JPP Journal of Pharmacy and Pharmacology

JPP 2001, 53: 1207–1215

Received December 19, 2000
Accepted May 21, 2001
ISSN 0022-3573

Colon-specific delivery of budesonide from microencapsulated cellulosic cores: evaluation of the efficacy against colonic inflammation in rats

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Abstract

Budesonide (BDS) is a potent corticosteroid that has important implications in the pharmacotherapy of inflammatory bowel disease, especially in the treatment of ulcerative colitis and Crohn's disease. BDS is available on the market in the form of enteric-coated preparations. However these products, similar to other available site-specific dosage forms, are not sufficiently selective to treat colonic inflammatory bowel disease. The objective of this study was to evaluate the efficacy of a new microparticulate system containing BDS, to treat experimentally induced colitis in rats. This microparticulate system consisted of BDS-containing hydrophobic cores, microencapsulated within an enteric polymer, which solubilizes at above pH 7, thus combining pH-sensitive and controlled-release properties. Colonic injury and inflammation were assessed by measuring colon/bodyweight ratio, myeloperoxidase (MPO) activity, and by scoring macroscopic and histological damage in colitic rats. Rats were treated orally with BDS, included in the developed system, once a day for 4 days after the induction of inflammation. A BDS suspension and BDS-containing enteric microparticles were included as control formulations in the experimental design. The administration of the new BDS delivery system significantly reduced the colon/bodyweight ratio compared with the administration of control formulations. Similarly, MPO activity and macroscopic and histological damage of the inflamed colonic segments decreased significantly when the BDS formulation was administered, compared with the results obtained after oral administration of the drug suspension. There were no significant differences, however, when the new treatment was compared with the control formulation consisting of simple enteric microparticles.

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Funding: This work was supported by the Xunta de Galicia, Spain, under project XUGA 20310B98.

Introduction

Inflammation and mucosal injury in the colonic region may be caused by several factors and are frequently associated with significant morbidity and a reduction in quality of life. The most prominent disorders of the large intestine are idiopathic inflammatory bowel diseases, such as ulcerative colitis, a mucosal inflammatory condition confined to the colon and rectum, and Crohn's disease, a transmural inflammation of gastrointestinal mucosa that may occur in any part of the intestine, but mainly occurs in the colon. The aetiology of such colonic injury may be generally well defined (as occurs in experimentally induced colitis in animals) or more obscure (as occurs with ulcerative colitis and Crohn's disease) (Hanuaer & Kirsner 1988; Jewell 1993).

Salicylates, corticosteroids and immunosuppressive drugs are commonly used to treat active disease and to lengthen remission from injury (DiPiro et al 1989). These drugs are currently administered orally, rectally or intravenously. Generally, the most efficient form of treating inflammatory bowel disease is the administration of corticosteroids by the parenteral route, but this use is limited to severe acute disease because of the important systemic side-effects of corticosteroids. To avoid this problem, pharmaceutical research has been focused on developing new oral dosage forms that allow maximal efficacy in the treatment of inflammatory bowel disease. With these formulations, the drug concentration in the affected region could be maximized and, thus, the ratio of drug absorbed into the systemic circulation would decrease considerably, as would its side-effects. Several approaches have been used in an attempt to achieve the targeting of drugs to the colonic region (Rubinstein 1995; Watts & Illum 1997). These systems were intended to fulfil the following requirements: protect the drug or the system itself during intestinal transit to the colon, normalize the time-of-stay into the colon, and ensure site-specific drug release. To achieve this specific colonic delivery, there are three main practical mechanisms by which a delivery system can be targeted into the colon after oral administration: pH-dependent coating, time-dependent coating, and biodegradation by colonic bacterial enzymes.

