Effects of diet on experimentally induced intestinal ulcers in rats: morphology and tissue leukotrienes

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Abstract

The effects of dietary pretreatment on longitudinal ulcers of the intestine induced by indomethacin given intracolonicly were investigated in rats. The rats were pretreated with either standard diet or liquid meals. Intracolonic indomethacin (24 mg/kg/day) given for two days produced longitudinal ulcers and small scattered ulcers in the small intestine in the control rats that were receiving standard pelleted formula. Three days pretreatment with one of two types of liquid meals, low residual diet (LRD) or elemental diet (ED), significantly reduced the incidence (3% in ED group and 0% in the LRD group) and the length of the longitudinal ulcers in the small intestine. The caecum was affected in each dietary pretreatment group (67% in controls, 80% in LRD group, and 69% in ED group). Colonic ulcers that were located in a longitudinal fashion were found in 42% of LRD group, while these ulcers were less frequently found in the ED group (13%) and controls (0%). Development of ulcers in the caecum and in the colon of rats in ED and LRD groups was more delayed than that of small intestinal ulcers of control rats. In another experiment, pretreatment by ED significantly increased colonic tissue leukotriene B₄ concentration when compared with that of controls. These findings suggest that the site of experimental enteropathy induced by indomethacin given intracolonicly can be modified by dietary pretreatment. This animal model can be available for investigating differences in the pathophysiology of enteropathy according to the site of involvement.

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Indomethacin is a well known cause of gastrointestinal damage both in humans¹ and in experimental animals.²⁻³ The mechanism of these damages remains unknown, but a decrease in prostaglandins, which induce impairment of cytoprotection in the mucosa is believed to be the main cause of the lesions.⁴⁻⁶ There remains some controversy, however, as to whether decreased prostaglandins directly result in damages in gastrointestinal mucosa.⁷⁻⁹

We have previously described the effects of indomethacin when it was intracolonicly given to the rat intestine, and we reported that

this agent caused ulcers, predominantly in the small intestine.¹⁰ Satoh *et al*¹¹ previously investigated the role of refeeding in the site of involvement within the gastrointestinal tract of the rat when indomethacin was given subcutaneously, and they found that liquid meals just before administration significantly reduced the degree of damage in the small intestine. The effect, however, of feeding both before and after treatment in the enteropathy has not been fully investigated.

In addition, based on the fact that the caecum was more frequently affected by intracolonic indomethacin in conventionally fed rats than in the previous investigations of oral or subcutaneous administration,¹⁰ we speculated that there may also be differences in the site of involvement in rats treated by this modified route of administration, according to the kind of food continuously given before and after treatment. We therefore compared the effect of solid meals and liquid meals. The liquid meals examined included both low residual type and elemental type. Both of these are commercially available and are known to be effective in the treatment of human Crohn's disease as enteral feeding.¹² In addition, an attempt was made to investigate whether any correlations can be found between prefeeding and tissue leukotrienes within the intestine.

Methods

ANIMALS

Male Wistar rats (150-200 g), aged from 6 to 8 weeks, purchased from Kyushu Animal Corporation (Tosu, Saga, Japan) were used in this study. They were kept in a room with restricted access at a controlled temperature. The animals were housed in wire cages with a maximum of six animals in each cage. Standard laboratory pelleted formula and tap water were provided *ad libitum*, until dietary pretreatment was begun.

STUDY PROTOCOL

The animals were divided into three groups according to the dietary pretreatment. Each diet was started three days before the induction of intestinal ulcers, and was continued until the animal was killed. The animals were killed 24, 48, or 72 hours after the administration of indomethacin, and damage to the intestine was investigated.

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TABLE I Composition of meal used in the study

	Standard diet	Liquid meal	
		Low residual diet (component)	Elemental diet (component)
Protein	18.0 g/100 g	3.2 g/100 g (casein, soybean protein)	4.2 g/100 g (amino acids)
Carbohydrate	60.0 g/100 g	12.2 g/100 g (dextrin, sucrose)	18.6 g/100 g (dextrin)
Fat	3.0 g/100 g	3.2 g/100 g (corn oil)	0.2 g/100 g (soybean oil)
Total energy	450 kcal/100 g	90 kcal/100 g	88 kcal/100 g

DIET

The control rats (SD group) ingested the standard pelleted formula and tap water throughout the investigation. Liquid meal pretreated rats ingested either the elemental type (ED group) (Elental, Roussel-Morishita, Osaka, Japan) or the low residual type (LRD group) (Ensure Liquid, Dainabot, Osaka, Japan) of liquid meal, diluted homogeneously in water. Table I shows the compositions of each liquid meal. Both of the liquid meals were diluted with water (1.0 kcal/ml), and kept in a plastic bottle that permitted the animals free access. The liquid meal was freshly prepared every 24 hours. The intake of meal by the rats was confirmed by the decrease in the quantity of remaining meal. Thus, the food intake in each dietary group was assessed by total weight ingested by rats in a similar cage.

