New Ulcerative Colitis Model Induced by Sulfhydryl Blockers in Rats and the Effects of Antiinflammatory Drugs on the Colitis

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ABSTRACT—We tried to produce a new ulcerative colitis model in rats by topical administration of sulfhydryl blockers. After male SD rats were fasted for 24 hr, 100 µl of 3% N-ethylmaleimide (NEM) or iodoacetamide (IA) was introduced into the colon via a Nelaton’s catheter. Both NEM and IA caused severe diarrhea with rectal bleeding and decreased body weight for about 7 days. At autopsy, adhesions and dilatation of the colon and severe mucosal lesions were observed. Both the weight and myeloperoxidase activity of the colon increased markedly. Maximum changes were observed within 1–3 days followed by gradual recovery, but even on day 21, some abnormalities were still observed. The ulceration and inflammation of the colon were confirmed by histological studies. Antiinflammatory drugs such as indomethacin inhibited the inflammation of the colon by NEM, but aggravated the ulceration. These results revealed that sulfhydryl blockers instilled into the colon caused ulcerative colitis in the rat. This model may be useful in studies on the pathogenesis of ulcerative colitis and the evaluation of drugs for therapy. Furthermore, it was suggested that antiinflammatory drugs may delay the healing of colonic ulcers.

Keywords: Antiinflammatory drug, Iodoacetamide, N-Ethylmaleimide, Sulfhydryl blocker, Ulcerative colitis model

Many animal models have been developed and used to study the etiology of inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease (1). Gastrointestinal mucosal damage is thought to be caused by an imbalance of aggressive factors such as intestinal flora, digestive juice and excessive motility of the intestine and defensive factors such as mucosal blood flow and mucus secretion. In inflammatory bowel diseases, it is suggested that immunological responses of the mucosa to the intestinal microflora, food residue and so forth are involved (2). We found that endogenous sulfhydryl (SH) compounds such as glutathione play an important role in protection of the gastric mucosa (3, 4). This implies that SH blockers may cause injury of the mucosa by decreasing the amount of defensive SH compounds. According to this hypothesis, we studied the effect of topical administration of SH blockers, N-ethylmaleimide (NEM) and iodoacetamide (IA), on the colonic mucosa and found that instillation of the blockers into the colon caused ulcerative colitis similar to that in humans, causing symptoms such as mucosal damage with inflammation, diarrhea with bleeding, and a decrease in body weight. We tried to elucidate the properties of this new ulcerative colitis model and the role of prostaglandins and leukotrienes in the pathogenesis.

MATERIALS AND METHODS

Induction of experimental ulcerative colitis

Male Sprague-Dawley rats weighing 200–250 g were used after a 24-hr fast. Various amounts (25–400 µl) and various concentrations (0.3–10%) of NEM and IA dissolved in 1% methylcellulose were introduced into the colon 6 cm from the anus via a Nelaton’s catheter. The animals were sacrificed 7 days after the administration of SH blockers by CO₂ asphyxiation, and macroscopic changes of the colon were observed. Administration of the blockers often caused diarrhea, dilatation and adhesion of the colon. These changes were graded according to the following criteria:

Diarrhea: 0, normal; 1, slight (wet around the anus); 2, moderate; 3, marked (lower part of the abdomen is soiled with soft feces or rectal bleeding)
Dilatation: 0, normal; 1, slight (less than 1.5 times the normal size); 2, moderate; 3, severe (more than 3 times the normal size)

Adhesion: 0, normal; 1, slight (torn off with slight pulling); 2, moderate; 3, marked (tightly bound to surrounding tissues)

Then the descending colon 7 cm from the anus was removed with the rectum and opened longitudinally. The contents were removed by washing the tissue with saline. The area (mm²) and severity (grade: 0, normal; 1, mucosal erosion; 2, moderate lesion; 3, deep lesion) of the mucosal damage were measured under a dissecting binocular microscope with a grid eyepiece (×10).

