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An animal model of longitudinal ulcers in the small intestine induced by intracolonically administered indomethacin in rats

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Summary: The ulcerogenic effect of intracolonically administered indomethacin was evaluated in rats. Conventionally fed rats aged from 5 to 10 weeks were treated by 8, 16, 24, or 32 mg/kg of intracolonic indomethacin for two days, and any damage to the stomach, small intestine and the colon was investigated. Longitudinal ulcers and scattered small ulcers were found in the small intestine at all doses of indomethacin, and the length of the longitudinal ulcers increase dose-dependently, but this was unrelated to the body weight of the rats. The cecum was frequently affected by irregularly shaped ulcers, and the incidence increased as the dose of indomethacin increased. The colon, other than the cecum, was not involved macroscopically. In contrast, the stomach was affected by only large doses of indomethacin (24 or 32 mg/kg), and the size of gastric ulcers increased according to the body weight of the rats. These findings suggest that intracolonic indomethacin in relatively young rats causes ulcers predominantly in the small intestine and the cecum, which are the frequent site of involvement of human Crohn's disease, and that this animal model may be suitable for investigation of the pathophysiology of inflammatory bowel disease. *Gastroenterol Jpn 1993;28:10-17.*

Key words: *non-steroidal anti-inflammatory drugs; indomethacin; small intestine; longitudinal ulcer; Crohn's disease.*

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) have been well known to cause gastroduodenal damage. In addition, they are also known to affect the small intestine in humans[1]. In experimental animals, large doses of indomethacin, administered orally or subcutaneously, led to ulceration or perforation of the small intestine[2-4].

Since the fact that refed rats treated by indomethacin manifest gastric antral ulcers without intestinal damage has been reported by Satoh et al[4], the practical extent of damage to the gastrointestinal tract by NSAIDs in experimental animals has been evaluated mainly focusing on gastric lesions.

Although much previous work exists on animal models for Crohn's disease, there are no studies in which longitudinal ulcers can be easily and constantly produced. In this study, we report on the ulcerogenic effect of indomethacin, which frequently induced typical longitudinal ulcers of the small intestine in rats. Intracolonic administration was chosen in consideration of possible occurrence of colonic damage.

Materials and Methods

Animals

Male Wistar rats (100-350 g), aged from 5 to 10 weeks, purchased from Kyushu Animal Corporation (Tosu, Saga, Japan), were used in this study. They were maintained in a restricted access room at a controlled temperature. The animals were housed in wire cages with a maximum of 6

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animals per cage. Standard laboratory pellet formula and tap water were provided at libitum.

Induction of gastrointestinal damage

The animals had free access to water or food prior to indomethacin administration. The rats were weighed, randomized into groups according to the dose of indomethacin treatment, and then anesthetized with intraperitoneal administrations of sodium amobarbital (100 mg/kg). A plastic catheter measuring 6 cm in length was inserted rectally (approximately as far as the sigmoid colon). Then, 8, 16, 24, or 32 mg/kg of indomethacin (Banyu, Tokyo, Japan) diluted in 1% of sodium carboxymethylcellulose solution (10 mg/ml) was injected via the catheter into the colon. In preliminary study, the solution, to which indigo carmine had been added for clear visualization, remained in the colon and cecum of the rats. On the next day, the same dose of indomethacin was again administered in the same manner. To avoid coprophagy, the animals were housed routinely in cages with raised mesh bases during the investigation.

The number of rats treated by each dose of indomethacin was 17 in 8 mg/kg, 15 in 16 mg/kg, 16 in 24 mg/kg, and 12 in 32 mg/kg respectively. In addition, three control rats which received only the vehicle intracolonically were also investigated. Thus, a total of 63 rats were included in this study.

Assessment of gastrointestinal lesions

Twenty-four hours after the second administration of indomethacin, the rats were sacrificed by an intraperitoneal amobarbital overdose. The stomach, small intestine, cecum, and the colon were removed, opened by a longitudinal incision, and pinned out on a wax block. The specimen was washed with saline, and any macroscopic change was checked. A precise evaluation of the lesions was made after each specimen was fixed in 10% formalin for two days.

Any damage to the stomach was evaluated according to whether ulcers were present or not. When any ulceration was macroscopically confirmed, the largest diameter of each ulcer was measured, then, the total diameter of all the ulcers in each animal was calculated.