Clinical use of enteric formulations for the treatment of inflammatory bowel disease is not new (Dew et al 1983). One disadvantage of these preparations is the possibility that the active molecule may be released before it reaches the target region. The potential for food effects associated with the use of large enteric-coated preparations intended for colonic targeting is another important drawback of these products (Ashford et al 1993; Ishibashi et al 1998). Both of these problems could be solved by combining enteric and controlled-release properties in a multiparticulate system, that is a formulation with a less variable gastric transit time that offers protection to the drug up to pH 7, and at the same time prevents the complete release of the drug in the ileum by means of a controlled-release polymer core.

We have designed a new microparticulate system consisting of a hydrophobic polymer core, with sustained-release properties, coated with an acrylic polymer with pH-dependent swelling and dissolution properties (Rodríguez et al 1998). For in-vivo evaluation of this colonic system, we selected budesonide (BDS), a drug of relatively recent application in colonic disorders (Greenberg et al 1994; Löfberg et al 1996; Hellers et al 1999). Although the drug is commercially available, mainly for the treatment of small intestine active Crohn's disease, it was found that less than 5% of the drug was available beyond the ileum and caecum (Edsbacker et al 1993), and therefore colonic delivery still needs to be optimized by a more reliable colon-specific system.

The aim of this work was to test the efficacy of the new microparticulate system containing BDS, in the treatment of experimentally induced colitis in rats. The therapeutic effects of BDS when administered in this system were compared with those obtained after the administration of the drug alone, or the drug included in simple enteric microparticles.

Materials and Methods

Materials

The following chemicals were obtained from commercial suppliers and used as received: cellulose acetate butyrate (CAB 171-15S; Eastman Chemical, Kingsport, TN); copolymer of methacrylic acid and methacrylic acid ester (Eudragit S100; Röhm Pharma, Darmstadt, Germany); BDS, horseradish peroxidase, 2,4,6-trinitrobenzenesulfonic acid (TNBS), hydrogen peroxide, *o*-dianisidine dihydrochloride and hexadecyltrimethylammonium bromide (Sigma Química, Madrid, Spain).

Preparation and characterization of Eudragit S microparticles containing BDS-loaded CAB cores

Eudragit S microparticles containing CAB cores were prepared by the oil-in-oil solvent evaporation technique, conveniently modified (Rodriguez et al 1998). BDS-loaded CAB microspheres (6%, w/v, polymer concentration) were encapsulated using a coat-to-core ratio of 5:1. Eudragit S microparticles containing BDS directly encapsulated were also prepared using a coat-to-core ratio of 10:1. Each formulation was prepared in duplicate and the batches were characterized separately to study batch-to-batch reproducibility.

The morphological examination of microparticles was performed by scanning electron microscopy (Jeol JSM-6400; Tokyo, Japan). Cross-sections of the microparticles were made using an ultracryotome (Cryocut 1800; Reitcher-Jung, Leica Instruments, Nussloch, Germany). The particle size distribution of the microspheres (volume diameter) was determined by a Coulter counter Multisizer II (Coulter Electronics, Luton, UK).

BDS loading was determined by HPLC (Rodriguez et al 1998) after drug extraction with methanol using a horizontal shaker (Promax 2020; Heidolph, Kelheim,

Germany) at room temperature for 24 h. In-vitro BDS release was performed using a continuous flow-through apparatus (Sotax, Basel, Switzerland) in a pH progression medium at 37°C, simulating gastrointestinal tract conditions (Rodriguez et al 1998). Determinations were performed in duplicate for each batch formulation.

Induction of colonic inflammation

All animal experiments were approved by the Ethical Committee of the Faculty of Medicine of the University of Santiago de Compostela. Animals were allowed free access to water and laboratory chow for the duration of the studies. To induce the model of chronic inflammation in the rat colon, we followed the method described by Morris et al (1989). Briefly, male Sprague-Dawley rats, 190–210 g, were arbitrarily separated into treatment groups, fasted for 24 h with free access to water and then lightly anaesthetized with ether. A graduated rubber cannula (Covaca, Madrid, Spain) was inserted rectally into the colon such that the tip was 8 cm proximal to the anus. TNBS (30 mg) dissolved in 50% ethanol (v/v) was instilled into the lumen of the colon through the rubber probe (total volume 0.25 mL). A control group received 0.25 mL 0.9% (w/v) saline, administered as before.

