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DIETS WITH NO OR LOW AMOUNTS OF DIETARY FIBER CAN REDUCE SMALL INTESTINAL ULCERS INDUCED BY NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN DOGS

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Recent progress in endoscopic techniques has revealed that non-steroidal anti-inflammatory drugs (NSAIDs) often cause ulcers in the small intestine in humans, but effective therapy is not available at present. In the present study, we investigated the effects of feeding condition and the amount of dietary fiber (DF) in the diet on the formation of gastrointestinal ulcers induced by NSAIDs in dogs. Several types of diets containing various percentages of DF were given to dogs. Indomethacin (1 or 3 mg/kg, p.o.), ketoprofen (2 mg/kg, s.c.), or fulnixin (1 mg/kg, s.c.) was administered once daily at 10 a.m. after a morning meal or without a morning meal (fasted condition) for 3 – 7 days. Gastrointestinal lesions were examined 24 h after the final dose of the drugs. When indomethacin (3 mg/kg) was administered after a morning meal (fed condition) for 7 days, it produced many lesions in the small intestine. However, when it was given in the fasted condition without the morning meal, the lesions were markedly decreased. All the NSAIDs given after feeding of regular dry food containing 6% DF once a day for 3 days produced many lesions in the small intestine. The lesions were decreased or increased in dogs given prescription diets containing low DF (1.1%) and high DF (15.4%), respectively. Furthermore, lesions were not observed in dogs given canned diet containing very low DF (< 0.1%), whereas lesions appeared again in dogs given canned diet supplemented with cellulose (3 or 10%) but not with pectin (10%). These results suggested that both feeding condition and insoluble DF, such as cellulose in the diet, play an important role in the formation of NSAID-induced small intestinal lesions, and that a diet with no or low amounts of DF may decrease gastrointestinal side-effects associated with the use of NSAIDs.

Key words: *dietary fiber, indomethacin, ketoprofen, non-steroidal anti-inflammatory drugs, small intestinal ulcer, diets, intestinal bacteria*

INTRODUCTION

Recent progress in endoscopic techniques, such as capsule endoscopy, has revealed that small intestinal ulcerations induced by non-steroidal anti-inflammatory drugs (NSAIDs) in humans are more frequent and more severe than previously thought (1-4). Furthermore, in contrast with upper gastrointestinal ulcers, effective therapy for the prevention of small intestinal damage is not available in patients (5), although it has been reported that some antiulcer agents and probiotics were effective in protecting the small intestinal mucosa against NSAIDs not only in experimental animals (6-10) but also in healthy volunteers (11-13). Therefore, how to manage NSAID-induced small intestinal damage is an important issue to be solved. We reported previously that both the feeding condition when NSAIDs are administered and the content of dietary fiber (DF) in the diet, especially insoluble DF such as cellulose, play important roles in the formation of small intestinal ulcers induced by NSAIDs in mice (14), rats (15, 16), and cats (17-19).

NSAIDs are often used in dogs and cats for the treatment of diseases, and they sometimes produce adverse effects on the gastrointestinal mucosa (20, 21). However, very few studies have examined the effects of NSAIDs on the small intestine in cats and dogs, except for our studies in cats (17-19, 22-24). In the present study, we investigated the effects of feeding condition and the amount of DF in the diet on the formation of gastrointestinal ulcers induced by NSAIDs in dogs using experimental doses of indomethacin and clinical doses of ketoprofen and fulnixin, which are commonly used NSAIDs in dogs. In addition, we examined the effects of various diets commercially available for dogs, such as canned diet with almost no DF and prescription diets containing low or high concentrations of DF, on gastrointestinal damage caused by NSAIDs.

MATERIALS AND METHODS

Animals

Seventy three (35 male and 38 female) mongrel dogs bred for the experiments in the animal house of Tottori University were used after feeding for > 6 months.

Experimental protocols were approved by the Animal Research Committee, Faculty of Agriculture, Tottori University, Tottori, Japan. In accordance with our institution's guidelines, the number of animals used in our studies was kept as low as possible; in most of the experiments, 2 male and 2 female dogs were included per group, and the same data from the control group was used in all ulcer experiments.

Drugs and diets

The following drugs were used: atropine sulfate (Wako, Osaka, Japan), fulnixin (Banamin®, Dainippon, Osaka, Japan), indomethacin (Sigma, St. Louis, MO, USA), ketoprofen (Ketofen®, Nihon Zennyaku Kogyo, Fukushima, Japan), pentobarbital sodium (Nembutal®, Dainippon), xylazine (Ceractal®, Bayer, Tokyo, Japan). Atropine, ketoprofen, and fulnixin were dissolved in physiological saline for subcutaneous injection (s.c.), and indomethacin for oral administration (p.o.) was suspended in 1% carboxymethylcellulose (Wako); 0.1 ml/kg of each drug was administered.

Dry food containing regular amounts of DF (RFD: Aijyo-Monogatari®, Petline, Gifu, Japan; DF: 6%), prescription diet containing low DF (LFD: canine i/d®, dry food for gastrointestinal disease such as dyspepsia, Hill's-Colgate Japan, Tokyo, Japan; DF: 1.1%) and prescription diet containing high DF (HFD: canine w/d®, dry food for metabolic syndrome such as obesity, Hill's-Colgate Japan; DF: 15.4%) were used.

Canned food containing almost no DF (NFD: Bistro-gonta®, Sunrise, Tottori, Japan; DF: <0.1%) was used. In experiments examining the effect of quality of DF on the formation of ulcers, α-cellulose (insoluble DF, Sigma) or pectin (soluble DF from citrus, Wako) was added to NFD.

The animals were given experimental diets containing DF at various percentages once daily in the morning (9 – 10 a.m.) or evening (5 – 6 p.m.) or twice daily in the morning and evening (*Fig. 1*). Nutritional composition, calories and amount of food intake per day of each diet are shown in *Table 1*. The amount of each diet per day were based on the manufacturer's information, and the diet was given once (total amount of diet) or twice (half amount of diet was given at each meal). When an animal stopped consuming the diet during the experimental period, the experiment was discontinued and the data were not used.

Induction and measurement of gastrointestinal lesions

NSAIDs were administered p.o. or s.c. at 10 a.m. just after the morning meal (*Fig. 1*, Fed-A or Fed-B) or without a morning meal (*Fig. 1*, Fasted) once daily for 3 – 7 days. Animals were sacrificed by bleeding from the carotid artery under deep anesthesia with a combination of xylazine (2 mg/kg, i.m.) and sodium pentobarbital (25 – 50 mg/kg, i.v.) 24 hours after the final dosing of NSAIDs. Then, the whole gastrointestinal tract (from esophagus to distal colon) was removed and cut along the longitudinal axis. The gastrointestinal tract was spread out on paper and mucosal lesions were observed macroscopically. In this study, lesions were not observed in the esophagus and colon. The location (corpus, antrum, duodenum, or small intestine), grade (erosion or ulcer), and area of lesions were measured. The location of the lesions in the small intestine was expressed as the distance (% of the whole small intestine) from the pyloric ring. The whole length of the small intestine from the duodenum to the ileum was divided into 10 equal-length parts. The first part was regarded as the duodenum, and segments of the small intestine was numbered from 2 to 10. The grade of lesions was estimated by at least 2 persons; 'ulcer' was defined as a severe lesion (deep with mucosal detachment) and 'erosion' was defined as a superficial or mild lesion. The lesion area (cm^2) was obtained from the product of the length and width of the lesion, and the total lesion area was obtained by summing the area of individual lesions in each part of the gastrointestinal tract (corpus, antrum, duodenum, and small intestine). Because the duodenum is a part of the small intestine, the total lesion area in the small intestine was calculated as the sum of the lesion areas in parts 1 to 10 of the small intestine.

Occult blood test

Feces samples were collected every morning, and kept –20°C until the end of the experiment for each dog. The degree of fecal occult blood in feces samples was examined qualitatively (none, mild, moderate, and severe) using hemo-test-Shionogi® (Shionogi, Osaka, Japan).

