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Colon-specific Delivery of Budesonide with Azopolymer-coated Pellets: Therapeutic Effects of Budesonide with a Novel Dosage Form against 2,4,6-Trinitrobenzenesulphonic Acid-induced Colitis in Rats

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Abstract

The objective of this study was to achieve colon-specific delivery of budesonide using azopolymer-coated pellets and to accelerate healing of 2,4,6-trinitrobenzenesulphonic acid sodium salt (TNBS)-induced colitis in rats.

After oral administration of azopolymer-coated pellets containing budesonide, a significant increase was observed in the therapeutic effects of the drug accompanied by a decrease in its systemic adverse effects when compared with oral administration in saline or rectal administration by enema. In addition, with the use of the colon-specific oral dosage form the dose of budesonide could be reduced.

These results suggested that azopolymer-coated pellets may be a useful dosage form for the colon-specific delivery of budesonide as an anti-inflammatory steroid drug to bring about the healing of TNBS-induced colitis in rats.

Inflammatory bowel disease is comprised of two distinct but similar conditions—Crohn's disease and ulcerative colitis. Ulcerative colitis affects the colon from the proximal rectum. The length of colon affected varies, but the disease always occurs in continuity in the mucosal layer only. On the other hand, Crohn's disease does not usually affect the rectal area, and lesions involve not only the mucosa but also the entire bowel wall. Both conditions are chronic, and although symptoms may spontaneously resolve, most patients will relapse (Podolsky 1991; Shanahan 1993; Spencer & McTavish 1995). Glucocorticoids are generally used as therapeutic drugs for patients with inflammatory bowel disease. Oral administration of these drugs is commonly used in the treatment of Crohn's disease and extensive ulcerative colitis. However, their use is sometimes limited because glucocorticoid therapy is associated with some potentially serious adverse effects such as Cushing's syndrome, hypertension, osteoporosis and diabetes mellitus. Furthermore, as suppression of pituitary-adrenal function can occur during long-term treatment with glucocorticoids and recovery of endogenous cortisol release is slow in patients who have received such therapy, adrenal insufficiency can result when treatment is discontinued. Budesonide is a nonhalogenated glucocorticoid which is used for the treatment of inflammatory respiratory disorders (Brogden & McTavish 1992). Its systemic bioavailability is 9·3–15% when administered orally in a sustained-release dosage form or rectally as an enema. This agent has greater topical anti-inflammatory activity but less systemic activity than other glucocorticoids, because of its high first-pass metabolism in the liver (Nilsson et al 1992). Therefore, budesonide is potentially an effective agent for the treatment of inflammatory bowel disease. However, it has been reported that oral administration of budesonide is accompanied by adverse effects such as gastrointestinal injury and Cushing's syndrome.

In a series of studies, we developed novel dosage forms such as azopolymer-coated pellets and chitosan capsules to achieve the colon-specific delivery of peptides and proteins (Tozaki et al 1997). Based on the successful results of colon-specific delivery of peptides, these new dosage forms might also be

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useful for the specific delivery of anti-inflammatory drugs to the large intestine. In the present study, therefore, we investigated specific delivery of the anti-inflammatory drug budesonide to the colon using azopolymer-coated pellets and its therapeutic effects against 2,4,6-trinitrobenzenesulphonic acid (TNBS)-induced colitis in rats. Here, we report that azopolymer-coated pellets containing budesonide were effectively delivered to the large intestine, especially the colon, and that this large intestine-specific delivery system showed a good therapeutic effect against TNBS-induced colitis. These findings suggest that azopolymer-coated pellets might be useful carriers for the colon-specific delivery of anti-inflammatory drugs, including steroid drugs, to bring about healing of TNBS-induced colitis in rats.

Materials and Methods

Materials

o-Dianisidine hydrochloride and hydrogen peroxide were purchased from Sigma Chemical Company (St Louis, MO). 2,4,6-Trinitrobenzenesulphonic acid (TNBS), carboxymethyl cellulose (CMC), hexadecyltrimethyl-ammonium bromide (HTAB), trichloroacetic acid, *t*-butylmethylether and triethylamine were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were of analytical grade.

Preparation of azopolymer-coated pellets

The azopolymer-coated pellets containing budesonide ($\sim$3·3 mg budesonide/capsule) were prepared by Ono Pharmaceutical Co. Ltd, (Osaka, Japan). The mean diameter and weight of the pellet were 1·0–1·2 mm and 1·0 mg, respectively (Tozaki et al 1998). The surface of the capsule was coated with Eudragit L-100 (10·7% w/w) as an enteric coating material and further coated with a straight-chain azopolymer (9·09% w/w) developed by Kimura et al (1992).

