J Pharmacol Sci **96**, 307 – 313 (2004)

Journal of Pharmacological Sciences

Full Paper

Oxazolone-Induced Colitis in BALB/C Mice: a New Method to Evaluate the Efficacy of Therapeutic Agents for Ulcerative Colitis

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Received March 30, 2004; Accepted September 27, 2004

Abstract. A number of experimental models of colitis have been proposed. However, few studies have presented T helper-2 (Th-2) type colitis models that substitute for human ulcerative colitis (UC). In recent years, the murine oxazolone (OXA)-induced colitis model came to be accepted as a Th-2 type model, but it has yet to be used in any pharmacological study. In the present study, we modified the OXA-induced colitis model in BALB/C mice to evaluate the efficacy of treatments for UC. Colitis was induced by intrarectal administration of OXA solution (7.5 mg/mL in 40% ethanol) in a BALB/C strain that is known to favor Th-2 immune responses. A lower mortality rate was obtained in the BALB/C strain than was found in the original method. Histological examination showed that there were morphological similarities to human UC. Increased mRNA expression of interleukin-13, a Th-2 cytokine, was observed in mesenteric lymph nodes. Intrarectal administration of 5-aminosalicylic acid or sodium prednisolone phosphate resulted in a significant improvement in the colitis. These results suggest that the OXA-induced colitis model in the BALB/C strain provides a new way to evaluate the efficacy of therapeutic agents for UC.

Keywords: ulcerative colitis, experimental model, mouse, oxazolone, 5-aminosalicylic acid

Introduction

Inflammatory bowel diseases (IBDs), which include ulcerative colitis (UC) and Crohn's disease (CD), are chronic inflammatory disorders of the gastrointestinal tract, and their pathogeneses are not clear. Recent studies suggest that disruption of the intestinal mucosal immune system is involved in the pathogenesis of IBDs (1). It is well recognized that T helper-1 (Th-1) immune responses may play an important role in CD, whereas Th-2 responses may be involved in UC (1 – 5). Incidentally, to evaluate the therapeutic effects of new drugs, an adequate animal model is necessary. Several methods have been reported to produce experimental models of colitis (6 – 10). However, many of these showed Th-1 type immune responses (10). A recent report showed that the use of the haptenating agent oxazolone (OXA) to induce colitis in SJL/J mice promotes the production of Th-2 type cytokines, resulting in lesions characterized by leukocyte infiltration limited to the superficial layer of the mucosa (11). However, no pharmacological studies using this model have been reported. Therefore, the objective of the present study was to modify the method of OXA induction of colitis in SJL/J mice in order to evaluate the efficacy of treatments for UC.

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Materials and Methods

Animals

All experiments were performed in accordance with the ethics code for animal experimentation of Nisshin Kyorin Pharmaceutical. Specific-pathogen-free male BALB/C mice (Charles River Japan, Kanagawa) weighing 20 – 24 g were used for the OXA-induced colitis model. Mice were housed in polycarbonate cages and fed a standard chow (Oriental Yeast, Tokyo) and tap water ad libitum. In the animal holding room, the temperature was maintained at 22 ± 2°C and the humidity at 55 ± 10% with a 12-h light/dark cycle.

Agents

4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (oxazolone sensitizing agent, OXA) was purchased from Sigma Chemical (St. Louis, MO, USA). Ethanol was purchased from Wako Pure Chemical Industries (Osaka). Injection-grade water was purchased from Otsuka Pharmaceutical Factory (Tokushima). Sodium carboxymethylcellulose (CMC) was purchased from Dai-ichi Kogyo Seiyaku (Kyoto). 5-Aminosalicylic acid (5-ASA) and sodium prednisolone phosphate (PDL) were obtained from our firm (Nisshin Kyorin Pharmaceutical, Tokyo). OXA was dissolved (7.5 mg/mL) in 40% (v/v) aqueous ethanol. 5-ASA was suspended in 2% CMC. PDL was diluted with saline (0.9% NaCl).

Induction of colitis

For induction of colitis, we modified a previously described method (11). Each BALB/C mouse was slightly anesthetized with ether. A metal catheter was inserted 4 cm into the lumen of the colon via the anus. OXA solution (0.15 mL/mouse) was administered into the colon through the catheter. After injection of the OXA solution, the catheter was removed, and the mouse was held vertically for 30 seconds.

