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Immunological control of drug absorption from the gastrointestinal tract: the mechanism whereby intestinal anaphylaxis interferes with the intestinal absorption of bromthymol blue in the rat

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Rats were immunized intraperitoneally with ovalbumin and the disappearance of bromthymol blue (BTB) from the intestinal lumen, its accumulation in the tissue, and its net absorption were examined by means of an in-situ recirculation technique during local anaphylaxis. The disappearance of BTB from the intestinal lumen and its net absorption were significantly reduced, but there was no significant effect on its accumulation in the tissue. The pH value of the luminal solution and the perfusate volume were not influenced by intraluminal challenge with the antigen in ovalbumin-immunized rats. In addition, no significant effect was observed on intestinal permeability to BTB in the in-vitro everted sac technique. The intestinal blood flow, measured by a hydrogen clearance method, was not reduced significantly by the intraluminal exposure to antigen. There was enhanced Evans Blue leakage and mucus release in the perfusate after intraluminal challenge with ovalbumin in ovalbumin-immunized rats, but not in non-immunized rats. A significant increase of BTB binding with macromolecular substances in the perfusate was observed during the local anaphylaxis. These findings suggest that the decreased absorption of BTB is due to the interaction with the macromolecular substances in the perfusate during local anaphylaxis.

We have recently found a decrease in the intestinal absorption of bromthymol blue (BTB) by ovalbumin-immunized rats during local anaphylaxis (Yamamoto et al 1985). The antibody formation was confirmed by a passive cutaneous anaphylaxis technique and the decreased absorption of BTB was shown to be dose-dependent and antigen-specific. In addition, there was a good correlation between an enhanced leakage of Evans Blue dye from the blood circulation and decreased absorption of BTB during local anaphylaxis. However, the mechanism by which local anaphylaxis interferes with the intestinal absorption of BTB has not been clearly demonstrated. Various physiological mechanisms such as changes in pH value, water and electrolyte transport, intestinal blood flow, mucosal permeability, and goblet cell mucus release could contribute. Perdue et al (1984) reported that intestinal challenge with the specific antigen led to a significant inhibition of Na^+, K^+, Cl^- and water absorption. Bloch & Walker (1981) demonstrated an alteration in vascular and mucosal permeability during intestinal anaphylaxis. Lake et al (1980) found an enhanced release of goblet cell mucus occurred after intraluminal exposure to the specific antigen. We have shown that during systemic anaphylaxis, intestinal absorption of salicylic acid was decreased due to the reduced blood flow (Nakamura et al 1982; Yamamoto et al 1984a, b, c).

The present research was aimed at the elucidation of the mechanisms by which there was a decreased absorption of BTB during local anaphylaxis.

MATERIALS AND METHODS

Materials

Ovalbumin was purchased from Sigma Chemical Co. St. Louis, Mo., USA. Freund's incomplete adjuvant was obtained from E. Merck, Darmstadt, Germany. BTB and all other reagents were of reagent grade from Nakarai Chemical Co., Ltd, Japan.

Preparation of drug solution

The isotonic buffer solution of pH 6·5 was prepared from 0·123 M Na_2HPO_4 and 0·163 M NaH_2PO_4. BTB was dissolved in this buffer solu-

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tion at the concentration of 0·1 mM for absorption studies.

Animals and immunization
Male Wistar albino rats, 150 to 200 g, were fed on a diet free of ovalbumin and immunized according to the following schedule. Ovalbumin (1 mg) dissolved in 0·25 ml of 0·9% NaCl (saline) was emulsified with an equal volume of incomplete Freund's adjuvant and given intraperitoneally to rats under light ether anaesthesia. Animals were immunized once and the absorption studies were carried out 10 days later.

Induction of intestinal anaphylaxis
Local intestinal anaphylaxis was elicited by intraluminal challenge with ovalbumin solution. Animals were anaesthetized with pentobarbitone given by intraperitoneal injection and the small intestine cannulated for the recirculation study. The entire length, from the pylorus to ileo-caecal junction, was used. The bile duct was ligated in all experiments. Ovalbumin dissolved in pH 6·5 buffer solution was recirculated through the intestine for 10 min at a rate of 5 ml min^{-1} using a peristaltic pump. In the control experiments, buffer solution was recirculated similarly.

Absorption studies
Absorption studies were performed using an in-situ recirculation technique and an in-vitro everted sac experiment.

