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ORIGINAL ARTICLE

Soluble Dietary Fiber Can Protect the Gastrointestinal Mucosa Against Nonsteroidal Anti-Inflammatory Drugs in Mice

Hiroshi Satoh[1] · **Tetsuro Urushidani**[1]

Abstract

Background Nonsteroidal anti-inflammatory drug (NSAID)-induced small intestinal damage is a serious problem in patients, but effective therapy is not available at present.

Aims The effects of feeding conditions and dietary fiber (DF) on NSAID-induced gastrointestinal lesions were examined in mice.

Methods NSAIDs (indomethacin, diclofenac, loxoprofen, aspirin) were administered to male mice in various feeding conditions. Gastrointestinal lesions were examined 24 h after NSAID dosing. Regular diets, dietary-fiber-free diet (FFD), and diets supplemented with various types of DF were given to mice.

Results NSAIDs produced marked ulcers and perforations selectively in the gastric antrum when they were administered after feeding of regular diet for 2 h after a 22-h fast. When NSAIDs, except for aspirin, were administered in unfasted conditions, they caused marked lesions in the small intestine. When mice were given FFD, antral ulcers and intestinal lesions induced by indomethacin (30 mg/kg, s.c.) markedly decreased, but when cellulose, an insoluble DF, was added to FFD, the lesions appeared again. The addition of pectin, a soluble DF, to regular diet containing 4.1 % crude fiber significantly inhibited the formation of antral ulcers as well as intestinal lesions caused by indomethacin or diclofenac (100 mg/kg, s.c.).

Conclusions The results indicated that NSAIDs given after feeding of diet produced ulcers selectively in the gastric antrum. The severity of the gastrointestinal lesions depended on the concentration of soluble or insoluble DF in food. Our results suggest that soluble DF such as pectin may be a safe means for protecting the gastrointestinal mucosa against NSAIDs.

Keywords Dietary fiber · Gastric antral ulcer · Indomethacin · NSAIDs · Pectin · Small intestinal ulcer

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Introduction

Recent progress in endoscopies such as capsule endoscopy has revealed that nonsteroidal anti-inflammatory drugs (NSAIDs) [1–4] and aspirin [5–7] often cause mucosal damage in the small intestine in patients, and these intestinal lesions are more common than previously thought. Although it has been reported that anti-ulcer agents, such as misoprostol, and probiotics protected the small intestinal mucosa against NSAIDs in healthy volunteers [8–10] and experimental animals [11–14], effective therapy for the prevention of intestinal damage is not available in patients. Therefore, it is important to understand how to protect the small intestine against damage caused by NSAIDs [15]. Recently, we reported that soluble dietary fiber (DF) such as pectin can prevent small intestinal damage caused by NSAIDs in cats [16, 17] and rats [18]. However, few studies have been done in mice, which are useful for the analysis of molecular mechanisms such as gene targeting or mutation.

On the other hand, although it has been reported that human gastric ulcers were often observed in the gastric antral area [19], there are few animal models of gastric

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antral ulcers. During a study on the role of food in the formation of gastrointestinal ulcers induced by NSAIDs, we found that indomethacin produced ulcers selectively in the gastric antrum when it was administered after refeeding of diet after a 24-h fast in rats [20]. In the present study, we examined in mice (1) the effect of feeding conditions (fasted, refed, and unfasted) on the location of gastrointestinal lesions induced by NSAIDs, and (2) the protective effect of soluble DF on the gastrointestinal mucosa.

Materials and Methods

Ethics Approval

Experimental protocols were approved by the Animal Research Committees at Doshisha Women's College of Liberal Arts, Kodo, Kyotanabe, Kyoto, Japan.

Animals

Seven-week-old male ddy mice (Shimizu Laboratory Supplies, Shizuoka, Japan) weighing 35–40 g were used.

Drugs and Diets

The following drugs and chemicals were used: aspirin, carboxymethylcellulose, diclofenac Na, indomethacin, and loxoprofen Na dihydrate (Wako, Osaka, Japan). Drugs for oral or subcutaneous administration were suspended in a saline containing 1 % carboxymethylcellulose. The drugs were prepared just prior to the experiments and administered in a volume of 0.05 ml/10 g body weight.

Various diets were used, i.e., regular chow pellet (CE-2 pellet; Clea Japan, Osaka, Japan), regular powder diet (CE-2 powder; Clea Japan), and dietary-fiber-free powder diet (FFD; F2LCP, KBT Oriental Bioservice, Kyoto, Japan). Cellulose (Sigma, MO, USA), pectin, and guar gum (Wako) were used as DF sources.

Composition of diets: Regular chow pellet and powder diet (CE-2, Japan Clea) contained 24.9 % crude protein, 4.4 % crude fat, 4.1 % crude fiber, 6.9 % crude ash, 2.5 % minerals, and 51 % soluble non-N materials such as starch and sucrose. FFD (F2LCP, Oriental Bioservice) contained 19.6 % protein, 17.1 % fat, 4.6 % minerals, 2.3 % vitamins, and 53.8 % sucrose.

Induction and Measuring of Gastrointestinal Lesions

NSAIDs were administered subcutaneously (s.c.) or orally (p.o.) in various feeding conditions, i.e., after a 24-h fast (Fig. 1, fasted), just after 2-h feeding of diet after a 22-h fast (Fig. 1, refed) or with continuous feeding without fasting (Fig. 1, unfasted).

The animals were killed by cervical dislocation after inhalation anesthesia with isoflurane (EscainR, Pfizer, Tokyo, Japan) at 24 h after NSAID administration. Then, both the stomach and small intestine were removed. The stomach was filled with 1.5 ml of 1 % formalin solution and then immersed in the same formalin solution for 15 min. The stomachs were opened along the greater curvature. The small intestine was immersed in 1 % formalin solution for 15 min, then spread out on paper, and opened along the anti-mesenteric side, and the contents were removed. The whole length of the small intestine from the