INDUCTION OF GASTROINTESTINAL DAMAGE

Indomethacin was given as described previously¹⁰ in which repeat administration uniformly produced longitudinal ulcers in the small intestine. The animals were allowed free access to food before indomethacin was given. The rats were anaesthetised with intraperitoneal sodium amobarbital (100 mg/kg). A plastic catheter measuring 6 cm in length was inserted rectally. Then, 24 mg/kg of indomethacin (Banyu, Tokyo, Japan) diluted in 1% of sodium carboxymethylcellulose solution (10 mg/kg) was injected through the catheter into the colon. Twenty four hours after the first dose, the same dose of indomethacin was again given in the same manner. To avoid coprophagy, the animals were housed in cages with raised wire mesh bases during the investigation.

ASSESSMENT OF INTESTINAL LESIONS

The rats were killed by an intraperitoneal amobarbital overdose 24, 48, or 72 hours after the second dose of indomethacin. The small intestine and the colon were removed, opened by a longitudinal incision, and pinned out on a wax block. The specimens were washed, and kept in 10% formalin for two days.

After the fixation, the specimen was investigated macroscopically. Because two types of ulceration (longitudinal ulcers and scattered small ulcers) occurred in the small intestine, they were assessed differently. A longitudinal ulcer was defined as an ulcer that was located on the mesenteric side of

the intestinal lumen and measured more than 10 mm in longitudinal length. The total lengths of the small intestine and of the longitudinal ulcers were measured, and the longitudinal ulcer index was defined as the ratio of the length of the measured ulcers *v* the length of the small intestine. Scattered small ulcers were defined as any ulcer smaller than 10 mm in its largest diameter, regardless of its location, and more than 5 mm distant from the longitudinal ulcers. The number of these ulcers was counted, and this figure was used as the small ulcer index.

The caecum and the colon were also examined. The largest diameter of those caecal ulcers that measured more than 5 mm in diameter, and the number of ulcers smaller than 5 mm both in the colon and the caecum were recorded.

SERUM INDOMETHACIN CONCENTRATION

In rats that were killed 24 hours after the second administration of indomethacin, blood samples were obtained from the inferior vena cava. These blood samples were centrifuged, and the serum concentrations of indomethacin were measured by high performance liquid chromatography.

TISSUE LEUKOTRIENES B₄ AND C₄ MEASUREMENT

In another experiment, tissue samples of the small intestine, the caecum, and the colon were obtained from the animals for the measurement of leukotrienes B₄ (LTB₄) and C₄ (LTC₄) before the induction of gastrointestinal damage. Samples of the small intestine were obtained from the upper jejunum (10 cm from the pylorus), from just the middle of the small intestine, and from the lower ileum (10 cm from the ileocaecal junction). The measurements were made 48 hours after each dietary pretreatment was started. Immediately after the animals were killed by amobarbital overdose, tissue samples were obtained, washed with saline, and kept in a refrigerator at -80°C. These samples were later weighed, finely minced with scissors, suspended in 1.0 ml of 20 mM TRIS buffer, and incubated in a water bath for 20 minutes. The samples were then centrifuged for 30 seconds and the supernatant was processed for the subsequent measurements of LTB₄ and LTC₄ using the commercial [³H] radioimmune LTB₄ and LTC₄ assay (TRK, 910; Asherman Research Corporation, Arlington Heights, IL).

STATISTICAL ANALYSES

The incidence of intestinal damage was compared using the χ^2 test. Parametric data were expressed as mean (SEM), and the values were compared among the groups, using one way analysis of variance (ANOVA). A p value <0.05 was considered significant.

Figure 1: Serial changes in body weight within the standard diet (SD) group, the elemental diet (ED) group, and the low residual diet (LRD) group.

Results

GENERAL FINDINGS
The rats constantly ingested each type of food both before and after the induction of gastro-intestinal damage. The average food intake before indomethacin was 10.2 g/day/rat (45.9 kcal/day/rat) in the SD group, 44 g/day/rat (39.6 kcal/day/rat) in the LRD group, and 42 g/day/rat (40.0 kcal/day/rat) in the ED

TABLE II Site and incidence of involvement for each diet group

	Small intestine			
	Longitudinal ulcer	Small ulcer	Caecum	Colon
SD group (n=24)	24/24 (100%)†	10/24 (42%)	16/24 (67%)	0/24 (0%)*
ED group (n=30)	1/30 (3%)†	13/30 (43%)	24/30 (80%)	4/30 (13%)*
LRD group (n=26)	0/26 (0%)†	11/26 (42%)	18/26 (69%)	11/26 (42%)*

SD=standard diet; ED=elemental diet; LRD=low residual diet. *p< 0.005, †p< 0.001.