Time course study of NEM-induced colitis

In the study to determine the time-dependent changes of experimental colitis, 100 µl of 3% NEM was introduced into the colon, and from 1 hr to 21 days later, the colon was removed. In addition to macroscopic observation, changes in histology, weight of the colon and myeloperoxidase (MPO) activity were measured.

Histological observation: The colon was cut longitudinally, and the contents were removed by washing with saline. The specimen was spread on hard paper, fixed in 10% formalin solution and then embedded in paraffin. Thin sections were made and stained with hematoxylin and eosin or with Azan.

MPO activity: The colon was opened longitudinally, washed with saline and spread on paper. After the extra water was absorbed with paper, a 3-cm length of the colon containing the lesion was removed and weighed. As a normal control, the colon from a rat that did not receive NEM was used.

According to the methods described by Krawisz et al. (5), MPO activity in the tissue was measured. The tissue was minced in a beaker containing 1 ml of 0.5% hexadecyltrimethylammonium bromide (HTAB) dissolved in 50 mM phosphate buffer. The tissue was then homogenated with a Polytron® (Kinematica, Lucerne, Switzerland) 3 times for 30 sec each on ice. After homogenization, the homogenizer was rinsed twice with 1 ml of HTAB. The pooled homogenate and washings were sonicated for 10 sec and then freeze-thawed three times. The supernatant was frozen and stored at −20°C until it was assayed.

Measurement of MPO activity: Samples were dissolved and centrifuged for 20 min at 3,000 rpm, and then 0.1 ml of supernatant was combined with 2.9 ml of 50 mM phosphate buffer containing 0.167 mg/ml O-dianisidine hydrochloride and 24 µl of 0.062% H₂O₂. The change in absorbance at 460 nm was measured with a spectrophotometer (Hitachi V-3200; Hitachi, Tokyo) every 6 sec for 2 min. One unit of MPO activity was defined as that degrading 1 µmol of peroxide per min at 25°C.

Effects of drugs on NEM-induced colitis

Rats were fasted for 24 hr, and experimental colitis was caused by introduction of 50 µl of 3% NEM into the colon 6 cm from the anus. One or 3 days later, the rats were autopsied; and diarrhea, distension and adhesion of the colon, mucosal lesions and weight of the colon (3 cm) were measured. In addition, MPO activity of the colon was also measured to see the effect on infiltration of neutrophils into the ulcerated mucosa. Drugs were suspended in 5% gum arabic or 10% ethanol (in case of prostaglandin E₂, PGE₂) and given orally 1 hr before and 24 and 48 hr after the administration of NEM in a volume of 0.2
ml/100 g body weight. In the control animals, the same volume of vehicle was given orally. In another study, PGE\textsubscript{2} dissolved in 10% ethanol was introduced into the colon 7 cm from the anus 30 min before NEM, and 24 hr later, the animals were autopsied. In the control group, the same volume of vehicle was given topically.

**Drugs and statistics**

The following drugs were used: N-ethylmaleimide, iodoacetamide, methylcellulose and indomethacin (Sigma, St. Louis, MO, USA); prostaglandin E\textsubscript{2} (Fuji Pharmaceutica, Toyama); dexamethasone and the 5-lipoxygenase inhibitor AA-861 (6) (Takeda, Osaka). AA-861 was synthesized at our division. Data are expressed as the mean±S.E. The statistical significance of the differences among groups was determined by Dunnett’s test (non-parametric for graded values) or by Student’s t-test for unpaired values. In all statistical analyses, an associated probability (P value) of <0.05 was considered significant.

**Approval by animal welfare committee**

This study was approved by the animal welfare committee of Takeda Chemical Ind., Ltd.