Fig. 1 Experimental schedule. Nonsteroidal anti-inflammatory drugs (NSAIDs) were administered orally or subcutaneously after a 24-h fast (fasted), just after refeeding of diet for 2 h after a 22-h fast (refed), or without fasting (unfasted). The mice were killed 24 h after the administration of NSAIDs
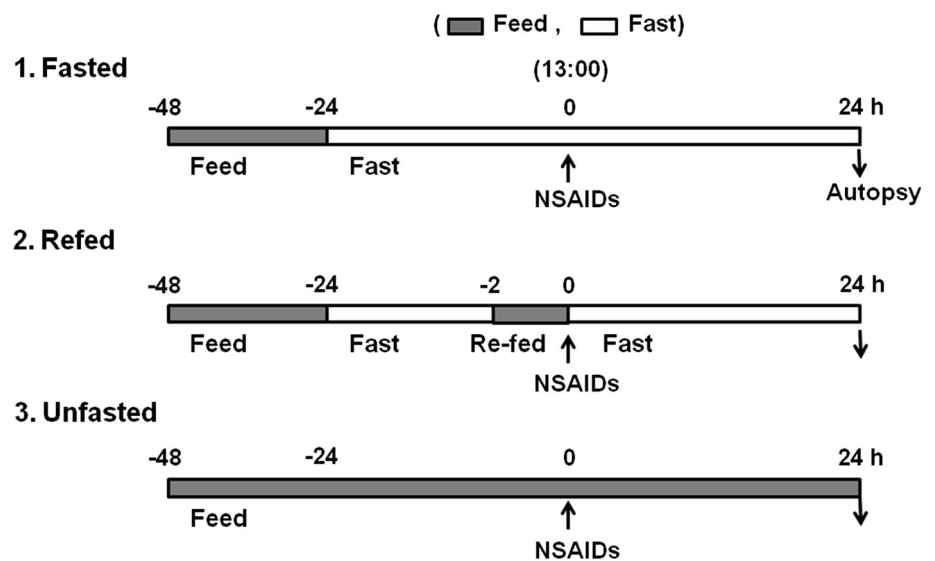
duodenum to the terminal ileum was measured, and then it was divided into 10 parts of equal length, and the segments of the small intestine were numbered from 1 to 10. Both the length (in mm, of the corpus and small intestine) and area (mm^2, of the antrum) of the individual lesions were measured with use of a dissecting microscope and a 1-mm square grid eyepiece (10×), and the sum of the lengths or areas of all the lesions in each corpus, antrum, and small intestine were used as the lesion index.

Histological Examination

The gastroduodenal preparations, including antral lesion area, were removed and immersed in 10 % formalin for histological examination. Serial sections (4 μm) of formalin-fixed, paraffin-embedded samples of each tissue were mounted on slides. Hematoxylin and eosin staining was then performed in accordance with conventional methods.

Statistics

All data are expressed as mean ± SEM. Differences between groups were analyzed using Student's t test for paired group comparisons, analysis of variance (Dunnett's multiple range test) if more than two variables were considered, or chi-square test (Fisher's exact probability test), with the significance level set at $P < 0.05$.

Results

Gastrointestinal Lesions Induced by NSAIDs in Various Feeding Conditions

When indomethacin (3–30 mg/kg, s.c.) was administered after a 24-h fast, it caused marked lesions selectively in the gastric corpus dose dependently (Figs. 2a, 3a, fasted). When indomethacin was administered after 2-h refeeding, it produced marked lesions selectively in the antrum. The lesions in the antrum were often observed in the posterior and anterior walls (Fig. 2a). The lesions increased dose dependently, and doses of indomethacin 10 and 30 mg/kg induced perforation in the antrum in 4 and 5 mice out of 7, respectively (Figs. 2a, 3a, refed). As shown in Fig. 2c, indomethacin produced true ulcers histologically in the gastric antrum. On the other hand, when indomethacin was administered without fasting, it caused marked lesions selectively in the small intestine (Figs. 2a, 3a, unfasted). Lesion indices in the corpus (fasted), antrum (refed) and small intestine (unfasted) caused by indomethacin (30 mg/kg, s.c.) were 25.1 ± 1.8 mm ($n = 7$), 7.0 ± 0.9 mm^2 ($n = 7$), and 144.9 ± 19.6 mm ($n = 7$), respectively.

Indomethacin (3–30 mg/kg) given orally caused very similar results to those observed when it was given s.c. Lesion indices in the corpus (fasted), antrum (refed) and small intestine (unfasted) caused by indomethacin (30 mg/kg, p.o.) were 26.6 ± 3.9 mm ($n = 8$), 5.7 ± 1.7 mm^2 ($n = 8$) and 158.6 ± 21.5 mm ($n = 8$), respectively. Indomethacin given after refeeding also produced perforation in the antrum in 2 out of 8 mice.

Both diclofenac (30 and 100 mg/kg, s.c.) and loxoprofen (100 and 300 mg/kg, s.c.) caused marked lesions as seen with indomethacin, i.e., selectively in the corpus, antrum, and small intestine in fasted, refed, and unfasted conditions, respectively. However, approximately 3 and 10 times higher doses of diclofenac and loxoprofen were needed to obtain similar ulcer indices to those of indomethacin, respectively. In addition, under refed conditions, perforation was also observed in the antrum with high doses of diclofenac (100 mg/kg) and loxoprofen (300 mg/kg) in 5 and 3 mice out of 7, respectively. On the other hand, aspirin (300 mg/kg) given s.c. did not cause any lesions in the stomach or small intestine. Therefore, in the subsequent study aspirin was given orally. In fasted conditions, aspirin (100 and 300 mg/kg, p.o.) caused marked lesions selectively in the corpus (Figs. 2b, 3b). Aspirin (300 and 1000 mg/kg) given after refeeding caused lesions selectively in the antrum (Figs. 2b, 3b). Perforation of the antrum was observed in 2 out of 8 mice given the high dose of aspirin (1000 mg/kg). Histological findings revealed that aspirin produced true ulceration in the antrum (Fig. 2c). In unfasted conditions, aspirin (300 and 1000 mg/kg, p.o.) did not produce severe lesions in the small intestine, except for reddish areas and mild erosions (Figs. 2b, 3b).