Experimental design and dosing

At 24 h after the induction of colonic inflammation, each treatment group (n = 6 rats) was administered one of the following formulations by oral gavage, once a day for 4 days: blank microparticles; BDS suspension; BDS-loaded Eudragit S microparticles; and Eudragit S microparticles containing BDS-loaded CAB cores. In all cases, drug or microparticles were suspended in 2 mL 0.5% hydroxypropylmethylcellulose (Methocel K15M; nominal viscosity of 15000 counts min^{-1}; Dow Chemical, Germany). Rats were administered a dose of 875 μg kg^{-1} day^{-1} BDS, or the amount of blank microparticles equivalent to this dose.

Determination of colon/bodyweight ratio

Five days after the intracolonic administration of TNBS, the animals were killed and the distal colon was rapidly excised and opened longitudinally along the mesenteric edge. The colon was washed with 0.9% (w/v) saline and placed with the mucosal surface upward over a glass plate chilled with ice. The ratio of the 8-cm segment distal colon weight to bodyweight was calculated as an index of colonic tissue oedema (Yue et al 1996).

Measurement of myeloperoxidase (MPO) activity

An assay of colonic MPO activity was used to measure neutrophil infiltration as a quantitative index of colonic inflammation (Bradley et al 1982). After weighing the distal colon, the mucosa was scraped off, suspended in 3 mL 0.5% hexadecyltrimethylammonium bromide in 50 mmol L^{-1} phosphate buffer (pH 6.0), and homogenized 3 times for 20 s (Potter-Elvejhem, IKA Werk, Germany) in an ice bath to maintain enzyme stability. The homogenate was then sonicated for 10 s with a Branson Sonifier 250 (Branson, USA), and centrifuged at 40000 *g* and 4°C for 15 min. The resulting supernatant was assayed spectrophotometrically for MPO content at 460 nm (Shimadzu UV-1603 spectrophotometer; Japan); 0.1 mL supernatant was mixed with 2.9 mL 50 mM phosphate buffer, pH 6.0, containing 0.167 mg mL^{-1} *o*-dianisidine dihydrochloride and 0.0005% hydrogen peroxide. One unit of MPO activity was defined as that degrading 1 μmol peroxide min^{-1} at 25°C. Results were expressed as U (mg protein)$^{-1}$. Total protein was determined using the method described by Lowry et al (1951).

Assessment of macroscopic ulceration and histological evaluation

Gross mucosal damage was scored on a 0–3 grade scale, by a single observer blind to the treatment. Damage was scored as follows: score 0 represented no damage; score 1, localized hyperemia with slight or minimal ulceration; score 2, linear ulcers and one or two regions with ulcers of 1–2 cm; score 3, severe ulceration (regions with ulcers $>$ 2 cm). After scoring, two tissue samples were excised from each colon and maintained in formaldehyde (10%, v/v) for microscopic studies. When visible ulceration or inflammation was present, at least one of the samples from the affected region was taken. These tissue samples were processed routinely and embedded in paraffin. Sections (5 μm) were stained with haematoxylin and eosin. Microscopic assessment by light microscopy was performed blind on coded slices. Histological damage was also scored on a 0–3 scale as follows: score 0, no damage; score 1, no significant inflammation, score 2, moderate inflammatory infiltrate (one or two regions affected with the number of neutrophils slightly in-

creased); score 3, severe inflammatory infiltrate (several inflamed regions with a lot of neutrophils). Finally, the global damage was represented by the numerical sum of two criteria (macroscopic and histological damage, range 0–6).

Statistical studies

Data were expressed as mean ± s.d. Data were statistically analysed by the Student's *t*-test when two treatments were compared, or one-way analysis of variance when more than two treatments were compared. When differences were significant, the Student-Newman-Keuls test was used for multiple comparisons between treatments. With all statistical analyses, an associated P value of $< 5\%$ was considered significant.