Statistical analysis

All data are expressed as mean ± standard error of the mean (S.E.M.). Differences between groups were analyzed using Student's *t*-test for 2-group comparisons, or analysis of variance (Dunnett's multiple range test) if more than 2 variables were considered, with the significance level set at 5% ($P < 0.05$).

RESULTS

Gastrointestinal lesions induced by indomethacin in various feeding conditions

When dogs were given RFD twice a day (*Fig. 1*, Fed-A), indomethacin given once daily for 7 days produced many lesions

Table 1. Nutritional composition of the experimental diets (%).

Diet	Dry food			Canned food
	RFD	LFD	HFD	NFD
Protein	>20.0 (22.2)	24.2 (26.3)	17.1 (18.8)	8.8 (50.3)
Fat	>5.0 (5.6)	12.7 (13.8)	8.0 (8.8)	5.1 (29.1)
CHO	51.0 (57.1)	47.9 (52.0)	46.3 (50.9)	<0.1 (0.6)
Fiber	<6.0 (6.7)	1.1 (1.2)	15.4 (16.9)	<0.1 (0.6)
Minerals	<10 (11.2)	<9 (9.8)	<5 (6.1)	1.9 (10.9)
H_2O	<10	<10	<11	82.5
Calories of diet (kcal/100 g)				
	290	385	290	80
Amount of diet given/day (g/10 kg. body weight)[a]				
	250	164	217	700

( ): % in dry condition, [a] According to the manufacturer's information.

in the small intestine as well as in the stomach and duodenum. Most of these were severe lesions (ulcers) (*Fig. 2a, 2b* and *3a*). In dogs given RFD once daily in the morning (*Fig. 1*, Fed-B), the lesions caused by indomethacin were obviously decreased (*Fig. 3b*). When indomethacin was administered in Fasted conditions (*Fig. 1*, Fasted), it caused only very mild lesions (erosions) both in the antrum and duodenum, and almost no lesions were observed in the rest of the small intestine (*Fig. 3c*). The mean

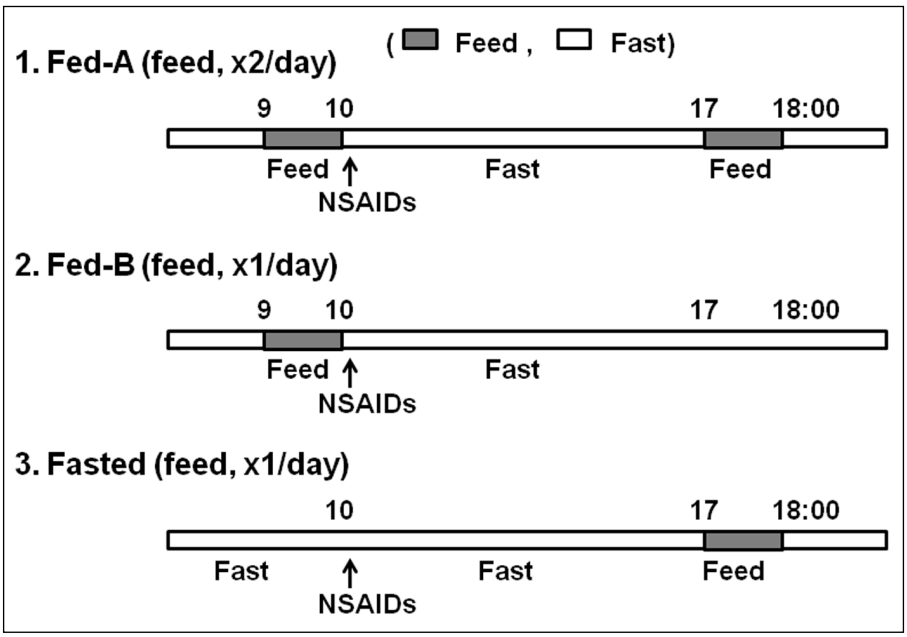

Fig. 1. Experimental schedule. The dogs were given the various diets twice daily in the morning (9 – 10 a.m.) and evening (5 – 6 p.m.) (Fed-A), or once daily in the morning (Fed-B) or evening (Fasted). Non-steroidal anti-inflammatory drugs (NSAIDs) were administered orally (p.o.) or subcutaneously (s.c.) at 10 a.m. just after feeding of diet for 1 h (Fed-A and Fed-B), or without diet (Fasted) for 3 – 7 days. Gastrointestinal lesions were examined 24 h after the final dose of NSAID.

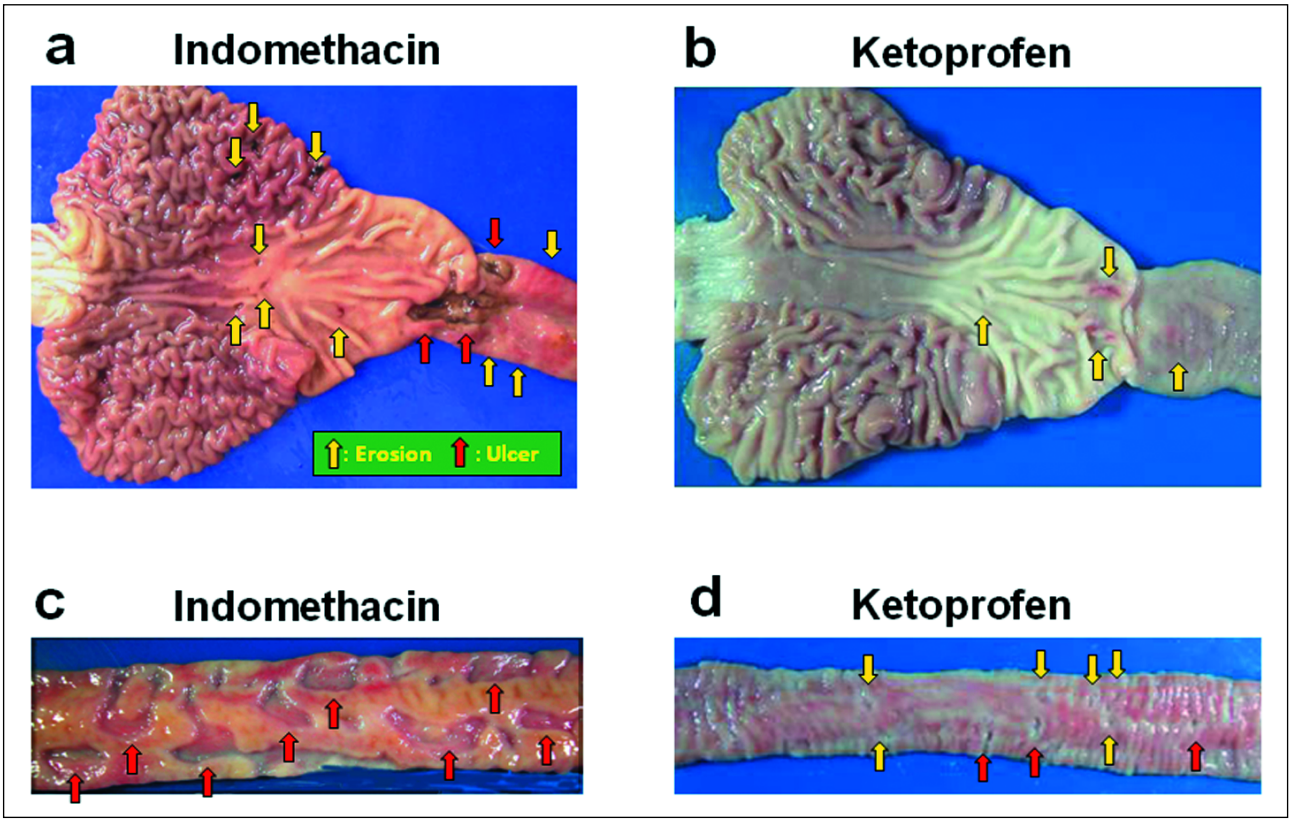

Fig. 2. Gastrointestinal mucosal lesions induced by non-steroidal anti-inflammatory drugs (NSAIDs) in dogs. (*a*), (*c*): Indomethacin (3 mg/kg, p.o.) was administered at 10 a.m. after feeding of diet twice daily for 7 days (Fed-A). Many lesions are seen in the stomach, duodenum, and small intestine; big, deep lesions are observed around the pyloric ring (*a*) and in the lower small intestine (*c*). (*b*), (*d*): Ketoprofen (2 mg/kg, s.c.) was administered at 10 a.m. after feeding of diet twice daily for 3 days. Several mild and moderate lesions are observed both in the antrum and duodenum (*b*) and in the lower small intestine (*d*). Arrows show the location of the lesions (yellow: erosion; red: ulcer).