Histological analysis

Colonic tissues from mice on days 0 (before colitis induction), 1, 2, 4, and 7 were fixed in 20% natural buffered formalin solution, embedded in paraffin, cut into tissue sections, and stained with hematoxylin and eosin (H and E). The stained sections were examined for evidence of colitis using the following criteria: the presence of inflammatory cell infiltration, crypts depletion, crypt abscesses, reduction in the number of goblet cells, ulceration, and edema formation.

Measurement of myeloperoxidase activity

Myeloperoxidase (MPO) activity in the colonic segment was analyzed by using a modification of previously described methods (12, 13). Briefly, the animals were sacrificed by decapitation at the indicated time points. A 4-cm segment of inflamed colon was removed and opened by a longitudinal incision, gently rinsed with ice-cold saline, and blotted dry. The segment was then homogenized in ice-cold potassium phosphate buffer (20 mM, pH 7.4). The homogenate was centrifuged at $4000 \times g$ for 20 min at 4°C. The pellet was rehomogenized in assay buffer (50 mM sodium phosphate, pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide). The homogenate was then freeze-thawed three times and centrifuged at $12{,}000 \times g$ for 10 min at 4°C. The supernatant was used for determination of MPO activity. MPO activity was measured spectrophotometrically using a microassay plate. The supernatant was diluted with assay buffer, and an aliquot (50 μL) was mixed with 25 μL of *o*-dianisidine (0.167 mg/mL) and 25 μL of 0.0005% H_2O_2. The change in absorbance at 490 nm was measured with a microplate reader (V max; Molecular Devices, CA, USA). When carrying out this method, at our facilities, an average value of 20 U/g protein was obtained for normal mice colon.

Analyses of cytokine mRNA expression levels by RT-PCR

Total RNA was extracted from mesenteric lymph node (MLN) on days 0 (before colitis induction), 1, 2, 4, and 7 using the ISOGEN reagent (Nippon Gene, Tokyo). Reverse transcription was performed using the ReverTra Ace cDNA synthesis kit with random hexamers (Toyobo, Osaka). PCR amplification of the cDNAs was carried out with primer sets for mouse IL-4, INF-gamma, IL-13, and IL-18 genes (Maxim Biotech, South San Francisco, CA, USA) and for housekeeping G3PDH gene (Toyobo) using Taq DNA polymerase (Toyobo). Amplification reactions consisted of 1 min at 94°C, 45 s at 60°C, and 1 min at 72°C, for 35 cycles for cytokines or for 28 cycles for G3PDH. PCR products were resolved on a 1% agarose gel containing ethidium bromide and the band intensities were quantified using Gel-Pro Analyzer software (MediaCybernetics, Silver Spring, MD, USA).

Treatment of mice with 5-ASA or PDL

Drug treatments were started 1 day before the induction of colitis (day −1) until the day of sacrifice (day 3). The mouse was slightly anesthetized with ether, and a metal catheter was inserted 4 cm into the lumen of the colon via the anus. The drugs (5-ASA or PDL) were administered into the colon through the catheter. 5-ASA was administered once a day at doses of 6.25, 12.5, and 25.0 mg/kg for a total volume of 5 mL/kg (14). PDL was administered once a day at doses of 0.1, 0.3, and 1.0 mg/kg for a total volume of 5 mL/kg (14). On day 3, the animals were sacrificed by decapitation. A 4-cm segment of distal colon (descending and rectum colon) was removed, and the MPO activity was measured as described above.

Statistical analyses

All results are expressed as the mean ± S.E.M. Statistical analyses were performed using Student's *t*-test and Dunnett's multiple comparison test. A P value <0.05 was considered statistically significant.

Results

The OXA-treated mice developed rapid-onset colitis marked by weight loss, diarrhea, and bloody stool. The mean body weight of the OXA-treated group was decreased transiently at day 1, but recovered to the normal level at day 8 (Fig. 1). Significant differences between OXA-treated and untreated mice were shown at days 1 (P<0.01), 2, and 3 (P<0.05). The diarrhea and/or bloody stool peaked at day 1 and was observed until day 4. Inflammation appeared in the distal colon (Fig. 2). The mortality of the OXA-treated group by the end of day 7 was approximately 15%.

