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Validation of a Pharmacokinetic Model of Colon-specific Drug Delivery and the Therapeutic Effects of Chitosan Capsules Containing 5-Aminosalicylic Acid on 2,4,6-Trinitrobenzenesulphonic Acid-induced Colitis in Rats

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Abstract

A pharmacokinetic model of colon-specific drug delivery developed in a previous study has been validated by use of 5-aminosalicylic acid (5-ASA) as a model anti-inflammatory drug.

The simulation curves obtained from the pharmacokinetic model were in good agreement with experimental data obtained after oral administration of 5-ASA-containing chitosan capsules. The concentrations of 5-ASA in the large intestinal mucosa after drug administration were higher than after administration of the drug in carmellose suspension. We then attempted colon-specific delivery of an anti-ulcerative colitis drug, in chitosan capsules, to accelerate healing of 2,4,6-trinitrobenzenesulphonic acid sodium salt (TNBS)-induced colitis in rats. To confirm this therapeutic model, salazosulphapyridine (SASP), a commercially available 5-ASA prodrug, was used as positive control. Colonic injury and inflammation were assessed by measuring myeloperoxidase activity and visual assessment (damage score), respectively. Because SASP is effective against TNBS-induced colitis in rats, use of the SASP-sensitive TNBS-induced colitis model validated the therapeutic effects of 5-ASA-containing chitosan capsules, which were significantly better than those of a suspension of the drug in carmellose.

These findings suggest that our pharmacokinetic model of colon-specific drug delivery can accurately evaluate this colon-specific delivery system and that 5-ASA-containing chitosan capsules are more effective than other 5-ASA formulations for treatment of TNBS-induced colitis in rats.

We have previously prepared chitosan capsules, a novel colon-specific drug-delivery system, containing insulin and a variety of additives including absorption enhancers and protease inhibitors, and demonstrated that this system improves the pharmacological availability of insulin after oral administration to rats (Tozaki et al 1997).

Ulcerative colitis and Crohn's disease are recurrent, idiopathic inflammatory disorders involving the mucosa and sub-mucosa of the colon. Recently, salazosulphapyridine (SASP) and steroidal drugs have been used for the treatment of ulcerative colitis and Crohn's disease. Reductive scission of the azo bond of SASP by gut bacteria yields sulphapyridine and 5-aminosalicylic acid (5-ASA), the putative active therapeutic agent (Khan et al 1977). SASP causes side-effects in some patients; some, e.g. anorexia and nausea, are dose-related whereas others, e.g. skin rash and blood dyscrasias, are idiosyncratic. Reversible infertility with a reduced proportion of abnormal spermatozoa, has been observed in men receiving SASP. Because it is

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believed that circulating sulphapyridine is the main cause of these unwanted side-effects (Peppercorn 1992), several approaches have been evaluated for delivery of 5-ASA to the colon in less toxic carriers (Vos et al 1992). However, 5-ASA is rapidly absorbed from the small intestine, and there is little localization of 5-ASA in the colon relative to the small intestine (Rijk et 1988). Therefore, our system delivered 5-ASA to the large intestine specifically, and we found it was therapeutic against 2,4,6-trinitrobenzenesulphonic acid (TNBS)-induced colitis in rats (Odoriba et al 1997; Tozaki et al 1998).

We recently developed a pharmacokinetic model of colon-specific drug delivery using chitosan capsules to simulate the in-vivo fate of salicylic acid delivered orally in chitosan capsules (unpublished work). In the current study we have analysed the in-vivo behaviour of 5-ASA after oral administration in chitosan capsules by use of the pharmacokinetic model developed previously, and compared this colon-specific delivery with 5-ASA-containing carmellose suspension. In addition to pharmacokinetic analysis we studied the therapeutic effect of 5-ASA-containing chitosan capsules against TNBS-induced colitis in rats; the effect was validated by comparison with the therapeutic effects of SASP, a commercially available 5-ASA prodrug, as a positive control.

Materials and Methods

Materials

5-ASA was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). TNBS, ethanol, carmellose sodium, salazosulphapyridine (SASP) and trichloroacetic acid were obtained from Nacalai Tesque (Kyoto, Japan). Other chemicals were of analytical grade.

