered the patients who dropped out because of clinical ineffectiveness. In the "placebo" group, five patients abandoned the study for this specific reason (7%), whereas all VSL#3 patients completed the study.

VSL#3 has proven to be effective by colonizing the host, changing the epithelial function and the immune response. Experimentally, in murine models of colitis, VSL#3 prevents redistribution and reduced expression of sealing tight-junction proteins (12) and specifically stimulates the expression of genes associated with lipid, xenobiotic, and peroxisome proliferator-activated receptor signaling (13).

The roles of probiotics in managing active UC have also been reported in literature. Studies have reported *Escherichia coli* 1917 Nissle to be as effective as low-dose mesalamine in preventing a relapse of quiescent UC (14–16), and treatment with *Saccharomyces boulardii* for 4 weeks was shown to induce clinical remission in 71% of patients with mild-to-moderate disease; however, very few patients were enrolled to draw any conclusions (17). Moreover, *S. boulardii* should be managed with caution, especially in immunocompromised patients (e.g., in patients under immunosuppressant treatment) (18).

Other studies have reported the efficacy of VSL#3 in patients affected by UC (19). An open-label study (20) showed that in 5-ASA allergic or nonresponsive UC patients, VSL#3 was able to colonize the intestine and suggested that the product may be useful in maintaining remission (15 out of 20 patients remained in remission during the 1-year study). Thereafter, an open-label study found that 77% of mild-to-moderate UC patients obtained remission with 3,600 billion CFU/day of VSL#3 at 6 weeks (6). An Italian randomized, controlled study found that VSL#3 900 billion CFU/day added to low-dose balsalazide shows better results in treating active UC than balsalazide or mesalazine alone (5). Two studies with VSL#3 in pediatric UC have recently been carried out; the first one is an open-label study showing that 56% of pediatric patients obtained remission, with a combined remission/response rate of 61% (21), and the second one is a double-blind placebo-controlled trial, showing that VSL#3 supplementation was only able to induce remission in 92.8% of UC children compared with 36.4% with steroid alone, and was effective in maintaining remission in 78.6% of patients during a 12-month follow-up compared with 26.7% in the placebo group (22).

A recent Indian multicenter placebo-controlled trial investigating VSL#3 in mild-to-moderate UC patients was published (23). Patients were given 3,600 billion CFU/day VSL#3 for 12 weeks. At week 6, the percentage of patients with an improvement in UCDAI >50% was significantly higher in the group given VSL#3 (25, 32.5%) than in the placebo group (7, 10%; $P=0.001$). At week 12, 42.9% of VSL#3 patients achieved remission, compared with 15.7% of placebo patients ($P<0.001$). Furthermore, significantly more number of patients given VSL#3 (40, 51.9%) achieved a UCDAI decrease of more than three points, compared with those given placebo (13, 18.6%; $P<0.001$).

Although the design of our study was similar, we recorded a higher placebo response compared with the Sood *et al.* (23) study (40% in our trial vs. 10% in Indian trial). The high "placebo" response rate of our study (40.8% of placebo patients had a 50% reduced UCDAI) may be easily explained by the continuous standard medical treatment provided to all the patients and allows for the statistically borderline results reached in this study for obtaining remission and mucosal healing. A possible suggestion for future studies, in addition to increasing the number of enrolled patients, may be to extend the study period to 12 weeks, expecting, as the Sood *et al.* (23) study proved, that a longer treatment with VSL#3 will offer more divergence from the placebo group. As stated by a recent review, another possible explanation for this high "placebo" response is that the country in which the study is conducted significantly influences the placebo response rate (24). In particular, studies carried out exclusively in Europe have a significantly higher placebo remission rate than studies outside Europe, ranging from 20.8% to 33.6% (24). Our placebo results are therefore in line with the literature estimates. This high percentage of placebo response may also account for some results of this study. For example, the failure to improve stool frequency vs. placebo may be very relevant to patients. We found VSL#3 better than placebo when we assessed the objective parameters (UCDAI,

rectal bleeding, remission, and mucosal healing). On the contrary, subjective parameters (stool frequency and physician rating of disease activity) do not seem to improve so significantly under VSL#3 treatment. Two reasons may explain these conflicting results. First, the "placebo" response may affect some subjective parameters (e.g., stool frequency). The second is that unchanged stool frequency may be related to overlapping irritable bowel syndrome, as this sometimes affects patients with inflammatory bowel disease (25).

An important point of discussion to be addressed is the rationale of this study. People may argue that a higher dose of 5-ASA therapy might be just as well tolerated and may be more convenient and less expensive for obtaining remission. This may be a rational and advisable approach. However, we need a new therapeutic approach to relapsing UC, especially when the patient is already under treatment with immunosuppressors. Increased doses of mesalazine formulations may be safe and effective in obtaining remission, but the azo-bonded formulations may be compromised by secretory diarrhea at doses providing >2–2.4 g/day of mesalazine (26). Moreover, biologics are at higher risk of severe side effects and are much more expensive than a high-dose probiotic treatment in obtaining remission in relapsing UC. On the contrary, VSL#3 is classified as a food or food supplement in most countries and is characterized by a very high safety profile that has also been confirmed throughout this study. The safety of VSL#3 has also been proven in pediatric inflammatory bowel disease and intensive care unit patients (21,22,27).

Of course, once remission has been obtained, physicians also need to know how these patients should be managed in the longer term, i.e., with maintenance doses of probiotic. A clinical trial assessing the optimal dose of VSL#3 in maintaining remission of UC is needed.

Another criticism may be that the VSL#3 dose used in this study is quite high, compared with other studies reporting an effect on remission of UC or pouchitis (7). This choice was based on the assumption that a high probiotic concentration is needed to treat an extensive and active colonic disease. Of course, the optimal dose to maintain remission may be much lower (e.g., one sachet daily for the maintenance of remission in pouchitis (3)), and, as stated, a further trial assessing the optimal dose of VSL#3 in maintaining remission of UC is needed.

In this trial, probiotics and 5-ASA seem to have a synergistic activity. It is unclear how the association between probiotic and 5-ASA may take effect. It is possible that VSL#3 may function in synergy with, or perhaps increases, the anti-inflammatory action of 5-ASA compounds. 5-ASA compounds are potent inhibitors of several inflammatory mediators, such as leukotrienes, prostaglandins, and platelet-activating factor, all of which have roles in the pathogenesis of UC (28). In addition, 5-ASA compounds inhibit the production of interleukin-1 and free radicals and have an intrinsic antioxidant activity (29). Probiotics reduce inflammation by a number of mechanisms, including alteration of the mucosal immune system, competitive exclusion of proinflammatory pathogens, production of antimicrobial factors such as bacteriocins and other metabolites (28,30), and support of increased intestinal barrier function (31,32). At present, on the basis of what has recently been published for acetaminophen, we cannot exclude the possibility that gut bacteria may be the principal target for drugs, and that by manipulating the gut flora in the drug treatment, the outcome can be improved (33).

We do not know whether similar results could have been obtained only by increasing the 5-ASA daily dosage by up to 4 g, provided that the incidence of 5-ASA-related side effects remains unchanged regardless of whether the dose is set at 2 g or 4 g. However, independent of any economic considerations (VSL#3, being a probiotic, is not covered by insurance policies), we believe that the association between 5-ASA and VSL#3 should be preferred, even to a high-dose 5-ASA regimen or to the 5-ASA/immunosuppressant association for the treatment of UC patients with mild-to-moderate UC. Our opinion is based on the fact that, because the mammalian genome does not encode for all functions required for proper immunological responses, it is therefore evident that humans depend on critical interactions with their microbiome for health (34,35).

In conclusion, our study found that the addition of the high-potency probiotic mixture VSL#3 to the standard UC treatment is able to induce significant symptomatic improvement of relapsing mild-to-moderate UC compared with the placebo group on standard treatment only. This double-blind, placebo-controlled study found that VSL#3 is also able to improve the clinical picture, reduce symptoms, and improve the endoscopic appearance of the colonic mucosa. Therefore, VSL#3 may be considered as a safe and effective option for patients suffering from relapsing mild-to-moderate UC, to avoid or delay the administration of steroids, immunosuppressants, and biologics.

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CONFLICT OF INTEREST

Guarantor of the article: Antonio Tursi, MD.

Specific author contributions: Antonio Tursi conceived the study and wrote the paper. Giovanni Brandimarte, Alfredo Papa, Andrea Giglio, Walter Elisei, Gian Marco Giorgetti, Giacomo Forti, Sergio Morini, Cesare Hassan, Maria Antonietta Pistoia, Maria Ester Modeo, Stefano Rodinò, Teresa D'Amico, Ladislava Sebkova, Natale Saccà, Emilio Di Giulio, Francesco Luzza, Maria Imeneo, Tiziana Larussa, Salvatore Di Rosa, Vito Annese, Silvio Danese, and Antonio Gasbarrini conducted the study and approved the paper before submission. Walter Elisei revised the statistical analysis.

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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Patients with ulcerative colitis (UC) may relapse even when under treatment.
- ✓ If UC is already being treated with mesalazine and/or immunosuppressants, the next therapeutic step is represented by a further course of steroids or by the use of biologics.

WHAT IS NEW HERE

- ✓ VSL#3 probiotic mixture seems to effect a significant improvement in the clinical picture of patients with relapsing UC.
- ✓ VSL#3 also seems to improve several other parameters, e.g., remission.
- ✓ VSL#3 may be a useful tool in the treatment of relapsing UC in patients already under treatment with mesalazine and/or immunosuppressants, because humans depend on critical interactions with their microbiome for health.

REFERENCES

1. Schwartz M, Cohen R. Optimizing conventional therapy for inflammatory bowel disease. Curr Gastroenterol Rep 2008;10:585–90.
2. Travis S, Stange EF, Lémann M *et al.* For the European Crohn's and Colitis Organisation (ECCO). European evidence-based consensus of the management of ulcerative colitis: current management. J Crohn Colitis 2008;2:24–62.
3. Gionchetti P, Rizzello F, Venturi A *et al.* Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. Gastroenterology 2000;119:305–9.
4. Gionchetti P, Rizzello F, Helwig U *et al.* Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. Gastroenterology 2003;124:1202–9.
5. Tursi A, Brandimarte G, Giorgetti GM *et al.* Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. Med Sci Monit 2004;10:PI126–31.
6. Bibiloni R, Fedorak RN, Tannock GW *et al.* VSL#3 probiotic-mixture induces remission in patients with active ulcerative colitis. Am J Gastroenterol 2005;100:1539–46.
7. Mallon P, McKay D, Kirk S *et al.* Probiotics for induction of remission in ulcerative colitis. Cochrane Database Syst Rev 2007;17:CD005573.
8. Moser G. How often do patients with IBD have symptom recurrence? Inflamm Bowel Dis 2008;14 (Suppl 2): S47.
9. Rizzello F, Gionchetti P, D'Arienzo A *et al.* Oral beclometasone dipropionate in the treatment of active ulcerative colitis: a double-blind placebo-controlled study. Aliment Pharmacol Ther 2002;16:1109–16.
10. Kornbluth A, Sachar DB, Practice Parameters Committee of the American College of Gastroenterology. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. Am J Gastroenterol 2004;99:1371–85.
11. Pardi DS, D'Haens G, Shen B *et al.* Clinical guidelines for the management of pouchitis. Inflamm Bowel Dis 2009;15:1424–31.
12. Mennigen R, Nolte K, Rijcken E *et al.* Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. Am J Physiol Gastrointest Liver Physiol 2009;296:G1140–9.
13. Reiff C, Delday M, Rucklidge G *et al.* Balancing inflammatory, lipid, and xenobiotic signaling pathways by VSL#3, a biotherapeutic agent, in the treatment of inflammatory bowel disease. Inflamm Bowel Dis 2009;15:1721–36.
14. Kruis W, Schütz E, Fric P *et al.* Double-blind comparison of an oral *Escherichia coli* preparation and mesalazine in maintaining remission of ulcerative colitis. Aliment Pharmacol Ther 1997;11:853–8.
15. Rembacken BJ, Snelling AM, Hawkey PM *et al.* Non-pathogenic *Escherichia coli* versus mesalazine for the treatment of ulcerative colitis: a randomised trial. Lancet 1999;354:635–9.
16. Kruis W, Fric P, Pokrotnieks J *et al.* Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. Gut 2004;53:1617–23.
17. Guslandi M, Giollo P, Testoni PA. A pilot trial of *Saccharomyces boulardii* in ulcerative colitis. Eur J Gastroenterol Hepatol 2003;15:697–8.
18. Riquelme AJ, Calvo MA, Guzmán AM *et al. Saccharomyces cerevisiae* fungemia after *Saccharomyces boulardii* treatment in immunocompromised patients. J Clin Gastroenterol 2003;36:41–3.
19. Famularo G, Trinchieri V, De Simone C. Inflammatory bowel disease. N Engl J Med 2002;347:1982–4.
20. Venturi A, Gionchetti P, Rizzello F *et al.* Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. Aliment Pharmacol Ther 1999;13:1103–8.
21. Huynh HQ, deBruyn J, Guan L *et al.* Probiotic preparation VSL#3 induces remission in children with mild to moderate acute ulcerative colitis: a pilot study. Inflamm Bowel Dis 2009;15:760–8.
22. Miele E, Pascarella F, Giannetti E *et al.* Effect of a probiotic preparation (VSL#3) on induction and maintenance of remission in children with ulcerative colitis. Am J Gastroenterol 2009;104:437–43.
23. Sood A, Midha V, Makharia GK *et al.* The probiotic preparation, VSL#3, induces remission in patients with mild-to-moderately active ulcerative colitis. Clin Gastroenterol Hepatol 2009;7:1202–9.
24. Garud S, Brown A, Cheifetz A *et al.* Meta-analysis of the placebo response in ulcerative colitis. Dig Dis Sci 2008;53:875–91.
25. Ansari R, Attari F, Razjouyan H *et al.* Ulcerative colitis and irritable bowel syndrome: relationships with quality of life. Eur J Gastroenterol Hepatol 2008;20:46–50.
26. Hanauer SB. Review article: high-dose aminosalicylates to induce and maintain remissions in ulcerative colitis. Aliment Pharmacol Ther 2006;24 (Suppl 3): 37–40.
27. Madsen K. Probiotics in critically ill patients. J Clin Gastroenterol 2008;42 (Suppl 3, Pt 1): S116–8.
28. Wallace JL, Vergnolle N, Muscará MN *et al.* Enhanced anti-inflammatory effects of a nitric oxide-releasing derivative of mesalamine in rats. Gastroenterology 1999;117:557–66.
29. Greenfield SM, Punchard NA, Teare JP *et al.* Review article: the mode of action of the aminosalicylates in inflammatory bowel disease. Aliment Pharmacol Ther 1993;7:369–83.
30. Schlee M, Harder J, Koten B *et al.* Probiotic lactobacilli and VSL#3 induce enterocyte beta-defensin 2. Clin Exp Immunol 2008;151:528–35.
31. Madsen K, Cornish A, Soper P *et al.* Probiotic bacteria enhance murine and human intestinal epithelial barrier function. Gastroenterology 2001;121:580–91.
32. Caballero-Franco C, Keller K, De Simone C *et al.* The VSL#3 probiotic formula induces mucin gene expression and secretion in colonic epithelial cells. Am J Physiol Gastrointest Liver Physiol 2007;292:G315–22.
33. Clayton TA, Baker D, Lindon JC *et al.* Pharmacometabonomic identification of a significant host-microbiome metabolic interaction affecting human drug metabolism. Proc Natl Acad Sci USA 2009;106:14728–33.
34. Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 2008;453:620–5.
35. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009;9:313–23.

Aliment Pharmacol Ther 2003; 17: 1153–1162. doi: 10.1046/j.0269-2813.2003.01558.x

Combined scintigraphic and pharmacokinetic investigation of enteric-coated mesalazine micropellets in healthy subjects

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SUMMARY

Background: There is a growing clinical trend to increase the daily dose of mesalazine, which leads to significant compliance issues associated with multiple dosings of current preparations.
Aim: To examine the gastrointestinal performance and systemic exposure of a 1.5 g sachet (micropellets) mesalazine formulation, compared with three enteric-coated tablets (500 mg each, Claversal).
Methods: A randomized, two-way, cross-over pharmacoscintigraphic (scintigraphy plus pharmacokinetics) study and a two-way, cross-over, pharmacokinetic-only study were performed in 24 healthy volunteers (12 subjects per investigation).
Results: The relative bioavailability of mesalazine was 92% comparing micropellets with Claversal tablets, and the cumulative urine excretion was *c.* 26% for both preparations, suggesting comparable systemic exposure for the two types of preparation. In the majority of subjects, drug release from the micropellet formulation occurred predominantly in the terminal ileum and ascending colon. The Claversal tablets disintegrated in comparable intestinal sites, albeit at slightly later time points than the micropellets, principally due to slower gastric emptying for the single-unit formulation.
Conclusion: The 1.5 g micropellet formulation offers comparable delivery properties to the marketed tablets, but with greater convenience of dosing.