**RESULTS**

**NEM-induced ulcerative colitis model**

**Effect of NEM concentration:** One hundred microliters of 1% methylcellulose solution containing 0.3%, 1%, 2%, 3%, 5% or 10% NEM was introduced into the colon; and the effect of each concentration on the symptoms such as diarrhea and mucosal lesion formation was examined. Seven days later the rats were autopsied. Vehicle (1% methylcellulose) alone or 0.3% NEM did not cause any changes, but NEM at concentrations of 1% or higher caused diarrhea beginning the next day. The grade of diarrhea increased concentration-dependently and reached a maximum at 5% (Fig. 1a). Rectal bleeding was often seen in the groups given NEM at concentrations higher than 3%. In the group given 10% NEM, 2 out of the 6 rats died within 5 days after administration. Body weight gain was significantly inhibited in the groups given NEM at concentrations higher than 1%, and the rats given 5% and 10% NEM lost weight (Fig. 1a). At autopsy, the part of the descending colon exposed to NEM was distended and adhering to surrounding tissues, mainly the seminal vesicles. These changes were observed in the rats given NEM at concentrations of 1% or more, increased in severity with the concentration of NEM, and reached a maximum at 5% or 10% (Fig. 1b). In the rats given NEM at concentrations higher than 3%, the colon was often distended severely, probably due to obstruction of the passage of the contents. The area and severity of the mucosal lesions in the colon were measured. Vehicle alone or 0.3% NEM did not cause any visible changes in the mucosa, but 1% and higher concentrations of NEM caused mucosal lesions (Fig. 2). The area and severity of the lesions increased depending on the concentration of NEM and reached a maximum at the concentration of 5% (Fig. 1c). Usually one large lesion was observed, but at

![Figure 2](attachment:figure2.png)

**Fig. 2.** Time-dependent changes in the colonic lesions induced by NEM in the rat (macroscopic observation). One hundred microliters of 1% methylcellulose solution containing 3% NEM was introduced into the colon (i.c.) 6 cm from the anus, and the animals were autopsied 1, 3, 7, 14 and 21 days later.
times, two or three lesions were observed, probably due to the feces remaining at the time of introduction of NEM. The lesions were often covered with grey or white-yellow, mud-like substances.

Effect of the volume of NEM: Various volumes (25, 50, 100, 200 and 400 µl) of 1% methylcellulose solution containing 3% NEM were introduced into the colon, and the effect of the volume on the symptoms such as diarrhea and mucosal lesion formation was examined. The rats were autopsied 7 days after administration. Under these conditions, administration of 25 µl of NEM solution caused diarrhea, and the severity of the diarrhea increased volume-dependently, reaching a maximum at 200 µl (Fig. 3a). In the group given 200 and 400 µl of NEM, 2 and 1, respectively, out of the 6 rats died within 7 days. Body weight gain was significantly inhibited in the groups given more than 25 µl of NEM, and the rats given 200 and 400 µl of NEM lost weight (Fig. 3a). Adhesion and dilatation of the colon were observed at a volume of 25 µl, and the severity increased with the volume of NEM solution, reaching a maximum at 200 µl (Fig. 3b). Mucosal lesions were also recognized at a volume of 25 µl. The severity of these increased with the volume of NEM solution, reaching a maximum at a volume of 400 µl (Fig. 3c).

Time-dependent changes in NEM-induced colitis

One hundred microliters of 3% NEM was introduced into the colon, and the time-dependent changes in experimental colitis were determined.

Diarrhea and macroscopic changes in the colon: The administration of NEM caused severe diarrhea, adhesion and dilatation in all 12 rats the next day. Diarrhea continued for 7 days followed by gradual recovery (Fig. 4a).
Seven out of the 12 rats showed moderate diarrhea on day 14, and one of the 12 rats still had slight diarrhea on day 21. The grades of both adhesion and dilatation increased with time, reaching a maximum on day 3, and the high grades continued for 2 weeks and then gradually decreased (Fig. 4b).