lesion areas (MLAs) of the stomach (corpus and antrum), duodenum and small intestine in the Fed and Fasted groups are shown in *Fig. 3d*. Among the three groups, large differences were observed in the lesions of the small intestine, i.e., the MLAs of the small intestine in the Fed-A, Fed-B, and Fasted groups were 11.5 ± 4.0 cm^2 (n = 5), 7.7 ± 2.0 cm^2 (n = 4), and 0.3 ± 0.1 cm^2 (n = 4, $P < 0.05$ vs. Fed-A and Fed-B), respectively. The distribution of individual lesions caused by indomethacin in the small intestine of each dog in the Fed-B and Fasted groups are shown in *Fig. 4a* and *4b*, respectively. The lesions are observed mainly in the duodenum and lower half of small intestine in the Fed-B group, and the small intestinal lesions were markedly decreased in the Fasted group. Time-dependent changes in the occult blood test in 4 dogs in the Fed-B and Fasted groups given indomethacin for 7 days are shown in *Fig. 4c* and *4d*, respectively. In the Fed-B group, on the starting day none of the dogs showed any positive responses. In line with study days, the number of dogs with positive responses increased, and severe occult response was observed in all 4 dogs at day 7 (*Fig. 4c*). On the other hand, in the group given indomethacin in the Fasted condition, mild responses were observed during 7 days' treatment (*Fig. 4d*).

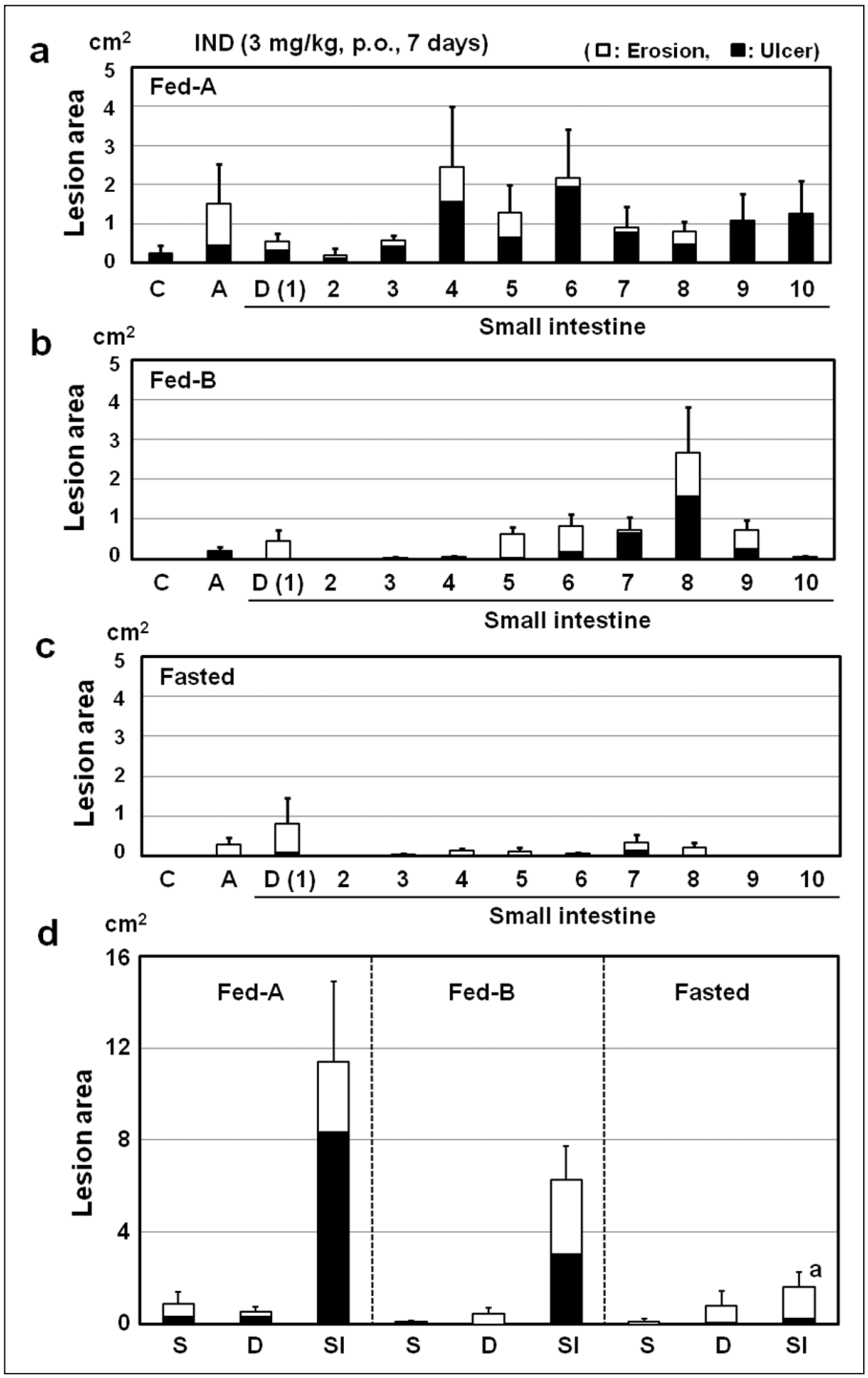

Fig. 3. Effect of feeding condition on indomethacin-induced gastrointestinal lesions. Indomethacin (3 mg/kg, p.o.) was administered at 10 a.m. for 7 days just after twice daily feeding of RFD (*a*, Fed-A), once daily in the morning (*b*, Fed-B) or once daily in the evening (*c*, Fasted). Figures in (*a*), (*b*),(*c*) show indomethacin-induced mean lesion area of each part of the stomach and small intestine (C, gastric corpus; A, gastric antrum; D, Duodenum; 2 – 10: parts of the small intestine). Figure (*d*) shows the mean value of the total lesion areas in the stomach (S), duodenum (D), and small intestine (SI) in the three feeding conditions. Data show the mean ± S.E.M. lesion areas for 4 (Fed-B, Fasted) or 5 (Fed-A) dogs. [a] $P < 0.05$ vs. Fed-A and Fed-B (Student's *t*-test).

Effect of dietary fiber on gastrointestinal lesions induced by indomethacin or ketoprofen

1. Effect of NFD, cellulose, and pectin on indomethacin-induced lesions

To examine the effects of DF on lesion formation, dogs were given RFD (DF: 6%), NFD (DF: < 0.1%), or NFD supplemented with cellulose (10%) or pectin (10%) once daily in the morning (Fed-B), and indomethacin (3 mg/kg, p.o.) was administered just after the morning meal (10 a.m.) once daily for 3 days. In dogs given RFD, indomethacin produced many lesions in the stomach, duodenum, and small intestine, and large MLAs were observed in the middle and lower parts of the small intestine (*Fig. 5a*). In the dogs given NFD, the lesions caused by indomethacin were markedly decreased, and almost no lesions were observed in the stomach, duodenum, or small intestine (*Fig. 5b*). In the group given NFD with 10% cellulose, indomethacin again produced lesions in the small intestine (*Fig. 5c*) but not in the stomach or duodenum. However, in the dogs given NFD supplemented with 10% pectin indomethacin did not cause any lesions in the gastrointestinal mucosa (*Fig. 5d*).