The distribution of intestinal lesions induced by indomethacin (30 mg/kg, s.c.), diclofenac (100 mg/kg, s.c.), and loxoprofen (300 mg/kg, s.c.) in unfasted mice is shown in Fig. 4a. Intestinal lesions were observed widely in the small intestine, but the lesion indices in the lower small intestine were higher than those in the upper small intestine with all of the NSAIDs. Figure 4b shows the effect of NSAIDs on the length of the small intestine. In the control group given vehicle, the length of the small intestine was 48.3 ± 1.0 cm ($n = 7$), and indomethacin (3–30 mg/kg, s.c.) decreased the length dose dependently, and the effects were significant ($P < 0.05$ and 0.01 vs. control). Both diclofenac (100 mg/kg) and loxoprofen (300 mg/kg, s.c.) also decreased the length significantly ($P < 0.01$ vs. vehicle), but aspirin (1000 mg/kg, p.o.) did not. The lesion index and intestinal length in each mouse treated with vehicle or indomethacin (3–30 mg/kg, s.c.) are plotted in Fig. 4c. There was a good correlation between these two values ($y = -6.6344x + 321.9$, $r = 0.862$; $P < 0.001$) where y is the lesion index (mm) and x is the intestinal length (cm).
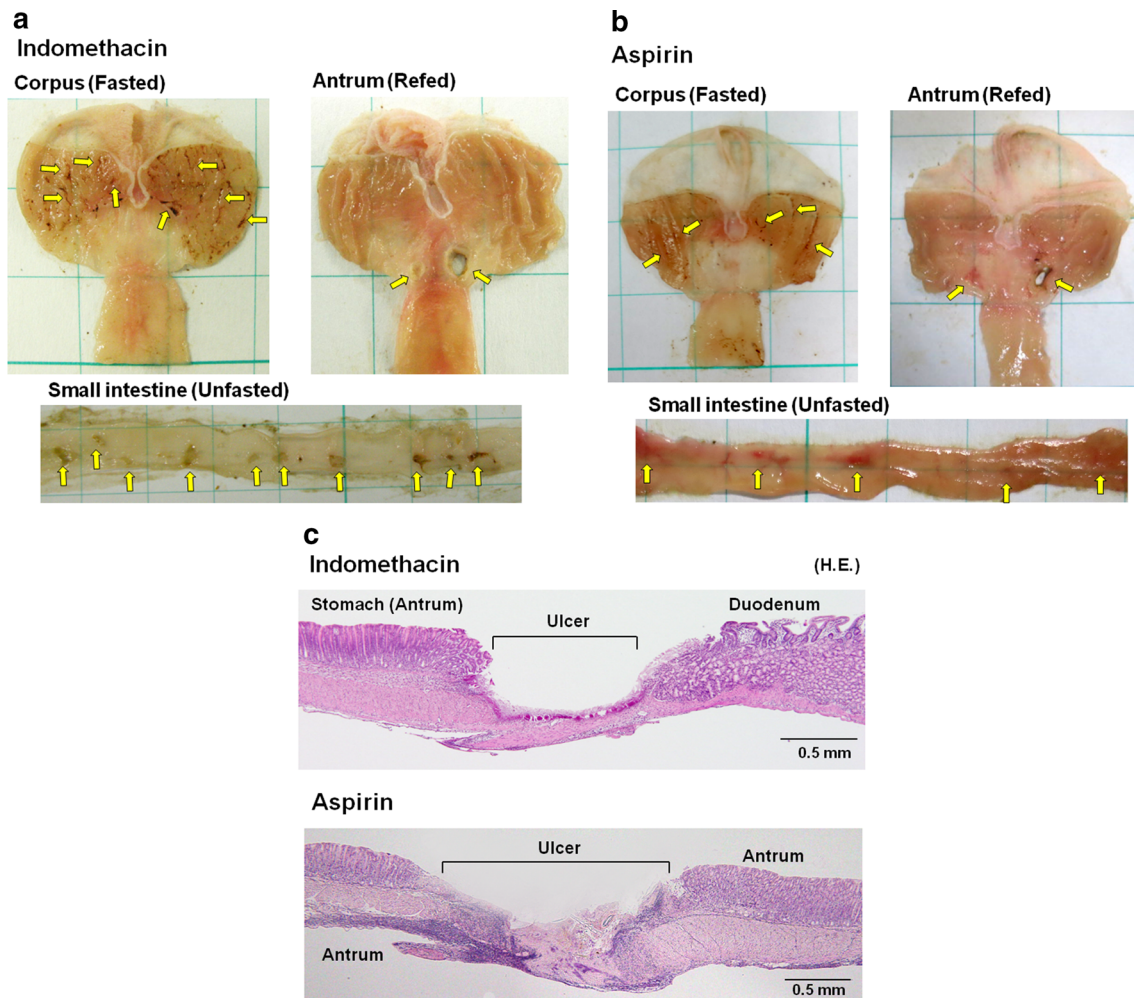
Fig. 2 Photographs of mucosal lesions of gastric corpus, gastric antrum, and small intestine: **a** indomethacin (30 mg/kg, s.c.) and **b** aspirin (300 or 1000 mg/kg, p.o.). *Each photograph* of mucosal lesions in the corpus, antrum, and small intestine was taken from mice given drugs in fasted, refed, and unfasted conditions, respectively. *Yellow arrows* show the lesions, and the grid in each photograph shows a scale (1 cm). In refed condition, drugs often caused perforation in the antrum. **c** Histological observation of gastric antral ulcer. Hematoxylin and eosin staining. Indomethacin (30 mg/kg, s.c.) and aspirin (1000 mg/kg, p.o.) were administered just after refeeding for 2 h, and the samples of gastric antrum were obtained 24 h after the administration of drugs

Role of Insoluble Dietary Fiber in the Formation of Gastrointestinal Lesions Induced by Indomethacin

In the refed mice, indomethacin (30 mg/kg, s.c.) produced marked lesions in the gastric antrum in the control group given powder diet, and perforation was observed in 6 out of 7 mice (Fig. 5a). The lesion index was 7.7 ± 1.6 mm^2 ($n = 7$). Lesions were markedly decreased in mice given FFD [lesion index 0.3 ± 0.1 mm^2 ($n = 8$); $P < 0.001$ vs. powder diet; Fig. 5a]. Perforation was not observed in these 8 mice. The amount of food intake during the 2-h refeeding period in the groups given powder diet and FFD was 1.5 ± 0.1 ($n = 7$) and 1.4 ± 0.2 g ($n = 8$), respectively. On the other hand, in mice given FFD supplemented

with cellulose (0.1–3 %), antral lesions appeared again, and the lesion index increased with increasing cellulose concentration. The lesion indices in the group given FFD with 1 and 3 % of cellulose were significantly larger than that of FFD alone, and antral perforation was observed 1 and 6 out of 8 mice, respectively (Fig. 5a). On the other hand, in mice given FFD supplemented with pectin (10 %), almost no lesions were observed in the antrum.