Macroscopic changes in the colonic mucosa: One hour after the administration of NEM, the colonic mucosa exposed to NEM was reddish, and hyperemia and edema were observed. The surface of the mucosa was covered with whitish materials like mucus. At 3 hr, the color of the mucosa was dark red probably due to hemostasis. The mucus-like substance on the mucosa disappeared at 6–12 hr, and the surface of the mucosa lost its smoothness. At 24 hr, necrotic mucosa was detached, and there was an obviously distinguishable border line between normal and damaged mucosa. The colon was dilated around the lesion site. These changes were more marked at 48 hr. At 3 days, the mucosa surrounding the lesion was elevated, and the colon seemed to be shortened longitudinally, distended transversely and looked like a barrel (Fig. 2). The ulcerated mucosa was covered with a grey or white-yellow, mud-like substance, probably the products of inflammation. The substance began to detach at 7 days and had almost disappeared at 2 weeks. The ulcerated area decreased with time, but was still observable at 3 weeks (Fig. 2). The changes in the area and severity of the mucosal lesions are shown in Fig. 4c. The area was almost the same until 3 days after administration. It then gradually decreased, but obvious lesions were observed even at 3 weeks. The severity was almost the same until 7 days after administration when it had started to decrease gradually.

Histological changes in the colon: Histological findings are summarized in Table 1. The administration of NEM induced hyperemia, hemorrhage, edema and necrosis in the mucosa within several hours (Fig. 5b). The mucosal lesions extended into the submucosa and muscle layers within 24 hr, and almost complete necrosis was recognized in all layers at 24 hr (Fig. 5c). Obvious infiltration of neutrophils was observed in the submucosa and muscle layers at 6–9 hr, and neutrophilic debris was increased in the same layers at 12 hr to 3 days. The surface of the ulcer crater was covered with a large cellular debris layer (necrotized tissue and products of inflammation) until day 7. Granulation tissue was observed in the ulcer bed at 3 days, was markedly increased on day 7 (Fig. 5d), and then gradually decreased. At 14 days, the damaged colonic tissue was replaced with granulation tissue (Fig. 5e). On the surface of the ulcer crater, there was a thin layer of necrotic tissue. Regeneration of the mucosa was seen in the surrounding tissue on day 14. This progressed with

### Table 1. Summary of histological findings in N-ethylmaleimide (NEM)-induced colonic lesions in rats

|                          | Control | Time after administration of NEM |
|--------------------------|---------|----------------------------------|
|                          |         | 1  | 3  | 6  | 9  | 12 | 24 hr | 3  | 7  | 14  | 21 days |
| **Mucosa**               |         |    |    |    |    |    |       |    |    |     |         |
| Hyperemia                | -       | ±  | +  | +  | +  | -  | -     | -  | -  | -    | -       |
| Hemorrhage               | -       | ±  | +  | +++| ++ | -  | -     | -  | -  | -    | -       |
| Necrosis                 | -       | +  | ++ | +++| +++| +++| +++   | +++| +++| +    | +       |
| **Muscularis mucosa**    |         |    |    |    |    |    |       |    |    |     |         |
| Necrosis                 | -       | +  | ++ | +++| +++| +++| +++   | +++| +++| +    | +       |
| **Submucosal layer**     |         |    |    |    |    |    |       |    |    |     |         |
| Edema                    | -       |    |    | ++ | ++ | +  | -     | -  | -  | -    | -       |
| Hemorrhage               | -       | -  | ±  | +  | +  | -  | -     | -  | -  | -    | -       |
| Infiltration of neutrophils | -    | -  | +  | +  | +  | +  | +++   | +++| +++| +    | +       |
| **Muscularis propria**   |         |    |    |    |    |    |       |    |    |     |         |
| Necrosis                 | -       | +  | ++ | +++| +++| +++| +++   | +++| +++| +    | +       |
| Infiltration of neutrophils | -    | -  | -  | -  | +  | +  | ++    | ++ | +  | -    | -       |
| **Proliferation of granulation tissue** | - | - | - | - | - | - | - | ± | +++ | +++ | + |
| Regeneration of mucosa   | -       | -  | -  | -  | -  | -  | -     | -  | ±  | +    | +       |

One hundred microliters of 1% methylcellulose solution containing 3% NEM was introduced into the colon 6 cm from the anus, and the animals were autopsied at the times indicated. Six rats were used in each group. The scores indicate - : negative, ± : slight, + : mild, ++ : moderate, +++ : severe. E*: Exfoliation of necrotic tissue.
time, but the ulcer crater was not completely covered with regenerated mucosa even on day 21 (Fig. 5f).