The MLAs of the stomach, duodenum, and small intestine in the groups given RFD, NFD, and NFD supplemented with cellulose or pectin are summarized in *Fig. 5e*, *5f*, and *5g*. The MLAs in the stomach, duodenum and small intestine given RFD were 1.9 ± 0.4 cm^2, 2.1 ± 0.7 cm^2, and 31.3 ± 2.5 cm^2 (n = 4),

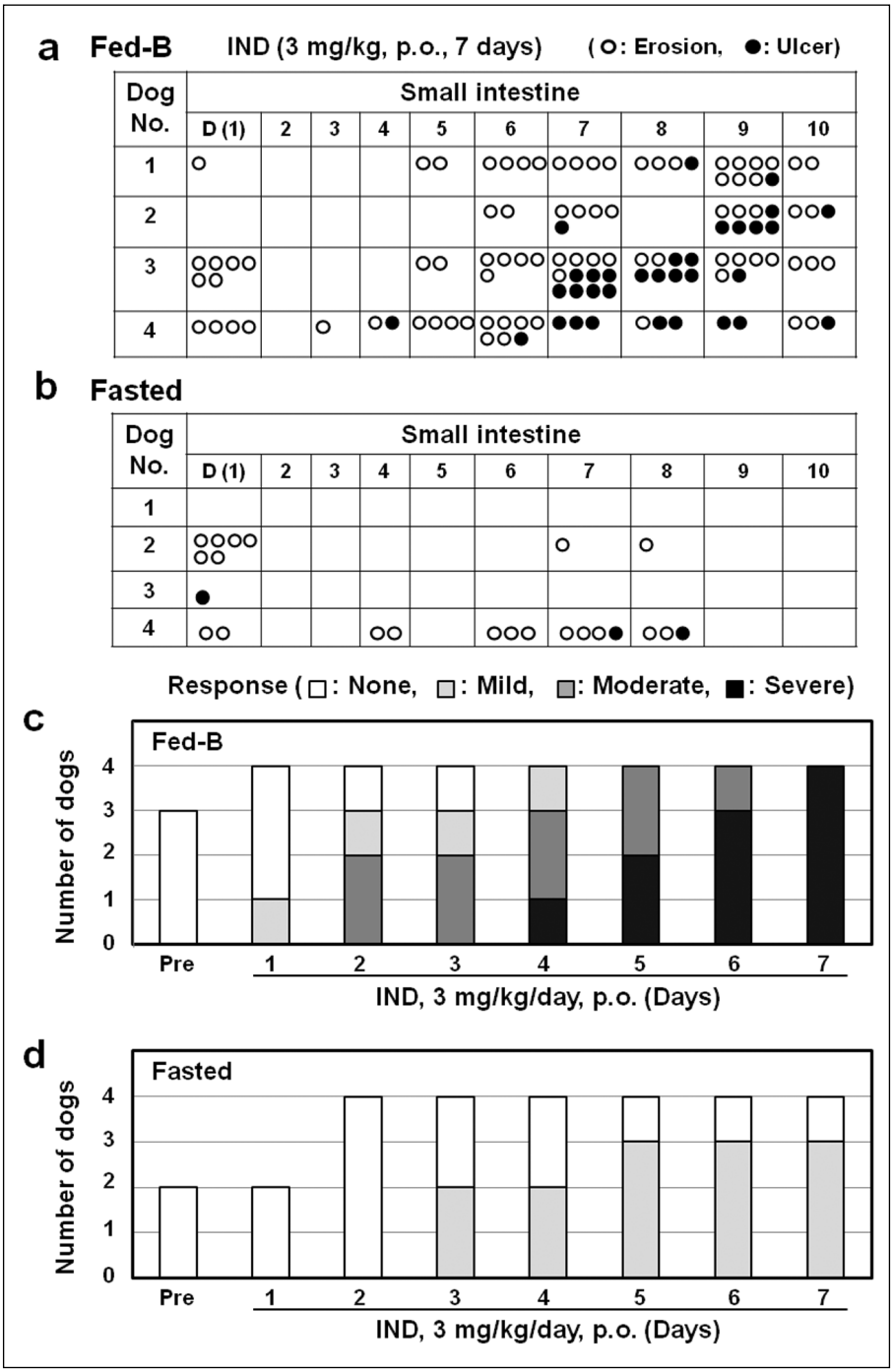

Fig. 4. Effect of feeding condition on indomethacin-induced small intestinal lesions and gastrointestinal bleeding. Indomethacin (3 mg/kg, p.o.) was administered at 10 a.m. for 7 days just after feeding of RFD once daily in the morning (Fed-B) or once daily in the evening (Fasted). (*a*), (*b*): Both the location and severity (erosion or ulcer) of each lesion observed in each of 4 dogs are shown. Many severe lesions are observed in the lower half of the small intestine in the group given indomethacin after feeding in the morning (*a*), and a few mild lesions are observed in the group given indomethacin in the Fasted condition (*b*). Gastrointestinal bleeding was examined in feces samples qualitatively (none, mild, moderate, or severe) by an occult blood test using hemo-test-Shionogi® (Shionogi, Osaka, Japan) (*c*), (*d*). Data show the results from 4 dogs in the Fed-B (*c*) and Fasted (*d*) groups. In the Fed-B group, positive responses were increased according to the number of treatment days, and severe occult blood response was observed at day 7 in all 4 dogs. On the other hand, in the group given indomethacin in Fasted conditions, a mild response was observed during 7 days' treatment.