Because two types of ulceration (longitudinal ulcers and scattered ulcers) occurred in the small intestine, they were assessed differently. A longitudinal ulcer was defined as an ulcer located on the mesenteric side of the intestinal lumen which measured more than 10 mm in longitudinal length. The total length of the small intestine (from the pylorus to the ileocecal junction) and the longitudinal ulcers were measured, and the longitudinal ulcers index was defined as the ratio of the length of the measured ulcers versus the whole length of the small intestine. Scattered small ulcers were defined as the ulcer smaller than 10 mm in its largest diameter, regardless of its location, and more than 5 mm apart from longitudinal ulcrs. The number of these ulcers was counted, and this figure was used as the small ulcer index. The cecum and the colon were also examined in a similar manner. If any ulcer was detected, the diameter was measured.

After these investigations, the severely damaged segments of the intestine was removed, fixed and stained with hematoxylin and eosin, and then evaluated by a pathologist who was blind to the treatment of each animal.

Serum concentration of indomethacin

Blood samples were obtained from the animals of each individual group from the inferior vena cava immediately after they were sacrificed. Each sample was centrifuged at 3000 rpm for 10 minutes at a temperature of 5°C. The concentration of indomethacin was measured by high-pressure liquid chromatography (HPLC) using Trirotar, Nippon Bunkoh, Tokyo, Japan.

Statistical analyses

All results, except for the occurrence of cecal ulcers, are expressed as mean±SEM. The extent of correlation between the size of gastric ulcers and body weight, and between the small intestinal ulcer index and body weight was determined by regression analysis. Overall significance was determined by a one-way analysis of variance (ANOVA) using F value for the length and number of small intestinal ulcers, and by χ^2 test for the

Table 1. Incidence of ulcers within the gastrointestinal tract

Dose of indomethacin	Site of involvement			
	Stomach	Small intestine		Cecum
		Longitudinal ulcer	Small ulcer	
8 mg/kg (n=17)	1/17 (6%)	3/17 (18%)	4/17 (24%)	0/17 (0%)
16 mg/kg (n=15)	4/15 (27%)	13/15 (87%)	8/15 (53%)	5/15 (33%)
24 mg/kg (n=16)	7/16 (43%)	16/16 (100%)	6/16 (38%)	13/16 (81%)
32 mg/kg (n=12)	7/12 (58%)	12/12 (100%)	6/12 (50%)	9/12 (75%)

Figures refer to the number of rats.

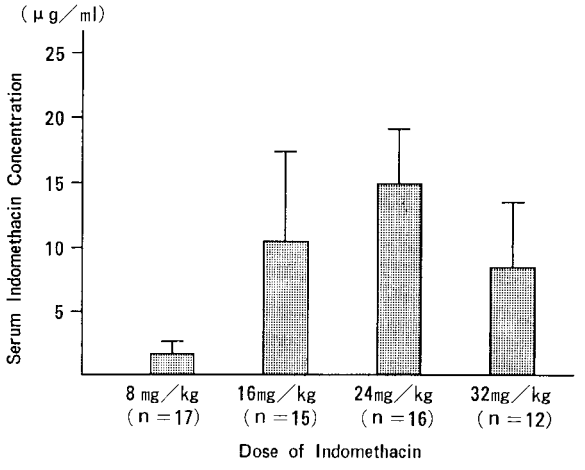

Figure 1. Serum concentration of indomethacin 24 hrs after the second intracolonic administration of indomethacin in rats, according to each dose. Values are mean±SEM. The serum concentration was different among the groups (one-way analysis of variance, $P<0.05$).

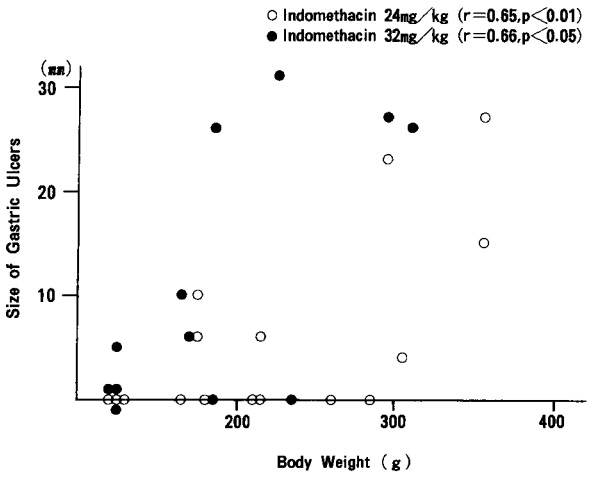

Figure 2. Correlation between body weight immediately prior to indomethacin administration and the size of gastric ulcers in 24 mg/kg (○) and 32 mg/kg (●) indomethacin-treated rats. The size of the gastric ulcer shows linear correlation with the body weight of rats (regression analysis).