In-situ recirculation technique. The procedure was as reported previously (Nakamura et al 1982; Yamamoto et al 1984a, b, c, 1985). After antigen pretreatment, 40 ml of a drug solution at 37 °C was recirculated through the intestine for 1 h at 5 ml min^{-1} using a peristaltic pump. At the end of an experiment, the perfused solution in the intestine was withdrawn and the lumen was washed with pH 6·5 buffer solution. The washings were combined with the perfused solution and made up to 100 ml with pH 6·5 buffer solution. The amount of drug absorbed from the lumen was calculated as the difference between the amount of the drug in the initial and the final solutions. The amount of net drug absorption during 1 h perfusion was calculated by the difference in the amount of dye between its disappearance from the lumen and accumulation in the tissue.

In-vitro everted sac experiment. The antigen pretreatment was carried out as in the in-situ recirculation experiments and then the small intestine was isolated and cut into two segments. The upper 20 cm segment (jejunum) and the lower 20 cm segment (ileum) were everted and ligated at one end. As serosal fluid, 3 ml of pH 7·4 phosphate buffer solution was introduced and the sac ligated, and placed in mucosal fluid (40 ml of pH 6·5 isotonic phosphate buffer solution containing 0·1 mM BTB) bubbled with 5% CO_2 in O_2 at 37 °C. At the end of 1 h, the serosal fluid was collected and assayed for BTB.

Volume and pH determination in the perfusate
The method and the antigen pretreatment were the same as in-situ absorption studies. After recirculation of pH 6·5 buffer solution, with or without ovalbumin, for 10 min at 5 ml min^{-1}, 40 ml of a buffer solution was recirculated for 1 h. The perfusate volume was recorded and the pH value in the perfusate determined.

Blood flow measurements
The intestinal blood flow before and after the challenge with ovalbumin in each animal was measured by a hydrogen clearance method (PHG 201, Unique Medical Co., Ltd) as described previously (Aukland et al 1964; Forrester et al 1980; Tepperman & Jacobson 1982).

Exsorption studies
The exsorption of Evans Blue to the gut lumen during local anaphylaxis was studied by the method described previously (Kakemi et al 1970; Tokunaga et al 1978; Yasuhara et al 1979; Muranushi et al 1980). The operation was the same as the in-situ absorption studies. After the intravenous administration of Evans Blue (1% w/v, 0·5 ml), the small intestine was perfused with pH 6·5 buffer solution with or without ovalbumin for 10 min at 5 ml min^{-1} using a peristaltic pump. Then, 40 ml of pH 6·5 buffer solution was recirculated through the intestine for 1 h in the same way. At the end of the recirculation, the amount of the dye in the perfusate was determined spectrophotometrically.

Mucus release determination in the perfusate
After recirculation of pH 6·5 buffer solution for 1 h in immunized and normal rats, an aliquot of the perfusate was centrifuged for 10 min at 3000 rev min^{-1} and the mucus concentration in the supernatant was assayed.

Binding of BTB with macromolecular substances in the perfusate

Ultrafiltration was adopted to estimate the binding of BTB with macromolecular substances that leaked in the perfusate. The operation and the procedure of ovalbumin challenge were the same as in-situ absorption studies. After the recirculation of BTB solution for 1 h, ultrafiltration was performed by micropartition system (MPS-1, Amicon Co., Ltd.).

Analytical methods

BTB in the perfused solution. The sample solution (2 ml) was made alkaline with 5 ml of 1 M NaOH and determined spectrophotometrically at 617 nm.

BTB in the tissue. The small intestine was homogenized in three times its weight of pH 6·5 buffer solution. A mixture of 5 ml of the homogenate and 5 ml of acetone was shaken for 15 min and then centrifuged for 10 min at 2500 rev min^{-1}. Supernatant (5 ml) was made alkaline with 1 ml of 1 M NaOH and determined spectrophotometrically at 621 nm.

Mucus. Mucus was determined by an anthrone method using D-glucose as a standard and a colorimetric reading at 620 nm (Roe 1955).

Evans Blue. After recirculation, the perfusate was centrifuged for 10 min at 2500 rev min^{-1} and the dye in the supernatant determined spectrophotometrically at 606 nm.

Statistical analyses

Results were expressed as the mean ± standard deviation. Statistical analyses were performed using Student's *t*-test.

RESULTS

In-situ intestinal transfer of BTB during local anaphylaxis

The disappearance of BTB from the intestinal lumen, its accumulation in the tissue, and its net absorption were examined by means of an in-situ recirculation technique during local anaphylaxis. As shown in Table 1, the disappearance from the intestinal lumen and the net absorption of BTB were significantly reduced compared with buffer treatment as the control; no significant effect was noted on the accumulation of BTB in the tissue.

Table 1. Intestinal transfer of BTB during local anaphylaxis.