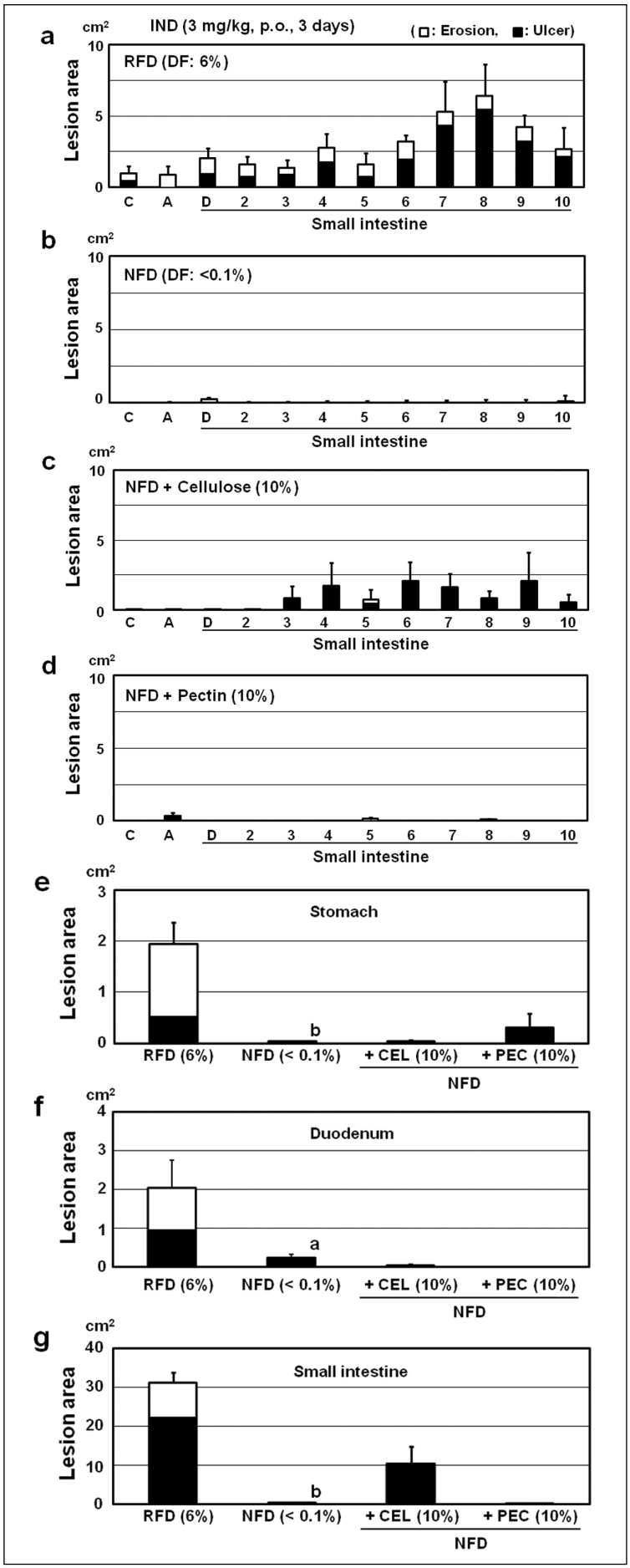

Fig. 5. Effect of dietary fiber (DF) on the formation of gastrointestinal lesions induced by indomethacin. Dogs were given RFD (DF: 6%) (*a*), NFD (DF < 0.1%) (*b*), NFD supplemented with cellulose (10%) (*c*) or pectin (10%) (*d*) once daily in the morning (9 – 10 a.m., Fed-B), and indomethacin (3 mg/kg, p.o.) was administered once daily at 10 a.m. for 3 days. The mean lesion areas of each part of the stomach, duodenum, and small intestine are shown. Figures (*e*), (*f*), and (*g*) show the mean value of total lesion areas in the stomach (*e*) duodenum (*f*), and small intestine (*g*) in the above feeding conditions. Data show the mean ± S.E.M. lesion areas for 4 dogs in each group. [a] $P < 0.05$; [b] $P < 0.01$ vs. RFD (Dunnett's test).

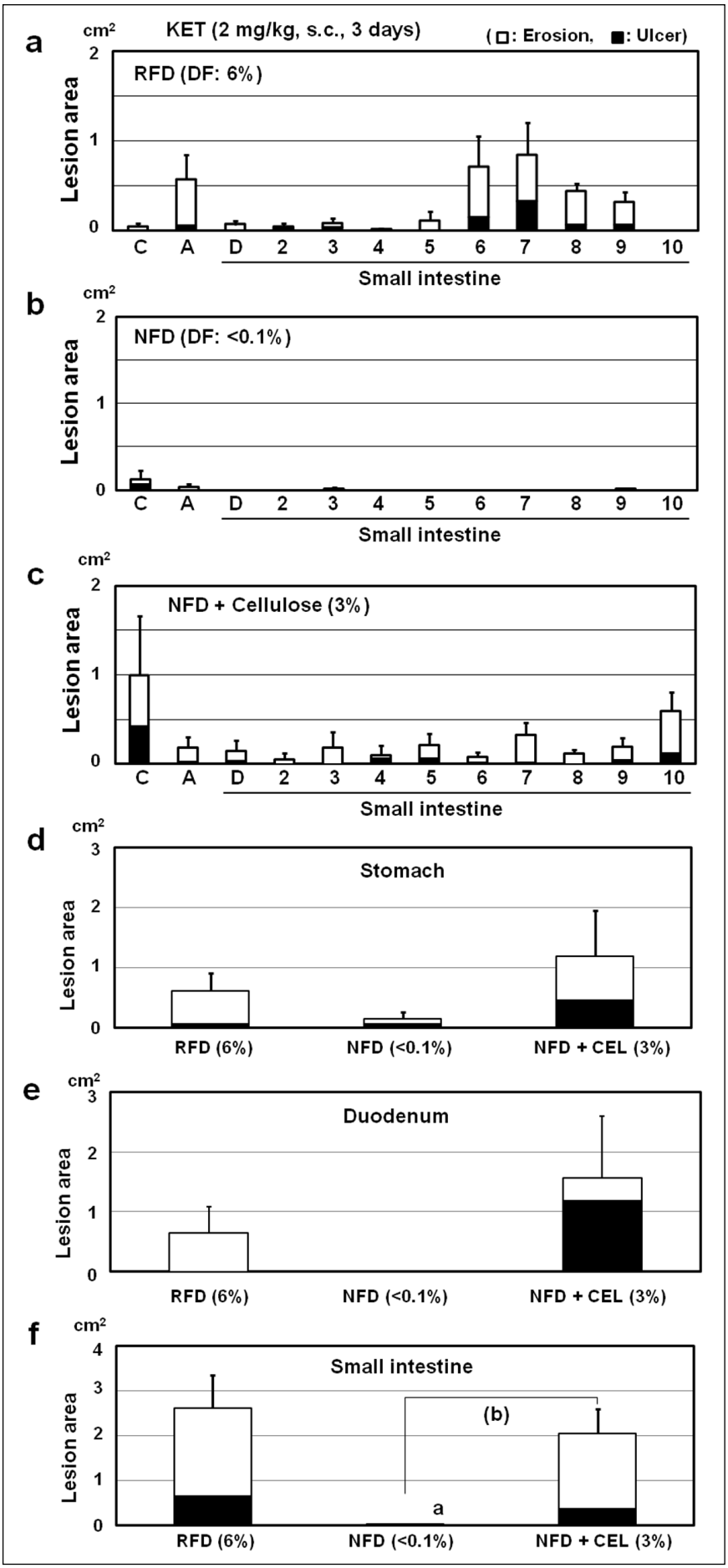

Fig. 6. Effect of dietary fiber (DF) on the formation of gastrointestinal lesions induced by ketoprofen. Dogs were given RFD (DF: 6%) (*a*), NFD (DF < 0.1%) (*b*) or NFD supplemented with cellulose (10%) (*c*) twice a day in the morning (9 – 10 a.m.) and evening (5 – 6 p.m.) (Fed-A), and ketoprofen (2 mg/kg, s.c.) was administered once daily at 10 a.m. for 3 days. The mean lesion areas of each part of the stomach, duodenum, and small intestine are shown. Figures (*d*), (*e*), and (*f*) show the mean value of total lesion areas in the stomach (*d*), duodenum (*e*), and small intestine (*f*) in the above feeding conditions. Data show the mean ± S.E.M. lesion areas for 4 dogs in each group. [a] $P < 0.05$ vs. RFD (Dunnett's test). [b] $P < 0.01$ vs. NFD (Student's *t*-test).

respectively. The MLAs in the group given NFD were very small, i.e. 0.1 ± 0.1 cm^2 (n = 4, P < 0.01 vs. RFD), 0.3 ± 0.1 cm^2 (n = 4, P < 0.05 vs. RFD), and 0.4 ± 0.2 cm^2 (n = 4, P < 0.01 vs. RFD), respectively. In the group given NFD with cellulose, the MLAs in the stomach and duodenum were not changed, but the MLA in the small intestine was increased (10.6 ± 4.2 cm^2, n = 4). Addition of pectin to NFD did not significantly affect the MLAs in the stomach, duodenum, or small intestine.

2. Effect of NFD and cellulose on ketoprofen-induced lesions

Dogs were given diet twice daily and ketoprofen (2 mg/kg, s.c.) was administered once daily for 3 days. In dogs given RFD, ketoprofen produced lesions mainly in the antrum and lower half of small intestine (*Fig. 6a*), and most were mild lesions (erosions). In the dogs given NFD, the lesions caused by ketoprofen were markedly decreased, and almost no lesions were observed in the stomach, duodenum, or small intestine (*Fig. 6b*). In the group given NFD with 3% cellulose, ketoprofen again produced many lesions in the stomach, duodenum, and small intestine (*Fig. 6c*). Although the MLA in the corpus was larger than those of the RFD or NFD groups, it was not significant.