Chitosan capsules (3·5 mm × 1·6 mm) containing 5-ASA (1·5 mg) were prepared at Aicello. The surface of these capsules was coated with hydroxypropylmethylcellulose phthalate as an enteric coating material (Tozaki et al 1997).

Pharmacokinetics of 5-ASA after intravenous or intestinal loop administration

Animal experiments were performed in accordance with the Guidelines for Animal Experimentation at Kyoto Pharmaceutical University. Male Wistar rats (Shimizu Laboratory Supplies, Kyoto, Japan), 200 g (approx.), were fasted for 16 h. Under anaesthesia induced by intraperitoneal injection of 32 mg kg^{-1} sodium pentobarbital, phosphate buffered saline (0·5 mL) containing dissolved 5-ASA (1·5, 2·3 or 3·0 mg) was intravenously administered by bolus injection. In-situ closed-loop absorption experiments were performed according to a method described elsewhere (Tozaki et al 1996). 5-ASA was dissolved in phosphate-buffered saline (pH 7·4; 1·0 mL) for the ileum loop and at pH 6·0 for the caecum loop. The drug solution was warmed to 37°C and 1 mL was injected into the loop. The gastrointestinal transit of chitosan capsules (4·5 mg/3 capsules) was determined by peritoneotomy at fixed times after oral administration.

Assay of 5-ASA

5-ASA was assayed by reversed-phase high-performance liquid chromatography (HPLC) (Vos et al 1991). Compounds were separated on a 150 mm × 4·6 mm i.d. ODS (C_{18}) column (Cosmosil AR-300; Nacalai Tesque, Kyoto, Japan). The mobile phase was 0·1 M acetic acid–acetonitrile–triethylamine, 92 : 8 : 0·2 (v/v); the flow rate was 0·8 mL min^{-1} at 37°C. Fluorescence detection at excitation and emission wavelengths of 315 and 430 nm was performed with a Shimadzu RF-10A detector.

Induction of colonic inflammation and treatment of ulcerative colitis

Colonic inflammatory lesions were induced by the method of Morris et al (1989) and Tozaki et al (1999). TNBS (20 mg in 0·25 mL 50% ethanol) was instilled into the colon via the anus of the rats. From one to four days after intracolonic administration of TNBS, 5-ASA (4·5 mg) or SASP (11·7 mg) was administered orally in chitosan capsules (three capsules) or carmellose suspension (1 mL) every 12 h. Five days after the administration of TNBS, the rats were killed and the distal colon was removed. Samples of inflamed tissue were excised to measure myeloperoxidase activity (Krawisz et al 1984) and damage score (Morris et al 1989).

Measurement of myeloperoxidase activity. The activity of myeloperoxidase, which is found in neutrophils, can be used to evaluate the extent of inflammation of the intestine. Specimens (200 mg) of distal colon were minced in a beaker containing hexadecyltrimethylammonium bromide (HTAB) buffer (0·5% HTAB in 50 mM phosphate buffer, pH 6·0; 1 mL) on ice, transferred to a test tube, and homogenized with a Polytron homogenizer (3 × 30 s, on ice). After homogenization, the

homogenizer (Kinematica AG, Switzerland) was rinsed with HTAB buffer (2 × 1 mL). The pooled homogenate and washes were sonicated for 10 s, freeze–thawed three times, and centrifuged at 10 000 rev min^{-1} for 1 min. The supernatant was assayed spectrophotometrically for myeloperoxidase activity. Supernatant (0·1 mL) was mixed with *o*-dianisidine hydrochloride (0·167 mg mL^{-1}) and hydrogen peroxide (0·44 mM). The absorbance change at 460 nm was measured with a microplate reader (Molecular Devices). One unit of myeloperoxidase activity degrades 1 μmol peroxidase min^{-1} at 25°C (Krawisz et al 1984).

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 0–5 by two independent observers blind to the treatment. A score of 0 represented no damage. A score of 1 represented localized hyperaemia, but no ulcers. A score of 2 represented linear ulcers with no significant inflammation. A score of 3 represented linear ulcer with inflammation at one site. A score of 4 represented two or more sites of ulceration or inflammation. A score of 5 represented two or more major sites of inflammation and ulceration or one major sites of inflammation and ulceration extending >1 cm along the length of the colon (Morris et al 1989).