Results

BDS-loaded CAB cores encapsulated in Eudragit S microparticles: in-vitro characterization

Eudragit S microparticles containing BDS-loaded CAB cores were spherical in appearance, regular in shape and had a smooth surface. Examination of these microparticles after sectioning revealed the successful encapsulation of the CAB cores within the Eudragit, resulting in a multinucleated structure (Figure 1). The mean particle size of the population of microparticles was 276.78 ± 19.45 μm and the drug content was 1.00 ± 0.02 %. A control formulation consisting of BDS-loaded Eudragit S microparticles was also prepared, with a mean particle size of 200.09 ± 36.33 μm and a drug loading of 7.67 ± 0.16 %.

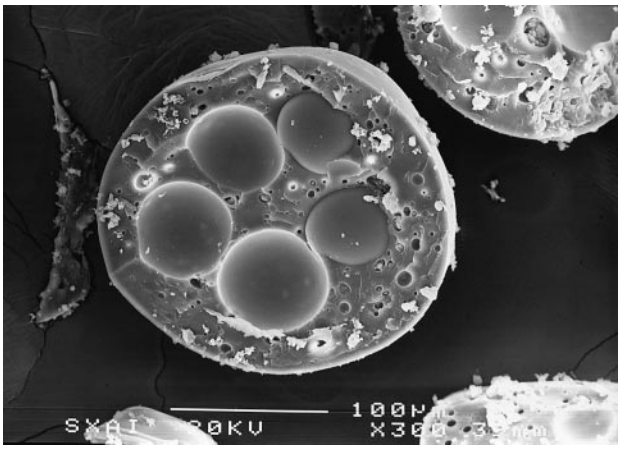

Figure 1 Scanning electron micrograph of a cross-section of Eudragit S microparticles containing budesonide-loaded cellulose acetate butyrate cores.

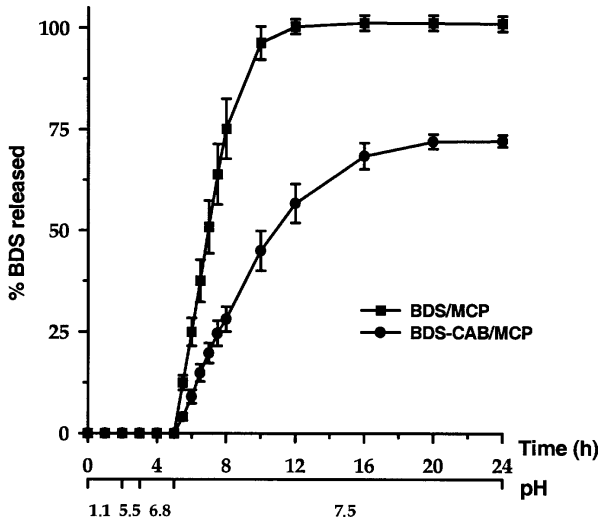

Figure 2 In-vitro release profiles obtained from Eudragit S microparticles containing budesonide (BDS) directly encapsulated (BDS/MCP) or included in CAB cores (BDS-CAB/MCP). Data are mean ± s.d., n = 4.

Figure 2 shows the in-vitro BDS release profiles from these two formulations. Once the enteric polymer had completely dissolved when the pH changed, BDS was immediately released from the control enteric formulation, but this did not occur when BDS was included in the CAB cores, which controlled the BDS delivery throughout the duration of the dissolution study.

Assessment of the induced colonic inflammatio

After administration of saline or TNBS in 50 % ethanol, the weight of the 8-cm segment proximal to the anus and the MPO activity were determined to ascertain the extent of the induced inflammatory process. When rats were treated with saline, there was no macroscopic or histological damage. Indeed, the colon/bodyweight ratio and the MPO activity were much lower (2.18 ± 0.20 and 0.091 ± 0.13, respectively) than those obtained in the TNBS-treated rats (8.05 ± 1.84 and 4.43 ± 1.77, respectively), both indices of damage being significantly higher for this group ($T_{(6)} = 21$; $P < 0.01$).