Induction of colonic inflammation

Male Wistar rats ($\sim$200 g) were fasted for 48 h before the induction of colitis. Colonic inflammatory lesions were induced by the method of Morris et al (1989). Briefly, 20 mg of TNBS was dissolved in 0·25 mL of 50% ethanol and instilled into the colon through a cannula inserted via the anus.

Treatment of colitis

From the day after intracolonic administration of TNBS, budesonide was orally administered using azopolymer-coated pellets or saline to rats via a polyethylene cannula under light ether anaesthesia twice a day. Alternatively, budesonide/CMC suspension was administered intracolonically to rats through a cannula via the anus under light ether anaesthesia twice a day. The orally administered doses (20, 100 and 500 μg per pellet or per 1 mL saline per day) and intracolonically administered doses (4, 10, 20 and 100 $\mu g\,mL^{-1}$ CMC suspension per day) of budesonide in this experiment were calculated from the clinical doses used for humans.

Assessment of colonic injury and inflammation

The rats were killed by intravenous administration of sodium pentobarbital (75 mg kg^{-1}) on day 5 after intracolonic administration of TNBS, and then the distal colon was removed. The samples of inflamed tissue were excised to measure the myeloperoxidase (MPO) activities (Krawisz et al 1984), the ratios of distal colon wet weight to body weight (C/B ratio) (Yue et al 1996) and the criteria for scoring the gross morphological damage (damage score) (Morris et al 1989). At the same time, the thymus was also removed from the rats. The systemic adverse effects of budesonide were evaluated by determination of atrophy of the thymus as thymus wet weight/body weight (T/B ratio) (Dahlberg et al 1983).

Measurement of myeloperoxidase (MPO) activity

The activity of MPO, which is found in neutrophils, can be used to evaluate the degree of inflammation in the intestine. The distal colon specimen (200 mg) was minced in a beaker containing 1 mL of HTAB buffer (0·5% HTAB in 50 mmol mL^{-1} phosphate buffer, pH 6·0) on ice, transferred to a test tube, and homogenized with a Polytron homogenizer (Kinematica AG, Switzerland) three times for 30 s, on ice. The pooled homogenate and washes were sonicated for 10 s in a bath-type sonicater, freeze-thawed three times, and centrifuged at 10 000 rev min^{-1} for 1 min. MPO activity in the supernatant was measured spectrophotometrically. Briefly, 0·1 mL of supernatant was added to 0·167 mg mL^{-1} *o*-dianisidine hydrochloride and 0·0005% hydrogen peroxide, then, the change in absorbance at 460 nm was measured with a microplate reader (Molecular Devices). One unit of MPO activity was defined as the amount which degraded 1 μmol of peroxidase per minute at 25°C (Krawisz et al 1984).

Determination of the C/B ratio

The C/B ratio (distal colon wet weight/body weight) was calculated as an index of colonic tissue

oedema (Yue et al 1996). The distal colon specimen was rinsed with isotonic saline. An 8-cm segment of distal colon including the major gross pathological changes was weighed.

Assessment of the damage score

The distal colon specimen was immediately examined under a stereomicroscope, and any visible damage was scored on a scale of 0 to 5 by two independent observers blind to the treatment regimen. Damage was scored as follows: score 0 represented no damage; score 1, localized hyperaemia, but no ulcers; scores 2 and 3, linear ulcers without and with significant inflammation in one site, respectively; score 4, two or more sites of ulceration, inflammation, or both; score 5, two or more major sites of inflammation and ulceration, or one major site of inflammation and ulceration extending >1 cm along the length of the colon (Morris et al 1989).

Determination of the T/B ratio

The T/B ratio (thymus wet weight/body weight) was calculated as an index of systemic adverse effects of budesonide. The thymus specimen was rinsed with isotonic saline, and a segment of the thymus was weighed.

Statistical analyses

Results are expressed as means $\pm$ s.e.m., and data obtained for therapy against TNBS-induced colitis were evaluated by comparing the values of groups treated with budesonide in azopolymer-coated pellets with those treated with budesonide in CMC suspension or PBS. Data were analysed by one-way analysis of variance followed by Dunnett's test.