INTRODUCTION

Mesalazine (5-aminosalicylic acid, 5-ASA) is commonly used in different formulations for the treatment of mild to moderate acute exacerbations of Crohn's disease and ulcerative colitis. The analysis of mesalazine in body fluids, its pharmacokinetics, metabolism and mode of action have been studied extensively.[1–3] However, the pathogenesis of inflammatory bowel disease, and thus the precise mechanism of action of mesalazine in subjects, is not fully understood.[4–15] It has been shown that the intra-individual variability in the therapeutic effectiveness of 5-ASA is directly correlated with the actual measured 5-ASA concentration in the target tissue.[16, 17] Therefore, the achievement of sufficiently high therapeutic drug doses at the affected regions of inflammation is an important determinant of topical efficacy in inflammatory bowel disease. The systemic availability of 5-ASA, however, is relevant to the assessment of tolerability and safety. In addition, it is an indirect measure of the systemic uptake of galenic formulations by mirroring the amount of mesalazine that has been in contact with the gut mucosa.[3]

Plasma concentrations of both the parent drug and the major metabolite, *N*-acetyl-5-ASA (N-Ac-5-ASA), are relatively high if the drug is released in the duodenum and proximal parts of the small intestine. 5-ASA absorption decreases progressively along the gastrointestinal tract.[18] After the oral administration of unmodified formulations, mesalazine is rapidly and almost

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completely absorbed from the small bowel,[19, 20] thereby preventing high and therapeutically effective lumen concentrations in typically disease-affected regions, such as the distal small intestine and colon. Various different forms of 5-ASA have been developed to combat this problem, allowing a delayed or, even better, a targeted release of the agent for the oral therapy of ulcerative colitis and Crohn's disease.[21]

To achieve the targeted delivery of orally administered 5-ASA preparations to the terminal ileum and the colon, several enteric acrylic resin coatings have been developed that dissolve at a range of pH values. Previous scintigraphic imaging has confirmed that disintegration generally takes place in the terminal ileum and ascending colon for such products.[21–23] For several 5-ASA preparations, a positive linearity between dose and therapeutic efficacy is well known, with a similar tolerability profile compared to that of placebo. As 5-ASA is, in general, well tolerated, and the incidence of observed adverse events does not appear to be dose related,[17] even at therapeutic levels of up to 4 g/day,[15] further efforts should be undertaken to improve the quality of life for patients.

Most patients with inflammatory bowel disease have to ingest high doses of 5-ASA, as remission maintenance therapy, for several years. Recent treatment guidelines recommend higher daily dosages of mesalazine (up to 4.8 g/day), making it difficult for many patients to comply with the recommended daily drug intake using currently available formulations.[24] Therefore, it is essential to offer patients a preparation in the future that combines all the benefits of the well-established oral treatment with the advantages of a less frequent and more comfortable-to-swallow preparation. An additional rationale for a new 5-ASA micropellet formulation is to enhance patient compliance, as high-dose pellet units are easier and more comfortable to take.

A product containing enteric-coated micropellets consisting of 1.5 g 5-ASA (i.e. equivalent to three 500 mg tablets) has been developed. The 1.5 g mesalazine micropellets can be used for those indications approved for Claversal tablets and are in line with current guidelines for the treatment of inflammatory bowel disease. Using the combined approach of pharmacoscintigraphy and pharmacokinetics, the objective of the present study was to determine the gastrointestinal transit profile of the new micropellet 5-ASA formulation (1.5 g mesalazine) with regard to *in vivo* drug delivery.

MATERIALS AND METHODS

Study design

The clinical study protocol was approved by an independent ethics committee (Quorn Research Review Committee, Nottingham, UK) and all applicable regulatory requirements, including the Administration of Radioactive Substances Advisory Committee requirements. The analytical parts of the study were performed in accordance with German and Organization for Economic Co-operation and Development guidelines of Good Laboratory Practice.

The aim of the study was to investigate the gastrointestinal transit and release properties of 1.5 g 5-ASA micropellets (Merckle GmbH, Germany) vs. 3 × 5-ASA tablets (Claversal 500 mg, Merckle GmbH, Germany) using pharmacoscintigraphic and pharmacokinetic analysis. The study was conducted as a randomized, two-way, cross-over, pharmacoscintigraphic (scintigraphic plus pharmacokinetic assessment) investigation and a two-way, cross-over, pharmacokinetic-only investigation in a target population of 24 healthy male and female subjects (12 subjects in each investigation). Subjects were allocated to the pharmacoscintigraphic or pharmacokinetic-only group following the sequence of enrolment.

Each volunteer allocated to the pharmacoscintigraphic group was scheduled to receive, orally, one sachet of radiolabelled 1.5 g 5-ASA micropellets on one occasion and three radiolabelled Claversal 500 mg tablets in the subsequent dosing period. Each volunteer allocated to the pharmacokinetic-only group was scheduled to receive, orally, one sachet of unlabelled 1.5 g mesalazine (5-ASA) micropellets on one occasion and three unlabelled Claversal 500 mg tablets in the subsequent dosing period.

Radiolabelling of dosage forms

Radiolabelled mesalazine micropellets were prepared by the inclusion of samarium oxide as excipient into the dosage form. Prior to administration, the non-radioactive ^{152}Sm was converted by neutron activation into the gamma-emitting radionuclide ^{153}Sm. Identical drug dissolution characteristics for the clinically used formulation and the samarium oxide-containing study preparation were verified by a validated BIO-DIS *in vitro* dissolution method. The micropellets were irradiated for 9 min in a neutron flux of 10^{12} cm^{-2}/s, 72 h prior to

dosing, and *in vitro* testing demonstrated that the neutron activation process did not adversely affect the performance of the dosage form or the stability of the drug. At the time of dosing, the sachet of mesalazine micropellets was radiolabelled with 1 MBq of ^{153}Sm.

The Claversal tablets were radiolabelled by the insertion of 5 mg ^{153}Sm oxide into each tablet through a drilled microhole, which was then sealed with cyanoacrylate. Irradiation of ^{152}Sm was carried out 24 h before tablet administration to the volunteers on the dosing day. At the time of dosing, each Claversal tablet contained 0.33 MBq of ^{153}Sm. Dissolution testing showed that the drill and fill procedure did not alter the release properties of the product.

Study procedures

Subjects were required to fast from midnight on the day prior to dosing and to remain fasting until 4 h post-dose, at which time a light lunch was provided. An evening meal was provided at approximately 9 h post-dose. On subsequent days, meals were provided at 24 h (breakfast), 28 h (lunch) and 33 h (dinner) post-dose. Each subject drank 200 mL of water at 2 h post-dose and decaffeinated fluids were permitted *ad libitum* after lunch.

For the 12 subjects who received the radiolabelled formulations, scintigraphic images were acquired at approximately 10-min intervals up to 12 h post-dose, and then at 20-min intervals until 16 h post-dose. Thereafter, images were obtained at 18, 20, 24, 36 and 48 h post-dose. For anatomical referencing, markers containing 0.1 MBq ^{99m}Tc were taped on to the skin, where the mid-clavicular line met the right costal margin, so that they lay in approximately the same transverse plane as the pylorus. Images were recorded using a gamma camera (General Electric Maxicamera) with a 40-cm field of view and fitted with a low-energy parallel-hole collimator. The volunteers remained moderately active during the study period and all images were acquired with the subjects standing upright in front of the gamma camera. On completion of the study, the scintigraphic images were analysed to obtain the parameters detailed in Table 1.

For all subjects participating in the study, venous blood samples (8 mL) were withdrawn via an in-dwelling cannula or by venepuncture at 0 (pre-dose) 1, 2, 3, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 36 and 48 h post-dose. The samples were centrifuged at approximately $1600 \times \boldsymbol{g}$ for 10 min at 4 °C. The resulting plasma fraction was frozen in labelled polypropylene tubes at − 20 °C until the end of each study period, and then at − 80 °C until required for assay. All subjects also collected all urine passed throughout the 48-h study period, according to the following sampling periods: 0 (pre-dose), 0–4, 4–8, 8–12, 12–16, 16–24, 24–36 and

Table 1. Scintigraphic variables for the two radiolabelled formulations

Variable	Micropellets	Tablets
Time post-dose of gastric emptying	Time when more than 50% (or 90%) of the radiolabel has left the stomach ($T_{50\%}$ or $T_{90\%}$)	Average between the time points for the two images between the transition
Time post-dose of arrival at the colon	Time when more than 50% (or 90%) of the radiolabel has reached the colon ($T_{50\%}$ or $T_{90\%}$)	Average between the time points for the two images between the transition
Duration of small intestinal transit	Difference in time between colon arrival and gastric emptying	Difference in time between colon arrival and gastric emptying
Anatomical location and time post-dose of initial tablet disintegration	Not scheduled	Time (post-dose) and anatomical location of radioactive marker release
Anatomical location and time post-dose of complete tablet disintegration	Not scheduled	Time (post-dose) and anatomical location when all of the radiolabelled marker has dispersed and no signs of a distinct 'core' remain

36–48 h post-dose. The total volume of urine collected for each time period was measured, and two 10 mL aliquots were taken and frozen in labelled polypropylene tubes at − 20 °C until the end of each study period, and then at − 80 °C until required for assay.

Plasma samples were analysed by high performance liquid chromatography with fluorescence detection. Frozen samples were thawed and plasma proteins were precipitated with perchloric acid. After centrifugation, the supernatant was neutralized with sodium hydroxide and an aliquot was subjected to high performance liquid chromatographic analysis. Both 5-ASA and N-Ac-5-ASA were quantified by external standardization in the established calibration range between 10 and 2500 ng/mL. Urine samples were mixed with four volumes of 50 mM ammonium acetate prior to high performance liquid chromatographic analysis. The method allowed the determination of 5-ASA and N-Ac-5-ASA in the concentration range between 0.5 and 100 μg/mL. Both methods were validated in accordance with internationally accepted recommendations.[25]

Plasma concentrations were used to determine the pharmacokinetic parameters C_{max} (ng/mL), T_{max} (h), AUC_{0-t} (ng.h/mL), $T_{1/2}$ (h) and $AUC_{0-\infty}$ (ng.h/mL). C_{max} and T_{max} values were directly taken from the plasma concentration–time profiles and AUC_{0-t} values were calculated by application of the linear trapezoidal rule. The elimination rate λ (1/h) was derived by log-linear regression analysis of the last 3–5 concentration/time point data pairs. $T_{1/2}$ (h) was calculated by ln $2/\lambda$ and $AUC_{0-\infty}$ by $AUC_{0-t} + C_{last}/\lambda$. C_{last} corresponds to the last quantifiable plasma concentration. Pharmacokinetic evaluation was performed employing the non-compartment analysis tool of WinNonlin Pro 3.0 (Pharsight Corporation). The cumulative urine excretion of 5-ASA and N-Ac-5-ASA was determined from the measured concentrations and documented urine volumes.

For statistical analysis, the computer program BIOQ V2.10 was used. With the inclusion of $n = 24$ subjects, the equivalence test had a power of 80%, when equivalence limits were set to 0.71 and 1.40. In order to achieve a better approximation to a normal distribution, AUC_{0-t} and C_{max}, as well as the excretion value *Ae*, were logarithmically transformed before analysis and tested parametrically by an analysis of variance (ANOVA). ANOVA was calculated by splitting the total variance into the components of subject and treatment. The component subject was further split into sequence and subject within a sequence.

From the result of this procedure, the two one-sided hypotheses at the $\alpha = 0.05$ level of significance were tested; 90% confidence intervals of two one-sided *t*-tests were calculated by re-transformation of the shortest confidence interval for the difference of the $\log_{10}$ transformed values. ANOVA and the determination of the confidence intervals were applied to AUC_{0-t}, C_{max} and *Ae*.

RESULTS

Pharmacokinetic evaluation

The mean pharmacokinetic parameters for 5-ASA and N-Ac-5-ASA after the single administration of mesalazine micropellets or Claversal tablets, both at a dose of 1500 mg 5-ASA, are summarized in Table 2. A relative

Table 2. Pharmacokinetic parameters of 5-aminosalicylic acid (5-ASA) and *N*-acetyl-5-aminosalicylic acid (N-Ac-5-ASA) for two mesalazine formulations with an administered dose of 1500 mg (mean ± s.d.; $n = 24$)

	5-ASA		N-Ac-5-ASA	
	Micropellets	Claversal tablets	Micropellets	Claversal tablets
C_{max} (ng/mL)	2026 ± 1508	1591 ± 1265	2965 ± 1616	2532 ± 1309
T_{max} (h)	5.4 ± 1.2	8.3 ± 1.6	5.9 ± 1.5	9.4 ± 1.9
AUC_{0-t} (ng.h/mL)	9865 ± 5692	10383 ± 3881	33179 ± 8352	32479 ± 6759
$AUC_{0-\infty}$ (ng.h/mL)	10798 ± 5651	11418 ± 4498	*	39810 ± 9330
$T_{1/2}$ (h)	19.5 ± 13.2	13.7 ± 11.4	*	20.4 ± 15.3
Ae (μmol)	121.9 ± 178.1	116.1 ± 149.7	2476 ± 661	2495 ± 577
(%)†	1.24	1.19	25.3	25.5

* Owing to difficulties in determining the elimination rate constant λ in a number of subjects, the descriptive statistics of the λ-derived parameters, $T_{1/2}$ and extrapolated $AUC_{0-\infty}$, are not reported.
† Percentage of 1500 mg administered dose of mesalazine (9803.92 μmol).

bioavailability of 92% for mesalazine micropellets vs. Claversal tablets was found. The AUC_{0-t} values of the micropellets and tablets were within the set equivalence limits for point estimates and 90% confidence intervals (Table 3).

Plasma concentrations

The AUC_{0-t} values for both 5-ASA and N-Ac-5-ASA did not differ significantly between micropellets and tablets. Overall, the concentration of 5-ASA in plasma from both preparations was low, as expected, whereas the plasma level of the metabolite N-Ac-5-ASA was three times higher due to the known, primarily presystemic, 5-ASA metabolization in the gut wall and the different systemic absorption and first-pass performance in the liver when compared with the parent drug (Table 2).

The plasma concentrations of 5-ASA and N-Ac-5-ASA after ingestion of the micropellet formulation increased earlier and showed an earlier peak than the curves for the tablet (Figure 1). After administration, the first time point with quantifiable 5-ASA concentrations varied between 1 and 4 h. The absorption profile of the metabolite N-Ac-5-ASA was even faster compared with the parent drug 5-ASA. However, there was considerable inter-individual variability in the absorption of the parent drug and metabolite for both formulations.

The 5-ASA micropellets produced a bi-phasic absorption profile, with a slow initial drug absorption phase followed by a rapid increase in plasma concentration. For the Claversal tablets, the initial slow phase of drug absorption was not observed. After a prolonged lag time, the plasma concentrations increased with inter-individual variability — more gradually in some subjects and more quickly in others. The mean plasma concentration–time curves (± S.E.M.) for 5-ASA and N-Ac-5-ASA are provided in Figure 1.

After the administration of 5-ASA micropellets, the maximum concentrations varied between 416 and 5893 ng/mL for 5-ASA and between 1249 and 6644 ng/mL for N-Ac-5-ASA [for individual values (scintigraphic plus pharmacokinetic assessment group only), see Table 4]. A similar variability was observed for Claversal tablets, with maximum plasma concentration ranges of 386–5522 ng/mL (5-ASA) and 1174–7011 ng/mL (N-Ac-5-ASA).

Table 3. Point estimates and 90% confidence intervals of pharmacokinetic parameters for the two mesalazine formulations (test = mesalazine micropellets; reference = Claversal 500 mg tablets)

Parameter	Compound	Point estimate	90% confidence interval
AUC_{0-t}	5-ASA	0.92	0.770–1.107
	N-Ac-5-ASA	1.01	0.929–1.104
C_{max}	5-ASA	1.24	0.867–1.782
	N-Ac-5-ASA	1.13	0.889–1.425
Ae	5-ASA	0.99	0.559–1.743
	N-Ac-5-ASA	0.98	0.853–1.134

5-ASA, 5-aminosalicylic acid; N-Ac-5-ASA, *N*-acetyl-5-aminosalicylic acid.