incidence of cecal ulcers. Student's t-test with one-sided test and χ^2 test with Yates's correction were used when the gastrointestinal damage was compared between each group. Probabilities <0.05 were considered significant.

Results

General observations

Indomethacin-treated rats frequently manifested diarrhea which mainly occurred after the second administration of the agent, decreased food and water intake and slight weight loss during three days' observation. Neither these manifestations nor practical gastrointestinal damage were found in any of the control rats. The overall incidence of damage within the gastrointestinal tract is indicated in **Table 1.**

Serum indomethacin concentration

Serum concentration 24 hours after the second indomethacin administration were 2.3±0.9 μg/ml in the 8 mg/kg-treated group, 10.7±6.9 μg/ml in the 16 mg/kg group, 15.9±4.2 μg/ml in the 24 mg/kg group, and 9.4±4.3 μg/ml in the 32 mg/kg group. Except for the 32 mg/kg group, the serum concentration of indomethacin increased in proportion to the intracolonically administered dose (**Figure 1**).

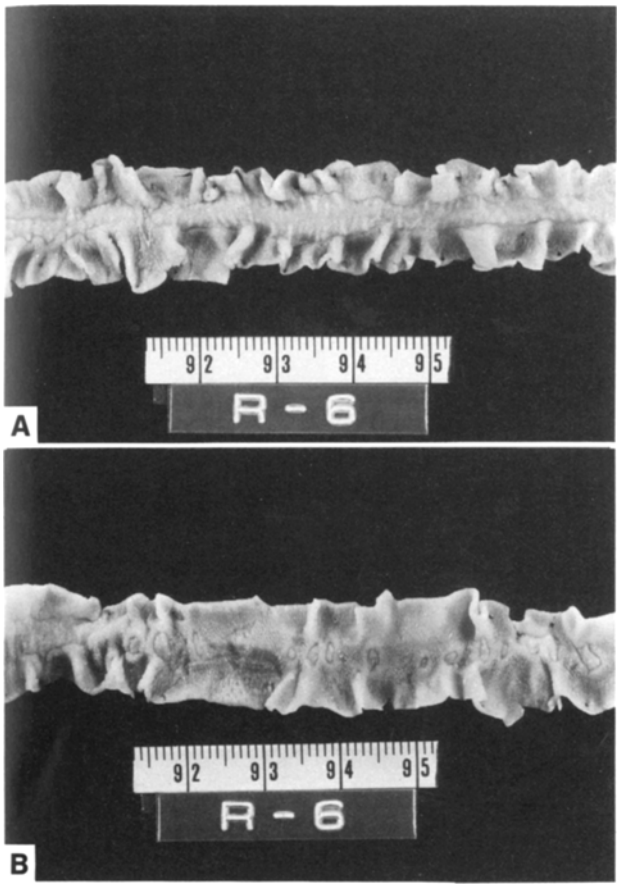

Figure 3. Macroscopic findings of small intestinal ulcers in an indomethacin-treated rat. A: A longitudinal ulcer can be seen on the mesenteric side of the small intestinal lumen. B: Small scattered ulcers can be seen. The ulcers are on the mesenteric side of the intestinal lumen, and are sharply demarcated from the surrounding mucosa.