In unfasted mice, indomethacin produced marked lesions in the small intestine in the control group (Fig. 5b). The lesion index was 168.3 ± 26.7 mm ($n = 7$). The lesions were decreased in mice given FFD [lesion index 8.7 ± 2.3 mm ($n = 8$); $P < 0.001$ vs. powder diet; Fig. 5b]. The food intakes during 24 h in the group given powder diet and FFD were 7.0 ± 0.5 ($n = 7$) and

Fig. 3 Ulcerogenic effects of indomethacin and aspirin on gastrointestinal mucosa in different feeding conditions in mice. Drugs were administered subcutaneously (s.c.) or orally (p.o.) after a 24-h fast (fasted), just after 2-h refeeding with chow pellets after a 22-h fast (refed) or without fasting (unfasted). Gastrointestinal lesions were examined 24 h after dosing of drugs. Lesion index in the corpus (*upper*), antrum (*middle*), and small intestine (*lower*) is shown versus the drug dose. Data show the mean values and the standard errors of 6–8 rats: **a** indomethacin (3–30 mg/kg, s.c.) and **b** aspirin (100–1000 mg/kg, p.o.). *Figures in parenthesis* show the number of mice with antral perforation versus total number of mice
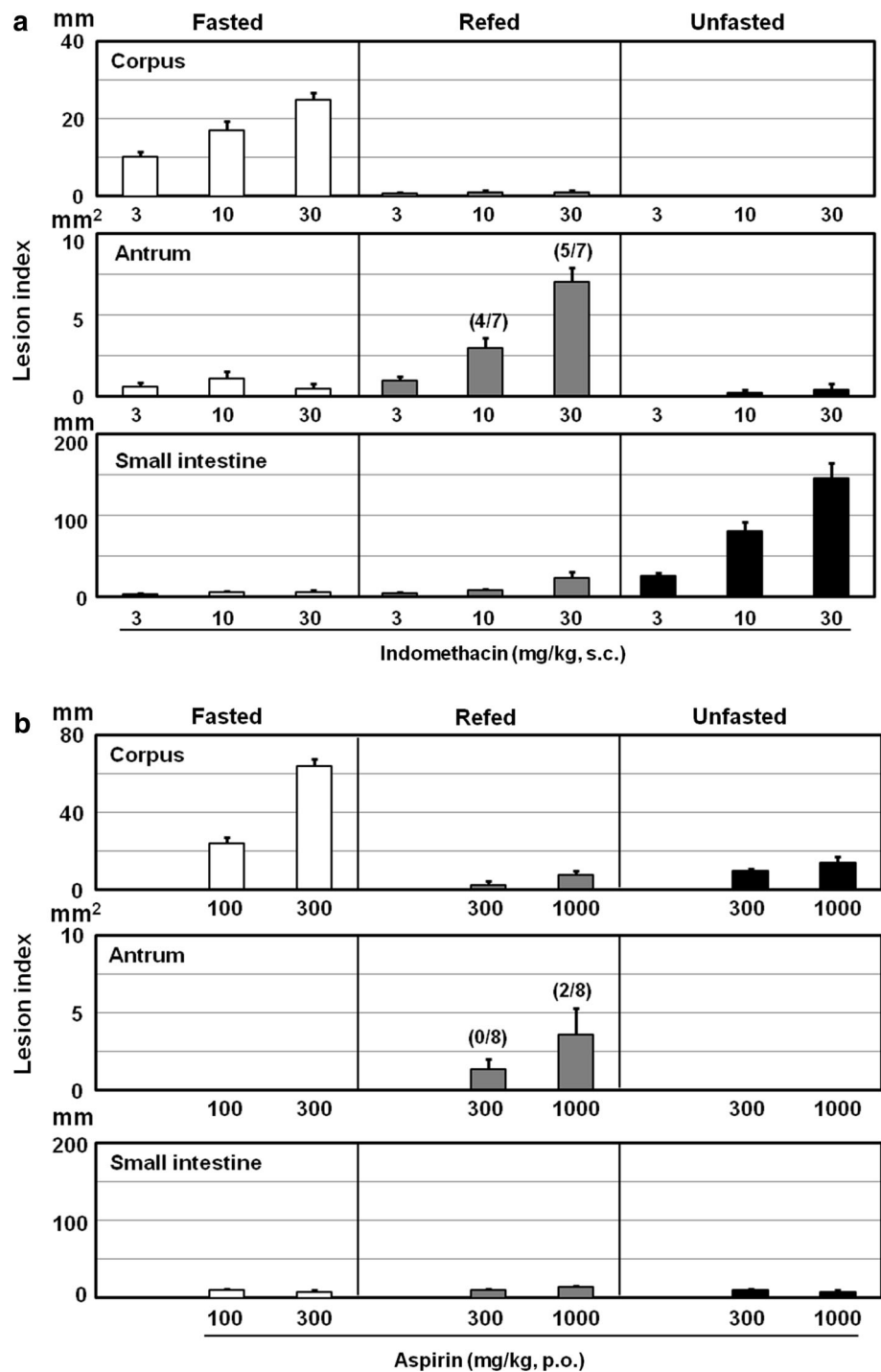
5.2 ± 0.2 g (n = 8), respectively. On the other hand, in the mice given FFD supplemented with cellulose, intestinal lesions appeared again and increased with increasing cellulose concentration. Although the lesion indices in the group given FFD supplemented with 3 % or more of cellulose were significantly larger than that with FFD alone, the lesion index for 10 % cellulose was about half that of

the control group [79.6 ± 6.3 mm (n = 8); Fig. 5b]. In mice given FFD supplemented with pectin (10 %), almost no lesions were observed in the small intestine. The length of the small intestine in the control group given vehicle was 47.1 ± 0.6 cm (n = 7). Indomethacin (30 mg/kg, s.c.) markedly decreased the length of the small intestine [38.0 ± 2.1 cm (n = 8); P < 0.01 vs. vehicle], but this

