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Site-specific delivery of anti-inflammatory drugs in the gastrointestinal tract: an in-vitro release model

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Abstract

Mesalazine and budesonide are anti-inflammatory drugs that are used to induce and maintain remission of inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis. Both drug substances are intended to act locally at the inflamed sites of the gastrointestinal tract. The therapeutic objective for per oral treatment with these drugs is to achieve a high concentration of the active drug at the sites of inflammation while minimizing systemic absorption. The aim of this study was to develop a test system able to reflect the changing environment that a dosage form incorporating the anti-inflammatory agent is exposed to as it moves through the gastrointestinal tract. The USP dissolution apparatus 3 was used for all experiments. Compendial, as well as biorelevant, media were used to simulate passage through the gastrointestinal tract under various physiological conditions. Different dosage forms of mesalazine (5-aminosalicylic acid, 5-ASA) and budesonide available on the German market were tested. Although all dosage forms were indicated for the same therapeutic objectives, each of the dosage forms exhibited a characteristic release pattern under in-vitro conditions simulating a passage through the fasted-state gastrointestinal tract. Results from this test series indicate that, in the case of various dosage forms of mesalazine and budesonide used for the therapy of Crohn's disease and ulcerative colitis, release patterns as the dosage form moves through the gastrointestinal tract may vary widely. As the various phenotypes of IBD have different requirements in terms of pattern of distribution of the inflamed sites, and because other aspects of gastrointestinal physiology vary within the patient population, the test methods and approach described here should be very useful in designing therapy tailored to the needs of each individual patient.

Introduction

Inflammatory bowel disease (IBD) is a chronic and debilitating illness prevalent in the Western population. The aetiopathogenesis has not been clearly elucidated but is thought to involve a complex interplay among genetic, environmental, microbial and immune factors (Lim & Hanauer 2004a). The inflammation process is most likely facilitated by defects in both the barrier function of the intestinal epithelium and the mucosal immune system (Podolsky 2002). IBD is characterized by chronic intestinal inflammation that often shows an intermittent course with acute attacks followed by periods of remission. IBD can manifest itself in a variety of forms, the most common being Crohn's disease and ulcerative colitis. These two diseases can present very similarly in terms of clinical symptoms even though their inflammation patterns are distributed differently in the gastrointestinal tract. The variability in extent and severity of disease have led to various diagnostic and therapeutic schemes (Stange et al 2003). Because IBD is chronic and typically presents initially before 30 years of age, patients generally require lifelong treatment. Most of the currently available agents act by downregulating the chronic inflammation in the intestinal mucosa, which is believed to underlie disease pathogenesis (Hanauer & Present 2003). The primary goal of anti-inflammatory therapy is induction and maintenance of remission (Hanauer 1996) using agents that are effective and cause minimal adverse events. This paper focuses on an in-vitro assessment of orally administered dosage forms of the anti-inflammatory drugs mesalazine and budesonide for local therapy of chronic inflammation in both Crohn's disease and ulcerative colitis with respect to the site specificity of release in the gastrointestinal tract.

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Crohn's disease is a chronic transmural inflammation of the bowel that can affect the whole gastrointestinal tract, usually in a discontinuous pattern. The initial location of Crohn's disease is most commonly in the lower ileum. From here, the inflammation typically spreads towards proximal parts of the small intestine. However, the colon is also often involved. Depending on location and extent of the disease, the clinical manifestations can vary markedly. Currently it is not possible to cure Crohn's disease; the main objective of therapy is to contain the inflammation. Therefore, treatment is dominated by anti-inflammatory drugs, including oral or topical preparations of corticosteroids and mesalazine, which are both prescribed in all phases of the disease.

Ulcerative colitis is a chronic IBD affecting only the colon and which shows a continuous distribution in the gastrointestinal mucosa. Most patients suffer from a proctitis wherein the focal point of the inflammation is found in the distal part of the colon and the rectum. From this origin, the inflammation often spreads proximally. If the disease affects only the left side of the colon, it is called limited or distal colitis. In the most severe cases, the whole colon is affected and one speaks of a pancolitis. About 30% of patients suffer from this severe form of ulcerative colitis. As with Crohn's disease, it is not possible to cure ulcerative colitis. General aims of treatment are, therefore, to bring acute attacks into remission and thereafter to prevent relapse. Here, too, anti-inflammatory drugs dominate medical treatment.

For many years mesalazine (mesalamine, 5-aminosalicylic acid, 5-ASA) has played an important role in the treatment and maintenance therapy of mild to moderately active Crohn's disease and ulcerative colitis (Hanauer 1996; Podolsky 2002; Biancone et al 2003). Its main principle of action is a topical reduction of inflammation in the mucosa (Azad Khan et al 1977). Mesalazine is poorly absorbed from the colon (Bondesen et al 1988) but since it is rapidly absorbed from the upper digestive tract (Myers et al 1987) and has limited activity when administered systemically (due to inefficient redistribution of the mesalazine to the sites of inflammation (Bondesen 1997)), it is desirable to minimize absorption across the gut wall. In addition, systemic absorption leads to unwanted systemic side-effects. Based on these considerations, oral mesalazine dosage forms should release the active substance selectively at the inflamed areas in the gastrointestinal tract. Because of the different disease patterns of Crohn's disease and ulcerative colitis, different formulations are required to adequately treat different patient subgroups. This means that treatment must begin with accurate diagnosis regarding location, severity and extent of the disease so that the appropriate drug delivery system can be selected for each patient. Currently marketed formulation concepts for oral treatment include tablets coated with gastric-resistant pH-sensitive polymers, microspheres that release the active drug via diffusion controlled mechanism and enteric-coated microspheres that are intended to release independently of pH once they have passed into the small intestine.