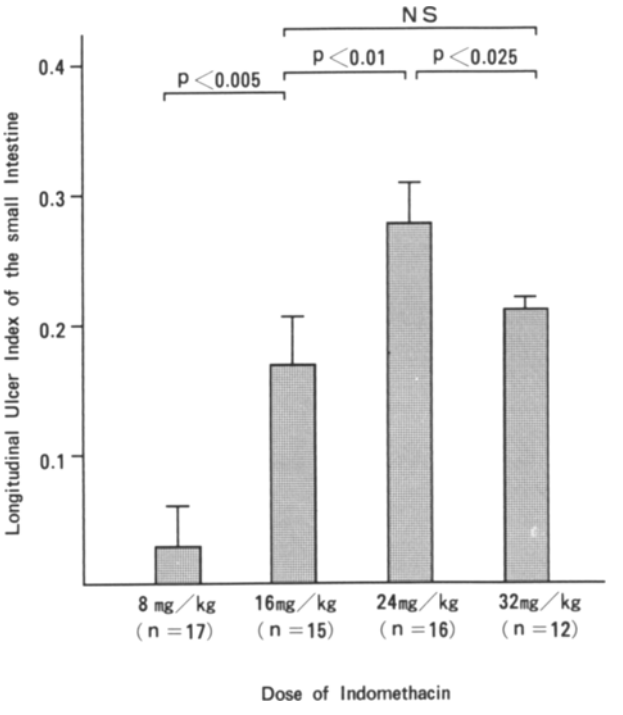

Figure 4. Longitudinal ulcer index of the small intestine in each group. Results are expressed as means±SEM. The ulcer index is statistically different among the groups divided according to the dose of indomethacin (one-way analysis of variance, $P<0.001$). The ulcer index was larger in 24 mg/kg group than in other groups (Student's test with one-sided test).

Damage to the stomach

In rats treated by large dose of indomethacin (24 or 32 mg/kg), various sizes of ulcers, which were located in the gastric antrum and in the gastric corpus, occurred. In those rats treated by small doses of indomethacin, however, there were minimal or no gastric ulcers developed. In addition, the size of the gastric ulcers was correlated with the body weight in either 24 mg/kg ($r=0.65$, $P<0.01$) or 36 mg/kg-treated rats ($r=0.66$, $P<0.05$) (**Figure 2**).

Damage to the small intestine

Figure 3 shows two types of ulcer, a longitudinal ulcer and scattered small ulcers, found in the small intestine. The longitudinal uclers was frequently found in the mid-small intestine and were always located on the mesenteric side. Average values for the longitudinal ulcer index are shown in **Figure 4**. The rats treated with $\geq$16 mg/kg of indomethacin had longer ulcers (0.17±0.03 in 16 mg/kg, 0.28±0.03 in 24 mg/kg, and 0.21±0.01 in 32 mg/kg-treated rats) than did those with 8 mg/kg (0.03±0.02) although the ulcer index in the 32 mg/kg group was smaller than that in 24 mg/kg group. The ulcer index of the small intestine did not correlate with the body weight as did the size of the stomach ulcer. Scattered small ulcers were found on both the mesenteric and the anti-mesenteric sides of the small intestine. The number of these small ulcers was not significantly different among the groups (**Figure 5**).

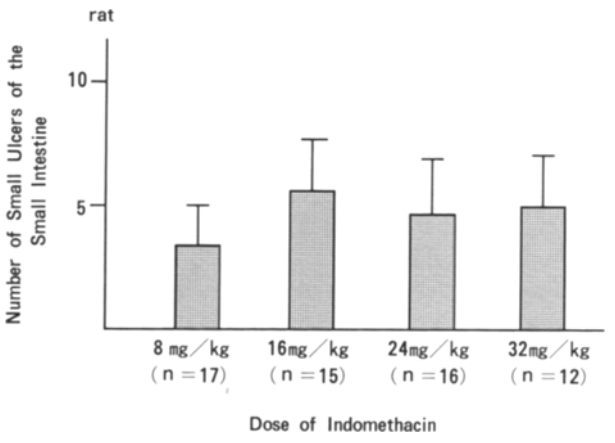

Figure 5. Number of scattered small ulcers of the small intestine in each group. There is no statistical significance in the number of small ulcers among the groups (one-way analysis of variance).

Figure 6. A typical cecal ulcer. An ulcer, the margin of which is irregularly separated from the surrounding mucosa, can be seen.