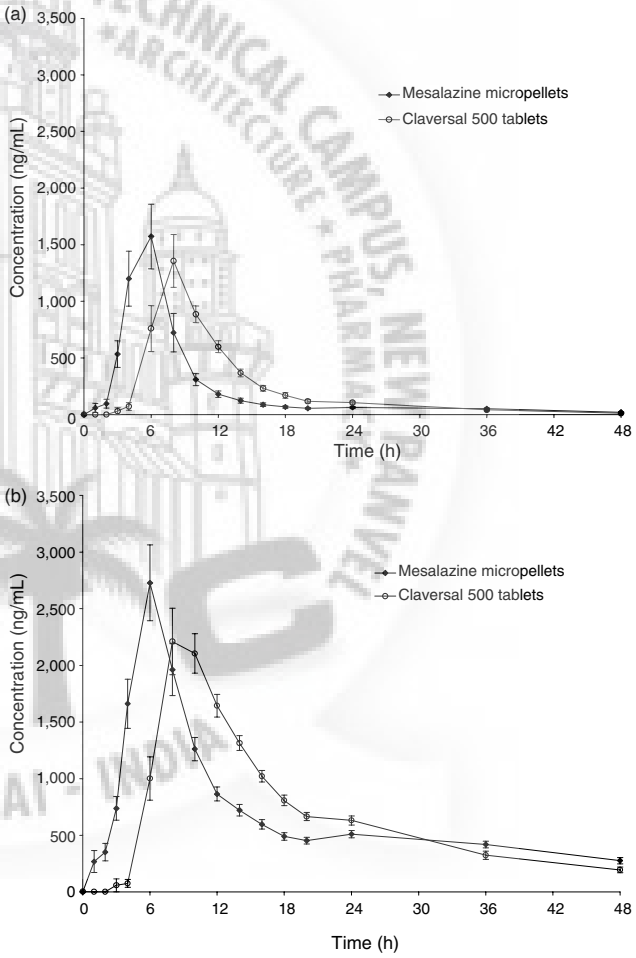

Figure 1. Mean concentration vs. time profiles of 5-aminosalicylic acid (5-ASA) and *N*-acetyl-5-aminosalicylic acid (N-Ac-5-ASA). Data presented as mean ± S.E.M. following cross-over administration of single doses of 1.5 g mesalazine as either mesalazine micropellets (total $n = 24$) or as Claversal tablets ($n = 24$). Top panel: 5-ASA; bottom panel: N-Ac-5-ASA.

Table 4. Correlation between pharmacokinetic parameters and transit characteristics for the micropellet formulation

Subject	5-ASA			N-Ac-5-ASA			Location of micropellets at T_{max}
	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng.h/mL)	C_{max} (ng/mL)	T_{max} (h)	AUC_{0-t} (ng.h/mL)	
1	622	8.0	6610	1347	8.0	26188	AC/TC
2	5893	6.0	29407	6644	6.0	53404	AC/TC
3	1503	4.0	6735	2331	4.0	36699	DSB/ICJ/AC
4	2243	4.0	10338	2817	4.0	34694	DSB/AC
5	1393	4.0	8628	1776	4.0	27380	DSB/AC
6	416	8.0	6117	1424	10.0	28131	AC/TC
7	2211	6.0	10058	3431	6.0	34758	DSB/ICJ/AC
8	805	6.0	5382	1718	6.0	21433	AC
9	1914	6.0	8500	2775	6.0	30126	DSB/ICJ/AC
10	1707	4.0	7000	1835	4.0	27481	DSB
11	893	6.0	7245	1477	6.0	24846	DSB/ICJ/AC/TC
12	917	4.0	4706	2016	4.0	30924	ICJ/AC/TC

5-ASA, 5-aminosalicylic acid; N-Ac-5-ASA, *N*-acetyl-5-aminosalicylic acid; AC, ascending colon; DSB, distal small bowel; ICJ, ileo-caecal junction; TC, transverse colon.

Urine concentrations and cumulative excretion

The drug was primarily excreted as N-Ac-5-ASA. Within 48 h, a mean cumulative drug excretion of 26.5% (5-ASA micropellets) and 26.6% (5-ASA tablets) was determined.

For both formulations, high inter-individual variability was observed, but for the parent drug the values were generally low.

Renal excretion of 5-ASA was limited to 16 h after dosing. In all samples collected after the end of the 16-h collection period, the contribution of 5-ASA to urinary drug excretion was negligible. In contrast, individual cumulative excretion–time profiles for N-Ac-5-ASA indicated that urine excretion was not complete after 48 h. The mean urine excretion profiles are shown in Figure 2.

Scintigraphic results

Scintigraphic images were analysed for all 12 subjects in line with the parameters described in Table 1, and the results are provided in Table 5.

Gastric emptying for Claversal tablets occurred, on average, at 0.52 ± 0.36 h post-dose, compared with gastric emptying times for the micropellets of, on average, 1.28 ± 1.10 h ($T_{50\%}$) and 2.29 ± 2.52 h ($T_{90\%}$) post-dose. Mean small intestinal transit times of 3.36 ± 0.78 h were recorded for Claversal tablets. This was similar to the mean small intestinal transit ($T_{50\%}$ and $T_{90\%}$) times of 2.96 ± 1.54 and 3.72 ± 2.80 h reported for the micropellets. The Claversal tablets disintegrated in all subjects. Initial and complete disintegration occurred at 4.75 ± 1.24 h and at 6.11 ± 1.53 h post-dose, respectively. For Claversal tablets, initial disintegration could be seen in the terminal ileum. In other subjects, disintegration was observed at the ileo-caecal junction and in the ascending colon.

DISCUSSION

High intraluminal drug concentrations at the site of inflammatory lesions have been shown to be clinically important. At the present time, mesalazine products on the market differ considerably with respect to their drug release behaviour[12] and their sensitivity to pH levels in the gut.[26–28] Gamma scintigraphy has been described as an 'elegant technique for phase-I investigation of the locality of *in vivo* release',[29] and has 'become the method of choice for investigating the fate of pharmaceutical dosage [forms] in the body'.[30]

The ability to visualize the drug delivery process in a non-invasive manner acts to fill a significant void in current understanding. In this study, gamma scintigraphy was used to evaluate and compare the gastrointestinal transit profile of the mesalazine micropellet formulation with the anatomical site of disintegration of Claversal tablets. In addition, the relationship between *in vivo* drug delivery and the subsequent pharmacokinetic profile was investigated. In the present study, gamma scintigraphic analysis demonstrated that, for Claversal tablets, gastric emptying occurred well within the 120–140-min migrating myoelectric

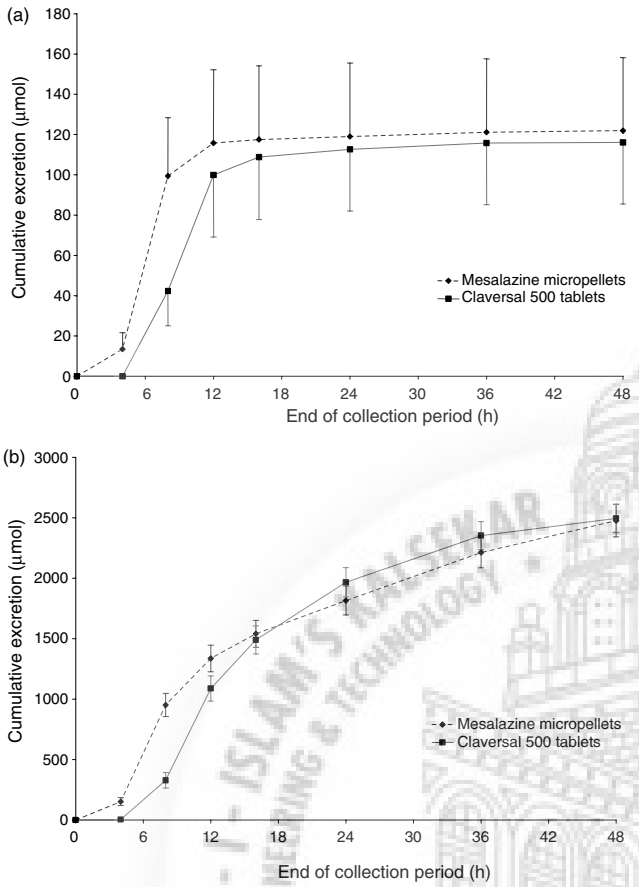

Figure 2. Mean cumulative excretion of 5-aminosalicylic acid (5-ASA) and *N*-acetyl-5-aminosalicylic acid (N-Ac-5-ASA). Data presented as mean ± S.E.M. following cross-over administration of single doses of 1.5 mg mesalazine as either mesalazine micropellets (total $n = 24$) or as Claversal tablets (total $n = 24$). Top panel: 5-ASA; bottom panel: N-Ac-5-ASA.

complex (MMC) cycle following administration in the fasted state.[31] Gastric emptying of micropellets was delayed when compared with Claversal tablets. This is due to the gradual emptying of the micropellets from the stomach, which is typical of a multi-particulate formulation. The Claversal tablets disintegrated in the terminal ileum, ileo-caecal junction and ascending colon. In previous studies, steady state concentrations of 5-ASA, observed in biopsy specimen homogenates with Claversal 500 mg tablets, correlated very well with these sites of disintegration.[32] Transit times suggest that the two dosage forms behave similarly in terms of the small intestinal residence times. There is always a high degree of intra- and inter-subject variability observed in gastrointestinal data.[31] However, for the majority of subjects dosed, the intestinal transit times were in general agreement with the 3 h (± 1 h; range, 1.3–6 h) reported previously for solutions, pellets and tablets.[33]

The combination of scintigraphic evaluation and conventional pharmacokinetic assessment to determine the intestinal performance of pharmaceutical formulations is a powerful approach to aid in the understanding of the interaction of drug, delivery system and gastrointestinal tract. This is even more important for drugs that demonstrate their efficacy as topical agents, and that are poorly absorbed from the human intestine, for which any measure of plasma concentration is critically influenced by the location of the formulation in the gut.[34]

This holds true for mesalazine, which is known to be well absorbed if released high up in the intestine, but poorly absorbed from the colon. Thus, for mesalazine, the location and integrity of the pharmaceutical product will significantly influence systemic exposure. The Committee for Proprietary Medicinal Products (CPMP) has indicated that for 'locally acting products' [pharmacokinetic] bioequivalence is generally not a suitable way to show therapeutic equivalence, as plasma levels are not relevant for local efficacy, although they may play a role with regard to safety.[35] Therefore, in the context of the current study, measurements of plasma levels and pharmacokinetics proved useful for the evaluation of safety, but provided little or no information on the *in vivo* fate of the therapeutic moiety at its target site.

Using the scintigraphic data to assess both initial and complete disintegration of the three individual Claversal tablets provides important information on the *in vivo* disposition of mesalazine. Disintegration was observed to occur whilst the preparation was in the terminal ileum, ileo-caecal junction or ascending colon. In those individuals for whom the enteric coating was observed to dissolve prior to arrival in the colon, increased absorption of mesalazine was to be expected. In summary, Claversal tablets deliver mesalazine to the distal ileal and colonic lumen, without exposing the subject to excessive drug plasma levels.

The micropellet formulation has different intestinal transit characteristics from the tablet product. For single-unit preparations, gastric emptying is essentially an all-or-nothing event, i.e. all the tablets are either in the stomach or in the small bowel. However, with multi-particulate formulations, there is a gradual gastric emptying of the drug-containing cores over an extended period of time. As a consequence, the pellets gradually arrive in an area in which the enteric coating can

Table 5. Gastrointestinal transit profiles (mean and individual values) for the two mesalazine formulations (h)

	Micropellets						Tablets*			
	Gastric emptying		Colon arrival		Small intestinal transit					
Subject	$T_{50\%}$	$T_{90\%}$	$T_{50\%}$	$T_{90\%}$	$T_{50\%}$	$T_{90\%}$	Gastric emptying	ICJ arrival	Colon arrival	Small intestinal transit
1	0.94	1.29	2.30	3.94	1.36	2.65	0.91	1.81	2.39	1.48
							0.91	1.81	2.39	1.48
							0.91	1.81	3.40	2.49
2	1.85	2.22	3.63	4.98	1.78	2.76	0.52	†	4.37	3.85
							0.52	†	4.37	3.85
							0.52	†	4.37	3.85
3	0.37	1.19	4.13	4.33	3.76	3.14	0.01	†	3.33	3.33
							0.01	†	3.33	3.33
							0.01	†	3.52	3.25
4	0.87	1.67	3.91	4.50	3.04	2.83	0.19	2.51	3.48	3.28
							0.19	2.51	3.48	3.28
							0.19	2.51	3.48	3.28
5	0.61	0.87	3.99	4.36	3.38	3.49	0.60	1.46	‡	‡
							0.60	2.03	‡	‡
							0.60	3.04	‡	‡
6	2.62	3.13	3.91	4.36	1.29	1.23	1.25	2.97	3.65	2.40
							1.25	2.97	3.65	2.40
							1.56	2.97	3.65	2.09
7	1.54	2.53	5.36	7.04	3.82	4.51	0.43	4.18	‡	‡
							0.69	4.18	‡	‡
							0.69	4.18	‡	‡
8	1.15	2.56	3.42	4.48	2.27	1.92	0.18	1.88	3.13	2.96
							0.18	1.88	3.13	2.96
							0.18	2.47	3.13	2.96
9	1.16	1.39	4.29	9.76	3.13	8.37	0.78	2.65	4.25	3.47
							0.78	3.12	4.25	3.47
							1.05	3.53	4.25	3.20
10	0.54	1.04	13.82§	43.63§	13.28§	42.59§	0.35	3.11	4.58	4.23
							0.35	3.66	4.58	4.23
							0.35	3.89	‡	‡
11	1.13	1.90	8.28	12.36	7.15	10.46	0.20	3.61	4.32	4.12
							0.68	3.61	4.32	3.63
							0.68	3.61	4.32	3.63
12	0.37	1.00	2.08	4.10	1.71	3.10	0.33	4.33	4.64	4.32
							0.33	4.33	4.64	4.32
							0.33	4.33	4.64	4.32
Mean	1.10	1.73	4.12	5.84	2.97	4.04	0.54	3.03	3.83	3.29
SD	0.66	0.73	1.65	2.77	1.67	2.82	0.37	0.90	0.66	0.79
Median	1.04	1.53	3.91	4.48	3.04	3.10	0.52	3.01	3.65	3.33
n	12	12	11	11	11	11	36	30	29	29

ICJ, ileo-caecal junction.
* Separate values for each tablet.
† The tablets moved from the small intestine to the colon in consecutive images; therefore the ICJ arrival time and ICJ residence time could not be determined.
‡ Initial and complete tablet disintegration occurred in the ICJ; therefore colon arrival and small intestinal transit times could not be determined.
§ Due to extended transit times compared with the rest of the data, these data were excluded from descriptive statistics.

dissolve. The plethora of micropellets spread evenly during transit through the gastrointestinal tract and release mesalazine gradually.

A careful review of the individual scintigraphic and pharmacokinetic relationships suggests that, in the majority of individuals, drug release occurs predominantly in the terminal ileum and ascending colon (Table 4) for the micropellet product.

CONCLUSIONS

The objective of the present study was to investigate the gastrointestinal transit and release properties of mesalazine micropellets vs. Claversal tablets using the combined approach of pharmacoscintigraphy and pharmacokinetics. The rationale of the micropellet development was to obtain an oral formulation that allows convenient higher doses and increased residence time at the target site than currently possible with the tablet product.

The overall findings suggest comparable delivery of the therapeutic moiety, mesalazine, to the target sites of likely disease for the two products. As a consequence, the study findings create confidence that, in a separate study, therapeutic equivalence for these two preparations will be shown. The availability of a substantially increased unit dose (1.5 g for the micropellets vs. 0.5 g for the tablet) would constitute a real benefit for subjects with inflammatory bowel disease.