The use of pH-sensitive acrylate-based polymers represents the leading formulation approach to the oral treatment of IBD, with three of the four products currently available on the German market for the treatment of Crohn's disease or ulcerative colitis utilizing poly(meth)acrylates (PMMA) to modify the release profile of mesalazine (Rudolph et al 2001) (Table 1). The fourth product is a tablet containing slow-release ethylcellulose-coated microgranules of mesalazine. Following administration, the tablet quickly disintegrates in the stomach and each microgranule then acts as a discrete, slow-release formulation.

Corticosteroids were the first medications to be evaluated systemically in patients with IBD (Hanauer & Present 2003). Orally administered corticosteroids are effective in patients with active Crohn's disease and ulcerative colitis. However, oral systemic corticosteroid therapy is typically connected with steroid-associated side-effects. Hence, corticosteroid therapy is indicated primarily for the short-term induction of a remission of severe flare-ups and not

Table 1 Mesalazine and budesonide dosage forms used in the study (all available in Germany)

Product	Dosage form	Polymer type	Brand name of polymer	Release pH
Mesalazine				
Claversal	Coated tablet	MA–MM (1:1)	Eudragit L	> pH 5.5
Salofalk	Coated tablet	MA–MM (1:1)	Eudragit L	> pH 5.5
Salofalk Granustix	Coated microgranules	Coating: MA–MM (1:1) Core: MM neutral esters	Eudragit L Eudragit NE	> pH 5.5
Pentasa	Immediate-release tablet containing coated microgranules	Ethylcellulose	Surelease	pH independent
Budesonide				
Budenofalk	Capsule containing coated microgranules	MA–MM (1:1/1:2) cationic MMs	Eudragit L/S (Eudragit RL/RS)	> pH 6.4
Entocort	Capsule containing coated microgranules	MA–MM (1:1) ethylcellulose	Eudragit L	> pH 5.5

MA, methacrylic acid; MM, methacrylate esters.

as maintenance therapy (Hanauer 1996; Podolsky 2002). In terms of dissociating the anti-inflammatory effects from the unwanted side-effects of oral glucocorticosteroids, topically active compounds such as budesonide have been developed. The pharmacokinetic profile of budesonide favours a high topical efficacy, because of rapid uptake by mucosal tissue and enhanced receptor-binding properties (Campieri 2002). During and following systemic absorption, budesonide is metabolized to inactive compounds, minimizing the corticoid-related systemic side-effects. Similarly to mesalazine, different formulations of budesonide would be required to provide an adequate topical treatment of the inflamed areas in different patient subgroups. To date, ulcerative colitis has been treated with budesonide solely in the form of enemas. By contrast, two different pH-dependent oral preparations of budesonide are available on the German market for the treatment of Crohn's disease located in the distal ileum, ileocaecal region and ascending colon (Table 1). Both oral formulations are capsules containing microgranules that consist of a sugar core with a polymer layer, in which budesonide is suspended, and an enteric coating. Entocort microgranules are coated with Eudragit L, which dissolves at pHs above 5.5, while Budenofalk microgranules are designed to release the active drug when the pH exceeds 6.4 (information provided by the manufacturer).

The aim of this study was to evaluate the ability of the different formulations containing mesalazine or budesonide to release drug under in-vitro conditions simulating a passage through different sections of the gastrointestinal tract, and subsequently to identify which of the formulations might be most suitable for the various distinct patient subgroups with Crohn's disease or ulcerative colitis. Special attention was paid to simulating physiological conditions regarding pH values, composition of the gastrointestinal fluids and passage times, taking the usual dosing conditions into consideration.

Materials and Methods

Materials

Mesalazine drug substance (lot no. 127H1055) was purchased from Sigma-Aldrich (Steinheim, Germany). Claversal 500 mg (lot no. B20295; Merckle GmbH, Ulm, Germany) and Pentasa 500 mg (lot no. FK326T; Ferring Arzneimittel GmbH, Kiel, Germany) were kindly donated by their manufacturers. Salofalk and Salofalk Granustix (lot no. 00L04904/E and lot no. 01G26001L; Dr Falk Pharma, Freiburg, Germany) were purchased commercially. Budesonide standard substance (lot no. 32450453) was purchased from Caelo (Hilden, Germany). Entocort (lot no. DH1365A2; AstraZeneca, Wedel, Germany) and Budenofalk (lot no. 02G25070L; Dr Falk Pharma, Freiburg, Germany) capsules were purchased commercially. Egg phosphatidylcholine, EPC (lot no. 105019-5), was kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Taurocholic acid sodium salt was purchased from Tiefenbacher (Hamburg, Germany). Potassium dihydrogen phosphate, potassium hydrogen phosphate, sodium chloride, sodium dihydrogen phosphate, sodium hydrogen phosphate, sodium hydroxide and hydrochloric acid were all of analytical grade and purchased commercially from Merck (Darmstadt, Germany).

Dissolution test set-up

Drug release experiments ($n = 3$ per formulation and set-up) were performed with a BIO-DIS Release Rate Tester (RRT 8; CALEVA Ltd, Dorset, UK). The volume of media was 220 mL (compendial media) or 200 mL (biorelevant media) at 37 ± 0.5°C. Mesh sizes of 420 μm were used for both the top and bottom mesh. For all experiments, a dip rate of 10 dips/min was used (Rohrs et al 1995). The samples were periodically removed using a plastic syringe and immediately filtered through a 0.45-μm Teflon filter (FP 030/2; Schleicher & Schuell GmbH, Dassel, Germany). No adsorption to these filters was observed for either mesalazine or budesonide. Experiments were run at least in triplicate and results expressed as mean % ($\pm$s.d.) dissolved at the given sampling time.