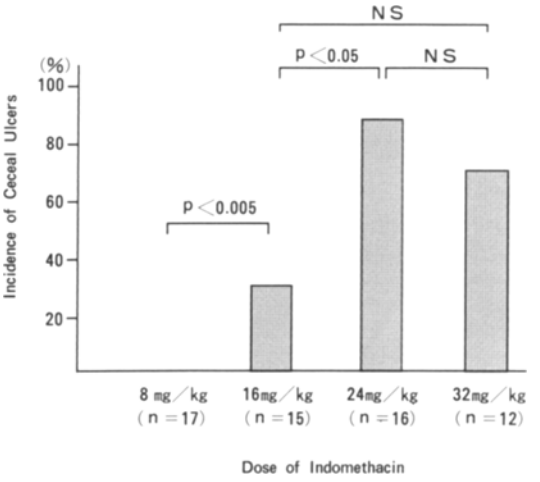

Figure 7. Incidence of cecal ulcers in each group. The incidence is different among the groups (χ^2 test, $P<0.001$), and it increases according to the increase of the dose of indomethacin up to 24 mg/kg.

Damage to the cecum and the colon

Indomethacin did not produce any visible changes in the colon of rats. In contrast, the cecum was frequently affected with shallow ulcers of varying sizes (**Figure 6**). The incidence of cecal ulcer increased as the dose of indomethacin increased among all groups, except for the 32 mg/kg group (**Figure 7**).

Histological features

Histologically, the affected segments of the stomach and the intestine were edematous with inflammatory exudates. Gastric ulcers and longitudinal ulcers of the small intestine were mainly restricted to the submucosal layer, focally extending to the proper muscular layer, covered with necrotic slough and accompanied by inflammatory infiltrate, which was mainly composed of neutrophils (**Figure 8**). Eosinophils, and a few of plasma cells and lymphocytes were also seen. Small ulcers of the small intestine and cecal ulcers tended to be shallow compared with other ulcers, and the submucosal layer of the affected cecum was slightly thickened with edema and dilated capilaries with frequent thrombi-formation, accompanied by mild inflammatory infiltrate (**Figure 9**). However, there were no specific inflammatory lesion either in the small intestine or in the cecum.

Discussion

There have been many reports describing ulcerogenic effects of indomethacin in the stomach and intestine in rats[2-7]. Generally, indomethacin is given orally or subcutaneously. One of the studies involving subcutaneous indomethacin loading demonstrated that the sites of gastrointestinal lesions were different according to the fed state of the animals at the time of administration[4]. In fast-