REFERENCES

1 Schröder H, Campbell DES. Absorption, metabolism and excretion of salicylazusulfapyridine in man. Clin Pharmacol Ther 1972; 13: 539–51.
2 Brogden RN, Sorkin EM. Mesalazine (5-ASA). An overview of its clinical pharmacological properties and therapeutic potential in inflammatory bowel disease. Drugs 1989; 38: 500–23.
3 Schwab S, Klotz U. Pharmacokinetic considerations in the treatment of inflammatory bowel disease. Clin Pharmacokinet 2001; 40(10): 723–51.
4 Shanahan F. Crohn's disease. Lancet 2002; 359: 62–9.
5 Farrell RJ, Peppercorn MA. Ulcerative colitis. Lancet 2002; 359: 331–40.
6 Greenfield SM, Punchard NA, Teare JP, *et al.* The mode of action of the aminosalicylates in inflammatory bowel disease. Aliment Pharmacol Ther 1993; 7: 369–83.
7 Mutschler E, Geisslinger G, Kroemer HK, Schaefer-Korting M. Arzneimittelwirkungen, 8. Auflage. Stuttgart: Wissenschaftliche Verlagsgesellschaft, 2001: 645–7.
8 Ireland A, Jewell DP. Mechanism of action of 5-aminosalicylic acid and its derivates. Clin Sci (Colch) 1990; 78: 119–25.
9 Allgayer H, Ahnfelt NO, Kruis W, *et al.* Colonic *N*-acetylation of 5-aminosalicylic acid in inflammatory bowel disease. Gastroenterology 1989; 97: 38–41.
10 Zhou SY, Fleisher D, Pao LH, *et al.* Intestinal metabolism and transport of 5-aminosalicylate. Drug Metab Dispos 1999; 27: 479–89.
11 Christensen LA, Fallingborg J, Abildgaard K, *et al.* Topical and systemic availability of 5-aminosalicylate: comparisons of three controlled release preparations in man. Aliment Pharmacol Ther 1990; 4: 523–33.
12 De Vos M, Verdievel H, Schoonjans R, *et al.* Concentrations of 5-ASA and Ac-5-ASA in human ileocolonic biopsy homogenates after oral 5-ASA preparations. Gut 1992; 33: 1338–42.
13 Klotz U, Seyffer R, Allgayer H, Maier KE. Pharmacokinetic properties of mesalazine (5-aminosalicylic acid). In: MacDermott RP, ed. Inflammatory Bowel Disease: Current Status and Future Approach. Amsterdam: Elsevier Science Publishers B.V. (Biomedical Division), 1988: 725–9.
14 Tromm A, Griga T, May B. Oral mesalazine for the treatment of Crohn's disease: clinical efficacy with respect to pharmacokinetic properties. Hepatogastroenterology 1999; 46: 3124–35.
15 Clemett D, Markham A. Prolonged-release mesalazine. A review of its therapeutic potential in ulcerative colitis and Crohn's disease. Drugs 2000; 59: 929–56.
16 Frieri G, Pimpo MT, Andreoli A, *et al.* Prevention of postoperative recurrence of Crohn's disease requires adequate mucosal concentration of mesalazine. Aliment Pharmacol Ther 1999; 13: 577–82.
17 Frieri G, Giacomelli R, Pimpo M, Palumbo G, Passacantando A, Caprilli R. Mucosal 5-aminosalicylic acid concentration inversely correlates with severity of colonic inflammation in patients with ulcerative colitis. Gut 2000; 47: 410–4.
18 Layer PH, Goebell H, Keller J, Gignass A, Klotz U. Delivery and fate of oral mesalamine mircogranules within the human small intestine. Gastroenterology 1995; 108: 1427–33.
19 Bondesen S, Bronnum-Schon J, Pedersen V, Rafiolsadat Z, Honore Hansen S, Huidberg EF. Absorption of 5-aminosalicylic acid from colon and rectum. Br J Pharmacol 1988; 25: 269–72.
20 Myers B, Evans DNW, Rhodes J, *et al.* Metabolism and urinary excretion of 5-aminosalicylic acid in healthy volunteers when given intravenously or released for absorption at different sites in the gastrointestinal tract. Gut 1987; 28(2): 196–200.
21 Gisbert JP, Gomollon F, Maté J, Pajares JM. Role of 5-aminosalicylic acid in treatment of inflammatory bowel disease: a systematic review. Digestive Dis Sci 2002; 3: 471–88.
22 Hardy JG, Healey JNC, Reynolds JR. Evaluations of an enteric coated delayed release 5-ASA tablet in patients with inflammatory bowel disease. Aliment Pharmacol Ther 1987; 1: 273–89.
23 Dew MJ, Ebden P, Kidwai NS, *et al.* Comparison of the absorption and metabolism of sulphasalazine and acrylic-

coated 5-aminosalicylic acid in normal subjects and patients with colitis. Br J Clin Pharmacol 1984; 17: 474–6.
24 Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med 1987; 317: 1625–9.
25 Shah VP, Midha KK, Dighe S, *et al.* Analytical methods validation: bioavailability, bioequivalence and pharmacokinetic studies. J Pharm Sci 1992; 81(3): 309–12.
26 Evans DF, Pye G, Bramley R, Clarke AG, Dyson TJ, Hardcastle JB. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. Gut 1988; 29: 1035–41.
27 Fallingborg J, Christensen LA, Ingeman-Nielsen M, *et al.* pH-Profile and regional transit times of the normal gut measured by a radiotelemetry device. Aliment Pharmacol Ther 1989; 3: 605–13.
28 Leopold CS. Coated dosage forms for colon-specific drug delivery. Pharmaceut Sci Technol Today 1999; 2: 197–204.
29 Nick C. Formulations on trial. Good Clinical Practice 1996; 3(5): 20–6.
30 Meseguer G, Gurny R, Buri P. In vivo behaviour of dosage forms: application of gamma scintigraphy to non-enteral routes of administration. J Drug Targeting 1994; 2: 269–88.
31 Coupe AJ, Davis SS, Wilding IR. Variation in gastrointestinal transit of pharmaceutical dosage forms in healthy subjects. Pharm Res 1991; 8: 360–4.
32 De Vos M, Verdievel HR, Schoonjans Praet M, Bogaert M, Barbier F. Concentrations of 5-ASA in human ileocolonic biopsy homogenates after oral 5-ASA preparations. Gut 1992; 33: 1338–42.
33 Davis SS, Hardy JG, Fara JW. Transit of pharmaceutical dosage forms through the small intestine. Gut 1986; 27: 886–92.
34 Wilding IR. Bioequivalence testing for locally acting gastrointestinal products: what role for gamma scintigraphy? J Clin Pharm 2002; 42: 1200–10.
35 The European Agency for Evaluation of Medical Products. CPMP/ENP/239/95 Final Note for Guidance on the Clinical Requirements for Locally Applied, Locally Acting Products Containing Known Constituents. London: Human Medicine Evaluation Unit, in operation from June 1996.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Colon specific drug delivery of mesalamine using eudragit S100-coated chitosan microspheres for the treatment of ulcerative colitis

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ABSTRACT

The purpose of the present study was to prepare, characterize and evaluate the colon-targeted microspheres of mesalamine for the treatment and management of ulcerative colitis (UC). Microspheres were prepared by the ionic-gelation emulsification method using tripolyphosphate (TPP) as cross linking agent. The microspheres were coated with Eudragit S-100 by the solvent evaporation technique to prevent drug release in the stomach. The prepared microspheres were evaluated for surface morphology, entrapment efficiency, drug loading, micromeritic properties and in-vitro drug release. The microspheres formed had rough surface as observed in scanning electron microscopy. The entrapment efficiency of microspheres ranged from 43.72%-82.27%, drug loading from 20.28%-33.26%. The size of the prepared microspheres ranged between 61.22-90.41µm which was found to increase with increase in polymer concentration. All values are statistically significant as $p<0.05$. Micromeritic properties showed good flow properties and packability of prepared microspheres. The drug release of mesalamine from microspheres was found to decrease as the polymer concentration increases. The release profile of mesalamine from eudragit-coated chitosan microspheres was found to be pH dependent. It was observed that Eudragit S100 coated chitosan microspheres gave no release in the simulated gastric fluid, negligible release in the simulated intestinal fluid and maximum release in the colonic environment. It was concluded from the study that Eudragit-coated chitosan microspheres were promising carriers for colon-targeted delivery of Mesalamine.

***Key Words*:** Ionic-gelation emulsification method, cross-linking, drug release, delivery, particle size, pH dependent.

INTRODUCTION

Pharmaceutical invention and research are increasingly focusing on delivery systems which enhance desirable therapeutic objectives while minimizing side effects. Oral drug delivery system represents one of the frontier areas of drug delivery systems. Such a dosage form manages common concern which exists in area of cost-efficient treatment, patient compliance, optimum drug delivery and bioavailability (Kumar *et al.*, 2012). The last two decades there has been a remarkable improvement in the field of novel drug delivery systems. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc, which modulates the release and absorption characteristics of the drug (Dehghan *et al.*, 2010).

Microspheres constitute an important part of this particulate drug delivery system by virtue of their small size and efficient carrier characteristics. However, the success of this novel drug delivery system is limited due to their short residence time at the site of absorption. It would therefore be advantageous to have means for providing an intimate contact of the drug delivery system with absorbing gastric mucosal membranes (Lohani and Gangwar, 2012). Microspheres are characteristically free powders consisting of proteins or synthetic polymers that are biodegradable in nature and ideally having a particle size less than 200µm (Alagusundaram *et al.*, 2009).

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Colon specific drug delivery systems have gained increasing attention for the treatment of diseases such as Chrohn's disease, ulcerative colitis and irritable bowel syndrome (Patel *et al.*, 2010). Ulcerative colitis is a type of inflammatory bowel disease (IBD) that affects the lining of the large intestine (colon) and rectum. Repeated swelling (inflammation) leads to thickening of the intestinal wall and rectum with scar tissue. Death of colon tissue or severe infection (sepsis) may occur with severe disease (Burger and Travis, 2011). Mesalamine (5-ASA) is an anti-inflammatory drug used to treat crohn's disease and ulcerative colitis. Since Mesalamine (5-ASA) is largely absorbed from the upper intestine, selective delivery of drugs into the colon may be regarded as a better method of drug delivery with fewer side effects and a higher efficacy (Swapna *et al.*, 2011).

In the present study, an attempt has been made to prepare mesalamine microspheres prepared by ionotropic gelation method using chitosan as polymer and sodium tripolyphosphate (TPP) as the cross-linking agent. Chitosan is a biodegradable natural polymer with great potential for pharmaceutical applications owing to its biocompatibility, non-toxicity and mucoadhesive properties. TPP is an extensively researched well established, charged, non-toxic, multivalent, anionic cross-linking agent with five bonding sites on the molecules.

MATERIALS AND METHODS

Materials

Mesalamine was obtained from Zydus Cadila, Ahmedabad, India. Chitosan was a gift sample from Central Institute of Fisheries Technology, Cochin. Eudragit S100 was obtained from Ranbaxy Laboratories Limited, New Delhi, India. TPP was purchased from Loba Chemicals. All other chemicals used in experiment were of analytical grade and used as such.

Preparation of microspheres

Cross linked chitosan microspheres were prepared using ionic-gelation emulsion method. Chitosan solution (4% w/v) was prepared in 5% aqueous acetic acid solution in which the drug was previously dissolved and dispersed in liquid paraffin containing span 80 (1%w/v) (Gawde and Agrawal, 2012). The dispersion was stirred using a specially fabricated stainless steel half-moon paddle stirrer and saturated aqueous solution of TPP (1 ml to 3 ml), a cross-linking agent was added with continuous stirring. The stirring was continued for 4 h, prepared microspheres were centrifuged, washed twice with hexane to remove oily phase from the solution and acetone and were then dried in vacuum desiccators for 48 hrs.

Coating of chitosan microspheres

Chitosan microspheres were coated with Eudragit S-100 solvent evaporation method (Vasir *et al.*, 2003). Chitosan microspheres (50 mg) were dispersed in 10 ml of coating solution prepared by dissolution of 500 mg of Eudragit S-100 in ethanol: acetone (2:1). This organic phase was then poured in 70 ml of light liquid paraffin containing 1% w/v Span 80. The system was maintained under agitation with speed of 1000 rpm at room temperature for 3 hours to allow for the evaporation of solvent. Finally, the coated microspheres were filtered, washed with n-hexane, and dried in desiccators (Jain *et al.*, 2012).

Identification by FT-IR spectrophotometer

FTIR studies of mesalamine and formulation was carried out to find any possible interactions between the drug and the polymers during formulation (Garud *et al.*, 2011a). FTIR spectra of drug and drug-polymer in formulation were obtained in KBr pellets using a Perkin Elmer model spectrum BX-FTIR spectrophotometer in the ranges, 4000- 400 cm^{-1}.

Morphology and particle size

Shape and surface morphology of microspheres were studied using Scanning Electron Microscope (SEM LEO 430, Leo Electron Microscopy Ltd., England). For determination of surface characteristics all the microspheres were coated uniformly with gold palladium by using sputter coater for 5 to 7 minutes, after fixing the sample in individual steps. All samples of microspheres were then randomly examined for surface morphology at different magnification ranges. Particle size of the microcapsules was evaluated using optical microscopy method (Lachman and Lieberman, 1991). Approximately 100 microspheres were counted for particle size determination using a calibrated optical microscope (Magnus MLX-DX). The experiments were performed in triplicate (n=3).

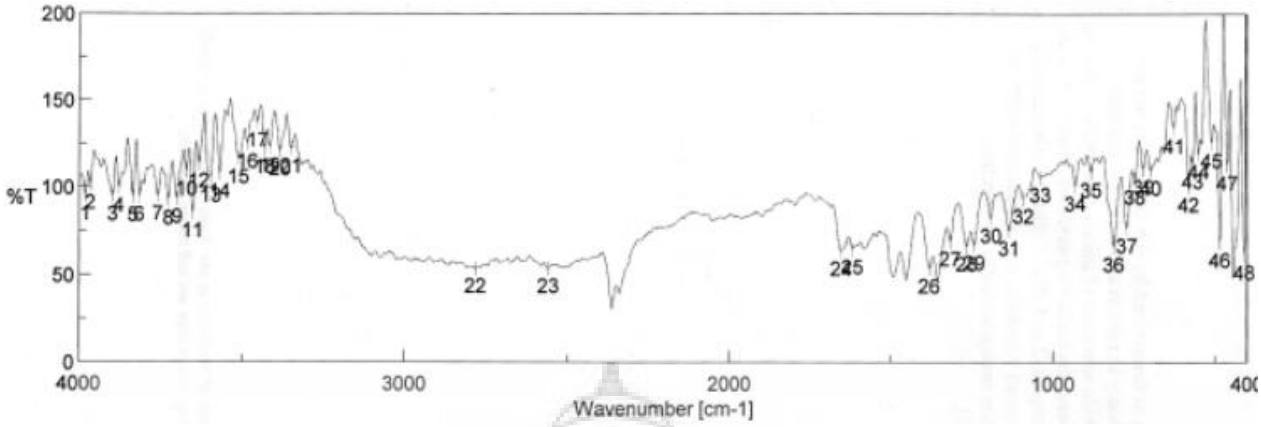

Figure 1: FT-IR Spectra of mesalamine.

Micromeretic properties

Accurately weighed microspheres were poured gently through a glass funnel into a graduated cylinder exactly to 10 ml mark. Initial volume was noted. Bulk density and tapped density were noted using tapping method using 10 ml measuring cylinder. Angle of repose (θ), Hausner's ratio (H) and Carr's index (% C) were calculated to study the flow properties of microspheres by using following formulas (Kancharla *et al.*, 2011):

$$\theta = \tan^{-1}\frac{h}{r}$$

where, h is height and r is radius of the pile, respectively.

$$H = \frac{Dt}{Db}$$

$$\%\,C = \frac{Dt - Db}{Dt} \times 100$$

where, Dt is tapped and Db is bulk density, respectively.

Entrapment efficiency, drug loading and % yield of microspheres

50 mg of microspheres were dispersed in 10 ml PBS pH 6.8 for 10 min with occasional shaking. The suspension was then centrifuged for 5 min and the supernatant was kept aside. The sediment microspheres were then incubated for 48 hrs with PBS pH 6.8 and the drug concentration was determined spectrophotometrically by UV at 334 nm (Shimadzu Pharmspec UV-1700, Japan). The entrapment efficiency, drug loading and % yield of microspheres (n=3) were calculated by using following formulas (Garud and Garud, 2011b):

$$\%\,EE = \frac{Dcal}{Dth} \times 100$$

where, Dcal is the calculated drug content and Dth is the theoretical drug content, respectively.

$$\%\,DL = \frac{Wd}{Wm} \times 100$$

where, Wd and Wm represents weight of drug and weight of microspheres, respectively.

$$\%\,Y = \frac{Wm}{Wt} \times 100$$

where, Wt represents total expected weight of drug and polymer

***In-vitro* release studies**

The drug release rate from the microspheres was studied in a medium of changing pH using the dissolution apparatus II at 37±0.5 °C with a rotation speed of 100 rpm. A weighed amount of mesalamine microspheres (equivalent to 50 mg of drug) were added to dissolution medium (350 ml of 0.1N HCl, pH 1.2) for the first two hours. At the end of second hour, the pH of the dissolution medium was raised to 4.5 by the addition of 250 ml solution composed of 3.75 g of KH_2PO_4 and 1.2 g of NaOH. At the end of fourth hour pH was raised to 7.4 by adding 300 ml of phosphate buffer concentrate (2.18 g of KH_2PO_4 and 1.46 g of NaOH in distilled water) (El-Bary *et al.*, 2012). At predetermined time intervals, 5 ml sample was withdrawn, passed through a 0.45 μm membrane filter (Millipore). After appropriate dilutions, the concentration of drug in samples was analysed spectrophotometrically at predetermined $\lambda_{max(s)}$. The initial volume of dissolution medium was maintained by adding 5 ml of fresh dissolution medium after each withdrawal.