The MLAs of the stomach, duodenum, and small intestine in the groups given RFD, NFD, and NFD supplemented with cellulose are summarized in *Fig. 6d*, *6e*, and *6f*. The MLAs in the stomach, duodenum, and small intestine in dogs given RFD were 0.62 ± 0.30 cm^2, 0.65 ± 0.43 cm^2, and 2.61 ± 0.73 cm^2 (n = 4), respectively. The MLAs in the group given NFD were 0.14 ± 0.10 cm^2, 0.0 ± 0.0 cm^2, and 0.01 ± 0.01 cm^2 (n = 4, P < 0.05 vs. RFD), respectively. In the group given NFD with cellulose, the MLAs in the stomach, duodenum, and small intestine were increased, and the increase in the small intestine was significant (2.05 ± 0.54 cm^2, n = 4, P < 0.01 vs. NFD alone).

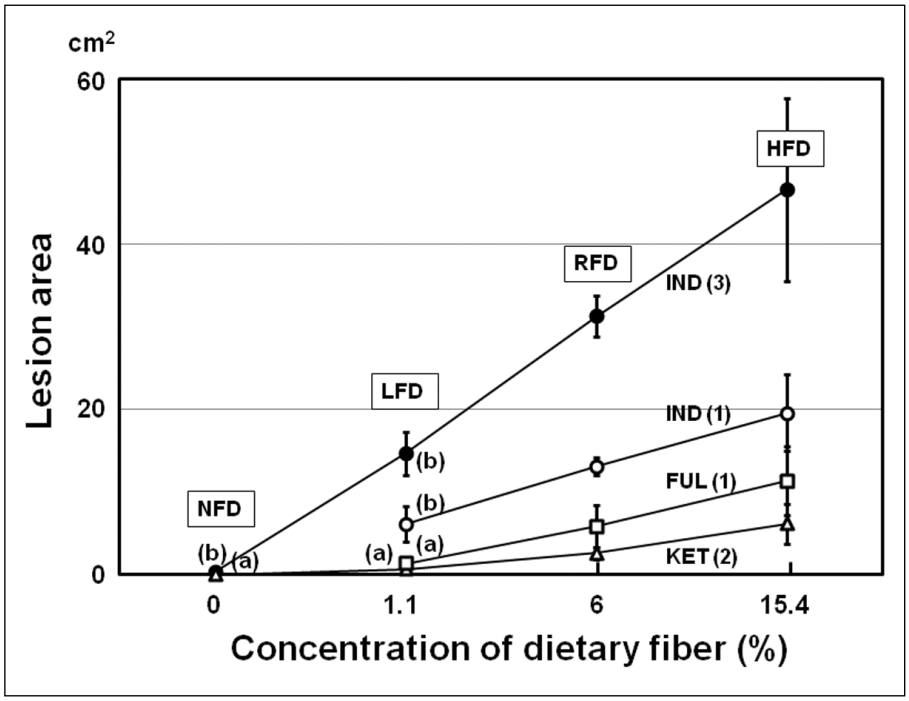

Fig. 7. Effect of prescription diets on the formation of gastrointestinal lesions induced by NSAIDs. Dogs were given NFD (DF < 0.1%), low-fiber diet (LFD; DF: 1.1%), RFD (DF: 6%), or high-fiber diet (HFD; DF: 15.4%) once (Fed-B) or twice (Fed-A) daily in the morning (9 – 10 a.m.) and evening (5 – 6 p.m.), and indomethacin (IND, 1 and 3 mg/kg, p.o.), ketoprofen (KET, 2 mg/kg, s.c.), or fulnixin (FUL, 1 mg/kg, s.c.) was administered once daily at 10 a.m. for 3 days (indomethacin and ketoprofen) or 5 days (fulnixin). Data show the mean ± S.E.M. lesion areas for 4 dogs in each group. [a] P < 0.05, [b] P < 0.01 vs. RFD (Student's *t*-test).

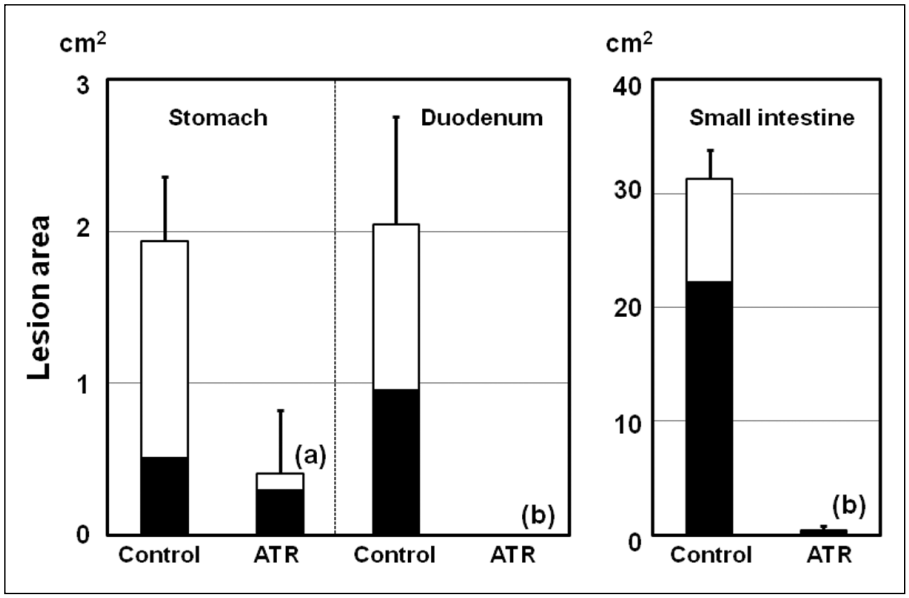

Fig. 8. Effect of atropine on the formation of gastrointestinal lesions induced by indomethacin. Dogs were given RFD once daily in the morning (9 – 10 a.m.) and indomethacin (3 mg/kg, p.o.) was administered once daily at 10 a.m. for 3 days. Atropine (ATR, 0.1 mg/kg) was administered subcutaneously twice daily, i.e. just before feeding of diet (9 a.m.) and 5 p.m. The mean lesion areas of each part of the stomach, duodenum, and small intestine are shown. Data show the mean ± S.E.M. lesion areas for 4 dogs in each group. [a] P < 0.05, [b] P < 0.01 vs. Control (Student's *t*-test).

Effect of prescription diets on small intestinal lesions induced by non-steroidal anti-inflammatory drugs

In this study, dogs were given commercially available prescription diets containing low (1.1%) or high (15.4%) concentrations of DF in addition to RFD, and low and high doses of indomethacin (1 and 3 mg/kg, p.o.) or clinical doses of ketoprofen (2 mg/kg, s.c.) and fulnixin (1 mg/kg, s.c.) were administered once daily for 3 days (indomethacin and ketoprofen) or 5 days (fulnixin). In dogs given RFD, all the NSAIDs caused lesions in the small intestine, although the MLA of the small intestine in the group given indomethacin even at low dose was clearly higher than those with ketoprofen and fulnixin (*Fig. 7*). In these studies, similar results were obtained by the administration of all the NSAIDs, i.e. the MLAs were decreased in the group given LFD and increased in the group given HFD. The MLAs in dogs given LFD were significantly decreased ($P < 0.05$ or <0.01 vs. RFD). As mentioned in the previous section, in the dogs given NFD the lesions caused by indomethacin and ketoprofen were markedly decreased ($P < 0.01$ and < 0.05 vs. RFD) (*Fig. 5g*, *6f*, and *7*).

Effect of atropine on gastrointestinal lesions induced by indomethacin

To examine the effect of atropine on gastrointestinal lesion formation, in this study dogs were given RFD once daily in the morning (Fed-B), and indomethacin (3 mg/kg, p.o.) was administered once daily for 3 days. Atropine (0.1 mg/kg) was administered subcutaneously twice daily, i.e., just before feeding of diet (9 a.m.) and 5 p.m. for 3 days. In the control dogs, indomethacin produced many lesions in the stomach, duodenum, and small intestine (*Fig. 8*). The MLAs in the small intestine as well as in the stomach and duodenum were significantly ($P < 0.01$) decreased in dogs given atropine (*Fig. 8*).