Histological examination of colonic sections from the OXA-treated mice revealed superficial inflammation characterized by epithelial cell loss and/or regenerative epithelium, depletion of goblet cells, inflammatory cell infiltration composed mainly of neutrophils and eosinophils, edema formation, hemorrhage, vascular dilatations, and occasionally crypt abscess. These observations peaked around day 2 (Fig. 3). These symptoms gradually declined, although they still remained on day 7.

The colonic MPO activity was measured on days 0 (before induction), 1, 2, 3, 4, and 7 after induction of colitis. A rapid increase in the activity of MPO was observed; this activity peaked around day 2 and decreased to normal level on day 7 (Fig. 4).

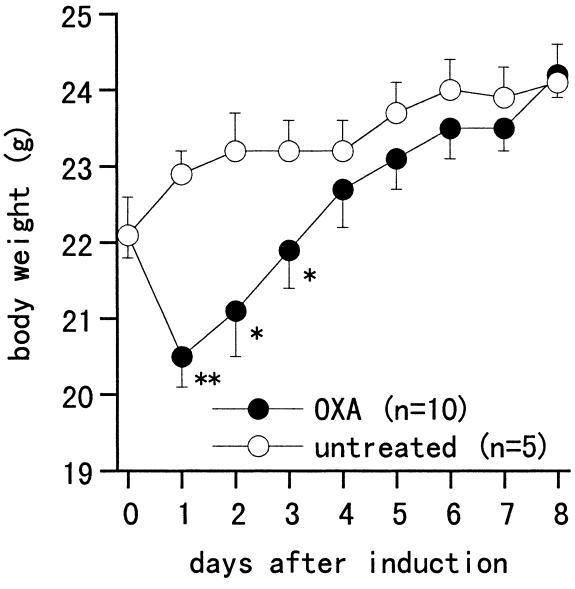

Fig. 1. Time-dependent changes of the body weight. Intrarectal administration of OXA solution induced a rapid weight loss on day 1, but recovered to the normal level at day 8. Significant differences were observed on days 1, 2, and 3. Each point represents the mean ± S.E.M. of 10 (OXA-treated group) or 5 (untreated group) mice. **P<0.01 and *P<0.05 *vs* untreated group (Student's t-test).

The expression of IL-13 mRNA in MLN increased according to the day (Fig. 5), whereas slight differences in IL-4, INF-gamma, and IL-18 gene expression were observed during the same period. The expression level of IL-13 on day 7 was approximately five times higher than on day 0.

Intrarectal administration of 5-ASA or PDL within the respective clinical dosage range resulted in a marked improvement in the colitis, as well as a reduction in the loss of body weight and in the frequency of diarrhea and/or bloody stool. In addition, a significant reduction in MPO activity in the colonic tissue was observed on day 3 after the induction of colitis (Figs. 6 and 7).

Discussion

The original OXA-induced colitis model in SJL/J mice was reported in 1998 (11). Their model was described as having a histologic resemblance to human UC. However, there is some difficulty in pharmacological use of the SJL/J strain. First, mice of this strain have a high mortality rate (approximately 50% in the first 4 days) (11). Second, it is difficult to keep two or more SJL/J mice together because they are aggressive. Recently, the researchers who originally created the model have modified it, as have another group, to evaluate the immune responses of gene-modified (knockout or transgenic) animals (15 – 17). For this purpose, the authors of those studies chose C57 background strains (C57/BL6 or C57/BL10), since these strains are generally used for producing gene-modified animals. However, these strains have also a tendency toward Th-1 immune responses (18). Furthermore, the induction of colitis in C57 strains requires a presensitizing treatment, since these strains are resistant to induction of colitis by a haptenating agent (6). On the other hand, the BALB/C strain is widely used in pharmacological research and is well recognized as a strain that develops polarized Th-2 immune responses (18 – 20).

A single intrarectal administration of OXA solution in BALB/C mice can produce colitis, just as it does with other strains. We set up optimal conditions for the induction of colitis that would be suitable for use in evaluating the drugs. The mortality rate of these mice was lower than that of the SJL/J strain (approximately 15% in BALB/C *vs* 50% in SJL/J). Histological examination showed morphological similarities between this model and human UC (21, 22); these similarities were characterized by epithelial cell loss and/or regenerative epithelium, depletion of goblet cells, and inflammatory cell infiltration of the mucosal superficial layers (Fig. 3).