Changes in wet weight and MPO activity: Wet weight of the colon in untreated animals was 0.36 ± 0.02 g/3 cm (n = 6). The administration of NEM into the colon caused a marked increase in the weight, i.e., about 4 times (1.44 ± 0.14 g/3 cm) at 7 days (Fig. 6a). The weight of the colon gradually decreased, but it was still higher than the pretreatment value even at 3 weeks (Fig. 6a). Time-dependent changes in the MPO activity of the colon after the administration of NEM are shown in Fig. 6b. MPO activity of the colon in untreated animals was 0.15 ± 0.06 U/g w.w. MPO activity had increased to 32.0 ± 2.0 U/g w.w. on the day after NEM administration. Thereafter, almost the same activity was observed for 7 days. The activity then gradually decreased and was 6.4 ± 2.0 and 0.7 ± 0.2 U/g w.w. at 2 and 3 weeks, respectively.

IA-induced ulcerative colitis model

One hundred microliters of 1% methylcellulose solution containing various concentrations (0.3–10%) of IA were introduced into the colon, and 7 days later, the rats were autopsied. The administration of IA caused severe diarrhea, inhibition of body weight gain, dilatation and adhesion of the colon and mucosal lesions. These changes were observed when IA was instilled into the colon at concentrations of 1% or more and increased concentration-dependently (Fig. 7). Three out of the 7 rats given 10% IA died within 7 days after administration.

Effects of drugs on NEM-induced ulcerative colitis

In this study, 50 μl of 3% NEM was used to induce ulcerative colitis. The administration of NEM caused diarrhea, dilatation and adhesion of the colon, an increase in MPO activity and obvious mucosal lesions in the colon.
Effect of indomethacin: Indomethacin (10 mg/kg, p.o.) significantly inhibited the increase in colon weight, mildly prevented diarrhea but did not affect the dilatation and adhesion of the colon observed 24 hr after NEM administration (Table 2). MPO activity per unit weight was significantly increased by pretreatment with indomethacin, but the increase in total activity in a 3-cm length of the colon was not significant. Indomethacin did not inhibit the lesion formation and rather aggravated it. Repeated administration of indomethacin (3 mg/kg, p.o.) for 3 days prevented the increase in colonic weight and aggravated the lesions (Table 3). These effects were significant.

Effect of dexamethasone: Dexamethasone (0.1 mg/kg, p.o.) inhibited the adhesion and increase in colon weight but did not inhibit the diarrhea, dilatation of the colon or lesion formation observed 24 hr after NEM administration (Table 2). MPO activity was not increased following the administration of dexamethasone but rather slightly decreased. The administration of dexamethasone (0.03 mg/kg, p.o.) for 3 days mildly inhibited the dilatation of the colon and the healing of mucosal lesions (Table 3).

Effect of AA-861: AA-861 (100 mg/kg, p.o.) did not affect the diarrhea, dilatation of the colon or mucosal lesions, but inhibited the adhesion and increase in colon weight observed 24 hr after the administration of NEM (Table 2). AA-861 significantly increased the MPO activity per unit wet weight of the colon. Repeated administration of AA-861 for 3 days slightly inhibited the dilatation of the colon (Table 3).

Effect of PGE₂: Oral administration of PGE₂ did not affect the changes observed in the colon 24 hr after the administration of NEM, except for a mild inhibition of...
adhesion at the dose of 1 mg/kg (Table 4). On the other hand, topical administration of PGE2 into the colon inhibited the mucosal lesion formation, diarrhea and adhesion of the colon and increased the weight of the colon (Table 4).