	Buffer	Ovalbumin (400 mg)
Disappearance (%)	62·2 ± 4·1 (8)	51·9 ± 3·2 (7)**
Tissue accumulation (%)	41·3 ± 4·4 (8)	36·6 ± 4·1 (7)†
Net absorption (%)	22·2 ± 4·2 (8)	15·3 ± 2·6 (7)**

Results are expressed as the mean ± s.d. with the number of experiments in parentheses. ** $P < 0·01$, † not significantly different, compared with the control.

The pH value and the perfusate volume during local anaphylaxis

The pH value and the perfusate volume after 1 h recirculation of pH 6·5 buffer solution in ovalbumin-immunized rats were not affected by local intestinal anaphylaxis.

Transfer rate of BTB through tissue in-vitro during local anaphylaxis

In both jejunum and ileum, no significant effect was obtained on the intestinal permeability of BTB by intraluminal challenge with ovalbumin in immunized rats.

The intestinal blood flow during local anaphylaxis

The intestinal blood flow during local anaphylaxis measured by the hydrogen clearance method was not significantly reduced by intraluminal exposure to 200 mg or 400 mg of the antigen.

The intestinal exsorption of Evans Blue in ovalbumin-immunized and non-immunized rats

A significant increase in leaks of the dye was observed by the intraluminal challenge with ovalbumin compared with the control value, but was not observed in non-immunized rats (Table 2).

Intraluminal mucus release in ovalbumin-immunized and non-immunized rats

There was a significant increase in mucus release on intraluminal exposure to 200 or 400 mg of ovalbumin in immunized rats (means with s.d., 26·2 ± 2·7 μg ml^{-1}, n = 6, and 33·5 ± 9·5 μg ml^{-1}, n = 5, respectively, $P < 0·01$, from control value of 18·7 ± 4·3 μg ml^{-1}, n = 7). However, the enhancement was not found in non-immunized rats challenged with 400 mg of ovalbumin intraluminally.

Binding of BTB with intraluminal macromolecular substances in the perfusate

There was a significant increase in the binding of BTB with intraluminal macromolecular substances

Table 2. The leakage of Evans Blue in ovalbumin-immunized and non-immunized rats

	Evans Blue concentration in the perfusate (μg ml^{-1})	
Animals	Buffer	Ovalbumin (400 mg)
Immunized rats	11·6 ± 5·3 (8)	43·4 ± 21·2 (7)**
Non-immunized rats	16·3 ± 8·4 (6)	10·6 ± 7·1 (8)†

Results are expressed as the mean ± s.d. with the number of experiments in parentheses. ** $P < 0{\cdot}01$, † not significantly different, compared with the control.

by the intraluminal challenge with 40, 200 or 400 mg of ovalbumin in ovalbumin-immunized rats, although no significant effect was noted in ovalbumin-immunized rats challenged with 4 mg of ovalbumin. Furthermore, there was no significant difference between ovalbumin-treated and buffer-treated groups on the binding of BTB with intraluminal macromolecular substances in non-immunized rats (Table 3).

Table 3. Binding of BTB with intraluminal macromolecular substances in the perfusate in ovalbumin-immunized rats and non-immunized rats

Ovalbumin (mg)	Immunized rats	Non-immunized rats
0	34·8 ± 6·3 (8)	40·7 ± 7·2 (6)
4	40·3 ± 6·6 (7)†	—
40	43·4 ± 6·6 (7)*	—
200	44·8 ± 3·7 (7)***	—
400	51·8 ± 7·6 (8)†	38·7 ± 4·2 (8)*

Results are expressed as the mean ± s.d. with the number of experiments in parentheses, † not significantly different, * $P < 0{\cdot}02$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$, compared with the control.

DISCUSSION

In the present experiments, we have investigated the mechanism whereby an intestinal immune response interferes with the intestinal absorption of BTB. Our previous results had suggested that some BTB was to be found in the intestinal tissue during the course of absorption (Nakamura et al 1976), therefore, the accumulation of the dye in the tissue was examined by means of an in-situ recirculation technique during local anaphylaxis. No significant effect was noted, suggesting that intestinal anaphylaxis does not interfere with the tissue accumulation of BTB and the net absorption of BTB correlated with the disappearance of the dye from the intestinal lumen.

As Nakamura et al (1976) had demonstrated that the binding to brush borders and the uptake by isolated epithelial cells of BTB was decreased with increasing the pH value, the pH value of the perfusion fluid after recirculating the intestine with local anaphylaxis for 1 h was examined and found not to be affected by the antigen challenge. Therefore, the decreased BTB absorption during local anaphylaxis is not due to the changes in pH value.

Recently, Perdue et al (1984) reported that intestinal challenge with the specific antigen led to a significant inhibition of water and electrolyte absorption. This is inconsistent with our present finding that the perfusate volume was not influenced by the intraluminal challenge with the antigen. The reason for the difference remains unexplained.