All enteric-coated dosage forms containing mesalazine or budesonide, respectively, were recommended for administration in the fasted state. Based on this consideration, the test set-up used in this study was created to simulate fasted conditions of the gastrointestinal tract.

Residence times

In the last two decades, various gamma-scintigraphic studies have been performed to examine the gastrointestinal transit time of different types of dosage forms (Davis et al 1984, 1986; Christensen et al 1985; Coupe et al 1991) and also to determine whether transit time is influenced by intake of food. From the results of these studies, it can be concluded that the average small intestinal transit time (SITT) of a dosage form is approximately 180 ($\pm$ 60) min (Coupe et al 1991) and is apparently unaffected by physical form (Davis et al 1986) or dietary condition (Christensen et al 1985). However, the gastric emptying time can be controlled by various influences, including the size and shape of the dosage form (Davis et al 1986), presence or absence of food (Davis et al 1986), exercise, stress and body posture. Data from more recent studies using newer test methods (e.g. magnetic marker monitoring, MMM) have confirmed these conclusions (Weitschies et al 1997, 1999; Weitschies 2001).

For the simulation of fasted-state residence times in the different regions of the gastrointestinal tract, mean transit times reported in several gamma-scintigraphy studies were utilized. The difference in gastric emptying time (GET) between tablets and small particles (≤ 2 μm) (Davis et al 1986; Hardy et al 1993; Weitschies 2001) was accounted for by using different gastric residence times for tablets and small particles in the release model (Table 2).

Table 2 Media and residence times used for the in-vitro release studies

	pH	Compendial media	Biorelevant media	Residence time (min)	
				Tablets	Granules
Stomach	1.8	SGFsp[a]	SGFplus	60	30
Proximal jejunum	6.5	SIFsp USP[a]	FaSSIF	15	45
Distal jejunum	6.8	SIFsp USP 26	FaSSIF[a]	15	45
Proximal ileum	7.2	SIFsp USP[a]	FaSSIF[b]	30	45
Distal ileum	7.5	SIFsp USP 23[a]	Blank FaSSIF[a]	120	45
Ascending colon	6.5	SIFsp USP[a]	Blank FaSSIF	360[c]	360[c]
Transverse colon	6.5	SIFsp USP[a]	Blank FaSSIF	240[c]	240[c]
Descending colon	6.8	SIFsp USP 26	Blank FaSSIF[a]	360 + 240[c]	360 + 270[c]

[a]pH modified; [b]pH and content of bile components modified; [c]residence times in the colon can vary strongly.

Dissolution media

Phase 1 of the test series entailed the testing of each product in compendial media adjusted to the pH of the region of maximal absorption (simulated intestinal fluid without enzymes, SIFsp, USP 26, pH 6.8) and the main site of inflammation in the case of Crohn's disease (SIFsp, USP 23, pH 7.5).

In phase 2, passage through the gastrointestinal tract was simulated using two different pH-gradient methods: the first was a gradient of compendial media to represent the pH profile, and the second a gradient of biorelevant media simulating both pH conditions and other aspects of the composition of fluids in the fasted gastrointestinal tract, to evaluate whether the release behaviour might be altered by the presence of small concentrations of bile salts and lecithin. An overview of the various media used is given in Table 2.

The pH values used to simulate the different sections in the gastrointestinal tract result from various clinical trials examining the pH profile in healthy subjects and patients with Crohn's disease or ulcerative colitis, respectively (Fallingborg et al 1998; Press et al 1998; Ewe et al 1999; Nugent et al 2001). In the studies cited, it could be clearly shown that gastric and small intestinal luminal pH profiles in both Crohn's disease and ulcerative colitis were similar to those in healthy subjects. The very low intraluminal colonic pH values in IBD that had been reported in the first studies (Fallingborg et al 1993; Sasaki et al 1997) could not be confirmed in later studies. Low intraluminal pH values do seem to occur in certain patients but the underlying mechanism is still uncertain. For testing the release behaviour of the dosage forms used in our study, colonic pH proved not to play a decisive role. Therefore the more often observed pH values of 6.5 and 6.8 were used to simulate proximal and distal parts of the colon. However, future attempts to develop dosage forms that exhibit pH-dependent release in the colon would need to take the incidence of patients with very low intraluminal colonic pH into consideration.

Biorelevant media to simulate conditions in the stomach and small intestine in the fasted state have been developed over the last decade (Galia et al 1998, 1999). To simulate passage through the fasted stomach, SGFplus (Galia et al 1999) was used. In contrast to the compendial medium, SGFsp of the United States Pharmacopeia (USP 2003), SGFplus reflects the mean physiological human gastric pH of about 1.8 (Evans et al 1988), as well as the reduced surface tension that has been repeatedly measured to be in the range of 35–50 $mN\,m^{-1}$ (Finholt & Solvang 1968; Finholt et al 1978; Fell & Mohammad 1995; Efentakis & Dressman 1998). To simulate passage through the small intestine, various modifications of Fasted State simulated Intestinal Fluid (Marques 2004) were used. With this biorelevant dissolution method for the small intestine, it was possible to simulate not only basal bile salt and lecithin concentrations in the duodenum but also their decreasing concentrations due to re-uptake during passage through the more distal parts of the small intestine. Table 3 shows the pH values and the corresponding concentrations of sodium taurocholate and lecithin used in the study.