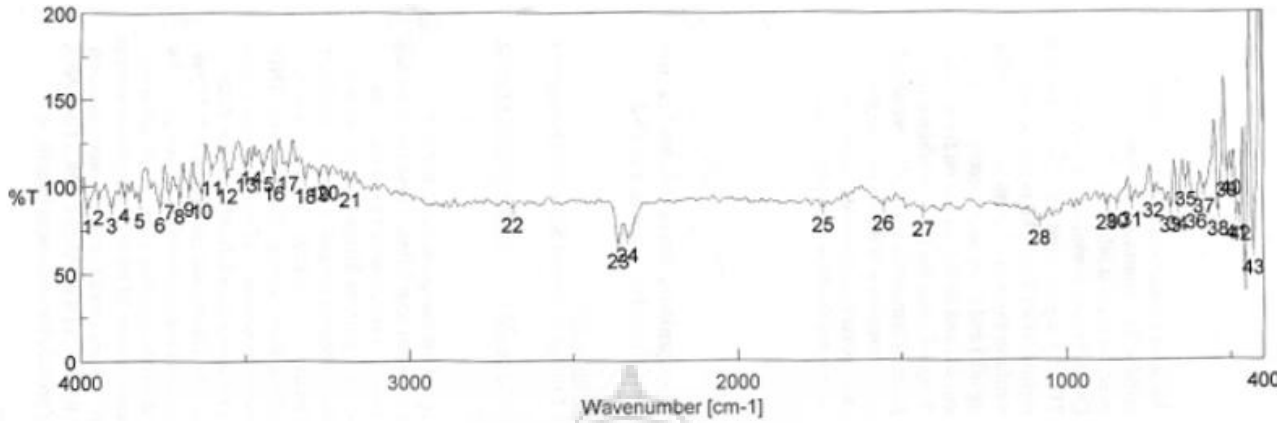

Figure 2: FT-IR Spectra of formulation.

Perfect sink conditions prevailed during the drug release studies.

Statistical analysis

The results were expressed in mean ± S.D. One way ANOVA (Analysis of Variance) was performed for studying the statistical significance using Minitab 15 software. Values of $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Identification by FT-IR spectrophotometer

FTIR studies of mesalamine and prepared formulation is shown in Figure 1 and 2. It is clear from the FTIR that the characteristic peaks of the drug are also present in the formulation depicting no incompatibility between the drug and polymers in the formulation.

Morphology and particle size

Visual examination of the SEM indicated that the microspheres of mesalamine were spherical with varied surface roughness (Figure 3). The particle size of microspheres ranged from 61.22-90.41 µm and were found to increase with increasing polymer content ($p<0.05$) (Table 2). As the emulsifier concentration was increased from 0.5 to 1.5 ml, the particle size was found to increase in the prepared formulations ($p<0.05$).

Table 1: Formulation composition of cross linked chitosan polymer.

Sl. No.	Formulation Code	Drug:Polymer	Emulsifier concentration (ml)
1	M1	1:1	0.5
2	M2	1:1	1.0
3	M3	1:1	1.5
4	M4	1:2	0.5
5	M5	1:2	1.0
6	M6	1:2	1.5
7	M7	1:3	0.5
8	M8	1:3	1.0
9	M9	1:3	1.5

Micromeretic properties

For the prepared formulations angle of repose (11.65-16.29°), Carr's index (6.72-22.16%) and Hausner's ratio (1.10-1.29), confirmed good flow properties of the microspheres (Table 2).

Entrapment efficiency, drug loading and % yield of microspheres

The microencapsulation efficiency for the different formulations was high (ranged from 43.72% to 82.27%) and significantly increased with increasing chitosan content ($p<0.05$). Drug loading of microspheres was found to be ranging from 33.26±1.04 to 20.28±0.96 and it significantly decreased with increasing chitosan content ($p<0.05$). The % yield of microsphere significantly increased with increasing chitosan content ($p<0.05$) and ranged from 63.99% to 84.94% for the prepared formulations (Figure 4). An increase in polymer concentration resulted in formation of larger microspheres entrapping greater amount of drug (Swapna *et al.*, 2011).

***In vitro* release studies**

In the *in-vitro* release studies, changing the pH conditions was attempted in lieu to mimic the GI conditions without enzymes. The pH condition used was pH 1.2 for a period of 2 h (stomach), pH 4.5

Table 2: Angle of repose, Carr's index, Hausner's ratio and particle size (μm).

Formulation codes	Angle of repose Mean±SEM	Carr's index Mean±SEM	Hausner's Ratio Mean±SEM	Particle Size(μm) Mean±SEM
M1	16.29±0.68	6.72±0.43	1.29±0.30	72.21±1.93
M2	16.16±0.65	8.75±0.42	1.10±0.28	65.22±0.94
M3	15.52±0.63	19.25±0.39	1.23±0.31	61.22±1.28
M4	13.65±0.54	20.45±0.53	1.22±0.27	80.02±1.80
M5	13.85±0.59	20.34±0.56	1.25±0.27	74.15±0.84
M6	14.34±0.53	20.28±0.39	1.31±0.26	72.05±1.12
M7	11.65±0.61	20.17±0.41	1.34±0.30	98.91±1.20
M8	11.98±0.59	21.64±0.41	1.27±0.29	93.41±1.43
M9	12.34±0.52	22.16±0.55	1.28±0.27	90.41±1.83

(duodenum) for 2 h followed by pH 7.4 (distal ileum and colon) for the remaining duration of the study. A successful colon targeted drug delivery should have minimum drug release during its transit in the stomach and upper intestine to ensure maximum drug release in the colon (Chandran *et al.*, 2009).

Eudragit S100 is an anionic copolymer of methacrylic acid and methyl methacrylate, the ratio of free carboxyl groups to the ester groups is approximately 1:2. It exhibits a dissolution threshold pH slightly above 7.2 (Sinha and Kumria, 2003). Due to the pH-sensitive property of this polymer, it was selected to avoid the rapid dissolution of mesalamine during the initial transit of the microspheres through the gastric cavity and the upper small intestine.

The retardation in drug release was found to be significant with increasing polymer concentration ($p<0.05$). The increased density of polymer matrix at higher concentration resulted in an increased diffusion pathlength. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release (Srivastava *et al.*, 2005). However, increase in emulsifier concentration in the formulations showed insignificant results in the drug release rate ($p>0.05$). Eudragit coating of chitosan microspheres prevented the release of drug in stomach and targeted the delivery of drug to colon.

It was found that formulations with drug-polymer ratio of 1:1 (M1 to M3) released complete drug at 12 hours. A comparison of percentage release of drug from cross-linked chitosan microspheres vs time without coating is shown in Figure 5. A comparative % drug release of chitosan microspheres (M3, M6 and

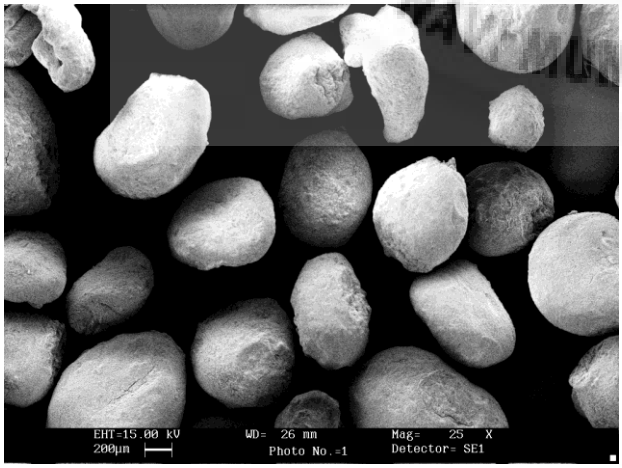

Figure 3: SEM of mesalamine-loaded chitosan microspheres.

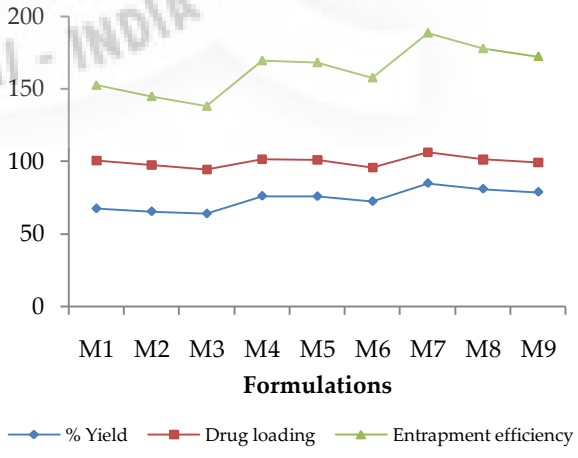

Figure 4: Percent yield, drug loading and entrapment efficiency of formulations.

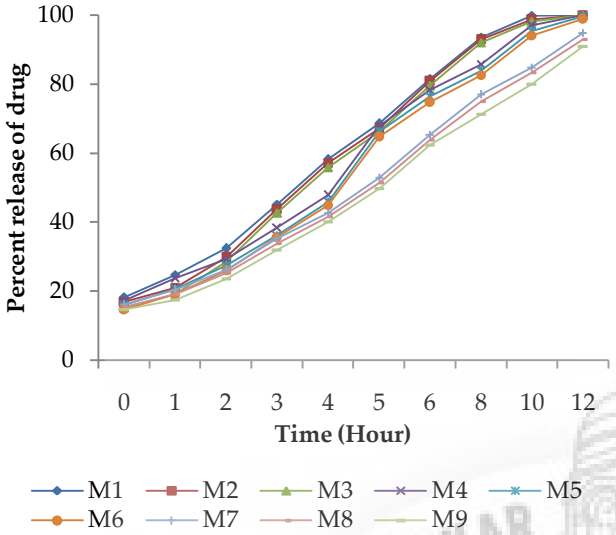

Figure 5: A comparison of percentage release of drug from cross-linked chitosan microspheres vs time (in hours) without coating.

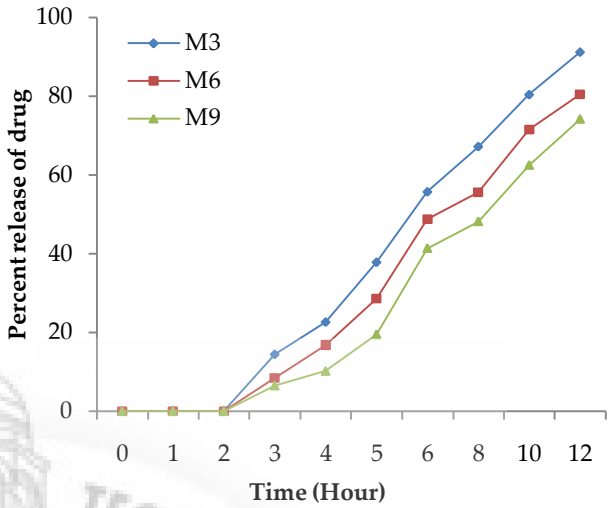

Figure 6: A comparative % drug release of chitosan microspheres (M3, M6 and M9) coated with Eudragit S-100 polymer.

M9) coated with Eudragit S-100 with drug-polymer ratio 1:1, 1:2 and 1:3, respectively at 1.5 ml emulsifier concentration is depicted in Figure 6. It was observed that Eudragit S100 coated chitosan microspheres gave no release in the simulated gastric fluid, negligible release in the simulated intestinal fluid and maximum release in the colonic environment.

CONCLUSION

Mesalamine microspheres were prepared successfully by using the ionic-gelation emulsification method. Prepared microspheres showed good % yield and drug loading. Encapsulation efficiency of microspheres was good for all formulations. The prepared microspheres with 1:3 ratio of drug-polymer coated with Eudragit S100 (M9) was found suitable for colonic release of mesalamine resisting drug release in gastric medium, minimizing release in the upper intestinal region and showing maximum release in the colonic region. Therefore, the developed formulation proves to be promising for the colon targeted drug delivery of mesalamine and thereby facilitating in the management of ulcerative colitis.

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REFERENCES

Alagusundaram M., Madhusudana C.C., Umasharkari K., Badrinath A.V., Lavanya C., Ramkanth S. (2009), Microspheres as a novel drug delivery system, International Journal of ChemTech Research. 1(3): 526-534.

Burger D., Travis S. (2011), Conventional medical management of inflammatory bowel disease, Gastroenterology. 140(6): 1827-1837. [DOI] PMid:21530749

Chandran S., Sanjay K.S., Ali Asghar L.F. (2009), Microspheres with pH modulated release: design and characterization of formulation variables for colonic delivery, Journal of Microencapsulation. 26(5): 420-431. [DOI] PMid:18821120

Dehghan S., Aboofazeli R., Avadi M., Khaksar R. (2010), Formulation optimization of nifedipine containing microspheres using factorial design, African Journal of Pharmacy and Pharmacology. 4(6): 346-354.

El-Bary A.A., Aboelwafa A.A., Al Sharabi I.M. (2012), Influence of some formulation variables on the optimization of pH dependent, colon targeted, sustained release mesalamine microspheres, AAPS PharmSciTech. 13(1): 75-84. [DOI] PMid:22130789 PMCid:3299443

Garud N., Garud A., Jain N. (2011a), Formulation design and *in-vitro* evaluation of metformin microspheres using ionotropic gelation technique, Journal of Pharmacy Research. 4(7): 2103-2106.

Garud N., Garud A. (2011b), Preparation and *in-vitro* evaluation of metformin microspheres using non-aqueous solvent evaporation technique, Trop J Pharm Res. 13(4): 577-583.

Gawde P., Agrawal S. (2012), Design and characterization of Eudragit coated chitosan microspheres of Deflazacort for

colon targeting, Journal of Pharmacy Research. 5(9): 4867-4870.

Jain V., Gupta S., Pandey G.K., Dubey B.K., Jain P.K., Saluja M.S. (2012), Design and characterization of eudragit coated chitosan microspheres of mesalazine for irritable bowel disease, International Journal of Drug Discovery and Herbal Research. 2(1): 301-307.

Kancharla K., Basavaraj B.W., Bharath S., Deveswaran R., Madhavan V. (2011), Formulation and evaluation of intragastric floating multiparticulate system of Aceclofenac, Der Pharmacia Lettre. 3(2): 238-245.

Kumar K.P.S., Bhowmik D., Srivastava S., Paswan S., Dutta A.S. (2012), Sustained release drug delivery system potential, The Pharma Innovation. 1(2): 48-60.

Lohani A., Gangwar P.C. (2012), Mucoadhesive microspheres: A novel approach to increase gastroretention, Chronicles of Young Scientists. 3(2):121-128. [DOI]

Lachman L., Lieberman H.A., Kanig J.L. (1991), The Theory and Practice of Industrial Pharmacy, 2nd Ed, Mumbai, India, Varghese Publishing House, 26-27.

Patel J.K., Patel R.P., Amin A.F., Patel M.M. (2005), Formulation and evaluation of mucoadhesive glipizide microspheres, AAPS Pharm Sci Tech. 6(1): E49-E55. [DOI] PMid:16353963 PMCid:2750411

Sinha V.R., Kumria R. (2003), Coating polymers for colon specific drug delivery: A comparative in vitro evaluation, Acta Pharm. 53(1): 41-47.

Srivastava A.K., Ridhurkar D.N., Wadhwa S. (2005), Floating microspheres of cimetidine: formulation, characterization and in vitro evaluation, Acta Pharm. 55(3): 277-285.

Swapna A., Mohd A.B., Wamorkar V., Swathimutyam P. (2011), Formulation and Evaluation of Mesalamine Microspheres for Colon Targeted Drug Delivery System, Journal of Pharmacy Research. 4(6): 1670-1672.

Vasir J.K., Tambwekar K., Garg S. (2003), Bioadhesive microsphere as a controlled drug delivery system, International journal of Pharmaceutics. 255(1-2): 13-32. [DOI]

PRODUCT MONOGRAPH

PrSALOFALK®

Mesalamine Suppositories
500 mg and 1000 mg

LOWER GASTROINTESTINAL TRACT ANTI-INFLAMMATORY

A07EC02

APTALIS PHARMA CANADA INC.
597, Laurier Boulevard
Mont-Saint-Hilaire, Québec
CANADA J3H 6C4

Date of Preparation:
January 11, 2013

Date of Revision:
December 30, 2014

Submission Control No: 178877

Table of Contents

[Pr]SALOFALK®

Mesalamine Suppositories
500 mg and 1000 mg

PART I: HEALTH PROFESSIONAL INFORMATION

SUMMARY PRODUCT INFORMATION

Route of Administration	Dosage Form / Strength	Clinically Relevant Non-medicinal Ingredients
Rectal	Suppositories 500, 1000 mg	None *For a complete listing see DOSAGE FORMS, COMPOSITION AND PACKAGING section.*

INDICATIONS AND CLINICAL USE

SALOFALK® (mesalamine suppositories) 500 and 1000 mg are indicated:
- in the management of ulcerative proctitis.
- as adjunctive therapy in more extensive distal ulcerative colitis (DUC).