Fig. 2. Macroscopic observation of colitis induced by intrarectal administration of OXA solution. A: normal BALB/C mouse. B: BALB/C mouse treated with 40% ethanol (day 2). C: BALB/C mouse treated with OXA solution (day 2), showing the presence of hemorrhagic edematous change.

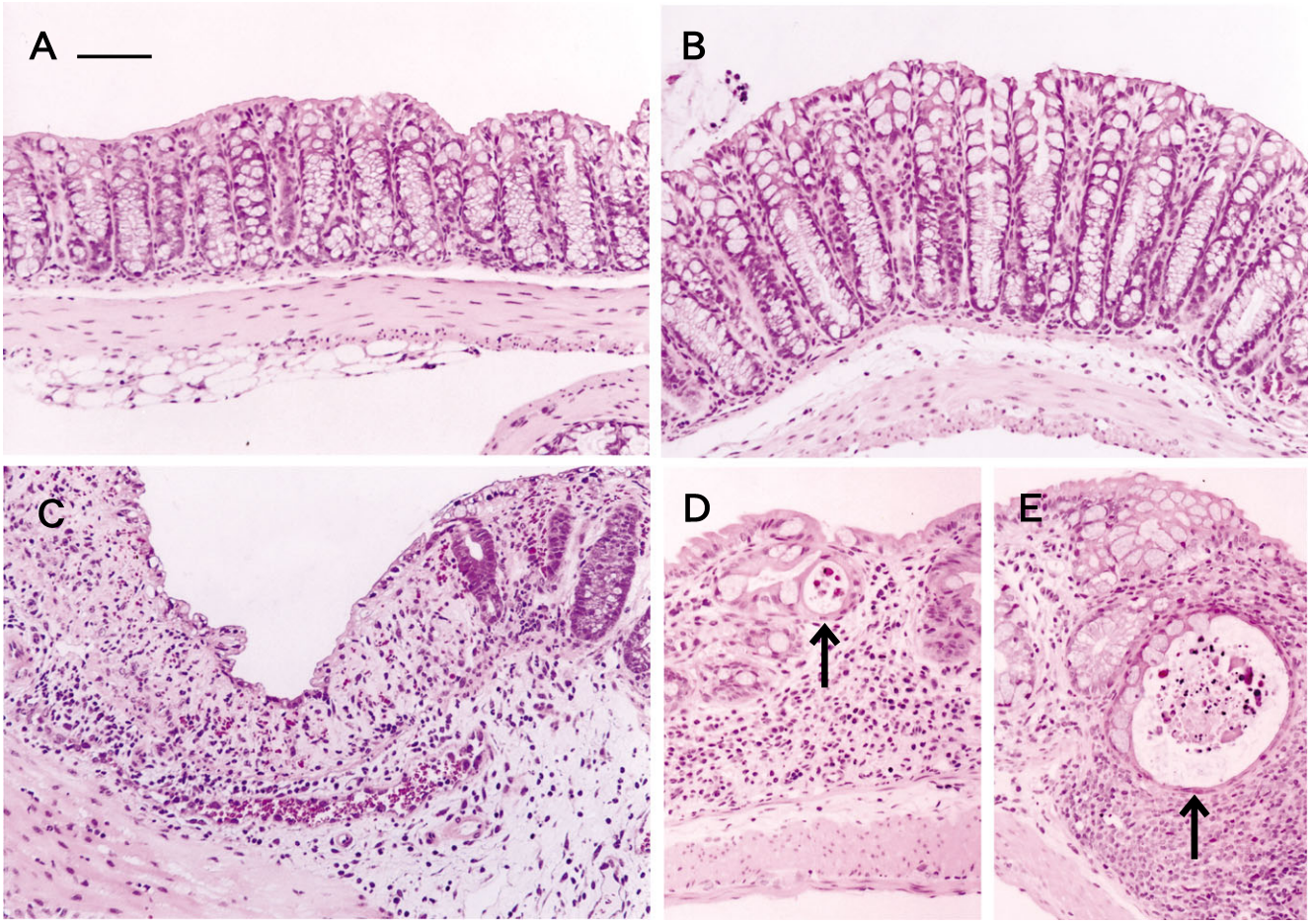

Fig. 3. Histologic examination of the colonic sections from BALB/C mice. Photomicrographs of hematoxylin and eosin stained section of distal colon from A: untreated mouse (×50, Scale bar indicates 20 μm); B: 40% ethanol-treated mouse on day 2 (×50); C: OXA-treated mouse on day 2, showing the presences of epithelial cell loss and/or regenerative epithelium, depletion of goblet cells, inflammatory cell infiltration, edema formation, hemorrhage, vascular dilatation (×50); and D, E: OXA-treated mouse on day 1, showing the presences of cryptitis (D, ×50) and crypt abscess (E, ×50).

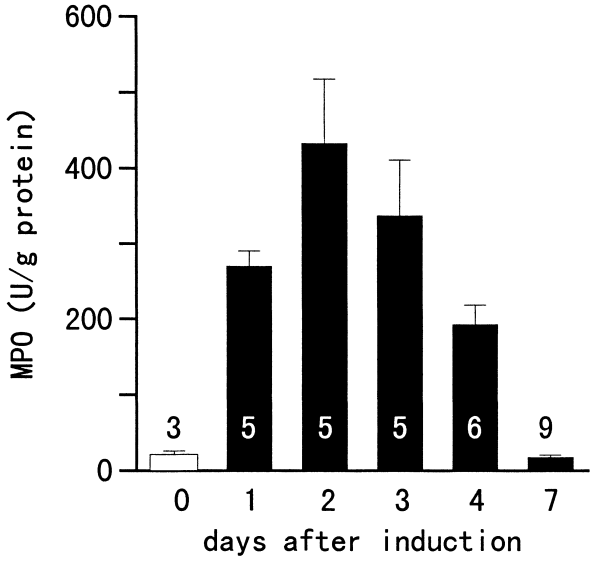

Fig. 4. Time-dependent change of the colonic MPO activity. Colitis was induced by a intrarectal administration of OXA solution (7.5 mg/mL in 40% ethanol). Colonic MPO activities were determined on days 0 (before colitis induction), 1, 2, 3, 4, and 7 after colitis induction. The MPO activity peaked on day 2. Each column represents the mean ± S.E.M. of the indicated number of mice.