In all the animals that received TNBS, an important decrease in corporal weight (between 10 and 15 %) was

observed, and they developed areas of visible bowel wall thickening, inflammation, hyperemia and ulcers. The sites of inflammation and ulceration were localized 2–6 cm proximal to the anus. Generally, no damage was detected in the splenic flexure. Transmural inflammation, fibrinous adhesions to the small bowel and pericolic accumulations of mesenteric fat were observed in a large number of rats. The colonic mucosa showed several hyperemic zones and the ulcers were surrounded by thickening and inflamed tissue. An extensive infiltration of the mucosa and submucosa, primarily by neutrophils, was also observed.

All rats suffered from diarrhoea after administration of TNBS, however this was not the case for those rats treated with saline. A narrowing of the lumen of the colon adjacent to the inflamed sites with a proximal dilation of the bowel was also seen in the TNBS-treated rats, but generally the colon was not perforated.

Efficacy studies of the new BDS microparticulate system

As shown in Table 1, the colon/bodyweight ratio decreased significantly ($P < 0.05$) when BDS was administered in the enteric-coated CAB cores to colitic rats, compared with blank microparticles, BDS suspension or BDS-loaded enteric microparticles. The administration of enteric microparticles containing BDS, either inside the polymeric cores or directly encapsulated, significantly reduced MPO activity ($P < 0.05$), compared with the values obtained when the groups were given blank microparticles or BDS suspension (Table 1).

Table 1 Colon/bodyweight ratios and myeloperoxidase (MPO) activity measured in TNBS-treated rats after oral administration of blank microparticles (blank MCP), budesonide (BDS) suspension, BDS-loaded Eudragit S microparticles (BDS/MCP) and Eudragit S microparticles containing BDS-loaded CAB cores (BDS-CAB/MCP).

Formulation	Colon/bodyweight ratio (mg g^{-1})	MPO activity (U (mg protein)$^{-1} \times 10^2$)
Blank MCP	11.20 ± 2.04	7.73 ± 1.70
BDS suspension	10.04 ± 4.17	8.78 ± 3.60
BDS/MCP	11.47 ± 3.29	4.25 ± 3.06*
BDS-CAB/MCP	5.84 ± 1.73*†	2.79 ± 2.70*

Data shown are the mean ± s.d., n = 6. *$P < 0.05$ compared with the groups given blank microparticles or BDS suspension. †$P < 0.05$ compared with the group given BDS-loaded Eudragit S microparticles.