UV analysis

Samples from the release tests in single media ($n = 2$ per vessel and sampling time point) were analysed at 231 nm (pH 1.8) and 331 ($pH \geq 6$) for mesalazine and at 247 nm for budesonide using a UV-spectrophotometer (U 2000; Hitachi Ltd, Tokyo, Japan) equipped with a 10 mm cuvette. The percentage of drug released was calculated using a standard curve ($R^2 \geq 0.999$) of appropriate standard solutions of mesalazine/budesonide daily prepared in the tested media.

HPLC analysis

The HPLC system consisted of a LaChrom L-7100 pump, a LaChrom L-4250 UV-VIS Detector, a LaChrom L-2000

Table 3 pH values and bile salt concentrations used to simulate passage through the fasted small intestine

	pH	Biorelevant media	Sodium taurocholate concn (mmol L^{-1})	Lecithin concn (mmol L^{-1})
Proximal jejunum	6.5	FaSSIF	3	0.75
Distal jejunum	6.8	FaSSIF[a]	3	0.75
Proximal ileum	7.2	FaSSIF[b]	1.5	0.375
Distal ileum	7.5	Blank FaSSIF[a]	—	—

[a]pH modified; [b]pH and content of bile components modified.

autosampler and EZChrom Elite Chromatography Data System software (Merck Hitachi, Darmstadt, Germany).

Mesalazine

Samples from the pH-gradient method and the biorelevant release tests were analysed using a validated method (Haney & Dash 1997) on a Lichrosorb RP-8, 5 μ, 125 × 4 mm column (Merck, Darmstadt, Germany) using methanol–phosphate buffer pH 7.4 (20:80%) as mobile phase. The flow rate was set at 1.0 mL min^{-1} and detection was at 331 nm. Mesalazine typically eluted after ~2 min.

Budesonide

Samples from the pH-gradient method and the biorelevant release tests were analysed with a validated in-house method (Rudolph 2002) using a Lichrocart RP-18, 5 μ, 125 × 4 mm column (Merck, Darmstadt, Germany) and acetonitrile–purified water (55:45%) as mobile phase. The flow rate was set at 1.0 mL min^{-1}, resulting in elution of budesonide after ~3 min. The amount of released drug was determined using a wavelength of 246 nm.

Results

Mesalazine

Release experiments at different pH values (Figures 1 and 2) show that although all four mesalazine dosage forms are intended for site-specific drug delivery in the small intestine and proximal colon, they exhibited release behaviours quite distinct from one another.

Testing in SIFsp pH 6.8, a medium that reflects pH conditions in the mid-jejunum, led to overt differences in drug release behaviour. With the two enteric-coated tablet formulations, Salofalk 500 mg and Claversal 500 mg, the active drug substance was abruptly and quantitatively released after lag times of 120 and 180 min, respectively (Figure 1). At first glance it seems remarkable that the lag times to onset of drug release differ from each other (about 60 min), because the product descriptions for Claversal and Salofalk are qualitatively identical. However, for enteric-coated products, characteristics of the coating, such as film thickness, are crucial for the onset of drug release. So it is not unreasonable that the release profiles vary at a pH that is representative for the mid-jejunum (pH 6.8). Increasing the pH of the test medium to pH values that are typical of conditions in the distal ileum led to a convergence (particularly in the lag time) of the release profiles. At pH 7.5 it was no longer possible to detect any difference between the two

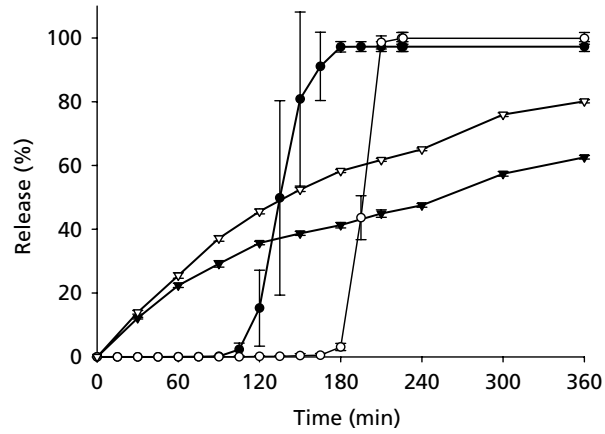

Figure 1 Dissolution profiles of different mesalazine dosage forms in simulated intestinal fluid (SIFsp) USP 26 at pH 6.8 (●, Salofalk 500 mg; ○, Claversal 500 mg; ▼, Pentasa 500 mg; ▽, Salofalk Granustix 500 mg). Data are means ± s.d, n = 3.

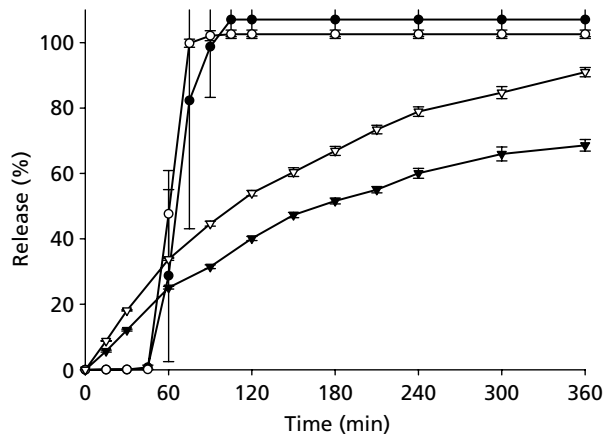

Figure 2 Dissolution profiles of different mesalazine dosage forms in simulated intestinal fluid (SIFsp) USP 23 at pH 7.5 (●, Salofalk 500 mg; ○, Claversal 500 mg; ▼, Pentasa 500 mg; ▽, Salofalk Granustix 500 mg). Data are means ± s.d, n = 3.

tablet formulations (Figure 2). Figures 1 and 2 indicated further that the drug release behaviour of Pentasa, in contrast to release from the enteric-coated products, was influenced little by pH changes within the small intestine. This is to be expected on the basis of its formulation, which is based on a diffusion-controlled release mechanism, as well as the lack of pH dependence of mesalazine solubility in the intestinal pH range. Despite the gastric-resistant (Eudragit L) coating, the overall drug release profile of Salofalk Granustix microgranules was also pH independent under pH conditions that reflected the mid-jejunum and ileum.