Fig. 4 Distribution of intestinal lesions caused by nonsteroidal anti-inflammatory drugs (NSAIDs) and length of the small intestine in the mice given NSAIDs. Either indomethacin (IND, 3–30 mg/kg, s.c.), diclofenac (DIC, 100 mg/kg, s.c.), loxoprofen (LOX, 300 mg/kg, s.c.), aspirin (ASA, 1000 mg/kg, p.o.), or vehicle (VEH) was administered in unfasted conditions, and both intestinal lesions and length were examined 24 h later. a Distribution of lesions. The small intestine from the duodenum to the terminal ileum was divided into equal 10 lengths, and the segments were numbered from 1 to 10. Data show the mean values and the standard errors of each part of the small intestine for 6 or 7 mice. b Length of the small intestine. Data show the mean values and the standard errors for 6 or 7 mice. (*a*), (*b*), (*c*) *P* < 0.05, 0.01 and 0.001 versus VEH (Dunnett's test). c Relationship between the length of the small intestine and lesion index in mice given VEH or indomethacin (IND, 3–30 mg/kg, s.c.). Each point shows the results for an individual mouse. A good correlation between the lesion index (*y*, mm) and length (*x*, cm) is observed ($y = -6.6344x + 321.9$, $r = 0.862$, $P < 0.001$)
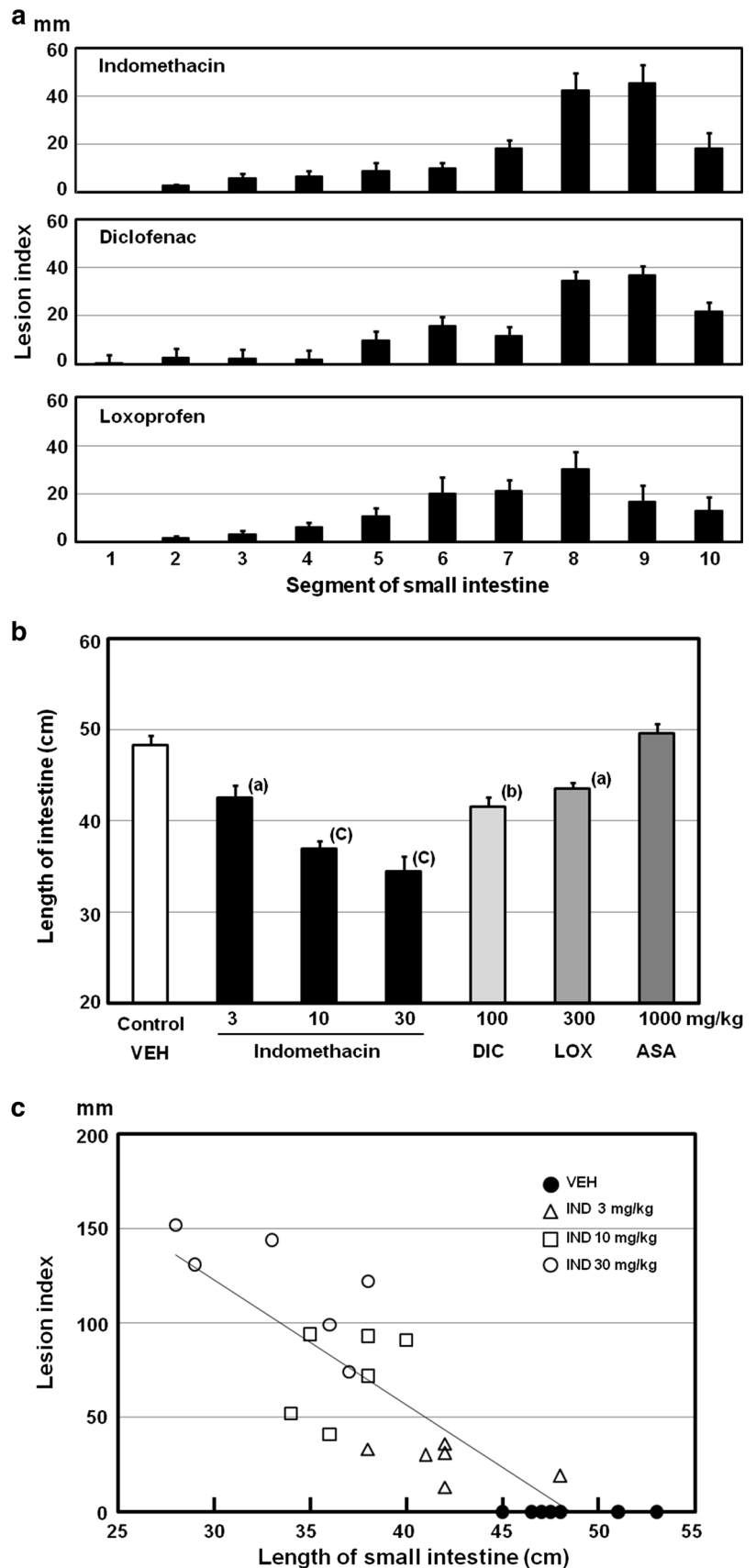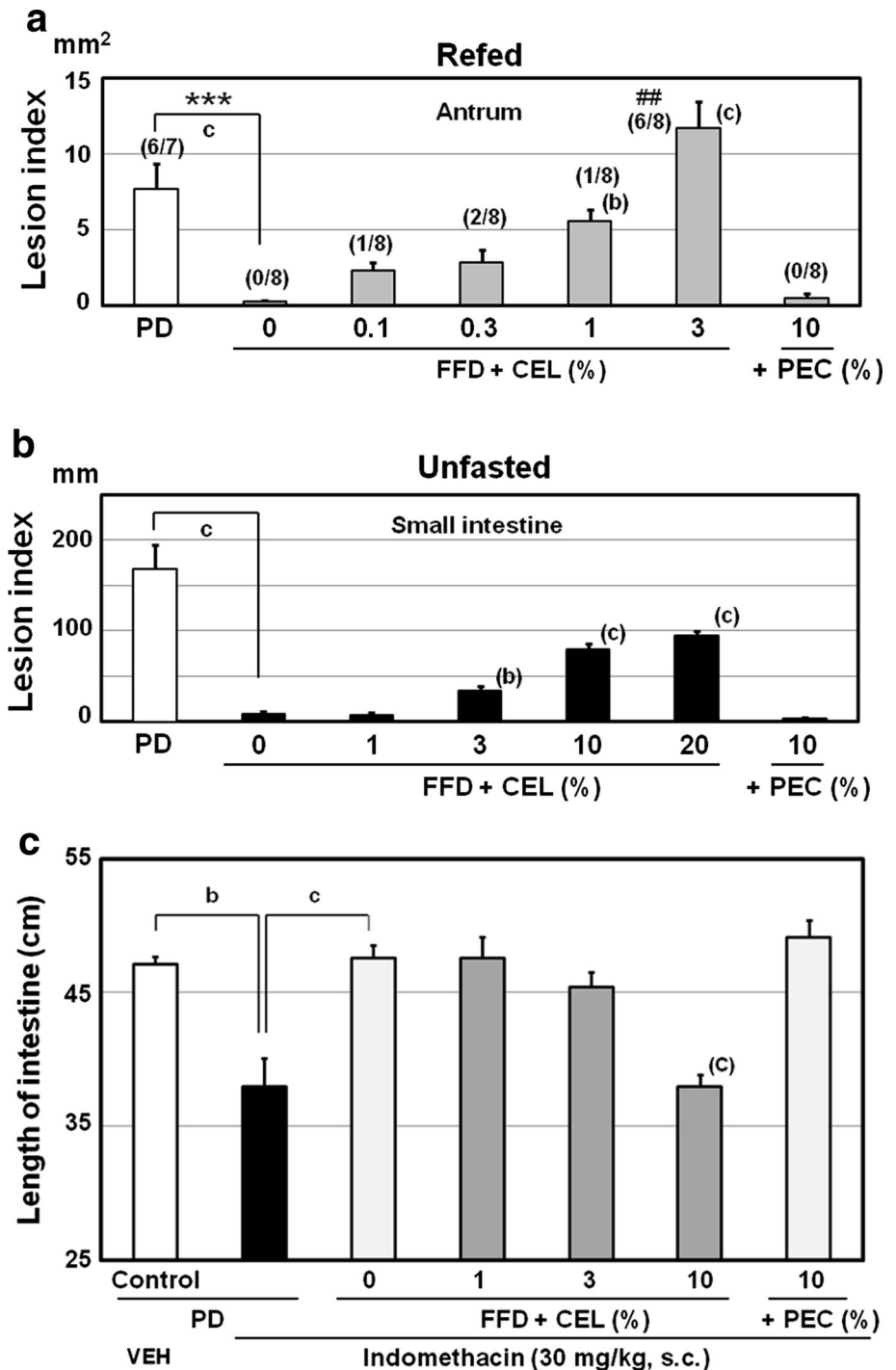
In the refed mice, indomethacin produced marked lesions in the gastric antrum in the control group given powder diet alone [lesion index 7.7 ± 1.6 mm^2 ($n = 7$); Fig. 6a]. The lesions decreased concentration dependently in mice