### DISCUSSION

Ulcerative colitis is still a poorly understood malignant disease in spite of the numerous clinical and basic studies. To develop a good experimental model is important not only for elucidation of the pathogenesis but also for

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**Table 2.** Effects of drugs on NEM-induced inflammation and ulceration of the colon in the rat (single administration)

| Treatment      | Dose (mg/kg, p.o.) | Diarrhea (0–3) | Adhesion (0–3) | Dilatation (0–3) | Weight of colon (g/3 cm) | MPO activity (U/g w.w.) | Mucosal lesion (Area (mm²), Severity (0–3)) |
|----------------|--------------------|----------------|----------------|------------------|--------------------------|--------------------------|------------------------------------------|
| Vehicle        | 2.1±0.1            | 0.9±0.2        | 0.9±0.1        | 0.74±0.04        | 19.95±2.90               | 14.82±2.25               | 382.1±29.6, 1.7±0.1                   |
| Indomethacin   | 10                 | 1.1±0.2        | 0.9±0.2        | 1.0±0.1          | 0.48±0.04*               | 35.22±5.21               | 15.90±1.51, 1.7±0.1                   |
| Dexamethasone  | 0.1                | 1.9±0.1        | 0.2±0.1        | 0.9±0.1          | 0.64±0.03*               | 19.81±2.08               | 12.34±1.02, 1.7±0.1                   |
| AA-861         | 100                | 2.1±0.3        | 0.4±0.1        | 0.7±0.1          | 0.59±0.02*               | 27.37±1.78               | 16.23±1.30, 1.8±0.1                   |
| Normal control | 0±0.0              | 0±0.0          | 0±0.0          | 0.19±0.01*        | 0.13±0.00                | 0.02±0.00                | 0±0.0                                   |

A drug or vehicle was given orally 1 hr before the administration of 3% NEM (50 µl/rat, i.c.), and the animals were autopsied 24 hr after NEM administration. Data show the mean values and the S.E.s of the 7 rats in each group. *P<0.05 vs Vehicle.

**Table 3.** Effects of drugs on NEM-induced inflammation and ulceration of the colon in the rat (repeated administration for 3 days)

| Treatment      | Dose (mg/kg, p.o.) | Diarrhea (0–3) | Adhesion (0–3) | Dilatation (0–3) | Weight of colon (g/3 cm) | Mucosal lesion (Area (mm²), Severity (0–3)) |
|----------------|--------------------|----------------|----------------|------------------|--------------------------|------------------------------------------|
| Vehicle        | 1.0±0.4            | 1.6±0.2        | 1.6±0.1        | 1.02±0.06        | 265.8±41.5               | 1.9±0.2                                  |
| Indomethacin   | 1.2±0.3            | 1.8±0.3        | 1.8±0.3        | 0.73±0.06*       | 440.8±66.3               | 2.3±0.1                                  |
| Dexamethasone  | 0.03               | 0.8±0.3        | 0.8±0.1        | 0.94±0.03        | 308.3±13.5               | 2.1±0.1                                  |
| AA-861         | 1.6±0.3            | 1.6±0.2        | 1.2±0.1        | 0.91±0.04        | 284.2±13.2               | 2.3±0.1                                  |
| Normal control | 0±0.0              | 0±0.0          | 0±0.0          | 0.22±0.01*       | 0±0.0                    | 0±0.0                                    |

A drug or vehicle was given orally once a day for 3 days; i.e., 1 hr before and 24 and 48 hr after the administration of 3% NEM (50 µl/rat, i.c.), and the animals were autopsied 3 days after NEM administration. Data show the mean values and the S.E.s of the 6 rats in each group. *P<0.05 vs Vehicle.