Results

To determine the optimal schedule for induction of colitis by TNBS, we examined the degree of colitis on different days after intracolonic administration of this agent. MPO activity, C/B ratio and damage score increased for 10 days after intracolonic administration of TNBS, and recovered to the control levels after 14 days. Maximal MPO activity, C/B ratio and damage score were observed on day 5 after intracolonic administration of TNBS (Tozaki et al 1998, 1999). We therefore evaluated the therapeutic effects of budesonide for treatment of colitis on day 5 after intracolonic administration of TNBS in the following experiments.

Effects of budesonide against TNBS-induced colitis after intracolonic administration in rats

We first investigated the therapeutic effects of budesonide against TNBS-induced colitis by intracolonic administration as an enema (Table 1). A 0·5% CMC solution was used as the vehicle. The CMC solution alone failed to reduce the MPO activity increased by TNBS. Intracolonic administration of budesonide ($4-20\,\mu$g) markedly decreased the MPO activity in TNBS-induced colitis in rats. However, at a high dose of budesonide ($100\,\mu$g) we found no good therapeutic effect as compared with other doses tested in the present

Table 1. Therapeutic and adverse effects of budesonide after intracolonic administration or oral administration in azopolymer-coated pellets or saline solution to rats with TNBS-induced colitis.

Group	MPO activity ((mg protein)$^{-1}$)	C/B ratio $\times 10^{-3}$ (w/w)	Damage score	T/B ratio $\times 10^{-1}$ (w/w)
Healthy rats	0·09 ± 0·05**	2·45 ± 0·15*	0·00 ± 0·00**	2·98 ± 0·04**
Colitis model				
No treatment	31·0 ± 2·52	9·40 ± 1·40	4·80 ± 0·20	1·99 ± 0·03
0·5% CMC (i/c)	25·2 ± 2·55*	7·30 ± 0·60	4·80 ± 0·20	1·80 ± 0·05
4 μg budesonide (i/c)	6·87 ± 2·23**	4·65 ± 0·52**	2·50 ± 0·99**	1·94 ± 0·03
10 μg budesonide (i/c)	9·36 ± 2·09**	4·70 ± 0·24*	2·00 ± 0·71**	1·82 ± 0·03
20 μg budesonide (i/c)	7·87 ± 2·56**	5·14 ± 0·13*	2·50 ± 0·89**	0·76 ± 0·01**
100 μg budesonide (i/c)	24·7 ± 3·64*	5·20 ± 0·60**	3·40 ± 0·50**	0·54 ± 0·00**
20 μg budesonide pellets (p.o.)	4·97 ± 2·31**††	4·90 ± 0·58**	1·83 ± 0·60**†	1·26 ± 0·03*††
100 μg budesonide pellets (p.o.)	2·69 ± 1·68**	4·46 ± 0·34**	0·80 ± 0·58**	1·40 ± 0·03*††
500 μg budesonide pellets (p.o.)	5·64 ± 4·75**	5·21 ± 0·62**	0·75 ± 0·48**††	0·73 ± 0·01**††
20 μg budesonide in saline (p.o.)	16·7 ± 3·37**	5·80 ± 0·43**	2·80 ± 0·37**	0·66 ± 0·01**
100 μg budesonide in saline (p.o.)	4·64 ± 2·25**	4·88 ± 0·26**	1·75 ± 0·75**	0·78 ± 0·02**
500 μg budesonide in saline (p.o.)	3·78 ± 3·01**	5·49 ± 0·48**	3·25 ± 0·63*	0·46 ± 0·01**

*$P < 0·05$, **$P < 0·01$ compared with TNBS-treated controls. †$P < 0·05$, ††$P < 0·01$ compared with the same dose of budesonide in saline.

experiment. The C/B ratios and damage scores were also significantly decreased in TNBS-induced colitis rats at all doses tested from 4–100 μg. At lower doses (4 and 10 μg), budesonide did not reduce the T/B ratio, which is an index of adverse effects. On the other hand, significant adverse effects were observed at doses of budesonide higher than 20 μg; i.e. thymus involution was induced by higher doses of budesonide (20 and 100 μg).