The mRNA expression levels of cytokines in MLN, a major lymphoid organ of the colon, were determined by RT-PCR. Remarkably increased IL-13 expression was observed, whereas IL-4, INF-gamma, and IL-18 were little affected (Fig. 5). It is thought that the increase in IL-13 in the BALB/C strain is equal to that in the SJL/J mice (15). It is generally recognized that Th-2 cytokines are involved in the etiology of UC (1 – 5), but IL-4, a typical Th-2 cytokine, is not changed in colonic lesions of UC patients with active disease (5, 23). Recently, the importance of IL-13 in the pathogenesis of UC has been clarified, with results indicating a relationship between IL-13 and destruction of the function of the colonic epithelial barrier (23, 24). Although the prominent role of INF-gamma in CD is well known (1 – 5), the relation of IL-18 to CD was also investigated (25 – 27). While the expression levels of IL-18 increase significantly in CD patients, IL-18 is not changed in UC patients (25 – 27). The results of the present study showed that there were immunological similarities between this model and UC.

Patients with IBD are usually treated with 5-ASA and/or corticosteroids (28 – 31). To confirm that this model can be used for drug evaluation, we investigated whether or not a basic treatment such as 5-ASA or PDL can improve this murine colitis. Intrarectal administration of each 5-ASA or PDL caused a marked improvement in the colitis (Figs. 6 and 7). We also tested some chemicals, such as immunosuppressants or specific inhibitors (e.g., azathioprine and MAPK inhibitors) on this colitis model and obtained the proper responses (our unpublished data).

In conclusion, the colitis model induced by OXA in BALB/C mice has some similarities to human UC.