**Table 4.** Effect of prostaglandin E2 (PGE2) on NEM-induced inflammation and ulceration of the colon in the rat

| Treatment      | Dose (mg/kg, p.o.) | No. of rats | Diarrhea (0–3) | Adhesion (0–3) | Dilatation (0–3) | Weight of colon (g/3 cm) | Mucosal lesion (Area (mm²), Severity (0–3)) |
|----------------|--------------------|-------------|----------------|----------------|------------------|--------------------------|------------------------------------------|
| Experiment I   |                    |             |                |                |                  |                          |                                         |
| Vehicle        | 9                  | 2.2±0.2     | 0.9±0.1        | 0.6±0.1        | 0.75±0.04        | 311.1±44.6               | 1.9±0.1                                  |
| PGE2           | 1                  | 2.4±0.2     | 0.4±0.2        | 0.4±0.1        | 0.83±0.05        | 298.6±31.6               | 1.9±0.1                                  |
| Experiment II  |                    |             |                |                |                  |                          |                                         |
| Vehicle        | 9                  | 1.9±0.2     | 0.8±0.2        | 0.5±0.2        | 0.80±0.08        | 294.3±55.2               | 1.7±0.1                                  |
| PGE2           | 0.1                | 1.9±0.2     | 0.3±0.1        | 0.4±0.1        | 0.81±0.05*       | 220.0±26.3               | 1.6±0.1                                  |
|                | 0.3                | 1.2±0.1**   | 0.1±0.1        | 0.3±0.1        | 0.80±0.07        | 162.1±26.6               | 1.5±0.2                                  |
|                | 1.0                | 1.4±0.2     | 0±0.0          | 0.4±0.1        | 0.78±0.04*       | 177.1±24.4               | 1.4±0.2                                  |

PGE2 or vehicle was given orally (Exp. I) 1 hr before or introduced into the colon (i.e., Exp. II) 30 min before the administration of 3% NEM (50 µl/rat, i.c.), and the animals were autopsied 24 hr after NEM administration. Data show the mean values and the S.E.s of the 7 or 9 rats in each group. *P<0.05, **P<0.01 vs Vehicle.
development of new drugs for this disease. Many experimental animal models have been developed (7–20) and have contributed to the progress in the understanding of this disease. In the present study, we developed a new model for this disease using SH blockers. In previous studies, we found that endogenous SH compounds play an important role in protection of the gastric mucosa against ulcerative stimuli (3, 4). This suggested that SH blockers may cause ulceration by decreasing the defensive potency of the mucosa. We tested this possibility in the rat colon and found that instillation of SH blockers into the colon induced both ulceration (mucosal lesions) and inflammation (increases in the weight and MPO activity of the colon and adhesion and dilatation of the colon). These results were also confirmed by histological observation. As symptoms, it caused severe diarrhea with rectal bleeding and a decrease in body weight. The changes in the colon and the symptoms are similar to those observed in human ulcerative colitis.

Among the many animal models, the properties of the present model using SH blockers are similar to those produced by instillation of necrotizing agents such as acetic acid (17, 18), formalin (19) and ethanol (20) into the colon, as the mucosal lesions appeared within several hr. However, the present model is further characterized by the following properties: 1) the mucosal lesions are produced easily without any operation and constantly in all animals; 2) relatively constant lesions of a suitable size can be produced as SH blockers are dissolved in viscous solutions (1% methylcellulose); 3) both the size and the severity of lesion can be regulated by changing the volume of the solution and concentration of SH blockers, i.e., it is possible to produce chronic ulcers; 4) inflammatory responses are also measured by means of the weight of the colon, MPO activity and adhesion and so on. This model having these properties will enable us to make a quantitative evaluation of the effects of drugs on the ulceration and inflammation.

Inflammation is a very important and characteristic feature of ulcerative colitis; therefore, most emphasis has been put on ways to inhibit the inflammation. This approach certainly caused the progression of therapeutics, and antiinflammatory drugs such as sulfasalazine, 5-aminosalicylic acid and steroids are often used in the treatment of ulcerative colitis. However, these drugs are not always satisfactory in the therapy of this disease, even though they inhibit the inflammation during the treatment period. Recently it has been suggested that products of the arachidonic acid cascade such as prostaglandins, leukotrienes, thromboxanes and platelet activating factor play some role in the pathogenesis of inflammatory bowel diseases not only in experimental animals (21–26), but also in patients (27–33). In the present study, we examined the role of prostaglandins and leukotrienes in the ulcerative colitis induced by NEM. Indomethacin, dexamethasone and AA-861, a 5-lipoxygenase inhibitor, all significantly inhibited the increase in the weight of the colon (one of the indicators of inflammation) induced by NEM, and both dexamethasone and AA-861 prevented the adhesion of the colon. These results suggest that both prostaglandins and leukotrienes are involved in NEM-induced inflammatory responses (edema and so forth) of the colon and support the idea of using antiinflammatory drugs for the treatment of ulcerative colitis from the viewpoint of inflammation. These drugs, however, did not prevent the increase in MPO activity in the colon caused by NEM, indicating a minor role of prostaglandins and leukotrienes in infiltration of neutrophils into the damaged tissue.