effect of indomethacin was not observed in mice given FFD. In mice given FFD supplemented with cellulose (1–10 %), indomethacin decreased the length concentration dependently, and the effect in mice given FFD with 10 % cellulose was significant ($P < 0.001$ vs. FFD alone; Fig. 5c). On the other hand, in the group given FFD supplemented with 10 % of pectin, indomethacin did not affect the length of the small intestine.

Effects of Soluble Dietary Fiber on the Formation of Gastrointestinal Lesions Induced by Indomethacin or Diclofenac

given pectin, and the inhibitory effect of pectin reached a maximum at a concentration of 1 % and the lesions were not decreased further at concentrations of 3 and 10 % (Fig. 6a). Perforation in the antrum was observed 6 out of 7 mice in the control group, and the number of mice with

perforation also decreased with increasing pectin concentration, and no perforation was observed in the group given powder diet supplemented with 1 and 3 % of pectin. On the other hand, guar gum showed a mild inhibitory effect at low concentrations of 0.03–0.3 %, but at higher

Fig. 6 Effect of pectin and guar gum on gastrointestinal lesions and the length of the small intestine in the mice given indomethacin. Animals were given powder diet (PD) or PD supplemented with pectin (PEC, 0.03–10 %) or guar gum (GG, 0.03–10 %), and indomethacin (30 mg/kg, s.c.) was administered just after 2-h refeeding (**a**, **b**) or without fasting (**c**, **d**, **e**). Both gastrointestinal lesions and the length of the small intestine were measured 24 h after dosing with indomethacin. **a**, **b** Antral ulcers, **c**, **d** intestinal lesions, and **e** length of the small intestine. Data show the mean values and the standard errors of 7–8 mice. *Figures in parenthesis* in (**a**) and (**b**) show the number of mice with antral perforation versus total number of mice. **a**–**d**, (*a*), (*b*), (*c*) $P < 0.05$, 0.01 and 0.001 versus PD alone (Dunnett's test), *, ***$P < 0.05$ and 0.001 versus PD alone (chi-square test), **e**, *c* $P < 0.001$ (*t* test), (*a*), (*c*) $P < 0.05$ and 0.001 versus PD alone with indomethacin (Dunnett's test)
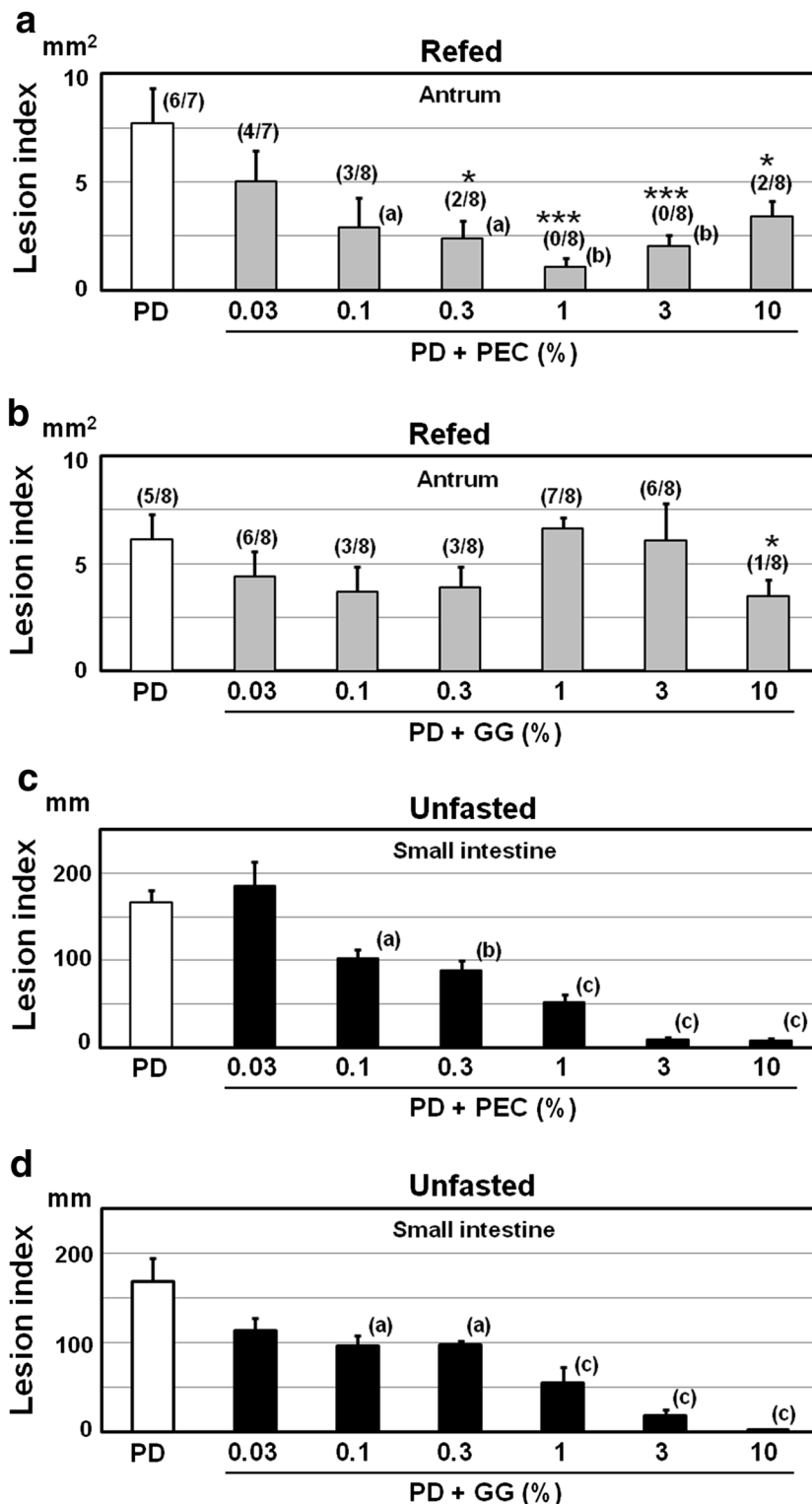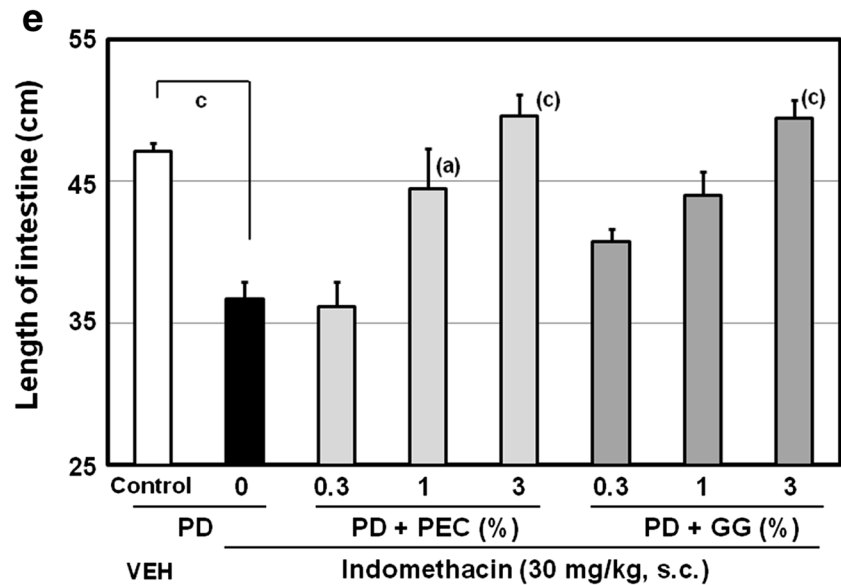
Fig. 6 continued

concentrations (1 and 3 %) it did not inhibit the formation of antral lesions (Fig. 6b). Diclofenac produced marked lesions in the gastric antrum in control mice given powder diet [lesion index 6.7 ± 1.2 mm^2 ($n = 8$)]. Addition of pectin (1 %) to the diet decreased the lesions significantly [lesion index 3.0 ± 0.7 mm^2 ($n = 8$); $P < 0.05$ vs. powder diet]. Diclofenac also caused perforation in the antrum in 6 out of 8 mice in the control group, and the number of mice with perforation was also decreased in the group given powder diet supplemented with 1 % pectin (3 out of 8 mice).