Figure 2: Typical longitudinal ulcer seen in the middle small intestine of a rat in the SD group (arrows). The ulcer was located in the mesenteric side of the small intestine.

group. The rats in each diet group commonly manifested diarrhoea after indomethacin. The food intake decreased to 4.2 g/day/rat (18.9 g/day/rat) in SD group, 19 g/day/rat (17.1 kcal/day/rat) in the LRD group, and 18 g/day/rat (15.8 kcal/day/rat) in the ED group. As Fig 1 shows, the body weight of the rats gradually decreased in each diet group. There were no significant differences in the decrease of body weight between the three groups. The serum concentration of indomethacin 24 hours after the second dose was not different among the groups (14.3(5.3) µg/ml in the SD group (n=8), 12.6(4.8) µg/ml in the ED group (n=8), and 13.8(4.5) µg/ml in the LRD group (n=8)).

SMALL INTESTINAL DAMAGE

While the small intestine was affected by longitudinal ulcers on the mesenteric side of the lumen in rats in the SD group (Fig 2), this type of ulcer was less frequently found in the ED and LRD groups. The incidence was lower in both the LRD group (0%, p<0.001) and the ED group (3%, p<0.001) than in the SD group (100%) (Table II). These differences in the incidence of longitudinal ulcers were seen at 24, 48 and 72 hours after induction (Fig 3). In contrast with the longitudinal ulcers, small scattered ulcers were found in each diet group. While the incidence of these small ulcers was

Figure 3: Incidence of longitudinal ulcers (A) and small ulcers (B) of the small intestine within the three diet groups. The incidence of longitudinal was significantly higher in the SD group than in the SD and LRD groups at 24, 48, and 72 hours after indomethacin. Although the incidence of small ulcers was similar among the groups, it gradually increased in the LRD group.

Figure 4: A large caecal ulcer seen in a rat belonging to the ED group (arrow).

not different between the three groups (Table II and Fig 3), the number of ulcers was less in the ED group (4(2)/rat) and in the LRD group (4(3)/rat) than in the SD group (9(3)/rat, $p<0.05$).

CAECAL AND LARGE INTESTINAL DAMAGE

The caecum was frequently affected in rats ingesting LRD or ED. While the caecal damage in rats in the SD group was characterised by small ulcers measuring less than 5 mm in diameter, the caecum of rats in the LRD group or in the ED group was more severely damaged by round or irregular ulcers that measured more than 5 mm in diameter, and the whole thickness of the caecal wall was severely inflamed (Fig 4). While caecal ulcers

Figure 5: Comparison of the incidence of caecal (A) and colonic ulcers (B) among the three groups. While caecal ulcers developed less frequently in both the ED and LRD groups 24 hours after indomethacin, the incidence gradually increased. Similarly, colonic ulcers developed 48 and 72 hours after indomethacin in the LRD group, while these ulcers were less frequently seen in the ED group. Colonic ulcers were not seen in the SD group.

developed within 24 hours in the SD group, the ulcer tended to develop later in the ED group and LRD group (Figure 5).

The colon of rats in the LRD group and in the ED group was frequently affected by small ulcers, whereas these colonic ulcers were not seen in the SD group. The incidence of colonic ulcers was higher in the LRD group than in the ED group (63% in the LRD group *v* 20% in the ED group after 48 hours, and 60% in the LRD group *v* 17% in the ED group after 72 hours) (Fig 5). The ulcers were located in a longitudinal fashion in the LRD group (Fig 6). The number of the colonic ulcers, however, was not significantly different between the ED group (5(4)/rat) and the LRD group (6(4)/rat).

TISSUE LEUKOTRIENES B₄ AND C₄

Figure 7 shows the tissue concentrations of LTB₄ and LTC₄ in rats pretreated by either SD, ED, or LRD for 48 hours. Six rats were investigated in each group. While tissue LTC₄ was not different among the groups at any site of the intestine, tissue LTB₄ was statistically higher in the ED group than in the SD or LRD group. In particular, LTB₄ of the caecum (1.68(0.16)pg/mg) and the colon (6.22(0.64)pg/mg) in the ED group was higher than those in the SD group (0.38(0.05)pg/mg and 0.55(0.05)pg/mg) and in the LRD group (0.12(0.04)pg/mg and 0.13(0.04)pg/mg).

Discussion

Since the original description of indomethacin induced enteropathy in experimental animals,^{2,3} some investigators have shown that low residual diet significantly prevents these lesions,^{11,13} and they therefore speculated that the solid components of a diet may play an important part in the pathogenesis of experimental enteropathy induced by this drug. In the investigation by Satoh *et al*,¹¹ one hour of refeeding by liquid meal just before the subcutaneous administration of indomethacin obviously prevented the small intestinal lesions.