Another important approach for the treatment of ulcerative colitis is to actively heal the ulcer. However, the antiinflammatory drugs did not accelerate the healing of mucosal lesions but rather aggravated them upon repeated administration for 3 days. These results suggest that antiinflammatory drugs may not be good in the healing of ulcerated mucosa, even though they are effective in inhibiting the inflammatory responses caused by NEM. In the other experiment using the same ulcerative colitis model, we found that healing of colonic lesions was obviously delayed by treatment with dexamethasone for 10 days (34). Also, it has been reported that both steroids and non-steroidal antiinflammatory drugs delay the healing of gastric ulcers induced by acetic acid in the rat (35–37). From all of these findings it may be concluded that drugs that inhibit inflammation delay the healing of ulcerated mucosa in the gastrointestinal tract. In other words, this implies paradoxically that inflammation might be one of the important factors in the healing of ulcer. This idea raises questions about the exclusive use of antiinflammatory drugs for the treatment of ulcerative colitis. However, there is not any drug available now for the therapy of ulcerative colitis that actively accelerates the healing of colonic ulcers. Wallace et al. (38, 39) have reported that leukotriene synthesis inhibitors accelerated the healing of colonic ulcers induced by trinitrobenzene sulfonic acid in the rat. In the present study, however, a leukotriene synthesis inhibitor (AA-861) did not affect the healing of ulcer upon 3-day treatment, although the compound showed an antiinflammatory effect. In the other study, we found that the healing of colonic ulcers induced by NEM was not affected by treatment with AA-861 for 10 days (unpublished observation). A clear conclusion about the role of leukotrienes in the healing of colonic ulcers would require more detailed studies. We have reported that recombinant human basic fibroblast growth factor (rhbFGF) and its mutein CS23 (TGP-580) acceler-
ated the healing of colonic ulcers induced by NEM in the rat (34). One of the big problems in the treatment of ulcerative colitis is recurrence of the disease as is seen in other ulcer diseases. It has also been reported that anti-inflammatory drugs activate quiescent inflammatory bowel disease (40). If the ulcerated mucosa is completely cured by drugs effective for ulcer healing, recurrence of the ulcer may be prevented. Progress in these studies may provide a new therapeutic approach to this disease.

We have reported that endogenous SH compounds play an important role in protection of gastric mucosa via prostaglandins (3, 4). In the present study, we found that instillation of SH blockers (NEM and IA) into the colon caused severe mucosal lesions, and PGE₂ given topically into the colon dose-dependently protected the mucosa against the effect of NEM. There is a possibility that PGE₂ decreased the formation of lesions by removing the NEM from the colon via the stimulation of colonic motility or causing diarrhea. However, PGE₂ did not inhibit the increase in colon weight caused by NEM. This indicates that the mucosa was sufficiently stimulated even in the presence of PGE₂. In addition, prostaglandins have been reported to protect the colonic mucosa in ulcer models utilizing ethanol (20), trinitrobenzene sulfonic acid (41) and acetic acid (42). These results suggest that prostaglandins as well as endogenous SH compounds play an important role in the protection of colonic mucosa as well as gastric mucosa and that a decrease in these substances may cause ulceration of the mucosa.

The results of the present study revealed that SH blockers instilled into the colon caused ulcerative colitis in the rat. This model may be useful in studies on the pathogenesis of ulcerative colitis and the evaluation of drugs for therapy. Furthermore, it was pointed out that ulceration and inflammation should be distinguished in the treatment of ulcerative colitis as anti-inflammatory drugs may inhibit the healing of the ulcer.

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