In unfasted mice, indomethacin produced marked lesions in the small intestine in the control group (Fig. 6c). The lesions decreased in mice given pectin in a concentration-dependent manner, and the inhibitory effects of pectin at concentration of 0.1 % and more were significant (Fig. 6c). Lesions were also prevented by the addition of guar gum to powder diet concentration dependently, and a significant effect of guar gum was observed at concentrations of 0.1 % and more (Fig. 6d). The inhibitory potency of guar gum was almost the same as that of pectin. Diclofenac produced marked lesions in the small intestine in control mice given powder diet (lesion index 144.3 ± 19.9 mm; $n = 7$). Lesions were markedly decreased in mice given powder diet supplemented with pectin [1 %; lesion index 48.7 ± 9.2 mm ($n = 7$); $P < 0.001$ vs. powder diet].

As mentioned in the previous section, indomethacin (30 mg/kg, s.c.) markedly decreased the length of the small intestine (Fig. 6e). Addition of pectin or guar gum (0.3–3 %) to the powder diet prevented this decrease in length induced by indomethacin concentration dependently, and the effects of 1 and 3 % pectin and 3 % guar gum were significant ($P < 0.05$ and 0.001 vs. powder diet alone; Fig. 6e). Diclofenac decreased the length of the small intestine in control mice given powder diet [length 40.1 ± 0.7 mm ($n = 7$)]. The effect of diclofenac was significantly inhibited in mice given powder diet supplemented with pectin (1 %) [length 43.9 ± 0.7 mm ($n = 7$); $P < 0.01$ vs. powder diet].

Discussion

From the present study, it was suggested that the location of gastrointestinal lesions induced by NSAIDs was highly dependent on feeding conditions (fasted, refed, or unfasted), and NSAIDs given after refeeding produced ulcers and perforations selectively in the gastric antrum in mice, supporting our previous findings in rats [20]. Because most patients take NSAIDs after a meal, the gastric antral ulcers could be a useful model for studying the pathogenesis of gastric ulcers in humans. On the other hand, in unfasted conditions, NSAIDs produced marked lesions in the small intestine predominantly in the lower half of the small intestine. A similar distribution of lesions has also been observed in rats [18, 21], cats [16, 22], and dogs (unpublished observation, Satoh et al.). The different distributions of lesions will be explained, at least in part, by an increased concentration of indigestive solid components of food, which play an important role in the formation of lesions [23], in the lower small intestine, as most of the liquid and digested soluble components of foods will be absorbed in the upper and middle intestine. In addition, it is plausible that a different distribution of pathogenic factors such as

intestinal microflora and bile acid [24] may contribute to the high incidence of lesions in the lower small intestine.