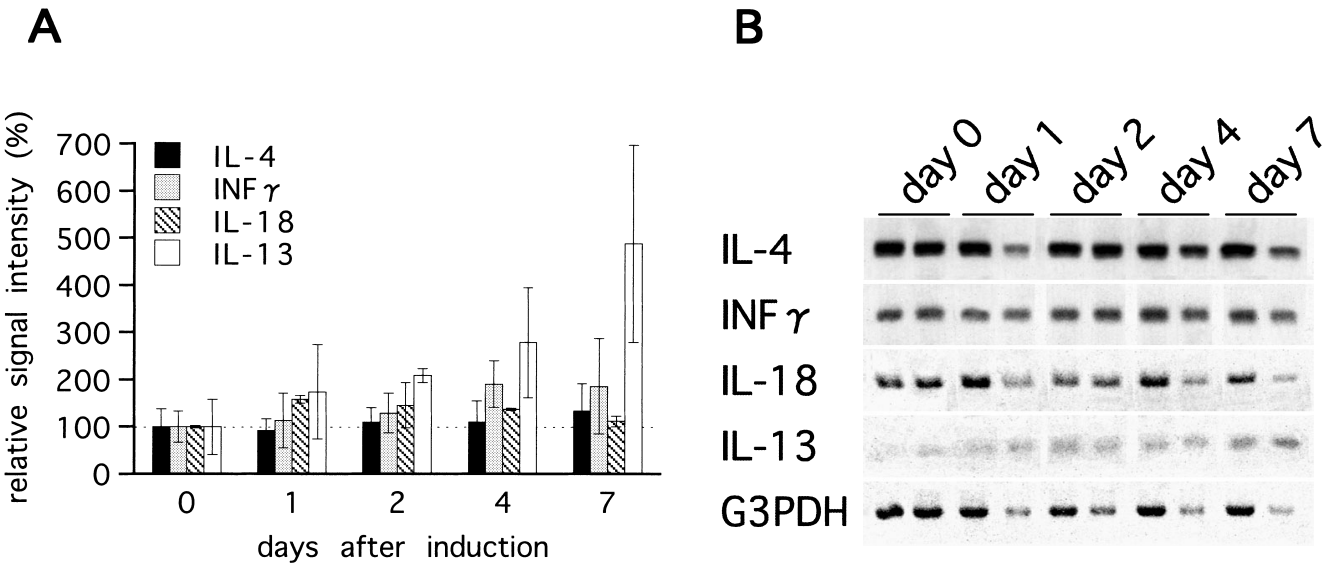

Fig. 5. Time-dependent changes of the mRNA expression levels of cytokines in MLN. A: Colitis was induced by an intrarectal administration of OXA solution, and total RNA was extracted from MLN. Cytokine gene expression levels on days 0 (before colitis induction), 1, 2, 4, and 7 after colitis induction were determined by RT-PCR. Optical density of each band was normalized by the G3PDH expression level and calculated as a relative signal intensity. Each column represents the mean ± S.E.M. of 4 to 6 mice. B: The photographic image of RT-PCR products (black/white reversed).