It was reported that intestinal anaphylaxis in the rat is accompanied by enhanced vascular and mucosal permeability (Murray et al 1971; Bloch et al 1979; Bloch & Walker 1981). Murray et al (1971) showed that the leakage of macromolecule (PVP) caused by the increase in permeability of the bowel wall was observed in rats infected with parasites. Bloch et al (1979) showed that in a parasite infection and with mild anaphylaxis, increase in uptake of protein antigen occurred in-vivo. They also demonstrated that the alteration in vascular and mucosal permeability which accompanied intestinal anaphylaxis was reflected by the increased retention of ^{125}I-labelled rat serum albumin in gut wall segments (Bloch & Walker 1981). Thus, intestinal anaphylaxis may cause an increase in uptake of macromolecules from the intestinal lumen. However, the effect of intestinal anaphylaxis on the intestinal uptake and transport of low molecular weight organic compounds has received little attention. We found no significant effect on the intestinal permeability of BTB after making an intraluminal challenge with ovalbumin in immunized rats suggesting that local anaphylaxis might not interfere with the permeability of low molecular weight compounds in spite of the enhanced permeability of macromolecules.

In a previous report, intestinal and gastric blood flow was found to be significantly reduced during systemic anaphylaxis and the reduced blood flow resulted in the decreased absorption of salicylic acid (Yamamoto et al 1984c). However, in the present study, intestinal blood flow was not affected by local anaphylaxis which is in agreement with our finding that local intestinal anaphylaxis does not interfere with the intestinal absorption of salicylic acid (Yamamoto et al 1985). It seems likely, therefore, that the blood flow does not contribute to the decreased absorption of BTB caused by local anaphylaxis.

An enhanced leakage of dye and mucus release were observed during local anaphylaxis as with systemic anaphylaxis. These phenomena may represent the intestinal immunological responses because there was no significant change in the leakage of the dye and mucus release by intraluminal antigen challenge in non-immunized rats. With systemic anaphylaxis, it has been shown that intestinal absorption of salicylic acid is decreased, but no significant change was obtained in the binding of salicylic acid with intraluminal macromolecular substances in the perfusate in spite of the enhanced leakage of the dye and the mucus release (Yamamoto et al 1984b). On the other hand, the present study has shown a decreased absorption and increased binding of BTB with macromolecular substances during local anaphylaxis, the binding almost correlating with the enhanced leakage of the dye and mucus. The increased binding of BTB may be due to an increase in macromolecular substances which may interfere with the intestinal absorption of BTB.

The enhanced leakage of the dye during local anaphylaxis has been widely reported. Ross et al (1976) demonstrated that after intraperitoneal sensitization of rats with rat serum containing reaginic antibody, intravenous injection of the dye and intraperitoneal challenge with antigen caused a release of the dye into the peritoneal fluid. As the dye combines preferentially with plasma albumin, its leakage represents the leakage of plasma protein into the intestinal lumen. This is consistent with the increased permeability of macromolecules during local anaphylaxis.

The release of goblet cell mucus caused by intestinal immune responses was demonstrated by Lake et al (1980) who found a dose-dependent and antigen-specific release of ^{35}S-labelled high molecular weight glycoprotein presumably from goblet cells during local anaphylaxis. Walker et al (1977) and Lake et al (1979) demonstrated that immune complexes prepared in antibody excess triggered a release of goblet cell mucus onto the intestinal surface and the release of goblet cell mucus was also enhanced by antigen stimulation in immunized animals. Furthermore, it was reported that intestinal mucus regulates the secretion and absorption of many molecules through the gastrointestinal mucosa (Saggers & Lawson 1966; Barry & Braybrooks 1974, 1975; Braybrooks et al 1975; Kellaway & Marriot 1975; Nakamura et al 1978; Nimmerfall & Rosenthaler 1980). Nakamura et al (1978) showed that the mucus layer has an important role in the absorption process of BTB. These findings suggest that the goblet cell mucus covering the epithelial surface of the gastro-intestinal tract during immune responses may represent a protective barrier against the penetration of BTB, a low molecular weight compound, as well as macromolecular antigens. In any case, these anaphylactic reactions are presumably triggered by the antigenic protein that is initially transported from the lumen and subsequently gains access to IgE-coated mast cells in the lamina propria, the antigen-antibody reaction being produced by mediators released from mast cells. As deficiency in the local intestinal immune system could severely impair mucosal barrier function, resulting in uptake of noxious substances that could contribute to the pathogenesis of intestinal or systemic disease, it is useful to know that the intestinal immune system may interfere with the transport of low molecular weight organic compounds as well as antigenic macromolecules.

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