DISCUSSION

In the present study, we first examined the effects of three different feeding conditions (Fed-A, Fed-B, and Fasted) on gastrointestinal lesions induced by indomethacin in dogs, and found that the lesions in the small intestine in the fasted condition (Fasted) was significantly ($P < 0.05$) smaller than those in fed conditions (Fed-A and Fed-B). The effect was also confirmed by a fecal occult blood test, i.e. indomethacin given after feeding of diet (Fed-B) produced severe responses, but there were very mild in the group given indomethacin in the fasted condition (Fasted). These results support our previous findings on the effect of feeding condition on the formation of small intestinal lesions in mice (14), rats (15, 16), and cats (17-19), i.e. NSAIDs caused no or few lesions in the small intestine when they were administered in the fasted condition, but produced many lesions when they were given just after feeding of diet (cats) or without fasting (mice and rats). Furthermore, in a previous study in cats (19) we found that the small intestinal lesions induced by enteric coated aspirin were markedly reduced when the drug was administered 3 hours after a meal, compared with the lesions caused by aspirin given just after a meal. All of the findings in mice, rats, and cats (14-19), and the results in the present study in dogs suggest that NSAID-induced small intestinal damage may be reduced by taking NSAIDs in the fasted condition, or practically, at least several hours after the meal, e.g. before go to bed in the evening.

We previously observed that small intestinal lesions induced by NSAIDs are dependent on the amount of insoluble DF in the diet in mice (14), rats (15, 16), and cats (17-19). Therefore, we next examined the effect of DF on the formation of small intestinal lesions induced by indomethacin in dogs. Indomethacin administered after feeding of RFD containing 6% crude DF produced many severe lesions in the small intestine after 3 days' treatment. The lesions were decreased substantially in dogs given NFD (canned diet containing almost no DF, i.e., < 0.1%). Furthermore, indomethacin caused lesions again in the small intestine in dogs fed NFD supplemented with 10% cellulose (insoluble DF), but not with 10% pectin (soluble DF Similar results were obtained when a clinical dose of ketoprofen (2 mg/kg, s.c., for 3 days) was used instead of indomethacin. In all species (mice, rats, cats, and dogs) tested to date, the formation of small intestinal lesions seems to depend on the involvement of insoluble DF in the diet, suggesting that insoluble DF may play an important role in the formation of small intestinal damage by NSAIDs also in humans. It will then be a question of how insoluble DF plays a role in the formation of small intestinal damage. It has been reported that NSAIDs cause hypermotility in the small intestine and decrease the mucus content of the small intestine in rats (25, 26). We also have reported that indomethacin markedly increased ileal motility in cats (17). In this study indomethacin-induced small intestinal lesions were almost completely diminished by treatment with low dose of atropine, an anti-cholinergic agent. It has been reported that indomethacin decreases mucosal protective activity *via* an increase of mitochondrial reactive oxygen species (ROS) in the mucosa before the formation of intestinal lesions in mice (27). Considering all of these findings together, it may be plausible that insoluble DF causes physical damage to the mucosal surface when the intestine is strongly contracted under fragile conditions of a decrease in surface mucus (26) and an increased concentration of mucosal ROS (27). This will explain the early stage of small intestinal damage caused by NSAIDs.

On the other hand, it is well known that NSAIDs often cause ulcers not only in the stomach but also in the duodenum in humans, but they hardly produced lesions in the duodenum in rodents such as mice and rats. In the present study NSAIDs produced obvious lesions in the duodenum in dogs, and we reported previously that NSAIDs caused duodenal ulcers in cats (17, 22). Although it is unknown why NSAIDs do not cause ulcers in the duodenum in rodents, the different feeding patterns (continuous vs. intermittent) of rodents and dogs as well as cats and humans may explain, at least in part, the different effect of NSAIDs on the duodenum (22). From these findings it was suggested that the gastrointestinal damage caused by NSAIDs in dogs may be closer to that of humans. In the present study, the lesions in the duodenum as well as in the stomach were markedly decreased in dogs given NFD, suggesting that DF plays an important role in the formation of ulcers not only in the small intestine but also in the stomach and duodenum. Various irritants, such as gastric acid, enteric bacteria, bile acids, and food substances, may then aggravate the gastrointestinal lesions caused by NSAIDs.

The above findings suggest that NSAID-induced small intestinal damage could be reduced by decreasing the amount of DF; therefore, we next examined this possibility further using prescription diets for dogs containing a low amount of DF (LFD, DF: 1.1%) or a high amount of DF (HFD, DF: 15.4%). In this study we used ketoprofen and fulnixin at clinical doses in dogs and low and high doses of indomethacin as NSAIDs. All the NSAIDs produced lesions in the small intestine in dogs given RFD (DF: 6%). NSAID-induced intestinal lesions were markedly decreased and increased in dogs fed LFD or HFD, respectively. In recent clinical studies on NSAID-induced intestinal lesions in humans, the incidence of lesions varied from 8.4% to 70% (1-4). The difference in incidence may be due, at least in part, to differences in the amount of DF in the diets. The results of the present study suggest that it will be useful to take a diet with low

amounts of DF in order to reduce the gastrointestinal side-effects of NSAIDs not only in dogs but also in humans. However, it has been reported that DF plays an important role in the pathology of inflammatory bowel disease such as ulcerative colitis and Crohn's disease, probably *via* influence on the microbial composition of the gut (28-30). On the other hand, we found that soluble DF such as pectin, in contrast to insoluble DF, could prevent the formation of small intestinal lesions induced by NSAIDs in mice (14), rats (16), and cats (18, 19). Therefore, it will be important to consider both the amount and quality (soluble or insoluble) of DF when we control the content of DF in the diet for the treatment of gastrointestinal diseases.

Recent studies using video capsule endoscopy showed that intestinal lesions induced by NSAIDs in humans are often seen in the middle and lower parts of the small intestine (31). In the present study both indomethacin and ketoprofen given after feeding of RFD produced lesions mainly in the lower half of the small intestine. We found a similar distribution of NSAID-induced small intestinal lesions in mice (14), rats (16), and cats (17). Although we did not examine the mechanism of this characteristic distribution of small intestinal lesions, several possibilities have been considered from the pathogenesis of small intestinal lesions (32). The first possibility may relate to the concentration of insoluble DF, i.e., the soluble components of the diet, such as water and nutrients, will be absorbed in the upper and middle parts of the small intestine, resulting in an increase in insoluble (undigested) solid components of food, including substances harmful to the mucosa, in the lower small intestine. The second possible mechanism may involve a different distribution of intestinal bacteria, especially Gram-negative bacteria (33), i.e., the distribution of bacteria in the upper small intestine will be less than that in the lower intestine because of the presence of bactericidal factors, such as gastric acid and bile acids. Furthermore, there will be a possibility that bacteria may be refluxed from the caecum and colon into the ileum, probably caused by abnormal hypermotility of the intestine caused by NSAIDs, although there is no direct evidence. Therefore, the lower intestine may be more vulnerable to bacterial invasion, which can aggravate the lesions. In addition, it is possible that high concentrations of bile acids and NSAIDs are achieved locally in the lower small intestinal mucosa because of enterohepatic circulation (34-36). Intestinal bacteria and bile acids may be not harmful to the mucosa in normal physiological conditions, but they will act to aggravate mild lesions in the mucosa.

From the results of present study it was suggested that both the timing of NSAID use with respect to food intake and insoluble DF, such as cellulose in the diet, play an important role in the formation of gastrointestinal lesions induced by NSAIDs. These results further suggested that a diet with no or low amounts of DF may decrease gastrointestinal side-effects associated with the use of NSAIDs not only in dogs but also in humans. We expect that the clinical advantages of diets with low DF in decreasing adverse events, especially in the small intestine, will be further explored in the near future.