Furthermore, indomethacin dose dependently decreased the length of the small intestine, and a good correlation between lesion index and length ($r = 0.862$, $P < 0.001$) was observed. A decrease in intestinal length was also observed in mice administered diclofenac and loxoprofen, which caused severe lesions in the small intestine, but not by aspirin, which did not cause severe lesions. These results suggested that the length of the small intestine may reflect the severity of intestinal lesions. A similar response (decrease in the length of the colon) has also been reported in an ulcerative colitis model induced by dextran sodium sulfate and changes in the length of the colon have been used as an indicator of the severity of ulcerated mucosa in the colon [25, 26]. Considering these findings together, it was suggested that the length of the small intestine may be used as an objective indicator of the severity of NSAID-induced damage in the small intestine, although the precise mechanism for the decrease in intestinal length has not been elucidated.

We reported previously that DF, especially insoluble DF such as cellulose, plays an important role in the formation of NSAID-induced intestinal lesions in rats [18, 21] and cats [17, 22]. In the present study, we examined the role of insoluble DF in the formation of antral ulcers as well as intestinal lesions in mice. Indomethacin produced severe lesions in the antrum and small intestine in control mice given powder diet containing 4.1 % crude fiber, in the refed and unfasted conditions, respectively. In mice given dietary-fiber-free powder diet (FFD) both antral and intestinal lesions were markedly decreased. However, in mice given FFD supplemented with cellulose (0.1–20 %), indomethacin again produced lesions in both the antrum and small intestine, although a big difference was observed in the cellulose dependency of lesion formation between antral ulcers and small intestinal lesions. These results supported our previous findings in the small intestine [18, 22] and suggested that insoluble DF plays an important role also in the formation of antral ulcers. It has been reported that NSAIDs caused hyper-motility in the gastrointestinal tract and decreased mucus content in the small intestine in rats [27–29]. Considering these findings and the results of the present study together, it was suggested that gastric antral ulcers as well as small intestinal lesions could be started by rubbing of the mucosa with indigested solid components of food such as cellulose in conditions of decreased mucus and increased hyper-motility caused by NSAIDs. The lesions could be aggravated by additional pathogenic factors such as gastric acid, intestinal microflora and bile acids [24].

Small intestinal damage caused by NSAID/aspirin is more common in patients who have taken the drugs for more than 3 months [1–7]. However, effective therapy for intestinal damage is not available at present. Recently, we reported that soluble DF such as pectin prevented small intestinal lesions induced by NSAIDs and enteric coated aspirin in cats [16, 17], and soluble DF prevented the formation of intestinal lesions induced by indomethacin without affecting its anti-inflammatory effect in arthritic rats [18]. In the present study, we examined the effects of pectin and guar gum on the formation of antral ulcers as well as small intestinal lesions induced by indomethacin or diclofenac in mice. Both pectin and guar gum (0.03–10 %) added to the control powder diet markedly prevented the formation of small intestinal lesions induced by indomethacin, and the efficacy of pectin was almost the same as that of guar gum. In addition, both pectin and guar gum significantly inhibited a decrease in the length of the small intestine caused by indomethacin. These results suggested that soluble DF could protect the intestinal mucosa against NSAIDs in mice.

Although we did not examine the protective mechanism of soluble DF on the intestinal mucosa in the present study, several possibilities were considered related to the pathogenic factors in the formation of intestinal damage caused by NSAIDs [24]. The first is related to intestinal mucin. It was reported recently that pectin stimulated intestinal mucin secretion in rat jejunum via up-regulation of Muc 2 expression [30]. Therefore, it is possible that soluble DF may protect the intestinal mucosa by increasing mucus content in the mucosa. The second is related to changes in intestinal microflora. It is well known that intestinal bacteria, especially Gram-negative bacteria, play an important role in the formation of NSAID-induced small intestinal lesions [31]. Recently, it has been reported that soluble DF such as pectin and guar gum caused favorable colonization of microflora in the intestine [32, 33], i.e., a decrease in Gram-negative bacteria and an increase in Gram-positive bacteria exerted a protective effect on the intestinal mucosa. In addition, soluble DF increased the growth of bacteria producing butyrate [32, 33], which plays an important role in the maintenance of epithelial barrier integrity (see an editorial by Dr. Wallace [34] in a recent issue of *Dig Dis Sci*). The third one is related to bile acids, i.e., it was reported that soluble DF can bind bile acids [35]. Therefore, it may be possible that soluble DF reduces NSAID-induced intestinal damage by preventing the aggravating effect of bile acids on mucosal lesions [36]. The fourth possibility is related to the viscosity of soluble DF, i.e., soluble DF protected the small intestine by compensating for a decrease in barrier function caused by NSAIDs owing to their viscous nature, as suggested in our previous study in cats [16].

On the other hand, a major difference was observed in the protective action of pectin and guar gum on the gastric antral mucosa, i.e., the formation of antral ulcers induced

by indomethacin was markedly prevented by pectin but not by guar gum. It has been reported that a high viscosity meal including guar gum, pectin, or agar delayed gastric emptying in healthy volunteers without affecting gastrointestinal motility [37–39], and the effect depended on the viscosity of the meal [40]. We reported that the severity of gastric antral ulcers was dependent on the amount of food intake (amount of gastric contents) in rats [21]. These findings suggest a possibility that guar gum can protect the gastric mucosa as seen in the intestinal mucosa, but the effect of guar gum may be masked in the stomach by a delay in gastric emptying, because the viscosity of guar gum is much higher than that of pectin [16].

From the present study, it was indicated that NSAIDs given after refeeding produced ulcers and perforations selectively in the gastric antrum. Furthermore, it was suggested that the length of the small intestine may reflect the severity of intestinal lesions caused by NSAIDs, and soluble DF such as pectin may be a novel and safe means for protecting the gastrointestinal mucosa against NSAIDs. The clinical usefulness of soluble DF in decreasing NSAID-induced gastrointestinal adverse events in patients will be clarified in the near future.

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