In this study, we expanded the time of dietary pretreatment, and in addition, further investigated the possible role of long periods of refeeding in the pathogenesis of indomethacin induced enteropathy. Because repeat intracolonic administration of indomethacin predominantly produces longitudinal ulcers,¹⁰ even in rats ingesting standard diet the numbers of small ulcers were comparatively small when compared with previous reports.²⁻⁴

Both ED and LRD significantly reduced the incidence of longitudinal ulcers of the small intestine, which have always been found in rats pretreated by the standard diet, and fewer small ulcers were seen in the small intestine. There may be a possibility that a difference in enterohepatic circulation of indomethacin³ among the dietary groups resulted in differences in the incidence of small intestinal lesions. Because the food intake after indomethacin was not, however, obviously different among the groups, our

Figure 6: Colonic ulcers in a rat from the LRD group. Small ulcers, which were located in a longitudinal fashion in three lines can be seen (arrows).

findings suggest that even in rats treated by intracolonic indomethacin, solid components of diet before and after treatment play an important part in the pathogenesis of enteropathies in the small intestine.

In contrast with the small intestine, the caecum and the colon of rats ingesting liquid meals were frequently affected by small ulcers. These colonic lesions were not seen in any of the rats treated by standard diet as confirmed by the previous investigation by Ligumsky *et al.*¹⁴ The development of colonic damage in the liquid diet groups may be partly explained

by the colonic administration of indomethacin applied in this study. Indomethacin may have directly damaged the colon, which contained less faeces because of the liquid meal pretreatment. Because the colonic ulcers (and also scattered small ulcers of the small intestine) occurred later, however, in the liquid diet groups, and as the food intake after treatment was similar among the groups, it seems reasonable to attribute the occurrence of these delayed lesions to the continuous feeding of liquid diet after indomethacin.

It has been recently reported that neutrophil-endothelial interaction¹⁵⁻¹⁷ and microcirculatory disturbances¹⁸ within the mucosa are the initial step in the pathogenesis of gastroenteropathy induced by non-steroidal anti-inflammatory drugs. In our experiment, both ED and LRD are considered to have suppressed the subsequent bacterial invasion^{2 5 6 19} or dietary stimuli^{11 13} in the small intestine. In the colon, however, the continuous stream and the specific composition of diarrhoea, but not the solid faeces or bacteria, seems the factor that enhances the mucosal damages.

The liquid meals used in this study have been known to change epithelial cell proliferation in the rat small intestine or in the colon.²⁰⁻²⁴ Maxton *et al.*²⁰ reported that elemental diet and complete liquid meals induced atrophy in the terminal ileum of rats. In other experiments,^{21 22 24} atrophy in the colonic mucosa induced by elemental diet has been confirmed. Thus, the difference in the frequency of colonic involvement between the ED and LRD groups in this study can be explained by the difference in the degree of proliferative activity within the colonic mucosa both before and after indomethacin treatment, which has been recently confirmed to accelerate cell losses from the epithelial surfaces.²⁵

The role of increased leukotrienes in the pathogenesis of experimental colitis induced by acetic acid²⁶ and trinitrobenzene sulphonic acid²⁷⁻²⁹ has been described. Although we evaluated tissue LTB₄ and LTC₄ in consideration of possible correlation between these metabolites and dietary pretreatment, we failed to obtain any conclusive data that can explain the difference in the site of enteropathy. Our results suggest, however, the possibility that diet pretreatment changes tissue concentrations of these metabolites. Further studies seem necessary from this point of view.

This study confirmed that intracolonic administration of indomethacin induced small intestinal ulcers in conventionally fed rats, while liquid meals significantly reduced the incidence of such ulcers. In addition, whereas treatment by the low residual type of liquid meal resulted in the occurrence of colonic ulcers, such damage was less frequently seen in rats ingesting the elemental diet. These findings partly conform to the clinical responses to liquid meals seen in human patients with Crohn's disease.^{12 30} While indomethacin induced enteropathy in experimental animals seems unavailable for grading human Crohn's disease, our results suggest that the rat model of colonic ulcers

Figure 7: Effects of pretreatment by SD, ED, and LRD on tissue leukotrienes B₄ (LTB₄) and C₄ (LTC₄). Each group consisted of six animals, and results are expressed as mean (SEM). While LTC₄ was not different among SD, LRD, and ED groups, LTB₄ was significantly different among the groups by one way analysis of variance. **p*<0.05, †*p*<0.005, ‡*p*<0.001.

can be intentionally produced by a method that includes dietary pretreatment. This may contribute to the understanding of the difference in pathophysiology of enteropathy within the intestine.

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