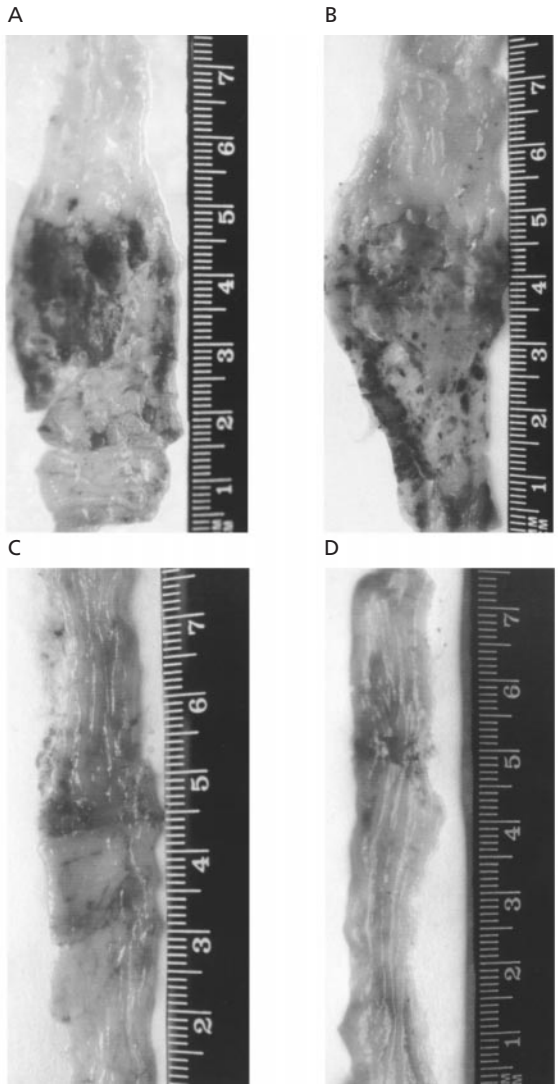

Figure 3 Photographs of the colon of TNBS-treated rats after oral administration of blank microparticles (A), budesonide (BDS) suspension (B), BDS-loaded Eudragit S microparticles (C), and Eudragit S microparticles containing BDS-loaded CAB cores (D).

Figure 3 shows the gross colonic macroscopic ulceration observed in colonic segments for each treatment group. As expected, when blank microparticles were administered, the damage induced was unaffected (Figure 3A). The BDS suspension did not lead to any appreciable improvement of ulceration and inflammation (Figure 3B). However, in the groups given BDS-loaded enteric control microparticles, or the new colonic system, only very small ulcers (not exceeding 1 cm) and less inflammation were detected (Figures 3C, D). The

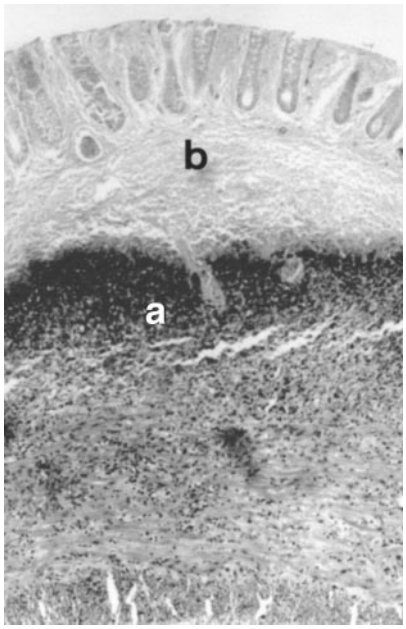

Figure 4 Optical micrograph of the colon of a TNBS-treated rat after oral administration of blank microparticles, showing mucosa with severe inflammatory infiltrate (a) and extensive areas of necrosis (b). This colon was given a damage score of 6 (magnification 40 ×).

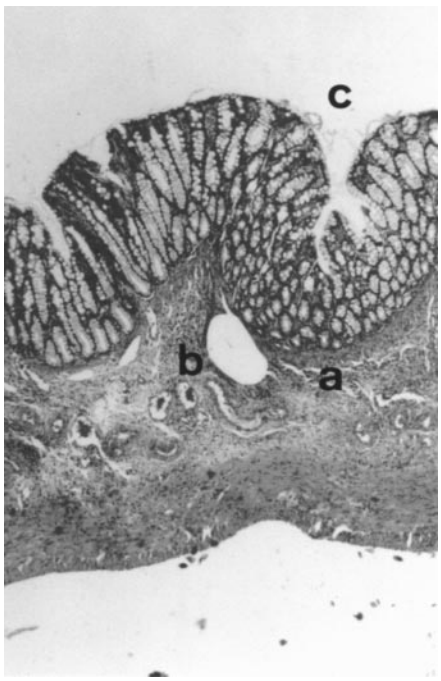

Figure 5 Optical micrograph of the colon of a TNBS-treated rat after oral administration of Eudragit S microparticles containing budesonide (BDS)-loaded CAB cores, showing mucosa with mild inflammatory infiltrate (a), vascular congestion (b), and well-conserved mucosa (c). This colon was given a damage score of 2 (magnification 40 ×).

Table 2 Scoring of macroscopic and histological damage measured in TNBS-treated rats after oral administration of blank microparticles (blank MCP), budesonide (BDS) suspension, BDS-loaded Eudragit S microparticles (BDS/MCP) and Eudragit S microparticles containing BDS-loaded CAB cores (BDS-CAB/MCP).