Geriatrics
Clinical studies with SALOFALK® suppositories, 500 mg and 1000 mg have not been performed in the geriatric population.

Pediatrics
Information on the safety and efficacy of SALOFALK® suppositories in children is limited. Therefore, use should be limited to situations where a clear benefit is expected. SALOFALK® suppositories should not be used in infants under two years of age.

CONTRAINDICATIONS

SALOFALK® (mesalamine suppositories) is contraindicated in:

- patients with severe renal impairment (GFR<30ml/min/1.73m^2) and/or severe hepatic impairment (see Warnings and Precautions).

- patients who are hypersensitive to this drug or to any ingredient in the formulation or component of the container. For a complete listing, see the Dosage Forms, Composition and Packaging section of the product monograph.

- cases of existing gastric or duodenal ulcer.
- patients with urinary tract obstructions.
- infants under two years of age.

Patients hypersensitive to salicylates, including acetylsalicylic acid (e.g. Aspirin®), may also be hypersensitive to this medication.

WARNINGS AND PRECAUTIONS

General

There have been reports of hepatic failure and increased liver enzymes in patients with pre-existing liver disease when treated with 5-ASA/Mesalazine products. Therefore, SALOFALK® (mesalamine suppositories) is contraindicated in patients with severe hepatic impairment (see Contraindications). In patients with mild to moderate liver function impairment, caution should be exercised and SALOFALK® (mesalamine suppositories) should only be used if the expected benefit clearly outweighs the risks to the patients.

SALOFALK® (mesalamine suppositories) should be used only if the benefits clearly outweigh the risks in patients with underlying, bleeding or clotting disorders as well as during pregnancy and lactation.

Patients with renal dysfunction, or elevated Blood Urea Nitrogen (BUN), or elevated serum creatinine, or with proteinuria, should be carefully monitored while receiving SALOFALK®.

Concomitant treatment with mesalamine can increase the risk of myelosuppression in patients receiving azathioprine or 6-mercaptopurine[1-3].

Effects on Ability to Drive and Use Machinery

There are no data available on the effects of mesalamine on ability to drive and use machines.

Carcinogenesis and Mutagenesis

Carcinogenicity studies in animals and mutagenicity tests were negative (see TOXICOLOGY)[4].

Cardiovascular

Cardiac side effects, including pericarditis and myocarditis have been uncommonly reported with the use of mesalamine[5].

Cases of pericarditis have also been reported as manifestations of inflammatory bowel disease. Discontinuation of mesalamine may be warranted in some cases, but rechallenge with mesalamine can be performed under careful clinical observation should the continued therapeutic need for mesalamine be present[6,7].

Gastrointestinal

Epigastric pain, also commonly associated with inflammatory bowel disease and prednisone or sulfasalazine (SAS) therapy (18%)[8],-should be investigated in order to exclude pericarditis and pancreatitis either as adverse drug reactions to mesalamine or secondary manifestations of inflammatory bowel disease.

Renal

Reports of renal impairment, including minimal change nephropathy, and acute or chronic interstitial nephritis have been associated with mesalamine products and pro-drugs of mesalamine. SALOFALK® (mesalamine suppositories) is contraindicated in patients with severe renal impairment (see Contraindications). In patients with mild to moderate renal dysfunction, caution should be exercised and SALOFALK® (mesalamine suppositories) should be used only if the benefits outweigh the risks.

Patients on mesalamine, especially those with pre-existing renal disease, should be carefully monitored with urinalysis, and BUN and creatinine testing. Initial assessment and periodic monitoring of the renal function is recommended since mesalamine is substantially excreted by the kidney, and prolonged mesalamine therapy may damage the kidneys.

Because elderly patients are more likely to have decreased renal function, closer monitoring of the renal function may be needed.

Sensitivity/Resistance

Caution should be exercised when mesalamine (5-ASA) is initially used in patients known to be allergic to sulfasalazine. These patients should be instructed to discontinue therapy if sign of rash or pyrexia become apparent. In case of an allergic reaction, appropriate measures (standard of care) should be taken.

Acute Intolerance Syndrome

Mesalamine has been implicated in the production of an acute intolerance syndrome characterized by cramping, acute abdominal pain and bloody diarrhoea, sometimes fever, headache and a rash; in such cases prompt withdrawal is required. The patient's history of sulfasalazine intolerance, if any, should be re-evaluated. If a rechallenge is performed later in order to validate the hypersensitivity, it should be carried out under close supervision and only if clearly needed, giving consideration to reduced dosage. The possibility of increased absorption of mesalamine and concomitant renal tubular damage as noted in the preclinical studies must be kept in mind. Patients on concurrent mesalamine products which contain or release mesalamine and those with pre-existing renal disease should be carefully monitored with urinalysis, and BUN and creatinine testing.

Special Populations

Pregnant Women

SALOFALK® should be used during pregnancy only if the benefits clearly outweigh the risks to the foetus. 5-ASA is known to cross the placental barrier, and no clinical studies have been performed in pregnant women[1,9].

Animal studies did not show evidence of impaired fertility or harm to the foetus due to mesalamine (see TOXICOLOGY), however, because animal reproduction studies are not always predictive of human response, SALOFALK® should be used during pregnancy only if clearly needed.

Nursing Women
There are no clinical trial studies in nursing women. SALOFALK® should be used in nursing women only if the benefits to the mother clearly outweigh the risks to the child. Mesalamine and its main metabolite N-acetyl-5-ASA are excreted in breast milk[9-12]. The concentration of mesalamine is much lower than in maternal blood, but the metabolite N-acetyl-5-ASA appears in similar concentrations.

When mesalamine is used in nursing women, infants should be monitored for changes in stool consistency as hypersensitivity reactions manifested as diarrhoea in the infants have been reported[1,13-15].

Pediatrics
Information on the safety and efficacy of SALOFALK® suppositories in children is limited.

SALOFALK® should not be used in infants/toddlers aged less than 24 months.

Geriatrics
Clinical studies of mesalamine did not include sufficient number of subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger patients. In general, dose selection for an elderly patient should be cautious, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and of concomitant disease or other drug therapy.

Mesalamine is substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function. Because elderly patients are more likely to have decreased renal function, it may be useful to monitor renal function.

ADVERSE REACTIONS

Adverse Drug Reaction Overview

Hypersensitivity reactions have been reported in a sub-group of patients known to be allergic to sulfasalazine including rash[16-19], pyrexia[16-19], and dizziness[16,18] with reactions occurring at the onset of therapy and resolving promptly following discontinuation[16,17].

Other manifestations of hypersensitivity reported with mesalamine include acute pancreatitis[19,20], hepatitis[19], pericarditis, interstitial nephritis, interstitial pneumonia and pleural effusion. Interstitial pneumonia, pancreatitis and pericarditis have also been reported as manifestations of

inflammatory bowel disease[22].

As with all 5-ASA products, exacerbations of ulcerative colitis characterized by cramping[18] acute abdominal pain[18-20] and diarrhoea[18,19,21] have been reported with mesalamine.

Other reported side effects include headache[18,19,21,23], flatulence[18], nausea[18,19,21,23], and hair loss[17,18], but do not appear to be common. Retreatment is not always associated with repeated hair loss. Aplastic anaemia has been reported in the literature with unspecified formulations of mesalamine.

Clinical Trial Adverse Drug Reactions

Because clinical trials are conducted under very specific conditions the adverse reaction rates observed in the clinical trials may not reflect the rates observed in practice and should not be compared to the rates in the clinical trials of another drug. Adverse drug reaction information from clinical trials is useful for identifying drug-related adverse events and for approximating rates.

Table 1: Clinical Trial Adverse Events Reported in > 0.1% of Patients

SYSTEM ORGAN CLASS Preferred Term	SALOFALK® N=841 %	Placebo N=176 %
Cardiac Disorders		
Pericarditis	0.1	0.0
Gastrointestinal Disorders		
Abdominal pain	7.9	7.9
Flatulence	6.0	4.5
Nausea	5.6	6.8
Diarrhoea	2.1	3.9
Abdominal distension	1.4	1.1
Haemorrhoids	1.3	0.0
Proctalgia	1.1	0.0
Constipation	0.9	2.2
Anorectal discomfort	0.5	1.7
Pancreatitis	0.1	0.0
Condition aggravated	0.1	0.0
General Disorders and Administration Site Conditions		
Fatigue	3.3	4.5
Pyrexia	3.0	0.0
Administration site reaction	1.3	0.5
Oedema peripheral	0.5	6.2
Asthenia	0.1	2.2

SYSTEM ORGAN CLASS Preferred Term	SALOFALK® N=841 %	Placebo N=176 %
Infections and Infestations		
Influenza	5.2	0.5
Urinary tract infection	0.5	2.2
Upper respiratory tract infection	0.1	0.5
Musculoskeletal, Connective Tissue and Bone Disorders		
Arthralgia	2.0	1.1
Back pain	1.3	0.5
Nervous System Disorders		
Headache	6.7	11.3
Dizziness	1.7	2.8
Insomnia	0.1	1.7
Respiratory, Thoracic and Mediastinal Disorders		
Pharyngolaryngeal pain	2.0	2.8
Skin and Subcutaneous Tissue Disorders		
Rash	2.8	2.2
Spots	2.2	5.1
Pruritus	1.1	0.5
Alopecia	0.8	1.1

Post-Market Adverse Drug Reactions

The following adverse reactions have been identified during the post-approval use of SALOFALK® suppository. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Cardiac Disorders: Myocarditis, Pericarditis

Eye Disorders: Eye swelling

Gastrointestinal Disorders: Abdominal pain (upper, lower), Abdominal cramps, Abdominal distension, Abnormal faeces, Anal pruritus, Anorectal discomfort, Constipation, Diarrhoea, Faeces discoloured, Flatulence, Frequent bowel movements, Mucus stools, Nausea, Painful defecation, Pancreatitis, Proctalgia, Rectal discharge, Rectal tenesmus, Stomach discomfort, Vomiting

General Disorders And Administration Site Conditions: Fatigue, Medication residue, Pain, Pyrexia

Nervous System Disorders: Burning sensation, Dizziness, Headache

Respiratory, Thoracic And Mediastinal Disorders: Dyspnoea

Skin And Subcutaneous Tissue Disorder: Alopecia, Erythema, Pruritus, Rash, Urticaria

The following adverse events have been identified during the post-approval use of mesalamine products:

Blood and Lymphatic System Disorders: Agranulocytosis

Immune System Disorder: Anaphylactic reaction, Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS), Stevens-Johnson Syndrome (SJS)

DRUG INTERACTIONS

Overview

Interaction between azathioprine, 6-mercaptopurine and aminosalicylates (including mesalamine) can increase the risk of leucopenia[1-3]. Other potential interactions with a number of drugs could occur (see Drug-Drug Interactions).

Drug-Drug Interactions

Interaction between azathioprine, 6-mercaptopurine and aminosalicylates including mesalamine, has been reported with oral mesalamine. Concomitant treatment with mesalamine can increase the risk of myelosuppression in patients receiving azathioprine or 6-mercaptopurine. An increase in whole blood 6-thioguanine nucleotide (6-TGN) concentrations has been reported although the mechanism of this interaction remains unclear[1-3].

Mesalamine could also increase renal and hematologic toxicity of methotrexate by additive effect and diminished absorption of folic acid[24].

The hypoglycemic effect of sulfonylureas may be enhanced. Interactions with coumarin, methotrexate, probenecid, sulfinpyrazone, spironolactone, furosemide and rifampicin cannot be excluded. Potentiation of undesirable glucocorticoid effects on the stomach is possible.

A theoretical interaction of salicylates with Varicella Virus Vaccine (chicken pox vaccine) might increase the risk of Reye's syndrome; as a result, the use of salicylates (including mesalamine) is discouraged for six weeks following Varicella vaccination[1,25].

Drug-food, drug-herb, or drug-laboratory interactions have not been studied.

Drug-Laboratories Test Interactions

Several reports of possible interference with measurements, by liquid chromatography, of urinary normetanephrine causing a false-positive test result have been observed in patients exposed to sulfasalazine or its metabolite, mesalamine/mesalazine.[54.55.56]

DOSAGE AND ADMINISTRATION

Recommended Dose and Dosage Adjustment

One 500 mg SALOFALK® rectal suppository is self-administered on a twice a day or three times a day basis. One 1000 mg SALOFALK® rectal suppository is self-administered on a once daily basis, at bedtime. The usual adult dose is 1.0 - 1.5 g/day and dosing is continued until a significant response is achieved or until the patient achieves remission. Dose tapering is recommended. Abrupt discontinuation is not recommended. Best results are expected with prolonged retention.

Missed Dose

If a dose of SALOFALK® is missed, it should be used as soon as possible, unless it is almost time for the next dose. A patient should not use two SALOFALK® doses at the same time to make up for a missed dose.

Administration

SALOFALK® suppositories are self-administered, one 500 mg suppository 2 or 3 times/day, and one 1000 mg suppository 1 time daily at bedtime. The suppository should be retained for 1 to 3 hours or longer to achieve the maximum benefit. While the effect of the suppositories may be seen within 3 to 21 days, the usual course of therapy would be from 3 to 6 weeks depending on symptoms and sigmoidoscopic findings.

Patient Instructions:

I. Detach one suppository from the strip of suppositories.
II. Hold suppository upright and carefully remove the plastic wrapper.
III. Avoid excessive handling of suppository, which is designed to melt at body temperature.
IV. Insert suppository completely into rectum with gentle pressure, pointed end first.
V. A small amount of lubricating gel may be used on the tip of the suppository to assist insertion.

In children, information on the safety and efficacy of mesalamine suppositories is limited. Therefore, use should be limited to situations where a clear benefit is expected.

OVERDOSAGE

There has been no clinical experience with mesalamine overdosage. However, because mesalamine is an aminosalicylate, the symptoms of overdose may mimic the symptoms of salicylate overdose; therefore, measures used to treat salicylate overdose may be applied to mesalamine overdose. Under ordinary circumstances, local mesalamine absorption from the colon is limited. There is no specific antidote and treatment is symptomatic and supportive.

For management of a suspected drug overdose, contact your regional Poison Control Centre.

ACTION AND CLINICAL PHARMACOLOGY

Mechanism of Action

The mechanism of action of mesalamine (5-aminosalisylic acid, 5-ASA), is not fully understood, but appears to be topical rather than systemic. Inflammatory intestinal disease is often accompanied by diffuse tissue reactions including ulceration and cellular infiltration of lymphocytes, plasma cells, eosinophils, polymorphonuclear cells and activated phagocytic cells[26].

The interference of mesalamine with either leukotriene or prostaglandin metabolism may play a major role in suppressing the inflammatory response mechanism[26-32]. 5-ASA prevents accumulation of thromboxane B2 and 6-keto-prostaglandin F1[27]. Both 5-ASA and SAS reverse H_2O, and Cl-secretion and increase Na+ secretion in experimentally-induced colitis in guinea pigs[33]. SAS and 5-ASA are known to inhibit polymorphonuclear cell migration possibly via lipoxygenase inhibition[32] at concentrations lower than those required to inhibit prostaglandin synthesis. It is thus possible that both SAS and 5-ASA are capable of inhibiting both pathways via lipoxygenase inhibition[26].

Intestinal secretion is stimulated not only by prostaglandins but also by the metabolites of arachidonic acid generated via the lipoxygenase pathway[26,32,34]. Upon phagocytic activation and arachidonic acid metabolism activation, reactive oxygen metabolites are generated[35]. 5-ASA acts as a dose dependent[35] antioxidant which scavenges oxygen derived free radicals produced by activated phagocytes[26,36]. In addition, 5-ASA associates with the membrane surface, allowing chain breaking anti-oxidant activity when peroxidation is initiated within the membrane. 5-ASA is able to block initiation of oxidation from solution as well as propagation within the membrane[37]. 5-ASA also inhibits the formation of both eicosanoids and cytokines[26,36].

Pharmacodynamics

SALOFALK® suppositories contain mesalamine (5-aminosalisylic acid, 5-ASA), the active principle of the prodrug sulfasalazine[17,38-42]. Although the 5-ASA mode of action is not clear, it appears to be multi-factorial. 5-ASA is thought to affect the inflammatory process through its ability to inhibit prostaglandin synthesis[27-32], interfere with leukotriene synthesis and consequent leukocyte migration[27,28] as well as act as a potent scavenger of free radicals[26]. Regardless of the mode of action, 5-ASA appears to be active mainly topically rather than systemically[43]. Rectal administration as 500 or 1000 mg suppositories of mesalamine (5-aminosalicylic acid) allows for direct targeting of free 5-ASA to the sites of inflammation along the mucosal lumen of the rectum, sigmoid and distal large bowel.