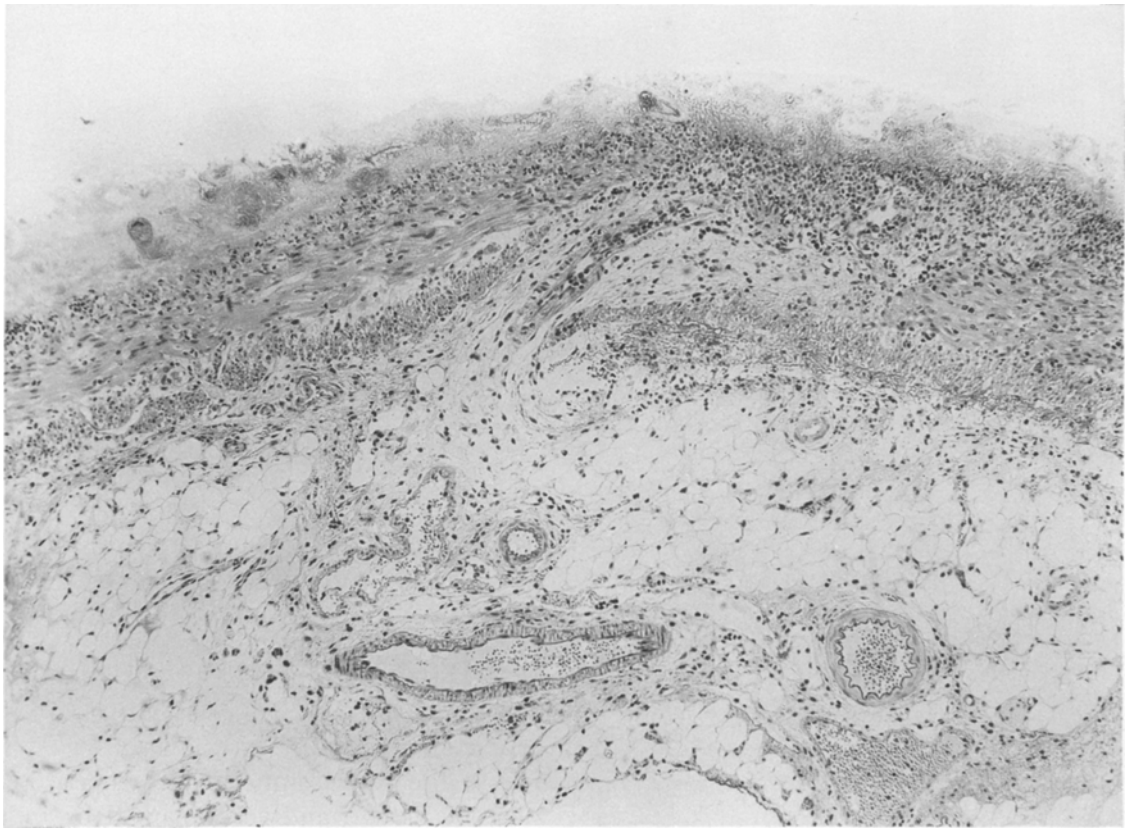

Figure 8. Histological appearance of the longitudinal ulcer of the small intestine. The penetrated muscularis propria and inflammatory infiltrate which is mainly composed of neutrophils, covered by necrotic slough, can be seen.

ing rats, ulcers developed predominantly in the gastric corpus, but in refed rats they occurred in the gastric antrum and in the small intestine. Conventionally fed rats had ulcers in the gastric corpus and the small intestine.

Intracolonic administration of indomethacin, which was applied in this study, was presumed to cause colonic damage, in addition to possible gastric and small intestinal ulcerations. On the contracy, however, the colon was macroscopically intact in all rats studied, while they had characteristic lesions, namely longitudinal ulcers in the small intestine.

The incidence and the length of small intestinal ulcers and cecal ulcers as well increased dose-dependently by indomethacin up to 24 mg/kg, but beyond this dose indomethacin caused less severe ulcers than 24 mg/kg. This can be explained by the level of serum concentration of indomethacin among the groups, which was rather lower in the 32 mg/kg-treated group than that in the 24 mg/kg-treated one. The total volume of indomethacin administered was higher in the former group, but the drug could be excreted due to an irritation of the colon before inducing ulcerogenecity.

There have been reports demonstrating oral or subcutaneous indomethacin to develop ulcers of the colon in animals[2,8]. However, neither direct contact of indomethacin with the colonic mucosa, nor the trasportation of indomethacin to the colon through the vessels, seem to give rise to ulcerogenecity in the colon of rats in this study. On the contrary, the prevelence of cecal ulcers in this study was higher than that in other studies[2,3,8]. This difference suggests that the direct contact of indomethacin with cecal mucosa may have affected the cecum adversely.