Formulation	Macroscopical damage	Histological damage	Total
Blank MCP	2.4 ± 0.9	2.6 ± 0.5	5.0 ± 1.4
BDS suspension	2.4 ± 0.5	2.0 ± 0.7	4.4 ± 1.1
BDS/MCP	1.5 ± 1.0	1.7 ± 1.2	3.2 ± 2.1
BDS-CAB/MCP	1.1 ± 0.8*	1.2 ± 0.8*	2.3 ± 1.6*

Data shown are the mean ± s.d., n = 6. *$P < 0.05$ compared with the groups given blank microparticles or BDS suspension.

microscopic aspect of colonic segments after administration of blank or enteric microparticles containing BDS-loaded CAB cores is shown in Figures 4 and 5, respectively. When blank microparticles were administered, a high number of neutrophils in the mucosa and submucosa were detected. However, a marked decrease in the number of these cells was observed after the administration of the coated BDS-loaded CAB cores, and the epithelial barrier with well-conserved cells was observed (Figure 5). The macroscopic and histological grades obtained for the different treatment groups are summarized in Table 2. Statistical analysis of the damage scores indicated that the new formulation significantly reduced the damage induced in the colonic segments, compared with those treated with blank microspheres or BDS suspension ($P < 0.05$). However, statistical differences were not significant when the groups given the new formulation or the enteric control microparticles were compared.

Discussion

In this work, we describe the in-vivo evaluation of the anti-ulcerative effects of a new colonic delivery system containing BDS, on experimentally induced colonic inflammation in rats. Corticosteroids have a broad anti-inflammatory effect in active inflammatory bowel disease and are unsurpassed by any other type of drug. The trade-off for high efficacy, however, has been the risk of systemic side-effects, which are sometimes severe and even irreversible, especially after long periods of treatment. To avoid this problem, several pharmaceutical

formulations have been designed, such as suppositories, enemas or foams, to deliver these drugs and to limit side-effects. However, these dosage forms can only access the distal regions of the colon and rectum. BDS has also been formulated into oral controlled ileal release capsules, which contain small pellets designed to begin the release of the drug in the small intestine. Unfortunately, less than 5% of the drug was found to be available beyond the ileum and caecum (Edsbacker et al 1993), and therefore it is not likely to have an appreciable effect on colonic inflammatory disease. An oral dosage form that allows drug delivery into the ascending and transverse colon could substantially improve the treatment of this pathology, by minimizing drug absorption in the small intestine and at the same time providing a high drug concentration in the inflamed colonic mucosa. We designed a new microencapsulated system consisting of a pH-dependent coating and a BDS-containing hydrophobic polymeric core, which have the special feature of releasing the encapsulated drug at colonic pH continuously over a prolonged period of time. Eudragit S microparticles containing BDS-loaded CAB cores were prepared by a microencapsulation procedure based on a modification of the oil-in-oil solvent evaporation technique (Rodriguez et al 1998). In-vitro release profiles obtained from these microparticles showed that they prevented BDS delivery at pH values below 7. Furthermore, a controlled-release of BDS from the cellulosic polymer matrix, once the enteric polymer had dissolved, was maintained over a prolonged period of time. This behaviour was explained by the slow diffusion of BDS through the cellulosic cores, which was the controlling step of drug release.

We then evaluated the therapeutic efficacy of the new microparticulate system using the TNBS-induced ulcerative colitis model in rats (Morris et al 1989), because of its simplicity and reproducibility. TNBS-induced colitis shares many of the histopathological and clinical features of human Crohn's disease and resembles human inflammatory bowel disease more closely than the ethanol-induced colitis model (Yamada et al 1992). The TNBS-induced colitis model in rats has proved to be useful for the evaluation of the therapeutic effects of various anti-inflammatory drugs included in colon-specific delivery systems (Tozaki et al 1999a, b). Five days after TNBS administration, colon/bodyweight ratio and MPO activity, used as indices of the colonic damage, were significantly higher than the levels observed in the control group that received saline. Typical features associated with colonic inflammation, such as important corporal weight loss and diarrhoea were observed in the TNBS-treated group, and all these animals showed areas of gross visible thickening of the colonic wall associated with cellular infiltration and ulcers.