The results described thus far, especially the results in SIFsp pH 6.8, are useful illustrations of the clear-cut differences in drug release mechanisms. But since it is likely that the in-vivo drug release of mesalazine dosage forms would be heavily influenced by both the passage time through the various segments of the gastrointestinal tract and the changing composition of the gastrointestinal contents to which the dosage form is exposed, a simple dissolution set-up like the one used to generate the results shown in Figures 1 and 2 is likely not useful to predict the in-vivo release pattern. Figures 3 and 4 show release profiles during a simulated passage through stomach, small intestine and proximal colon. Several carry-over effects were observed for the enteric-coated tablets. Both tablet formulations exhibited release patterns that were influenced by the change in pH between the stomach and small intestine, as well as the residence times at each location. These aspects are important considerations for predicting release behaviour in-vivo. In contrast, release rates of the microgranules in the small intestine appeared not to be influenced by carry-over effects associated with changing media from gastric to intestinal conditions within the course of the experiment. However, also for dosage forms with pH-independent drug release it is necessary to interpret results from a biorelevant gradient as it is well described that drug release can be altered by various physicochemical parameters of the test medium or the gastrointestinal contents, respectively.

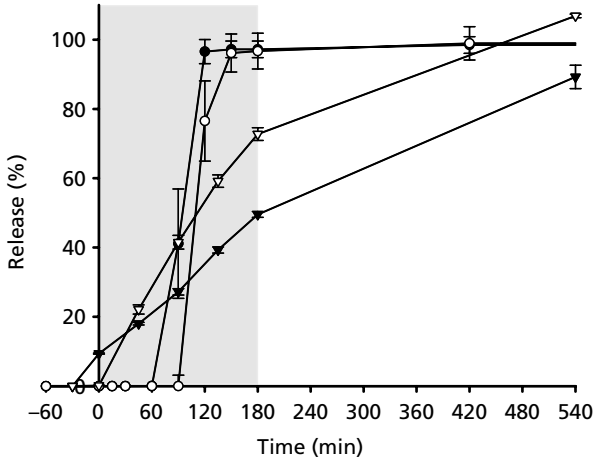

Figure 3 Dissolution behaviour of Salofalk 500 mg (●), Claversal 500 mg (○), Pentasa 500 mg (▼) and Salofalk Granustix 500 mg (▽) in a physiological-based pH gradient prepared from compendial media (negative values represent gastric residence time; shaded part represents drug release during passage through the small intestine). Data are means ± s.d, n = 3.

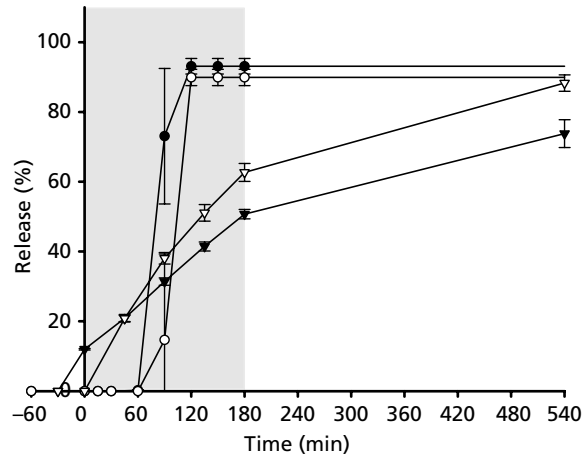

Figure 4 Dissolution behaviour of Salofalk 500 mg (●), Claversal 500 mg (○), Pentasa 500 mg (▼) and Salofalk Granustix 500 mg (▽) in a biorelevant pH gradient (negative values represent gastric residence time, shaded part represents drug release during passage through the small intestine). Data are means ± s.d, n = 3.

Results from both the compendial and the biorelevant pH-gradient method confirm that three of the formulations did not release any drug under gastric conditions within 30 (microgranules) or 60 (tablets) min, respectively. Since release from Pentasa is diffusion-, not pH-controlled, the Pentasa microspheres released a considerable amount of drug even in this medium. In contrast to Pentasa, the microspheres of Salofalk Granustix did not show any drug release in simulated gastric fluid under test conditions and started to release the drug at a constant rate only after passing into the small intestine.