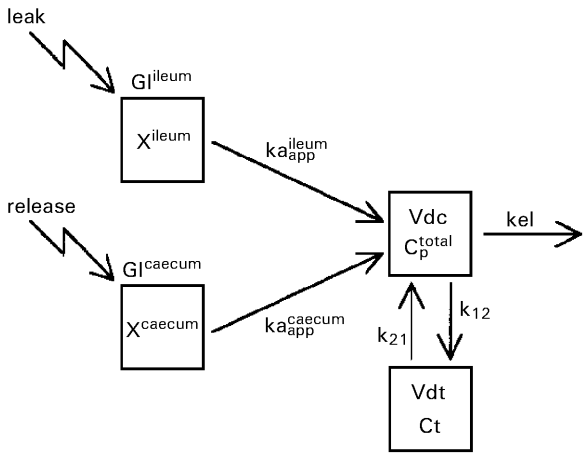

Figure 1. A pharmacokinetic model of colon-specific drug delivery by use of chitosan capsules. GI^{ileum} and GI^{caecum} are the ileum and the caecum gastrointestinal tracts, X^{ileum} and X^{caecum} the drug content of the ileum and the caecum, ka_{app}^{ileum} and ka_{app}^{caecum} the apparent first absorption rate constants in the ileum and the caecum, Vdc the volume of distribution of the central compartment, C_p the plasma concentration, Vdt the volume of distribution of peripheral tissue, C_t the concentration in peripheral tissue, k_{12} and k_{21} the distribution rate constants, and k_{el} the systemic elimination rate constant.

Data analysis

Our pharmacokinetic model of colon-specific drug delivery with chitosan capsules will be reported elsewhere (Figure 1). The intralumninal 5-ASA content and concentrations of the drug in plasma after oral administration in chitosan capsules have also been reported (Tozaki et al 1998). Pharmacokinetic parameters after intravenous administration or in-situ intestinal administration of 5-ASA were calculated by non-linear least-squares regression analysis by means of the Gauss–Newton algorithm in MULTI (Yamaoka et al 1981).

Statistical analysis

Results are expressed as means ± s.e.m. Data were analysed by one-way analysis of variance then Dunnett's test.

Results

Pharmacokinetics of 5-ASA

The pharmacokinetics of 5-ASA were investigated after intravenous and in-situ closed-loop administration at doses ranging from 1·5–3·0 mg. Plasma concentration profiles of 5-ASA followed the two-compartment model (Figure 2A), and the areas under the plasma concentration time curves (AUC) increased linearly with increasing doses (Figure 2B). Furthermore, the apparent absorption rate constants did not change at the doses tested (data not shown). We therefore used the pharmacokinetic parameters obtained at a dose of 1·5 mg for subsequent studies.

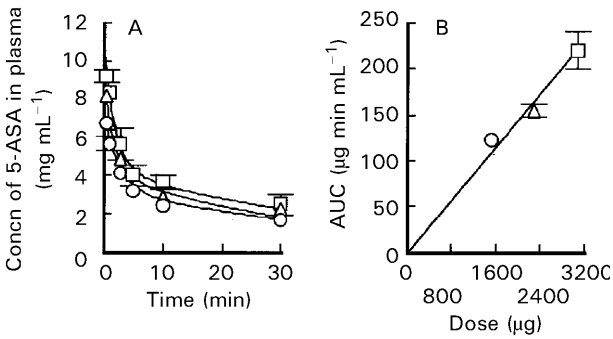

Figure 2. The plasma 5-aminosalicylic acid concentration–time profile (A) and the area under the curve of plasma concentration against time (AUC) (B) after intravenous administration of 5-aminosalicylic acid (○ 1·5 mg, △ 2·3 mg, □ 3·0 mg) to the rat. Results are expressed as means ± s.e.m. from three experiments.