The high values of the colon/bodyweight ratio obtained after administration of drug-containing control formulations (BDS suspension and BDS-loaded enteric microparticles; Table 1) indicated that there was still considerable inflammation in the colonic region. This indicated that the BDS levels attained in the colon were not sufficient to have an effect on the condition, probably because BDS was partially absorbed in the small intestine or the amount released was not sufficient to treat the damaged tissues appropriately. However, after administration of enteric microparticles containing BDS-loaded CAB cores, a significant decrease in the colon/bodyweight ratio was obtained. This finding could be explained by the fact that the hydrophobic cores prevented immediate drug delivery at pH 7 (terminal ileum); once the enteric polymer had completely dissolved, BDS was released throughout the entire colon, the process being controlled by the CAB matrix of the cores.

MPO activity is proportional to the number of neutrophils localized in the mucosa and submucosa over a wide range of neutrophil concentrations. These cells are present in inflammatory processes. Therefore, the determination of MPO activity can be used as an estimate of intestinal inflammation, and it is very useful as a method of assessing the efficacy of different drugs in animal models of inflammation (Krawisz et al 1984; Morris et al 1989). The fact that MPO activity values obtained after administration of BDS-containing control microparticles, or the new BDS formulation, were significantly lower than those obtained for the other controls (Table 1), but not different in themselves, might indicate that ulceration repair (as represented by the decrease in colon/bodyweight ratio) occurred not as a consequence of neutrophil migration inhibition. It is more likely that it occurred at another stage of the inflammatory process. Similar discordant results between parameters related to intestinal repair and MPO activity during colitis in rats have been described in other efficacy studies carried out with corticosteroid drugs (Jacobson et al 1993; Cui et al 1994; Fedorak et al 1995).

Macroscopic and histological damage indices (Table 2) were lowest in rats dosed with microparticles containing BDS-loaded CAB cores. However, as occurred with the MPO activity results, although damage scores after treatment with the new formulation or enteric control microparticles were significantly different compared with those obtained after administration of blank

microparticles or BDS suspension, the differences were not significant when the indices from the two enteric microparticulate systems were compared.

In general, these data confirm that the administration of the new microparticulate system containing BDS-loaded CAB cores may represent an effective tool for the treatment of human colonic inflammatory bowel disease. This colonic delivery system, the site-specificity of which is based on combining pH-sensitive and controlled drug delivery properties, caused a significant decrease in inflammation in the colon of colitic rats after oral administration, compared with the same dose of drug administered as an oral suspension. Similar studies carried out with novel colonic delivery systems containing BDS, such as glucuronide prodrugs (Cui et al 1994) or azopolymer-coated pellets (Tozaki et al 1999a), revealed the increased efficacy of the systems compared with oral administration of the free drug. However, results from those works indicated that not all the parameters measured (i.e. MPO activity, colon/bodyweight ratio) were significantly different from those obtained after administration of the same dose of the free drug and, in some cases, not all the doses tested led to significant changes in the responses. In our work, all the parameters changed significantly when BDS was administered in the new colonic system. Furthermore, when compared with a reference formulation, consisting of pH-sensitive microparticles, although not all the differences were significant, the general pattern of the data allowed us to conclude that the new system developed was more effective against the TNBS-induced colitis model in rats.

In conclusion, the new colonic delivery system significantly improved the efficacy of BDS in the healing of induced colitis in rats. Our results also demonstrated that the effects of BDS were generally improved compared with those obtained with simple BDS-loaded enteric microparticles. The described system may therefore be useful for clinical treatment of human colonic inflammatory bowel disease.

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