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REFERENCES

1. Laine L, Connors LG, Reicin A, *et al*. Serious lower gastrointestinal clinical events with nonselective NSAID or coxib use. *Gastroenterology* 2003; 124: 288-292.
2. Maiden L, Thjodleifsson B, Theodors A, Gonzalez J, Bjarnason I. A quantitative analysis of NSAID-induced small bowel pathology by capsule enteroscopy. *Gastroenterology* 2005; 128: 1172-1178.
3. Goldstein JL, Eisen GM, Lewis B, *et al*. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. *Clin Gastroenterd Hepatol* 2005; 3: 133-141.
4. Fortun PJ, Hawkey CJ. Nonsteroidal anti-inflammatory drugs and the small intestine. *Curr Opin Gastroenterd* 2007; 23: 134-141.
5. Satoh H, Takeuchi K. Management of NSAID/aspirin-induced small intestinal damage by GI-sparing NSAIDs, anti-ulcer drugs and food constituents. *Curr Med Chem* 2012; 19: 82-89.
6. Mizoguchi H, Ogawa Y, Kanatsu K, Tanaka A, Kato S, Takeuchi K. Protective effect of rebamipide on indomethacin-induced intestinal damage in rats. *J Gastroenterol Hepatol* 2001; 16: 1112-1119.
7. Kamei K, Kubo Y, Kato N, Hatazawa R, Amagase K, Takeuchi K. Prophylactic effect of irsogladine maleate against indomethacin-induced small intestinal lesions in rats. *Dig Dis Sci* 2008; 53: 2657-2666.
8. Watanabe T, Nishio H, Tanigawa T, *et al*. Probiotic Lactbacillus casei strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. *Am J Physiol Gastrointest Liver Physiol* 2009; 297: G506-G513.
9. Satoh H, Amagase K, Takeuchi K. Mucosal protective agents prevent exacerbation of NSAID-induced small intestinal lesions caused by antisecretory drugs in rats. *J Pharmacol Exp Ther* 2012; 348: 227-235.
10. Hayashi S, Kurata N, Kitahirachi E, *et al*. Cinacalcet, a calcimimetic, prevents nonsteroidal antiinflammatory drug-induced small intestinal damage in rats. *J Physiol Pharmacol* 2013; 64: 453-463.
11. Bjarnason I, Smethurst P, Fenn CG, Lee CE, Menzies IS, Levi AJ. Misoprostol reduces indomethacin-induced changes in human small intestinal permeability. *Dig Dis Sci* 1981; 34: 407-411.
12. Watanabe T, Sugimori S, Kameda N, *et al*. Small bowel injury by low-dose enteric-coated aspirin and treatment with misoprostol: a pilot study. *Clin Gastroenterol Hepatol* 2008; 6: 1279-1282.
13. Fujimori S, Seo T, Gudis K, *et al*. Prevention of nonsteroidal anti-inflammatory drug-induced small-intestinal injury by prostaglandin: a pilot randomized controlled trial evaluated by capsule endoscopy. *Gastrointest Endosc* 2009; 69: 1339-1346.
14. Satoh H, Urushidani T. Soluble dietary fiber can protect the gastrointestinal mucosa against non-steroidal anti-inflammatory drugs in mice. *Dig Dis Sci* 2016; 61: 1903-1914.
15. Satoh H, Guth PH, Grossman MI. Role of food in gastrointestinal ulceration produced by indomethacin in the rat. *Gastroenterology* 1982; 83: 210-215.
16. Satoh H, Matsumoto H, Hirakawa T, Wada N. Soluble dietary fibers can protect the small intestinal mucosa without affecting the anti-inflammatory effect of indomethacin in adjuvant-induced arthritis rats. *Dig Dis Sci* 2016; 61: 91-98.
17. Satoh H, Shiotani S, Otsuka N, Hatao K, Nishimura S. Role of dietary fibres, intestinal hypermotility and leukotrienes in the pathogenesis of NSAID-induced small intestinal ulcers in cats. *Gut* 2009; 58: 1590-1596.
18. Satoh H, Hara T, Murakawa D, Matsuura M, Takata K. Soluble dietary fiber protects against nonsteroidal anti-inflammatory drug-induced damage to the small intestine in cats. *Dig Dis Sci* 2010; 55: 1264-1271.

19. Satoh H, Amagase K, Takeuchi K. The role of food for the formation and prevention of gastrointestinal lesions induced by aspirin in cats. *Dig Dis Sci* 2013; 58: 2840-2849.
20. Khan SA, McLean MK. Toxicology of frequently encountered nonsteroidal anti-inflammatory drugs in dogs and cats. *Vet Clin North Am Small Anim Pract* 2012; 42: 289-306.
21. Bergh MS, Budsberg SC. The coxib NSAIDs: potential clinical and pharmacologic importance in veterinary medicine. *J Vet Intern Med* 2005; 19: 633-643.
22. Satoh H, Amagase K, Ebara S. Akiba T, Takeuchi K. Cyclooxygenase (COX)-1 and COX-2 both play an important role in the protection of the duodenal mucosa in cats. *J Pharmacol Exp Ther* 2013; 344: 189-195.
23. Takata K, Hikasa Y, Satoh H. Therapeutic and adverse effects of flunixin-meglumine in adult and young cats. *J Vet Med Sci* 2011; 73: 1591-1596.
24. Takata K, Hikasa Y, Satoh H. Comparison of gastrointestinal adverse effects of ketoprofen between adult and young cats. *J Vet Med Sci* 2012; 74: 1545-1550.
25. Takeuchi K, Miyazawa T, Tanaka A, Kato S, Kunikata T. Pathogenic importance of intestinal hypermotility in NSAID-induced small intestinal damage in rats. *Digestion* 2002; 66: 30-41.
26. Kunikata T, Tanaka A, Miyazawa T, Kato S, Takeuchi K. 16, 16-Dimethyl prostaglandin E_2 inhibits indomethacin-induced small intestinal lesions through EP3 and EP4 receptors. *Dig Dis Sci* 2002; 47: 894-904.
27. Tomita T, Sadakata H, Tamura M, Matsui H. Indomethacin-induced generation of reactive oxygen species leads to epithelial cell injury before the formation of intestinal lesions in mice. *J Physiol Pharmacol* 2014; 65: 435-440.
28. Ananthakrishnan AN, Khalili H, Konijeti GG, *et al*. A prospective study of long-term intake of dietary fiber and risk of Crohn's disease and ulcerative colitis. *Gastroenterology* 2013; 145: 970-977.
29. Konturek PC, Haziri D, Brzozowski T, *et al*. Emerging role of fecal microbiota therapy in the treatment of gastrointestinal and extra-gastrointestinal diseases. *J Physiol Pharmacol* 2015; 66: 483-491.
30. Owczarek D, Rodacki T, Domagala-Rodacka R, Cibor D, Mach T. Diet and nutritional factors in inflammatory bowel diseases. *World J Gastroenterol* 2016; 22: 895-905.
31. Chutkan R, Toubia N. Effect of nonsteroidal anti-inflammatory drugs on the gastrointestinal tract: diagnosis by wireless capsule endoscopy. *Gastrointest Endoscopy Clin N Am* 2004; 14: 67-85.
32. Takeuchi K, Satoh H. NSAID-induced small intestinal damage - roles of various pathogenic factors. *Digestion* 2015; 91: 218-232.
33. Satoh H, Takeuchi K. Role of food and enterobacteria in the formation and prevention of small intestinal damage induced by NSAIDs. *Front Gastrointest Res* 2012; 30: 52-60.
34. Jacob M, Foster R, Sigthorsson G, Simpson R, Bjarnason I. Role of bile in pathogenesis of indomethacin-induced enteropathy. *Arch Toxicol* 2007; 81: 291-298.
35. Duggan DE, Hooke KF, Noll RM, Kwan KC. Enterohepatic circulation of indomethacin and its role in intestinal irritation. *Biochem Pharmacol.* 1975; 24: 1749-1754.
36. Reuter BK, Davies NM, Wallace JL. Nonsteroidal anti-inflammatory drug enteropathy in rats: role of permeability, bacteria, and enterohepatic circulation. *Gastroenterology* 1997; 112: 109-117.

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