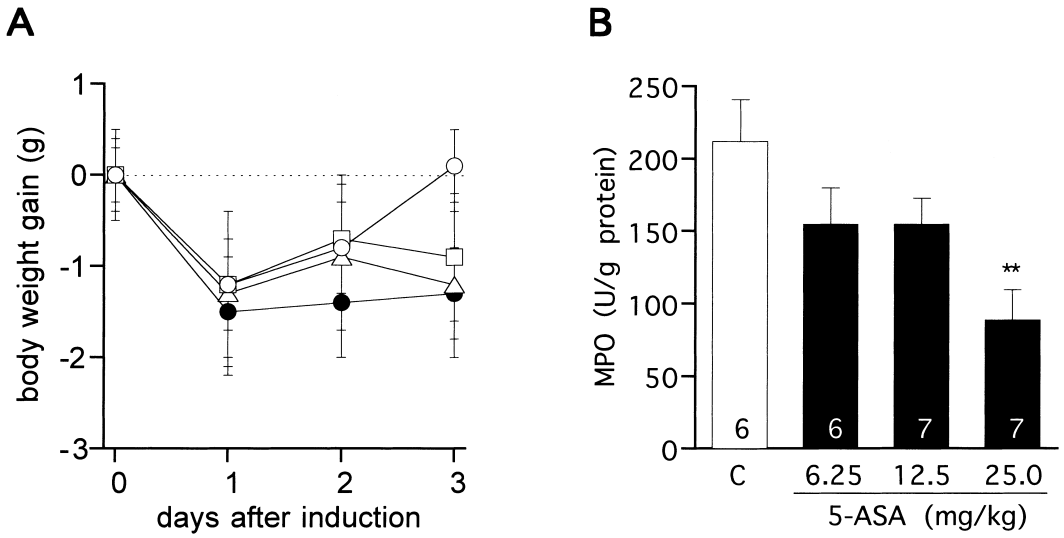

Fig. 6. Effect of 5-ASA on this model. A: Effect of 5-ASA on body weight changes. 5-ASA at 6.25 (open triangle, n = 6), 12.5 (open square, n = 7), or 25.0 (open circle, n = 7) mg/kg or vehicle (closed circle, n = 6) was intrarectally administered once a day for 4 days (from day −1 to day 2). The mean ± S.E.M. value of each group is reported. B: Effect of 5-ASA on colonic MPO activities. The colonic MPO activities were measured on day 3 after colitis induction. 5-ASA reduced the MPO activity in the dose dependent manner, and a significant difference was observed at 25.0 mg/kg. Each column represents the mean ± S.E.M. of the indicated number of mice. "C" means vehicle control. $^{**}P<0.01$ *vs* vehicle control (Dunnett's test)

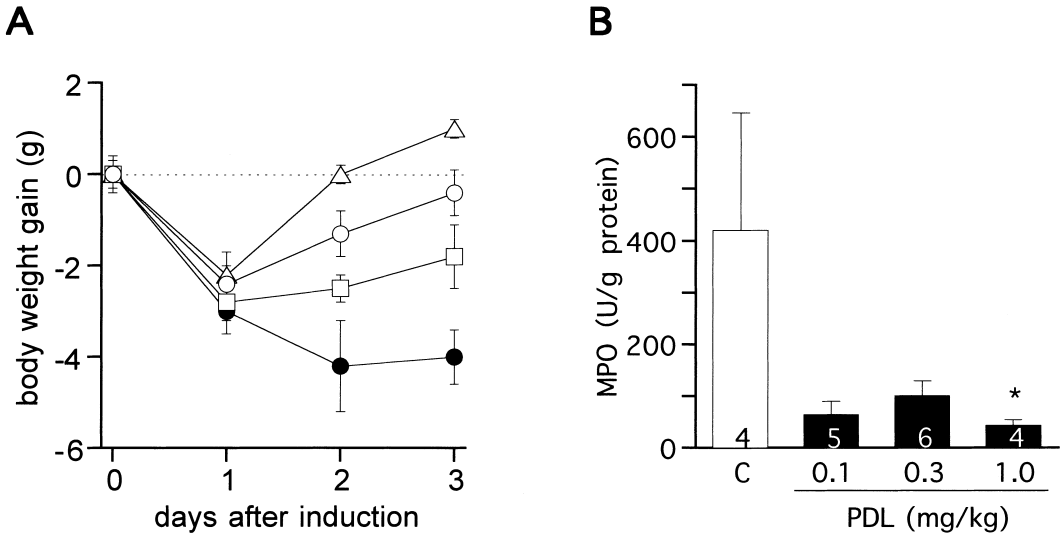

Fig. 7. Effect of PDL on this model. A: Effect of PDL on body weight changes. Mice were treated intrarectally with PDL at 0.1 (open triangle, n = 5), 0.3 (open square, n = 6), or 1.0 (open circle, n = 4) mg/kg or vehicle (closed circle, n = 4) once a day for 4 days (from day −1 to day 2). The mean ± S.E.M. value of each group is reported. B: Effect of PDL on colonic MPO activities. The colonic MPO activities were measured on day 3 after colitis induction. PDL reduced the MPO activity, and a significant difference was observed at 1.0 mg/kg. Each column represents the mean ± S.E.M. of the indicated number of mice. "C" means vehicle control. $^{*}P<0.05$ *vs* vehicle control (Dunnett's test)

Colonic MPO activity was ameliorated by treatments with either 5-ASA or PDL. Thus, the OXA-induced colitis model provides a new way to evaluate the efficacy of new therapeutic agents for UC.

Acknowledgments

The authors would like to thank Fumiko Nakagawa and Yuka Ueno for their technical assistances.

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