Effects of budesonide on TNBS-induced colitis after oral administration of azopolymer-coated pellets in rats

Next, we prepared azopolymer-coated pellets containing budesonide and investigated their therapeutic effects against TNBS-induced colitis after oral administration. The MPO activities after oral administration of budesonide with azopolymer-coated pellets were markedly decreased at the doses used in this experiment (20–500 μg). Administration of budesonide solution also reduced the MPO activity at doses of budesonide higher than 100 μg. We found a good therapeutic effect after oral administration of 20 μg of budesonide with azopolymer-coated pellets as compared with the same dose in the saline solution group, as evaluated by the MPO activity (Table 1). Budesonide in both oral dosage forms, i.e. saline solution and azopolymer-coated pellets, reduced the C/B ratios at all doses used in this study. However, no significant differences were observed in C/B values between azopolymer-coated pellets and saline solution at each dose. The oral administration of budesonide with azopolymer-coated pellets significantly reduced the damage scores at all doses examined in the present study.

The T/B ratios might reflect the therapeutic adverse effects of budesonide after oral administration with azopolymer-coated pellets or solution. The T/B ratios were decreased after oral administration of budesonide in azopolymer-coated pellets and saline solution at all doses examined. However, the T/B ratios for the group treated with budesonide in saline solution were higher than for those observed with administration of azopolymer-coated pellets. Taken together, the optimal dose of budesonide with azopolymer-coated pellets was concluded to be $\sim$20 μg per rat ($\sim$100 μg kg^{-1}).

Discussion

Kimura et al (1992) examined the degradation characteristics of the azopolymer-coating and azopolymer-coated pellets in cultures of human intestinal flora using scanning electron microscopy, UV spectroscopy and in-vitro release experiments. These results suggested that the azopolymer coating would be degraded and the drugs contained therein would be released in the large intestine after oral administration of azopolymer-coated pellets.

The steroidal drug budesonide is a non-halogenated glucocorticoid and has high affinity for glucocorticoid receptors. In addition, it was reported that its bioavailability after intracolonic administration is $\sim$15% and after first-pass metabolism in the liver it is converted to pharmacologically less active metabolites, 6β-hydroxy-budesonide and 16α-hydroxy-prednisolone (Spencer & McTavish 1995). Budesonide, therefore, has a greater topical anti-inflammatory activity and less systemic effects than many other glucocorticoids. Based on these characteristics, we determined the enhanced therapeutic effects of budesonide with azopolymer-coated pellets, which have been reported to be a useful colon-specific drug delivery dosage form, against TNBS-induced colitis in rats.

There have been numerous attempts to induce inflammatory and ulcerative colonic syndromes in laboratory animals that resemble human disease forms, but none have been entirely successful. In this study, we selected the TNBS-induced colitis model using rats because of its simplicity and reproducibility (Morris et al 1989). TNBS induced colitis may be a good model of inflammatory bowel disease because it involves the use of an immunological hapten and is followed by a more chronic phase of inflammation than ethanol-induced acute mucosal injury (Yamada et al 1992).

To confirm the topical effects of budesonide and determine the effective dose, we first investigated its therapeutic effects against TNBS-induced colitis after intracolonic administration. In general, inflammation induces vascular hyperpermeability. Then, neutrophils are activated and invade the inflammatory region through the blood vessels. As activated neutrophils exhibit high MPO activity, we determined the MPO activity as an inflammatory marker. Budesonide administered by enema significantly reduced the MPO activity in the dose range of 4–20 μg, while no significant inhibition of the enhanced MPO activity was observed at a high dose (100 μg). Although the reason for this is not clear, one possible mechanism is suppression of the generation of various eicosanoids by the inhibition of phospholipase A_2. An alternative mechanism is the balance between the attacking and defending factors at the colitis regions caused by various eicosanoids. MPO is also involved in the metabolism of reactive oxygen species which enhance inflammation due to DNA damage, inactivation of various proteins and enzymes and peroxidation of membrane lipids. Thus, similar therapeutic effects

of budesonide were observed when examining other indicators such as damage score, as were found when examining MPO activity.

The T/B ratio is considered to reflect the adverse effect of budesonide. No significant decrease in T/B ratio was observed after intracolonic administration of budesonide at doses of 4 or 10 μg (Table 1). Considering both the therapeutic and the adverse effects of budesonide, we concluded that the effective dose was in the range of 4–10 μg per rat ($\sim$20–50 $\mu g\,kg^{-1}$) for intracolonic administration. Based on the above results and the previous report that the bioavailability of budesonide after oral administration was $\sim$10%, we used three doses, 20, 100 and 500 μg per rat for oral administration.