Comparing results from the compendial with those from the biorelevant gradient method, no substantial differences could be found. Therefore the pH and the residence time in the different segments of the gastrointestinal tract are the main determinants of the drug release from the site-specific delivery systems of mesalazine. Based on the release profiles using a biorelevant pH gradient, the amount of mesalazine released from the dosage forms tested was estimated for different sites in the gastrointestinal tract. Assuming human gastrointestinal pH profiles and passage times similar to those used in this study, both tablet formulations are likely to release nearly the whole amount of drug in the proximal ileum, whereas both of the microgranules are expected to release the active drug partly in the small intestine and the proximal colon. As mentioned before, release from Salofalk Granustix starts only after the granules pass into the small intestine, whereas the Pentasa granules start releasing the active drug immediately upon contact with the gastrointestinal fluids. Hence, these two products can be

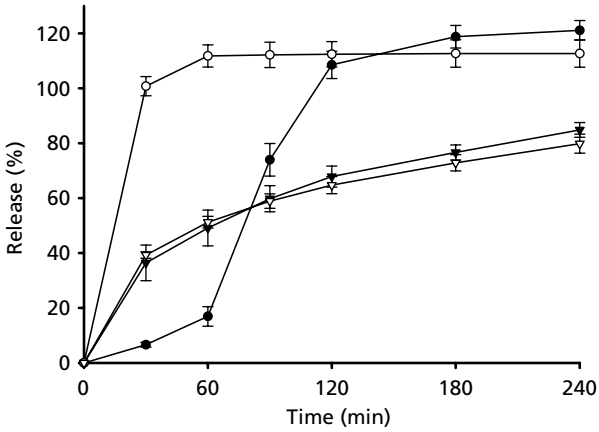

Figure 5 Dissolution profiles of different budesonide dosage forms in simulated intestinal fluid (SIFsp) USP at pH 6.8 (●, Budenofalk 3 mg; ▼, Entocort 3 mg) and pH 7.5 (○, Budenofalk 3 mg; ▽, Entocort 3 mg). Data are means ± s.d, n = 3.

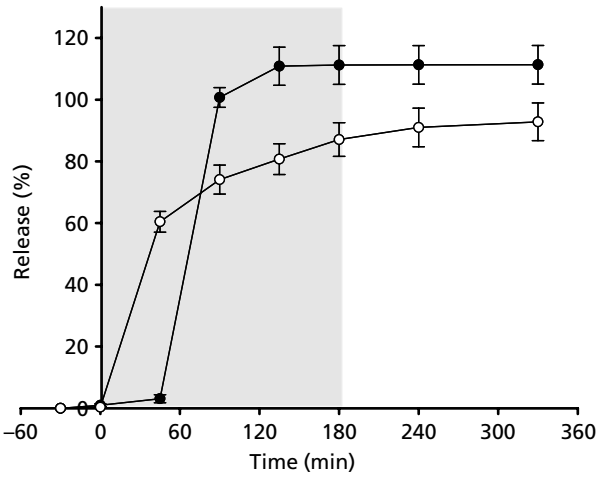

Figure 6 Dissolution behaviour of Budenofalk 3 mg (●) and Entocort 3 mg (○) in a physiological-based pH gradient prepared from compendial media (negative values represent gastric residence time, shaded part represents drug release during passage through the small intestine). Data are means ± s.d, n = 3.

differentiated by their ability, or lack thereof, to release drug in the stomach.

Budesonide

Both marketed budesonide dosage forms are intended to treat Crohn's disease located in the distal ileum, ileocecal region and ascending colon. As for mesalazine, the first series of tests was therefore conducted under two different pH conditions that reflected the mid-jejunum (pH 6.8) and the distal ileum (pH 7.5). Dissolution results from tests in single media indicated that, once the enteric coating has dissolved, drug release from the Entocort microgranules is controlled by the pH-independent ethylcellulose layer. In contrast to the pH-independent behaviour of Entocort, nearly the whole amount of drug was released within 30 min from Budenofalk microgranules at pH 7.5. At pH 6.8, the enteric coating needed much more time to dissolve. This was reflected in a substantial lag period before the main amount of drug was released (Figure 5).

Results from the single-media set-up indicated that the two modified-release formulations of budesonide may not be interchangeable with each other. However, single media do not reflect the passage through the gastrointestinal tract. Using the physiological-based pH-gradient method should therefore enable better prediction of the in-vivo behaviour of the two marketed dosage forms (Figures 6 and 7).

Results from both the compendial and the biorelevant pH-gradient method reflected the release behaviour of Budenofalk and Entocort during a simulated gastrointestinal passage. Neither dosage form released any drug in the stomach. While a controlled release of the active drug from Entocort microgranules is hypothesized to begin as early as the duodenum, the coating of the Budenofalk granules needs a higher pH to completely dissolve. As for mesalazine, bile components did not seem to have a substantial effect on the rate of drug release from the dosage forms tested. Hence, drug release from Budenofalk and Entocort is clearly controlled by the gastrointestinal pH and the

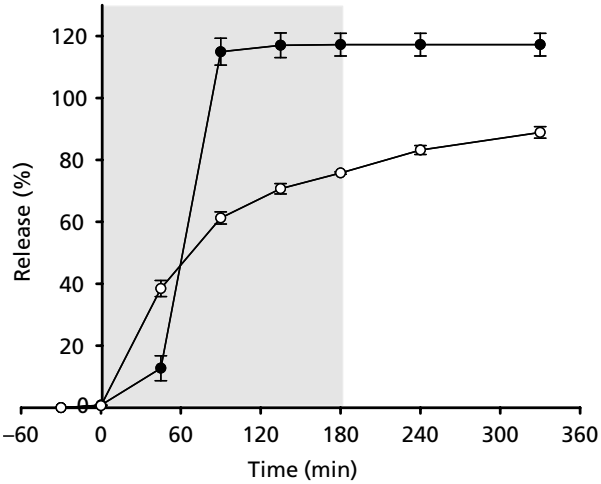

Figure 7 Dissolution behaviour of Budenofalk 3 mg (●) and Entocort 3 mg (○) in a biorelevant pH gradient (negative values represent gastric residence time, shaded part represents drug release during passage through the small intestine). Data are means ± s.d, n = 3.

passage time through the gastrointestinal tract. Estimating the mean percentage of drug release in the gastrointestinal tract based on the release profiles, Budenofalk seems to release nearly the whole amount of drug in the proximal small intestine, whereas the release rate of Entocort is somewhat slower and there is still some drug available for release in the ileum and proximal colon.