In-vivo oral administration of 5-ASA in chitosan capsules

The distribution of 5-ASA along the intestine was examined after oral administration of chitosan capsules containing 5-ASA (Figure 3). Chitosan capsules reached the caecum 3 to 5 h after administration (T^{caecum}, i.e. gastrointestinal transit times to caecum) and disintegrated in the caecum (Figure 3). To confirm colon delivery of chitosan capsules and to predict the effect of 5-ASA in the colon, we determined the intraluminal content of 5-ASA in the caecum at fixed times after oral administration of chitosan capsules. Large amounts of 5-ASA were observed in the caecum 4 to 6 h after administration (Figure 4A). Two hours later, 5-ASA appeared in the plasma (Figure 4B). These results and the pharmacokinetic parameters analysed by intravenous and closed-loop experiments were used to calculate the simulation curves for intraluminal content and plasma concentration profiles, using the pharmacokinetic model shown in Figure 1. Both simulation curves were consistent with the observed data after oral administration of 5-ASA-containing chitosan capsules (Figure 4).

In-vivo therapeutic experiments

The TNBS-induced colitis model was validated by use of SASP, a commercially available 5-ASA prodrug, as positive control. Myeloperoxidase activity and damage score after intracolonic administration of TNBS were significantly higher than for the saline and 50% ethanol groups used as controls (Table 1). SASP–carmellose suspension reduced the enhanced myeloperoxidase activity and damage score after intracolonic administration of TNBS. Therefore, the TNBS-induced colitis model in rats was demonstrated to be useful for evaluation

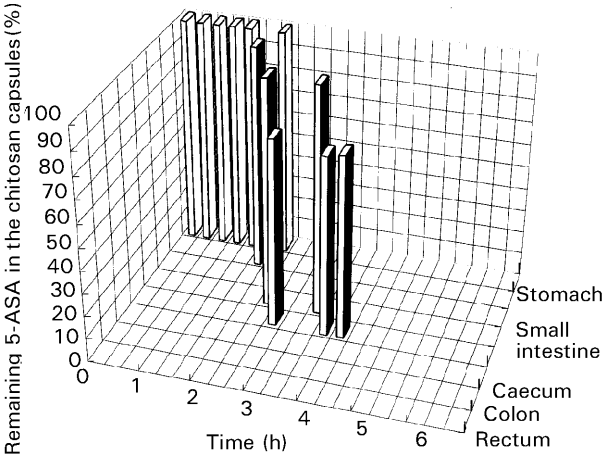

Figure 3. Gastrointestinal transit and amount of 5-aminosalicylic acid remaining after oral administration to rat in chitosan capsules. Results are from a single experiment.

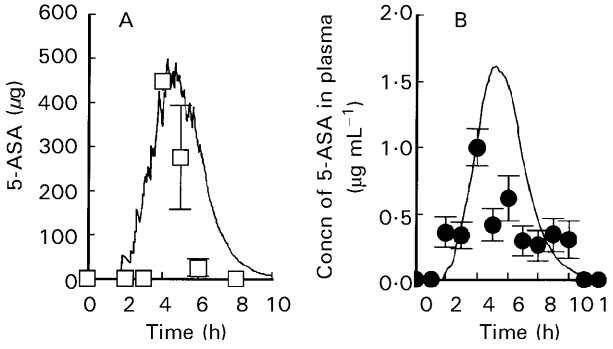

Figure 4. Amount of 5-aminosalicylic acid in the caecum (A) (– simulation curve, □ experimental data) and plasma concentration of 5-aminosalicylic acid (B) (– simulation curve, ● experimental data) after oral administration of chitosan capsules containing 5-aminosalicylic acid. Simulation curves were calculated by use of the pharmacokinetic model to be reported elsewhere. Experimental data are cited from Tozaki et al (1998a). Results are expressed as means ± s.e.m. from five experiments.

of the therapeutic effects of 5-ASA. The therapeutic effect of 5-ASA was enhanced by use of chitosan capsules compared with 5-ASA-containing carmellose suspension. 5-ASA-containing chitosan capsules and SASP–carmellose suspension had similar therapeutic effect.

We confirmed the effectiveness of 5-ASA-containing chitosan capsules at the morphological level (Figure 5). Substantial inflammation and ulceration were caused by intracolonic administration of TNBS compared with saline. 5-ASA was considerably more effective against inflammation and ulceration than carmellose suspensions. These morphological results were consistent with the myeloperoxidase activity and the damage score results (Table 1).