Pharmacokinetics

Absorption

Mesalamine (5-ASA) administered as a rectal suppository is variably absorbed. Systemic absorption of rectally administered 5-ASA is low as shown by urinary recoveries which range

from 5% to 35% of the daily dose adminstered[40,44]. In patients with ulcerative colitis treated with mesalamine 500 mg rectal suppositories, administered once every eight hours for six days, the mean mesalamine peak plasma concentration (C_{max}) was 353 ng/mL (CV=55%) following the initial dose and 361 ng/mL (CV=67%) at steady state. The mean minimum steady state plasma concentration (C_{min}) was 89 ng/mL (CV=89%).[45] Absorbed mesalamine does not accumulate in the plasma.

Distribution
Mesalamine administered as rectal suppositories distributes in rectal tissue to some extent. In patients with ulcerative proctitis treated with mesalamine 1000 mg rectal suppositories, rectal tissue concentrations for 5-ASA and N-acetyl-5-ASA have not been rigorously quantified.

Metabolism
Mesalamine is extensively metabolized by acetylation[9,38]. The only major metabolite of 5-ASA identified in man is N-acetyl-5-aminosalicylic acid (N-Ac-5-ASA)[38]. The site of metabolism has not been elucidated. In patients with ulcerative colitis treated with one 500 mg mesalamine rectal suppository every eight hours for 6 days, peak concentrations (C_{max}) of N-acetyl-5-ASA ranged from 467 ng/mL to 1399 ng/mL following the initial dose and from 193 ng/mL to 1304 ng/mL at steady state.[45]

The influence of renal and hepatic impairment on pharmacokinetics of mesalamine has not been evaluated.

Excretion
Mesalamine is eliminated from plasma mainly by urinary excretion, predominantly as N-acetyl-5-ASA. In patients with ulcerative proctitis treated with one mesalamine 500 mg rectal suppository every eight hours for six days, ≤ 12% of the dose was eliminated in urine as unchanged 5-ASA and 8-77% as N-acetyl-5-ASA following the initial dose. At steady state, ≤ 11% of the dose was eliminated as unchanged 5-ASA and 3-35% as N-acetyl-5-ASA. The mean elimination half-life was five hours (CV=73%) for 5-ASA and six hours (CV=63%) for N-acetyl-5-ASA following the initial dose. At steady state, the mean elimination half-life was seven hours for both 5-ASA and N-acetyl-5-ASA (CV=102% for 5-ASA and 82% for N-acetyl-5-ASA).[45]

STORAGE AND STABILITY
SALOFALK® (mesalamine, 5-aminosalicylic acid, 5-ASA) suppositories must be stored below 25°C (77°F). Can be refrigerated. Keep away from direct heat, light and humidity.

SPECIAL HANDLING INSTRUCTIONS
SALOFALK® (mesalamine suppositories) will cause staining of direct contact surfaces, including but not limited to fabrics, flooring, painted surfaces, marble, granite, vinyl, and enamel.

DOSAGE FORMS, COMPOSITION AND PACKAGING
Each smooth light tan to grey, bullet-shaped SALOFALK® suppository contains 500 mg or 1000

mg mesalamine (5-aminosalicylic acid) that are available in strips of 6 suppositories; boxes of 30 suppositories. Non-medicinal ingredients: Witepsol H-15 (suppository wax base). SALOFALK® (mesalamine suppositories) are gluten-free and phthalate-free.

PART II: SCIENTIFIC INFORMATION

PHARMACEUTICAL INFORMATION

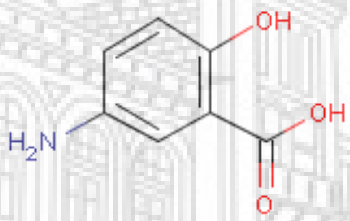
Drug Substance

Proper name: 5-aminosalicylic acid, mesalamine

Chemical name: 5-aminosalicylic acid (5-ASA)

Molecular formula and molecular mass: $C_7H_7NO_3$ 153.14

Structural formula:



Physicochemical properties:

Description: 5-aminosalicylic acid is a light tan to pink, needle shaped, crystalline powder.

Solubility: Slightly soluble in water, very slightly soluble in methanol and practically insoluble in chloroform; soluble in diluted HCl and diluted alkali hydroxides

Melting Range: 272°-280°C

CLINICAL TRIALS

A double-blind, placebo-controlled multicenter study was conducted in North America in patients with mild to moderate active proctitis. The primary measures of efficacy were clinical disease activity index [DAI], sigmoidoscopic and histological evaluations. The dosage regimen: was 500 mg mesalamine three times daily (1.5 g/day). A total of 79 patients were studied (39 patients received mesalamine suppositories, and 40 patients received placebo). Patients were evaluated clinically and sigmoidoscopically after three and six weeks of suppository treatment. Patients were 17 to 73 years of age (mean = 39 years), 57% were female, and 97% were white. Patients had an average extent of proctitis (upper disease boundary) of 10.8 cm and 84% of patients had multiple prior episodes of proctitis.

Compared to placebo, mesalamine suppository treatment was statistically ($p < 0.01$) superior with respect to improvement in stool frequency, rectal bleeding, mucosal appearance, and disease severity. Mesalamine-treated patients had an 80.4% mean reduction in DAI ($p < 0.05$) compared to placebo (36.8%) and 84.4% of mesalamine patients were considered 'much improved' by the investigator ($p < 0.01$) at 6 weeks. Daily diary records revealed a significant improvement in rectal bleeding in the mesalamine-treated patients ($p < 0.05$) within the first week compared to placebo indicating a faster onset of action. The effectiveness of mesalamine suppositories was statistically significant irrespective of sex, extent of proctitis, duration of current episode or duration of disease.

DETAILED PHARMACOLOGY

Animal Studies

5-ASA (mesalamine) is the active moiety of the prodrug sulfasalazine which acts to suppress inflammatory bowel disease. Animal pharmacology tests were conducted on 5-ASA using the oral route of administration for most tests, at a dose of 500 mg/kg in order to simulate practice relevant conditions. No adverse effect of 5-ASA on the following parameters or in the following animal pharmacology tests could be established: tremorine antagonism, hexobarbital sleep time, motor activity, anticonvulsant action (metrazol and electric shock), blood pressure, heart rate, respiratory rate (up to 10 mg/kg, i.v.), tocolysis (antispasmodic assay), local anaesthesia, antihyperthermal and antipyretic effects. In the paw-edema test with carrageen injection, 200 mg/kg *per os* proved ineffective, but 500 mg/kg 5-ASA *per os* exhibited mild antiphlogistic action.

In the animal renal function tests (natriuresis and diuresis), no biologically relevant effects of 200 mg/kg *per os* were demonstrated. After 600 mg/kg, marked functional changes were observed: increases in total urinary output, natriuresis and proteinuria. The urinary sediment contained an increased number of erythrocytes and epithelial cells. Both potassium elimination and specify weight were reduced. It can be concluded from these experiments that even high doses of 5-ASA have no effect on vital parameters. Disturbances in renal function are to be expected only at dosages equivalent to a single dose at least 8 to 10 times the daily dose in man.

Human Studies
See Action and Clinical Pharmacology.

TOXICOLOGY

Long-term Toxicity
Animal studies to date show the kidney to be the only significant target organ for 5-ASA toxicity in rats and dogs. At high doses, the lesions produced consisted of papillary necrosis and multifocal proximal tubular injury. In rats, the no-effect levels were 160 mg/kg/day for females and 40 mg/kg/day for males (minimal and reversible tubular lesions seen) after 13 weeks of oral administration. In dogs, the no-effect level in both males and females was 40 mg/kg/day after 6 months of oral administration. In this six-month oral toxicity study in dogs, doses of 80 mg/kg/day (about 1.4 times the recommended human intra-rectal dose, based on body surface area) and higher, caused renal pathology similar to that described for the rat. In a rectal toxicity study of mesalamine suppositories in dogs, a dose of 166.6 mg/kg (about 3.0 times the recommended human intra-rectal dose, based on body surface area) produced chronic nephritis and pyelitis. Aside from gastric lesions, heart lesions and bone marrow depression seen in some of the rats at the 640 mg/kg level and considered secondary effects of kidney damage, no other signs of systemic toxicity were noted at daily doses up to 160 mg/kg in rats and 120 mg/kg in dogs for 13 weeks and six months, respectively.

In the 12-month oral toxicity study in dogs, keratoconjunctivitis sicca (KCS) occurred at oral doses of 40 mg/kg/day (about 0.72 times the recommended human intra-rectal dose, based on body surface area) and above[46].

Carcinogenicity
Administration of doses of 0, 50, 100 and 320 mg/kg/day for 127 weeks in rats did not result in significant differences in unscheduled deaths, clinical signs, nodules or masses, between groups. Ophthalmoscopic investigations revealed no treatment-related changes. Treatment with SALOFALK® was not associated with oncogenic changes or an increased tumor risk. The assessment of hematology, clinical biochemistry and urinalysis indicated no changes of toxicological significance at 13, 26 and 52 weeks of treatment.

After 127 weeks, analysis of the lesions indicated slight substance-related and dose-dependent toxic changes as degenerative kidney damage and hyalinization of tubular basement membrane and Bowman's capsule in the 100 mg and 320 mg/kg/day groups. Ulceration of the gastric mucosa and atrophy of the seminal vesicles were also more frequent in the 320 mg/kg/day group.

Mutagenicity
5-ASA was not mutagenic in the Ames test, *E. coli* reverse mutation assay, mouse micronucleus test, sister chromatid exchange assay, or in a chromosomal aberrations assay. In contrast, sulfapyridine, which is the other primary metabolite of salicylazosulfapyridine, has tested positive in certain mutagenicity tests[4].

Reproduction Studies

Teratology studies with 5-ASA have been performed in rats at oral doses up to 320 mg/kg/day and in rabbits at oral doses up to 495 mg/kg/day (about 1.7 and 5.4 times the recommended human intra-rectal dose, respectively).The battery of tests completed to date has shown that 5-ASA is devoid of embryotoxicity and teratogenicity in rats and rabbits; that it does not affect male rat fertility after five weeks of oral administration at 296 mg/kg/day; and that it lacks the potential to affect late pregnancy, delivery, lactation or pup development in rats.

Other Studies

Nephrotoxic potential of 5-aminosalicylic acid:

Owing to its structural relationship to phenacetin, the aminophenols and salicylates, 5-ASA was included in a series of compounds studied following identification of antipyretic-analgesic nephropathy in humans. Calder *et al*. has reported in rats that in addition to the proximal tubule necrosis seen with acetylsalicylic acid (e.g. Aspirin®) and phenacetin derivatives, 5-ASA produced papillary necrosis, following single intravenous doses ranging from 150 mg/kg to 872 mg/kg[47-49].

Diener *et al.*[50] have shown that oral doses of 5-ASA of 30 mg/kg and 200 mg/kg daily for four weeks failed to produce any adverse effects on kidney function or histology in rat.

In a 13-week rat study, there were no renal lesions after four weeks in the animals receiving up to 160 mg/kg orally per day, but severe papillary necrosis and proximal tubular injury were seen in most animals receiving 640 mg/kg orally per day. At 13 weeks, the female animals were free of pathology up to 160 mg/kg; minimal and reversible lesions in the tubules occurred in a few males (with no changes in renal function) at the 40 mg/kg/day level. After six months of oral administration in dogs, no toxicity was seen in the 40 mg/kg/day group. At 80 mg/kg/day, two of eight treated dogs showed slight to moderate renal papillary necrosis. These dogs as well as two others showed minimal to moderate tubular lesions. At 120 mg/kg/day, two females had slight papillary necrosis. These and two others showed minimal to moderate tubule injury.

5-ASA rectal irritation challenge in dogs:

A rectal mucosa irritation study was designed and conducted to determine if 5-ASA rectal suspension causes any mucosal tissue stress either histologically or macroscopically. The test was carried out blind against placebo, administering one rectal suspension per day.

Treated dogs (n = 10) received 2.0 g of 5-ASA which was retained for an average of 5.5 hours over the 27-day study. The placebo group (n = 6) received suspensions of the same vehicle composition, but without 5-ASA. Calcium carbonate and food colouring were used in place of 5-ASA to mimic its appearance in the suspension formula. The rectal suspension control group (n = 2) received physiological saline enemas of equivalent volume daily. Seven days prior to dosing and after Days 15 and 30, all animals were given a proctologic examination with rectal biopsy. The histopathology data revealed no signs of significant irritation in either the treated or the control group. There was an increased incidence of edema of the lamina propria of the rectum in both the treated and control groups. These lesions represent the mildest form of

inflammation normally expected in the rectum. This mucosal inflammation is a completely reversible alteration and is probably the result of mild superficial irritation. There was no significant difference in the incidence and or severity of these changes between the treated and control groups.

The anorectal examination data revealed no signs of irritation in either treated or control group animals. The amount of mucus present in the rectum increased with time in all dogs, but did not exceed minimal severity. There was no significant difference between treated and control groups in the incidence and/or degree of severity of anorectal examination.

In conclusion, these data indicated no significant rectal mucosal tissue irritation in dogs related to the daily rectal administration of 2 g of 5-aminosalicylic acid rectal suspension over a period of 27 days.

REFERENCES

1. MICROMEDEX®: Mesalazine. DRUGDEX® Evaluations. April 2010.

2. Lowry PW, Franklin CL, Weaver AL, Szumlanski CL, Mays DC, Loftus EV, et al. Leucopenia resulting from a drug interaction between azathioprine or 6-mercaptopurine and mesalamine, sulphasalazine, or balsalazide. Gut 2001; 49(5):656-64.

3. Dewit O, Vanheuverzwyn R, Desager JP, Horsmans Y. Interaction between azathioprine and aminosalicylates: an in vivo study with Crohn's disease. Aliment Pharmacol Therap 2002; 16(1):79-85.

4. Witt KL, Bishop JB, McFee AF, Kumaroo V. Induction of chromosomal damage in mammalian cells in vitro and in vivo by sulfapyridine or 5-aminosalicylic acid. Mutat Res 1992; 283(1):59-64.

5. Amin HE, Della Siega AJ, Whittaker JS, Munt B. Mesalamine-induced chest pain: A case report. Can J Cardiol 2000; 16(5):667-9.

6. Stasinopoulou P, Kaziani A, Mantzaris G, Roussos A, Skoutelis A. Parallel manifestation of Crohn's disease and acute pericarditis: A report of two cases. Int J Colorectal Dis. 2007; 22:1123-25.

7. Cappell MS, Turkieh A. Chronic pericarditis and pericardial tamponade associated with ulcerative colitis. Dig Dis Sci 2008; 53:149-54.

8. Singleton JW, Law DH, Kelley ML, Mekhjian HS, Sturdevant RA. National Cooperative Chron's Disease Study: Adverse reactions to study drugs. Gastroenterology 1979; 77(4Pt 2):870-82.

9. Christensen LA. 5-Aminosalicylic acid containing drugs. Delivery, fate and possible clinical implications in man. Dan Med Bull 2000; 47(1):20-41.

10. Klotz U, Haring-Kaim A. Negligible excretion of 5-aminosalicylic acid in breast milk. Lancet 1993; 342(8871):618-9.

11. Jenss H, Weber P, Hartmann F. 5-Aminosalicylic acid and its metabolite in breast milk during lactation. Am J Gastroenterol 1990; 85(3):331.

12. Silverman DA, Ford J, Saw I, Probert C. Is mesalazine really safe for use in breastfeeding mothers? Gut 2005; 54:169-73.

13. Ito S, Blajchman A, Stephenson M, Eliopoulos C, Koren G. Prospective follow-up

of adverse reactions in breast-fed infants exposed to maternal medication. Am J Obstet Gynecol 1993; 168(5):1393-9.

14. Nelis GF. Diarrhoea due to 5-aminosalicylic acid in breast milk. Lancet 1989; 1(8634):383.

15. American Academy of Pediatrics. The transfer of drugs and other chemicals into human milk. Pediatrics 2001; 108(3):776-89.

16. Campieri M., Lanfranchi G, Brignola C, Bazzocchi G, Miguzzi M, Calari M. 5-Aminosalicylic acid as rectal enema in ulcerative colitis patients unable to take sulfasalazine. Lancet 1984; 1(8373):403.

17. Campieri M, Lanfranchi GA, Brignola C, Bazzocchi G, Miguzzi MR, Giochetti P. High-dose 5-aminosalicylic acid enemas in the treatment of ulcerative colitis. Intern Med Specialist 1984; 5:164-171.