Discussion

Results from this study clearly indicate that each drug/dosage form combination exhibits a distinct release

pattern. For selected patients this pattern may be very useful, while for others it may result in non-therapeutic levels of drug combined with an increased risk of systemic side-effects. Therefore, product substitution either among or between mesalazine and budesonide preparations could potentially cause problems for the patient.

Mesalazine

To date, no pharmacokinetic equivalence studies of mesalazine dosage forms have been conducted for treatment of either Crohn's disease or ulcerative colitis. Additionally, the optimal daily dose for inducing or maintaining remission, respectively, has not been clearly established (Baker 2004).

From this study it is obvious that the selection of the dosage form to be administered can strongly influence the outcome in the individual patient. The criteria mentioned here may therefore be an important factor in interpreting results from various clinical studies that have been performed over recent decades. After Azad Khan and his co-workers established in 1977 that mesalazine is the active component of sulfasalazine (Azad Khan et al 1977), mesalazine became the ASA (aminosalicylic acid) derivative of choice for the treatment of IBD. Over the years, new formulations of mesalazine have been developed. Lacking a sulfa moiety, mesalazine dosage forms facilitated administration of higher doses by avoiding the sulfasalazine-related side-effects. Based on the experience that mesalazine is a safe and effective drug for the treatment of ulcerative colitis, Singleton et al (1993) performed a clinical study to find a mesalazine dose that was applicable for inducing and maintaining remission in active Crohn's disease. A daily dose of 4 g, applied as a multiparticulate dosage form (Pentasa), was assumed to be adequate for the long-term treatment of Crohn's disease. On the basis of this study, oral mesalazine formulations became the treatment of choice in mild and moderate Crohn's disease (Feagan 2004). More recently, though, it has become obvious that the clinical significance of oral mesalazine for treatment of active Crohn's disease is not clear. In a recently published meta-analysis of double-blind, placebo-controlled trials, Hanauer & Stromberg (2004) concluded that daily mesalazine doses of less than 4 g daily seem to be completely ineffective for the treatment of Crohn's disease. In two further systematic reviews concerning the effectiveness of the usual treatments for Crohn's disease and ulcerative colitis, Bebb & Scott (2004a, b) also evaluated all placebo-controlled trials of commonly used drugs, including the oral mesalazine dosage forms of Claversal, Salofalk and Pentasa, for both inducing and maintaining remission of Crohn's disease. Results from these studies confirmed the observations of Hanauer & Stromberg and it was concluded that, at most, a modest effect in achieving remission could be reached with a daily dose of 4 g of mesalazine. For the treatment of ulcerative colitis, Bebb & Scott concluded from 9 placebo controlled trials, involving more than 1200 patients, that mesalazine alone is unsatisfactory in inducing remission in active disease (Bebb & Scott 2004a) but that based on the results from 8 placebo-controlled trials of mesalazine in maintaining remission in ulcerative colitis, it can be recommended for maintenance therapy.

The clinical studies that have been performed to assess applicability of mesalazine dosage forms for the treatment of different types and phases of IBD are extremely heterogeneous, so the question of whether mesalazine treatment is useful for treating Crohn's disease still cannot be definitively answered. It is not clear whether the borderline results to date are attributable to the suboptimal design of the clinical studies, the application of suboptimal dosage forms or to poor performance of the drug itself. As there was no attempt in any of these studies to match the most appropriate dosage form of mesalazine to the therapeutic needs of the individual patients, the influence of the dosage form on the therapeutic outcome remains the biggest question mark in the interpretation of the study results.

The release profiles generated in this study clearly indicate that the enteric-coated or slow-release preparations deliver variable amounts of drug to different regions of the gastrointestinal tract. These results are in good agreement with those from various clinical studies measuring the luminal concentration of mesalazine after oral administration (Myers et al 1987; Devos et al 1992; Christensen et al 1993). Further, these observations correspond well with recent studies (Hanauer 2004), in which Hanauer concluded that the variety of mesalazine formulations available for the treatment of IBD results in substantial differences in pharmacokinetic profiles and systemic drug load. Therefore, it seems reasonable that dosage form has been a confounding factor in therapeutic success and in the interpretation of clinical studies.

On the basis of the poor outcomes of the meta-analysis studies and the results presented here, it seems that patient-specific medication based on a precise diagnosis regarding localization, extent and severity of the inflammation should be invoked. Whenever an oral treatment is feasible, an appropriate delivery system with an appropriate, site-specific release pattern should be selected on the basis of the results from the biorelevant release tests. Based on their release behaviour, Salofalk and Claversal are likely to be most effective if the main site of inflammation is found in the ileum. By contrast, if only the colon is inflamed, nearly the whole amount of drug will be released from the two tablet formulations well before reaching the inflamed areas. A significant amount of drug will therefore be prematurely absorbed in the small intestine, resulting in an increased risk of side-effects and a lack of drug substance at the inflamed areas in the colon. Pentasa and Salofalk Granustix are intended for the treatment of inflammation that spreads throughout the whole small intestine and proximal colon. In contrast to Salofalk Granustix, Pentasa immediately starts to release the active drug in the stomach and therefore is particularly appropriate for those patients that suffer from a gastric inflammation. However, in most patients, a substantial drug release in the stomach represents drug wastage (loss of active drug due to systemic absorption) combined with an increased risk of adverse effects.