Discussion

To confirm the usefulness of chitosan capsules for colon-specific drug delivery, we analysed the intestinal absorption of salicylic acid using a pharmacokinetic model. This model enables us to understand the in-vivo fate of the drug after its oral administration in chitosan capsules. To achieve colon-specific drug delivery and increase the therapeutic effect of local effect drugs, the amount of drug in the caecum, X^{caecum}, should be increased.

Using data on the gastrointestinal behaviour and the plasma concentration profiles of 5-ASA after oral administration of chitosan capsules containing 5-ASA, we analysed the in-vivo fate of 5-ASA by the pharmacokinetic model to be described elsewhere. As shown in Figure 4, the observed intraluminal content and plasma concentration profile

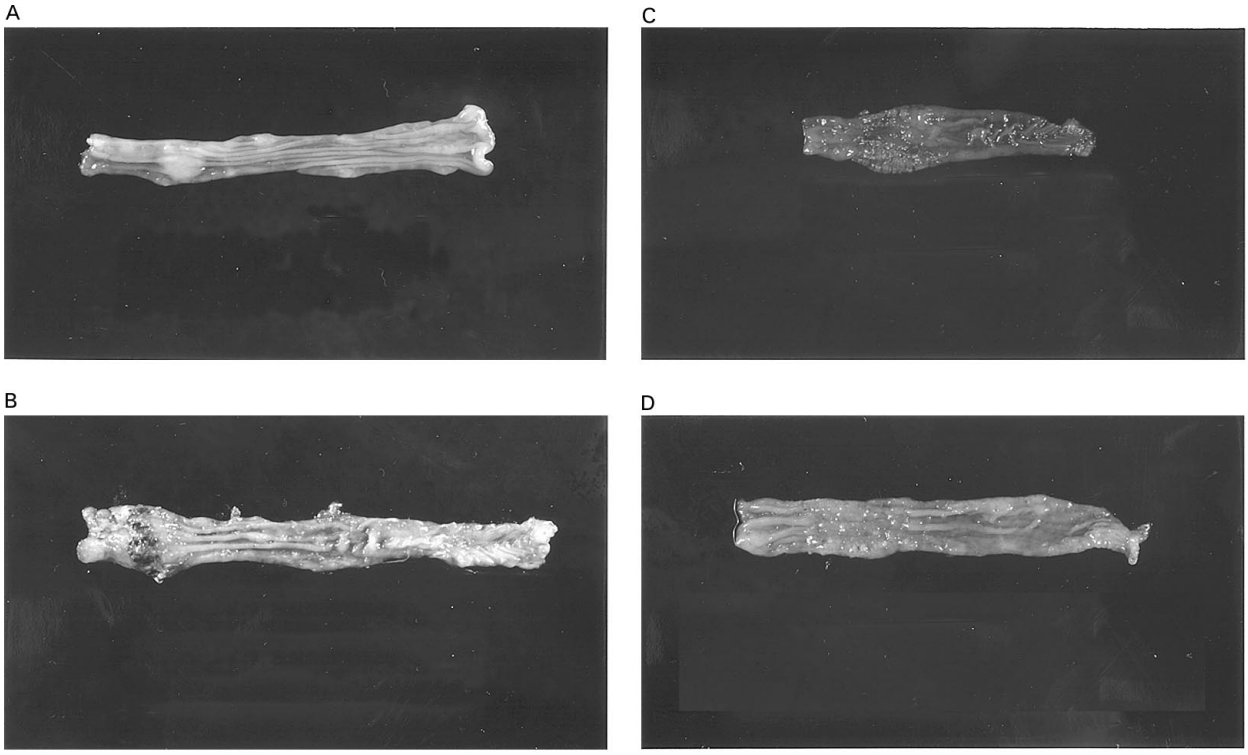

Figure 5. Photographs of the colon from rats five days after oral administration of 5-aminosalicylic acid in different dosage forms: A. Saline (0·2 mL), B. 2,4,6-trinitrobenzenesulphonic acid sodium salt (20 mg/0·25 mL), C. 2,4,6-trinitrobenzenesulphonic acid sodium salt (20 mg/0·25 mL) then 5-aminosalicylic acid-containing carmellose suspension (4·5 mg mL^{-1} after 12 h), D. 2,4,6-trinitrobenzenesulphonic acid sodium salt (20 mg/0·25 mL) then 5-aminosalicylic acid-containing chitosan capsules (4·5 mg in three capsules after 12 h).