The stomach of heavy rats tended to be more

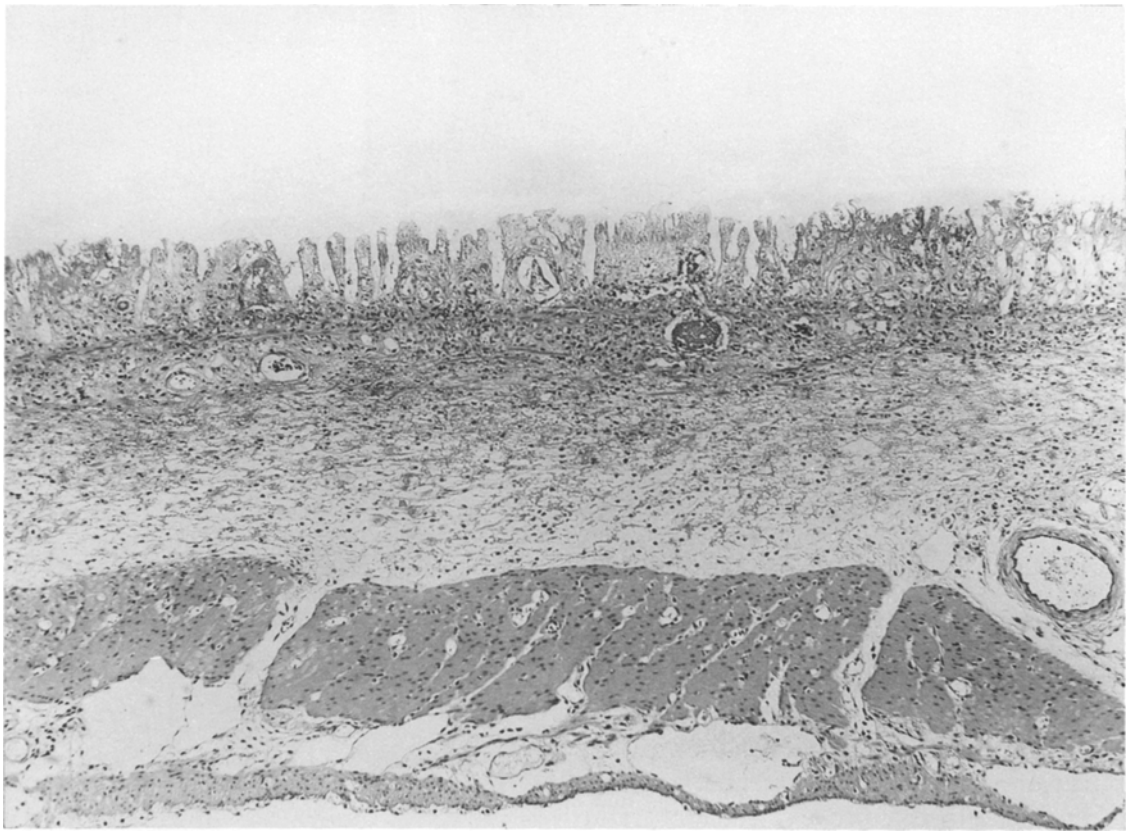

Figure 9. Microscopic view of the cecal ulcer. The ulcer is shallow, and restricted to the mucosa or submucosa. Marked submucosal edema and dilated capilaries, accompanied by mild inflammatory infiltrate, can be seen. Thrombus-formation can also be seen in the submucosal layer. These features resemble ischemic changes.

severely damaged than that of light rats. Ligumsky et al[9] recently reported the effects of various NSAIDs, including indomethacin, on the rat stomach, and found that there was a dose dependency of indomethacin involved in the induction of gastric damage. Our findings suggest that the severity of NSAID gastropathy in experimental animals may depend on the maturity of the animals. At present, there is no understandable mechanism which explains this phenomenon, but differences in gastric mucosal proliferative activity according to the age of the rat[10] may be involved.

Intracolonic indomethacin constantly produced longitudinal ulcers, which were located on the mesenteric side of the small intestine, dose-dependently, regardless of the body weight of rats. However, the number of small ulcers did not vary among the groups receiving different doses. In previous studies, a single oral or subcutaneous administration of indomethacin, ranging from 10 to 40 mg/kg, caused intestinal perforation which was accompanied by adhesion of intestinal loops[2,6] or multiple small ulcers on the mesenteric side of the small intestine[2,5]. In addition, Kent et al[2] reported that in some rats the ulcers were longitudinally oriented and were measuring from a few millimeters to many centimeters. Because the intestinal ulcers are reported to develop within 6 hours after administration of indomethacin[6], the high incidence and enlarged length of longitudinal ulcers in this study can be explained by double administration 24 hours apart from each other.

Recently, increased intestinal permeability, which is the word used for the transfer across the mucosa[11], in NSAID-induced enteropathy in both human[12] and experimental animals[12], and Crohn's

disease[13] has been described. Based upon these findings and their own study[14], in which intestinal permeability detected by ^{51}chrominium-labelled ethylenediamine tetra-acetate and intestinal myeloperoxidase activity were measured in indomethacin-treated rats, Banarjee and Peters[15] speculated that NSAID enteropathy and Crohn's disease may share some common pathogenic pathway.

Our findings confirmed that intracolonic indomethacin administration causes longitudinal ulcers, accompanied by small scattered ulcers, predominantly in the small intestine and in the cecum, and less frequently in the stomach of rats. Longitudinal ulcers are the typical feature in Crohn's disease[16], and in addition, small ulcers have often been recognized as early or accompanying lesions in this disease[16,17]. The similarities of the site of involvement and the characteristics of the ulcers between intracolonic indomethacin-treated rats and Crohn's disease patients suggest that this animal model may be valuable for investigating the pathophysiology of inflammatory bowel disease. Further efforts to prove epithelioid granuloma histologically using this model may provide some clues concerning the pathogenesis of Crohn's disease.

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