None of the mesalazine dosage forms tested in the study represents an optimal drug delivery system for colonic delivery. Nevertheless, they belong to the standard therapeutic regimes for induction and maintenance therapy of ulcerative colitis. Most recently, Lim & Hanauer (2004b) remarked on the lack of information regarding an optimal dose for the treatment of ulcerative colitis and also the insufficient data to support the superiority of any one mesalazine formulation over another. A possible reason for the high doses of mesalazine required for the treatment of ulcerative colitis to date may be that the drug delivery systems currently available are suboptimal for this purpose. Further, it is clear that the dosage forms studied here cannot be substituted for one another but after a clear diagnosis regarding type, localization, severity and extent of the inflammation in Crohn's disease, it may be feasible to design a patient-specific treatment using results from the dissolution method, as has been most recently proposed by Lim & Hanauer (2004b).

Budesonide

Oral glucocorticosteroids like prednisone have proven highly effective in the treatment of active Crohn's disease, but, as already mentioned, they are usually associated with a high incidence of side-effects (Andus et al 2003). In the search for a medication of comparable potency but with fewer side-effects, budesonide became a focus of interest. Several clinical studies have recently been performed to investigate the efficacy and safety of budesonide in the therapy of IBD (Rutgeerts et al 1994; Campieri et al 1997; McKeage & Goa 2002). In these studies, budesonide has been shown to be effective in the treatment of both Crohn's disease and ulcerative colitis. However, to date, oral budesonide has only been used for the treatment of mild to moderate active Crohn's disease. On the German market, two controlled-release drug delivery systems, Budenofalk and Entocort, are available: Both are intended to release the active drug in the ileum and proximal colon.

In a double-blind dose-finding study using an oral-controlled release preparation of budesonide (Entocort), Greenberg et al (1994) were able to demonstrate that a daily dose of 9 mg budesonide is most effective against active Crohn's disease. The results regarding remission rate were comparable with these from prednisone but the steroid-dependent systemic side-effects could clearly be reduced. In the meantime, a once-daily dose of 9 mg administered as Entocort capsules belongs to the first-line medications for treatment of patients with mild to moderate active Crohn's disease involving the ileum or ascending colon (Sandborn & Feagan 2003).

In the simulation of the gastrointestinal passage of Entocort micropellets in the biorelevant dissolution test system, it was observed that the enteric coating of the micropellets dissolves quickly as soon as the pH exceeds 5.5. In terms of in-vivo behaviour, this would mean that after entering the small intestine, the Entocort micropellets start to release the active drug by a diffusion-controlled mechanism. Assuming a mean small-intestinal transit time of 3 h, about 80% of the dose is released in this section, while only about 20% of the active drug is available for release in the proximal colon. Therefore, in cases where the inflammation is restricted to the ileocaecal junction and the proximal colon, a large percentage of the dose will not come into contact with the inflamed areas, primarily due to proximal drug absorption and subsequent first-pass hepatic biotransformation (Campieri 2002).

With a somewhat different composition, especially in the enteric coating, the micropellets of the more recently launched Budenofalk are intended to release the active drug when the pH exceeds 6.4. A 3-mg dose of Budenofalk administered before meals three times daily was shown to be effective in the treatment of mild to moderate Crohn's disease in the double-blind placebo-controlled study of Bar-Meir et al (1998). Results were very similar to those generated in a study of Campieri et al (1997) administering Entocort. Compared with a once-daily dose of 9 mg, the administration of budesonide divided in three doses seemed to have no effect on the response rate. Nevertheless, differences of Entocort and Budenofalk with respect to the pharmacokinetic profiles, especially t_{max}, could be observed (Bar-Meir et al 1998). Budesonide is released from Entocort with a time to maximal concentration of 2.7 h, whereas maximal release from the pH-modified release preparation occurs later, as reflected by a t_{max} of 4.3 h (Bar-Meir et al 1998). These observations are in good agreement with results from the Budenofalk release study. Using the biorelevant pH-gradient method, the micropellets from Budenofalk release the complete dose of budesonide abruptly under conditions that reflect the mid-jejunum.

Based on the release profiles, the Entocort product seems better suited to the therapy of cases where the inflammation is spread more widely through the small intestine and proximal colon, while Budenofalk seems more appropriate for the distal ileum and proximal colon. However, neither dosage form appears to be optimal for treating inflammation that is restricted to the proximal colon and they are hypothesized to be virtually useless for the treatment of the transversal or distal colon as release tests indicate that release would occur too early in the gastrointestinal tract and result in too much drug wastage (absorption into the body with subsequent metabolism).

Conclusion

Results from this study indicate that the biorelevant pH-gradient method is a convenient and discriminating method for comparing the drug-release behaviour from site-specific dosage forms of both mesalazine and budesonide. Specific drug-release profiles during gastrointestinal passage, as well as pH-dependency of drug release, could be clearly shown. From the release profiles of the mesalazine dosage forms studied here, it is obvious that the profile of the anti-inflammatory effects of mesalazine depends not only on the drug itself but also on the type of drug delivery system prescribed. Since clinical studies to date have not attempted to match the most appropriate

dosage form of mesalazine to the therapeutic needs of the individual patient, the disappointing outcome of the meta-analysis may be dosage form rather than drug related. In the future, mesalazine therapy should be tailored to the needs of the individual patient. Only when individually optimized therapeutic regimes are invoked, it will be possible to put to rest the question of whether mesalazine is a safe and effective drug for the therapy of both Crohn's disease and ulcerative colitis.

In recent decades, both budesonide and mesalazine have been shown to be effective in the treatment of ulcerative colitis. Oral dosage forms that are optimized for this therapy appear to be still lacking. The biorelevant pH-gradient method described here provides a valuable tool for developing new types of dosage forms that are intended for the treatment of colonic inflammation.

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