Table 1. Therapeutic effects of 5-aminosalicylic acid (4·5 mg) in a carmellose suspension or in a chitosan capsule on myeloperoxidase activity and damage score on day 5 in TNBS-induced colitis in rats.

Experiment	Myeloperoxidase activity	Damage score
Saline-ethanol control	1·18 ± 0·00**	0·45 ± 0·22**
Saline-saline control	5·96 ± 2·20**	2·0 ± 0·0**
TNBS induction		
Saline	31·37 ± 2·43	4·8 ± 1·9
Suspension	20·31 ± 1·41**	3·8 ± 0·4**
Capsule	13·33 ± 0·63** ##	2·2 ± 0·4** ##
Salazosulphapyridine	19·29 ± 0·63**	3·2 ± 0·5**

Results are expressed as means ± s.e.m. from five experiments. $**P < 0·01$ compared with the group given 2,4,6-trinitrobenzenesulphonic acid sodium salt (TNBS) and saline control. $\#\#P < 0·01$ compared with the group receiving 5-aminosalicylic acid in carmellose suspension. Data are from Odoriba et al (1997).

of 5-ASA are consistent with the simulation curves calculated by use of the pharmacokinetic model described in Figure 1. In this work we used pharmacokinetic parameters obtained after intravenous (Figure 2) and closed-loop intestinal (data not shown) administration. In this study the intestinal administration experiments were studied using the caecum, rather than the colon, to clarify 5-ASA kinetics in rats, because chitosan capsules disintegrate in the caecum after oral administration to the rat. This pharmacokinetic model might enable estimation of the pharmacokinetics of different colon-specific delivery systems, including chitosan capsules, and the optimization of colon-specific delivery dosage forms containing drugs which have a narrow therapeutic window.

We also tested chitosan capsules containing lactose as a placebo and found they had no therapeutic effect on TNBS-induced colitis in rats, showing that chitosan capsules themselves do not effect colitis (Odoriba et al 1997; Tozaki et al 1998). On the other hand, we observed a marked decrease in relative myeloperoxidase activity and morphological damage when 5-ASA was orally administered in chitosan capsules, rather than as a suspension in carmellose (Table 1).

We have previously reported the mucosal concentration of 5-ASA in different regions of the gastrointestinal tract 4 h after oral administration of different dosage forms. We found 5-ASA in the mucosae of stomach and small intestine after oral administration as a suspension in carmellose, but

none in the large intestinal mucosa. In contrast 5-ASA was observed only in the large intestinal mucosa after oral administration of 5-ASA as chitosan capsules. The increased effectiveness of 5-ASA-containing chitosan capsules is probably because of the large amounts of 5-ASA in the large intestine (Figure 3) and large intestinal mucosae (Tozaki et al 1998).

Moreover similar decreases in myeloperoxidase activity and damage scores were observed after administration of 5-ASA-containing chitosan capsules and SASP suspensions in carmellose (Table 1). SASP is widely administered as a clinical anticolitis drug despite adverse effects such as anorexia and nausea attributed to sulphapyridine. Our results indicate that 5-ASA-containing chitosan capsules might be as effective as SASP but with fewer side-effects. In our assumption, 5-ASA-containing chitosan capsules might have better therapeutic effect than other 5-ASA formulations, e.g. an enteric coating dosage form, because chitosan capsules might transport more 5-ASA to the region of inflammation in the colon than does the enteric coating formulation.

In conclusion, we have confirmed the usefulness of our pharmacokinetic model of colon-specific delivery in chitosan capsules. 5-ASA was delivered to the colon as expected by the pharmacokinetic model and the therapeutic effects of 5-ASA were significantly improved by use of 5-ASA-containing chitosan capsules in the TNBS-induced colitis model in rats. These results demonstrated that 5-ASA-containing chitosan capsules are more effective than other 5-ASA formulations in both aspects of the pharmacokinetics and therapeutics of TNBS-induced colitis in rats.

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