After oral administration of azopolymer-coated pellets, the MPO activity was markedly reduced at all doses used in this experiment. It was thus demonstrated that sufficient amounts of budesonide were delivered to the inflammatory region using orally administered azopolymer-coated pellets and its therapeutic effect was observed even at a dose of 20 μg. On the other hand, in the group given budesonide in saline solution no marked reduction of the MPO activity (which had been enhanced by TNBS) was observed at the low dose of 20 μg per rat because budesonide may be relatively well absorbed at the small intestine (Spencer & McTavish 1995). Increasing the dose of budesonide administered in saline solution not only increased the therapeutic effect but also caused reduction of the T/B ratio, which indicated an adverse effect. Therefore, it was concluded that the optimum therapeutic dose of budesonide administered in saline solution was 100 μg per rat ($\sim 500\,\mu g\,kg^{-1}$). When budesonide was administered orally in azopolymer-coated pellets, the dose required to produce a therapeutic effect was reduced by one-fifth ($\sim 100\,\mu g\,kg^{-1}$).

In conclusion, we demonstrated that an increase in topical therapeutic effects and decrease in systemic adverse effects were obtained following oral administration of budesonide in a novel dosage form, azopolymer-coated pellets. Such azopolymer-coated pellets containing anti-inflammatory drugs, including budesonide, may be useful for clinical treatment of colitis.

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References

Brogden, R. N., McTavish, D. (1992) Budesonide: an updated review of its pharmacological properties, and therapeutic efficacy in asthma and rhinitis. Drugs 44: 375–407

Dahlberg, E., Thalen, A., Brattsand, R., Gustafsson, J. A., Johansson, U., Roempke, K., Saartok, T. (1983) Correlation between chemical structure, receptor binding, and biological activity of some novel, highly active, 16α,17α-acetal-substituted glucocorticoids. Mol. Pharmacol. 25: 70–78

Kimura, Y., Makita, Y., Kumagai, T., Yamane, H., Kitao, T. (1992) Degradation of azo-containing polyurethane by the action of intestinal flora: its mechanism and application as a drug delivery system. Polymer 33: 5294–5299

Krawisz, J. E., Sharon, P., Stenson, W. F. (1984) Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity. Gastroenterology 87: 1344–1350

Morris, G. P., Beck, P. L., Herridge, M. S., Depew, W. T., Szewczuk, M. R., Wallace, J. L. (1989) Hapten-induced model of chronic inflammation and ulceration in the rat colon. Gastroenterology 96: 795–803

Nilsson, M., Edsbacker, S., Conradson, T.-B. (1992) Rectal and oral bioavailability of budesonide in man. Hell. J. Gastroenterol. 5 (Suppl.): 297

Podolsky, D. K. (1991) Inflammatory bowel disease. N. Engl. J. Med. 325: 928–937

Shanahan, F. (1993) Pathogenesis of ulcerative colitis. Lancet 342: 407–411

Spencer, C. M., McTavish, D. (1995) Budesonide: a review of its pharmacological properties and therapeutic efficacy in inflammatory bowel disease. Drugs 50: 854–872

Tozaki, H., Komoike, J., Tada, C., Maruyama, T., Terashima, H., Suzuki, T., Yamamoto, A., Muranishi, S. (1997) Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon. J. Pharm. Sci. 86: 1016–1021

Tozaki, H., Odoriba, T., Fujita, T., Murakami, M., Terabe, A., Suzuki, T., Okabe, S., Muranishi, S., Yamamoto, A. (1998) Colon delivery of anti-inflammatory drugs accelerates healing of TNBS-induced colitis in rats. Drug Delivery System 13: 165–171

Tozaki, H., Fujita, T., Odoriba, T., Terabe, A., Suzuki, T., Tanaka, C., Okabe, S., Muranishi, S., Yamamoto, A. (1999) Colon-specific delivery of R68070, a new thromboxane synthase inhibitor, using chitosan capsules: therapeutic effects against 2,4,6-trinitrobenzenesulfonic acid-induced ulcerative colitis in rats. Life Sci. In press

Yamada, T., Marshall, S., Specian, R. D., Grisham, M. B. (1992) A comparative analysis of two models of colitis in rats. Gastroenterology 102: 1524–1534

Yue, G., Sun, F. F., Dunn, C., Yin, K., Wong, P. Y.-K. (1996) The 21-aminosteroid tirilazad mesylate can ameliorate inflammatory bowel disease in rats. J. Pharmacol. Exp. Ther. 276: 265–270