18. Young BA, McLeod RS, Cohen Z. Exacerbation of ulcerative colitis following administration of 5-ASA enemas. Can J Gastroenterology 1989; 3(2):50-2.

19. Rachmilewitz D. Coated mesalazine (5-aminosalicylic acid) versus sulphasalazine in the treatment of active ulcerative colitis: A randomized trial. Br Med J 1989; 298(6666):82-86.

20. Sachedina B, Saibil F, Cohen LB, Whittey J. Acute pancreatitis due to 5-aminosalicylate. Ann Inter Med 1989; 110(6):490-2.

21. May B. Treatment of chronic inflammatory bowel diseases. A study of mesalazine under conditions of practice with more than 1700 patients.. Münch Med Wschr 1987; 129(43):786-789 (Published in German).

22. Welte T, Hamm H, Fabel H. Mesalazine alveolitis. The Lancet 1991; 338:1273.

23. Dew MJ, Hughes PJ, Harries AD, Williams G, Evans BK, Rhodes J. Maintenance of remission in ulcerative colitis with oral preparations of 5-aminosalicylic acid. Br Med J 1982; 285: (6347):1012 & 1355.

24. Malonne H, Fontaine J. Drug interactions: II. Interactions encountered in digestive diseases. J Pharm Belg 1997; 52(2):65-68.

25. World Health Organization. Weekly Epidemiological Record. 1998; 73:241-8. http://www.who.int/docstore/wer/pdf/1998/wer7332.pdf

26. Ahnfelt-Ronne, Nielsen OH. The anti-inflammatory moiety of SAS 5-aminosalicylic acid is a radical scavenger. Agents and Actions 1987; 21(1-2):191-4.

27. Ligumsky M, Karmeli F, Sharon P, Zor U, Cohen F, Rachmilewitz D. Enhanced thromboxane A2 and prostacyclin production in ulcerative colitis and its inhibition by steroids and sulfasalazine. Gastroenterology 1981; 81:444-9.

28. Goldin E, Rachmilewitz D. Prostanoids cytoprotection for maintaining remission in ulcerative colitis. Failure of 15(*R*), 15-methylprostaglandin E_2. Digest Dis Sci 1983; 28:807-811.

29. Sharon P, Ligumsky M, Rachmilewitz D, Zor U. Role of prostaglandins in ulcerative colitis. Enhanced production during active disease and inhibition by sulfasalazine. Gastroenterology 1978; 75(4):638-40.

30. Smith PR, Dawson DJ, Swan CH. Prostaglandin synthetase activity in acute ulcerative colitis: Effects of treatment with sulphasalazine, codeine phosphate and prednisolone. Gut 1979; 20(9):802-5.

31. Harris DW, Smith PR, Swan CH. Determination of prostaglandin synthetase activity in rectal biopsy material and its significance in colonic disease. Gut 1978; 19(10):875-7.

32. Sircar JC, Schwender CF, Carethers ME. Inhibition of soy bean lipoxygenase by sulfasalazine and 5-aminosalicylic acid: A possible mode of action in ulcerative colitis. Biochem Pharmacol 1983; 32(1):170-2.

33. Patterson DJ, Colony PC. Anti-secretory effects of sulfasalazine and 5-aminosalicylic acid in experimental colitis. Gastroenterology 1983; 1271.

34. Musch MW, Miller RJ, Field M, Siegel MI. Arachidonic acid metabolism and colonic secretion. Gastroenterology 1983; 84(5):1062-3.

35. Gionchetti P, Guarnieri C, Campieri M, Belluzzi A, Brignola C, Iannone P, et al. Scavenger effect of sulfasalazine, 5-aminosalicylic acid and olsalazine on superoxide radical generation. Dig Dis Sci 1991; 36(2):174-8.

36. Nielson OH, Bouchelouche PN, Berild D, Ahnfelt-Ronne I. Effect of 5-aminosalicylic acid and analogous substances on superoxide generation and intracellular free calcium in human neutrophilic granulocytes. Scan J Gastroenterol 1993; 28(6):527-32.

37. Pearson DC, Jourd'heuil, Meddings JB. The anti-oxidant properties of 5-aminosalicylic acid. Free Radic Biol Med 1996; 21(3):367-73.

38. Schroder H, Campbell DES. Absorption, metabolism, and excretion of salicylazosulfapyridine in man. Clin Pharmacol Ther 1972; 13:539-551.

39. Azad Khan AK, Piris J, Truelove SC. An experiment to determine the active therapeutic moiety of sulfasalazine. Lancet 1977; 2(8044):892-5.

40. van Hees PAM, Bakker JH, van Tongeren JHM. Effect of sulfapyridine, 5-aminosalicylic acid, and placebo in patients with idiopathic proctitis: A study to determine the active therapeutic moiety of sulfasalazine, in man. Gut 1980; 21(7):632-635.

41. Fischer C, Maier K, Stumpf E, von Gaisberg U, Klotz U. Disposition of 5-aminosalicylic acid, the active metabolite of sulfasalazine, in man. Eur J Clin Pharmacol 1983; 25(4):511-515.

42. Sutherland LR, Martin F. 5-Aminosalicylic acid enema in the maintenance of remission in distal ulcerative colitis and proctitis. Can J Gastroenterology 1987; 1(1):3-6.

43. Klotz U. Clinical pharmacokinetics of sulfasalazine its metabolites and other prodrugs of 5-aminosalicylic acid. Clin Pharmacokinetic 1985; 10(4):285-302.

44. Williams CN, Haber G, Aquino JA. Double-blind, placebo-controlled evaluation of 5-ASA suppositories in active distal proctitis and measurement of extent of spread using ^{99m}Tc-labeled 5-ASA suppositories. Digestive Diseases and Sciences 1987; 32(12):71S-75S.

45. Aumais G, Lefebvre M, Tremblay C, Bitton A et al. Rectal tissue, plasma and urine concentrations of mesalazine after single and multiple administrations of 500 mg suppositories to healthy volunteers and ulcerative proctitis patients. Aliment Pharmacol Therap 2003; 17:93-97.

46. Barnett KC, Joseph EC. Keratoconjunctivitis sicca in the dog following 5-aminosalicylic acid administration. Human Toxicol 1987; 6:377-83.

47. Calder IC, Funder CC, Green CR, Ham KN, Tange JD. Comparative nephrotoxicity of aspirin and phenacetin derivatives. Br Med J 1971; 4(786):518-521.

48. Calder IC, Funder CC, Green CR, Ham KN, Tange JD. Nephrotoxic lesions from 5-aminosalicylic acid. Br Med J 1972; 1:152-154.

49. Calder IC, Williams PJ, Woods RA, Funder CC, Green CR, Ham KN, et al. Nephrotoxicity and molecular structure. Xenobiotica 1975; 5(5):303-307.

50. Diener U, Tuczek HV, Fischer C, Maier K, Klotz U. Renal function was not impaired by treatment with 5-aminosalicylic acid in rats and man. Arch Pharmacol 1984; 326(3):278-82.

51. Hanauer S. Ulcerative Proctitis. Practical Gastroenterology 1991; Special Suppl:10-14.

52. Schroeder KW. Role of mesalazine in acute and long-term treatment of ulcerative colitis and its complications. Scan J Gastroenterol 2002; 37(Suppl 236):42-7.

53. Williams CN. Efficacy and tolerance of 5-aminosalicylic acid suppositories in the treatment of ulcerative proctitis: A review of two double-blind, placebo controlled trials. Can J Gastroenterol 1990; 4(7):472-475.

54. Bouhanick B, Fauvel J. Pont F. Biochemical misdiagnosis of pheochromocytoma in patients treated with sulfasalazine. JAMA (2010) 304: 1898-1901.

55. Ito T, Imai T, Kikumori T, Shibata A, Horiba T, Kobayashi H, Sawaki M, Watanabe R, Nakao A, Kiuchi T. Adrenal incidentaloma: review of 197 patients and report of a drug-related false-positive urinary normetanephrine result. Surg. Today (2006) 36: 961-965.

56. Walsh N. Sulfasalazine induced falsely positive urinary catecholamines. Rheumatology New'5(8), 11.2006

PART III: CONSUMER INFORMATION

Pr SALOFALK®
Mesalamine Suppositories
500 mg, 1000 mg

This leaflet is part III of a three-part "Product Monograph" and is designed specifically for Consumers. This leaflet is a summary and will not tell you everything about SALOFALK®. Contact your doctor or pharmacist if you have any questions about the drug.

ABOUT THIS MEDICATION

What the medication is used for:
SALOFALK® suppositories 500 or 1000 mg, are used in the management of ulcerative proctitis (inflamed rectum) and as adjunctive therapy in more extensive distal ulcerative colitis (inflammation of the lining of the large bowel and rectum).

What it does:
SALOFALK® is believed to work by interfering in the activity of certain mediators of inflammation (e.g., prostaglandins) which helps reduce the inflammation (swelling and pain) in the rectum and lower part of the large bowel.

When it should not be used:
SALOFALK® should not be used if:

- patients with severe kidney (renal) impairment (GFR<30ml/min/1.73m^2) and/or severe liver (hepatic) impairment (see WARNINGS AND PRECAUTIONS)
- You are allergic to mesalamine or to any ingredient in the formulation (see **What the non-medicinal ingredients are**)
- You have a sensitivity to salicylates, for example acetylsalicylic acid (Aspirin®)
- You have stomach or small intestinal ulcers
- You have urinary tract obstructions
- The patient is an infant under two years of age

What the medicinal ingredient is:
SALOFALK® contains mesalamine (me-SAL-a-meen), also known as 5-aminosalicylic acid, 5-ASA or mesalazine.

What the non-medicinal ingredients are:
SALOFALK® suppositories contain Witepsol H-15 (suppository wax base). SALOFALK® Suppositories are gluten-free and phthalate-free.

What dosage forms it comes in:
SALOFALK® suppositories 500 or 1000 mg are available in strips of 6 suppositories; boxes of 30 suppositories.

WARNINGS AND PRECAUTIONS

BEFORE you use SALOFALK® talk to your doctor or pharmacist if:

- You have a pre-existing liver disease. There have been reports of hepatic failure and increased liver enzymes in patients treated with 5-ASA or mesalazine (=mesalamine) products
- You have mild to moderate liver function impairment. Your doctor will decide if this product is right for you
- You ever had any unusual or allergic reaction to mesalamine (5-ASA), sulfasalazine (SAS), or salicylates (Aspirin®)
- You have liver or kidney disease
- You have bleeding or clotting disorders
- Your doctor has said you have higher than normal blood urea nitrogen (BUN) levels (renal function test)
- You are pregnant or breastfeeding. Mesalamine is excreted in human breast milk. Discuss with your doctor.

WHILE using SALOFALK®:

- Discontinue use at first sign of rash or fever.

You may have your blood or urine tested regularly to monitor your kidney function since prolonged use of SALOFALK® may damage your kidneys.

INTERACTIONS WITH THIS MEDICATION

Interaction between azathioprine, 6-mercaptopurine and aminosalicylates (such as SALOFALK®) has been reported.

Drug interactions with coumarin, methotrexate, probenecid, sulfinpyrazone, spironolactone, furosemide, rifampicin and Varicella Virus Vaccine (chicken pox vaccine) may be possible.

Possible interference with measurements, by liquid chromatography, of urinary normetanephrine causing a false-positive test result have been observed in patients exposed to sulfasalazine or its metabolite, mesalamine/mesalazine.

PROPER USE OF THIS MEDICATION

Usual adult dose:
One 500 mg SALOFALK® suppository is self-administered on a twice a day or three times a day basis. One 1000 mg SALOFALK® suppository is self-administered on a once daily basis, at bedtime. The usual adult dose is 1.0 - 1.5 g/day and dosing is continued until a significant response is achieved or until the patient achieves remission. Dose tapering is recommended. Abrupt discontinuation is not recommended. Best results are expected with prolonged

retention.

The suppository should be retained in the rectum for one to three hours or longer, if possible, to achieve the maximum benefit.

How to use SALOFALK® suppositories:

NOTE: SALOFALK® suppositories will cause staining of direct contact surfaces, including but not limited to fabrics, flooring, painted surfaces, marble, granite, vinyl, and enamel.

Patient Instructions:

- Detach one suppository from the strip of suppositories.
- Hold suppository upright and carefully remove the plastic wrapper.
- Avoid excessive handling of suppository, which is designed to melt at body temperature.
- Insert suppository completely into rectum with gentle pressure, pointed end first.
- A small amount of lubricating gel may be used on the tip of the suppository to assist insertion.

Overdose:

If you believe you have used too much, or in case of accidental oral ingestion, contact your doctor, hospital emergency department or regional Poison Control Centre immediately, even if there are no symptoms.

Missed Dose:

If you miss a dose of SALOFALK®, use it as soon as possible, unless it is almost time for the next dose. Do not use two SALOFALK® suppositories at the same time to make up for a missed dose.

SIDE EFFECTS AND WHAT TO DO ABOUT THEM

Rash, fever, and dizziness are common in patients allergic to sulfasalazine. Stop therapy at the first sign of a rash and contact your doctor.

Worsening of ulcerative colitis may occur and may include the following symptoms: abdominal or stomach cramps or pain (severe) and diarrhoea.

Other reported side effects reported with SALOFALK® suppositories include abdominal cramps, abdominal pain or discomfort, anal itching, anorectal discomfort, bloating, constipation, cough, diarrhoea, dizziness, flatulence, fever, frequent bowel movements, hair loss, headache, itching, lower back pain, mucus in stools, nausea, pain, painful bowel movements, rash, rectal discharge, rectal pain, stools discoloured and vomiting.

SIDE EFFECTS, HOW OFTEN THEY HAPPEN AND WHAT TO DO ABOUT THEM

Symptom / effect		Talk with your doctor or pharmacist		Stop taking drug and call your doctor or pharmacist
		Only if severe	In all cases	
Uncommon	Chest pain			✓
Unknown	Pancreatis (inflammation of the pancreas) with symptoms such as abdominal pain, nausea, vomiting, fever, rapid heartbeat, and feeling tired.			✓
	Allergic (hypersensitivity) reaction with symptoms such as rash, itching, fever, swelling of the mouth and throat, difficulty in breathing.			✓
	Myocarditis/ Pericarditis (inflammation of the heart muscle and lining around the heart) with symptoms such as abnormal heartbeat, fatigue, fever, difficulty in breathing, accumulation of fluid in the lung, and coughing.			✓
	Liver problems with symptoms such as severe abdominal pain, nausea, vomiting, yellowing of the skin and eyes, drop in appetite, bloating and distension.			✓

SIDE EFFECTS, HOW OFTEN THEY HAPPEN AND WHAT TO DO ABOUT THEM

Symptom / effect		Talk with your doctor or pharmacist		Stop taking drug and call your doctor or pharmacist
		Only if severe	In all cases	
Unknown	Acute intolerance syndrome with symptoms such as cramping, stomach pain, bloody and excessive stools, fever, headache and rash.			✓
	Interstitial pneumonia (lung abnormality with scarring) with symptoms such as difficulty in breathing, dry cough, fever, and persistent unwell feeling.			✓
	Aplastic anaemia (shortage of one or more types of blood cells) with symptoms such as fatigue, difficulty in breathing with exertion, rapid or irregular heartbeat, pale skin, frequent or prolonged infections, unexplained or easy bruising, nosebleeds and bleeding gums, prolonged bleeding from cuts, skin rash, dizziness, and headache.			✓

This is not a complete list of side effects. For any unexpected effects while taking SALOFALK®, contact your doctor or pharmacist.

HOW TO STORE IT

SALOFALK® suppositories must be stored below 25°C (77°F). Can be refrigerated. Keep away from direct heat, light and humidity. Keep out of reach of children.

REPORTING SUSPECTED SIDE EFFECTS

You can report any suspected adverse reactions associated with the use of health products to the Canada Vigilance Program by one of the following 3 ways:

- **Report online at www.healthcanada.gc.ca/medeffect**
- **Call toll-free at 1-866-234-2345**
- **Complete a Canada Vigilance Reporting Form and:**
  - **Fax toll-free to 1-866-678-6789, or**
  - **Mail to: Canada Vigilance Program**
    Health Canada
    Postal Locator 0701D
    Ottawa, Ontario
    K1A 0K9

Postage paid labels, Canada Vigilance Reporting Form and the adverse reaction reporting guidelines are available on the MedEffect™ Canada Web site at www.healthcanada.gc.ca/medeffect.

NOTE: Should you require information related to the management of side effects, contact your health professional. The Canada Vigilance Program does not provide medical advice.

MORE INFORMATION

This document plus the full product monograph, prepared for health professionals can be found at:
http://www.aptalispharma.com
or by contacting the sponsor, Aptalis Pharma Canada Inc., at: 1-800-565-3255

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