

Mucosal Protective Agents Prevent Exacerbation of NSAID-Induced Small Intestinal Lesions Caused by Antisecretory Drugs in Rats

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ABSTRACT

Antisecretory drugs such as histamine H₂-receptor antagonists and proton pump inhibitors are commonly used for the treatment of upper gastrointestinal mucosal lesions induced by nonsteroidal anti-inflammatory drugs (NSAIDs). However, it has recently been reported that these drugs exacerbate NSAID-induced small intestinal lesions in rats. Unfortunately, there are few effective agents for the treatment of this complication. We examined the effects of mucosal protective agents (MPAs) (misoprostol, irsogladine, and rebamipide) and mucin of porcine stomach on diclofenac-induced intestinal lesions and the exacerbation of the lesions by ranitidine or omeprazole. The effects of the drugs on intestinal motility and mucus distribution/content were also examined. Male Wistar rats (180–220 g) were used. Each drug was administered orally under fed conditions. Diclofenac (1–10 mg/kg) produced multiple lesions in the small intestine dose-

dependently. Both ranitidine (30 mg/kg) and omeprazole (100 mg/kg) significantly increased the intestinal lesions induced by low doses (3 and 6 mg/kg) of diclofenac. Misoprostol (0.03–0.3 mg/kg), irsogladine (3–30 mg/kg), and rebamipide (30–300 mg/kg), as well as mucin (30–300 mg/kg) inhibited the formation of intestinal lesions caused by a high dose (10 mg/kg) of diclofenac alone and prevented the exacerbation of diclofenac-induced lesions by antisecretory drugs. Diclofenac (10 mg/kg) markedly increased the intestinal motility and decreased the mucosal mucus, and the decrease of mucus was significantly inhibited by the MPAs. These results indicate the usefulness of the MPAs for the treatment of intestinal lesions induced by NSAIDs alone or by coadministration with antisecretory drugs, and suggest that mucus plays an important role in the protection of intestinal mucosa by the MPAs.

Introduction

Recent progress in endoscopic techniques, such as capsule endoscopy (CE) and double-balloon (push) endoscopy, has revealed that nonsteroidal anti-inflammatory drugs (NSAIDs) often cause mucosal lesions not only in the stomach and duodenum but also in the lower small intestine in humans (Goldstein et al., 2005; Graham et al., 2005; Maiden et al., 2005; Matsumoto et al., 2008). Antisecretory drugs such as proton pump inhibitors (PPIs) and histamine H₂-receptor antagonists (H₂-RAs) are commonly used for the treatment of upper gastrointestinal (GI) mucosal lesions induced by NSAIDs (Leandro et al., 2001; Peura, 2004; Yeomans et al., 2006; Scarpignato and Hunt, 2010; Sugano et al., 2011). These drugs have been thought to be ineffective in treating NSAID-induced small intestinal lesions because acid does not seem to be

involved in the formation of intestinal lesions. However, it has recently been reported that the antisecretory drugs exacerbated the small intestinal lesions induced by NSAIDs in rats via dysbiosis (Wallace et al., 2011) and intestinal hypermotility (Satoh et al., 2012). Furthermore, there are few effective agents for the treatment of small intestinal lesions induced by NSAIDs. Therefore, identification of effective therapies for the treatment of NSAID-induced small intestinal lesions remains an urgent priority (Satoh and Takeuchi, 2012).

It is well known that NSAID-induced side effects in the GI are caused primarily by a decrease in the levels of prostaglandins (PGs) in the GI mucosa. It has therefore been proposed that compensation for the reduction in mucosal PGs by the administration of exogenous PGs may be able to minimize NSAID-induced GI side effects. Misoprostol (MIS), a prostaglandin E₁ derivative, is only one compound available commercially among many derivatives of PGs that have been synthesized. The likelihood that MIS would be effective in protecting the small intestine was suggested by Bjarnason et al. (1989) and recent clinical studies using CE (Watanabe et al., 2008; Fujimori et al., 2009). However, it is well known

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ABBREVIATIONS: ATR, atropine; CE, capsule endoscopy; CI, confidence interval; DIC, diclofenac sodium; GI, gastrointestinal; H₂-RA, histamine H₂-receptor antagonist; IRS, irsogladine; LI, lesion index; MIS, misoprostol; MPA, mucosal protective agent; MUC, mucin; NSAID, nonsteroidal anti-inflammatory drug; OPZ, omeprazole; PAS, periodic acid-Schiff; PG, prostaglandin; PO, by mouth; PPI, proton pump inhibitor; RAN, ranitidine; REB, rebamipide; VEH, vehicle.

that MIS sometimes causes diarrhea and abdominal pain as side effects, as reported by Watanabe et al. (2008), which represents a significant impediment, especially for long-term treatment.

On the other hand, compounds with different (i.e., non-PG) chemical structures but similar mucosal protective activity to PGs [mucosal protective agents (MPAs)] have been developed in Japan, and some of the MPAs, irsogladine (IRS) (Ueda et al., 1984a,b) and rebamipide (REB) (Uchida et al., 1985; Yamasaki et al., 1987), are now used as antiulcer drugs for the treatment of gastric ulcer and gastritis as gastroprotective agents in Japan and other Asian countries (Arakawa et al., 2005; Hiraishi et al., 2010; Naito Yoshikawa, 2010; Akagi et al., 2013). It has been reported that IRS protects the gastric mucosa by enhancing mucosal integrity through facilitation of gap junctional intracellular communications (Ueda et al., 1995) and suppression of superoxide production by increasing the cAMP level via inhibition of phosphodiesterase (Kyojima et al., 2004). REB is known to protect the gastric mucosa by acting as a free radical scavenger (Yoshikawa et al., 1993; Sakurai et al., 2004). Furthermore, it has been reported that both IRS (Kamei et al., 2008) and REB (Mizoguchi et al., 2001) prevented indomethacin-induced small intestinal lesions in rats.

In the present study, we examined the effects of both IRS and REB on 1) the formation of small intestinal lesions induced by diclofenac sodium (DIC), an NSAID commonly used in humans, and 2) the exacerbation of the lesions by antisecretory drugs, ranitidine (RAN) and omeprazole (OPZ), in rats. In addition, the results were compared with those of MIS, a well known MPA, and mucin (MUC) from porcine gastric mucosa. The possible mechanism of the protection by IRS and REB on the small intestine was examined from the perspectives of intestinal motility and intestinal mucus [periodic acid–Schiff (PAS)–positive substance] distribution. The results suggest that the MPAs can protect the intestinal mucosa against NSAIDs with or without antisecretory drugs, and that mucus plays at least a partial role in the protection of intestinal mucosa by the MPAs.

Materials and Methods

Ethics Approval

Experimental protocols were approved by the Animal Research Committee at Kyoto Pharmaceutical University, Kyoto, Japan.

Animals

Seven-week-old male Wistar rats (Shimizu Laboratories, Shizuoka, Japan) weighing 180–220 g were used. The animals were given rat chow pellets (Type MF diet; Oriental-Yeast, Tokyo, Japan) and had free access to water during the experimental periods, unless otherwise specified.

Drugs

The following drugs and chemicals were used: atropine (ATR), carboxymethyl cellulose, DIC, diethyl ether, OPZ, RAN, NaHCO₃, and urethane (Wako, Osaka, Japan). Drugs for oral or subcutaneous administration were suspended in a 1% carboxymethyl cellulose solution. OPZ was suspended in 1% carboxymethyl cellulose containing 1% NaHCO₃ for oral administration. The drugs were prepared just prior to the experiments and administered in a volume of 0.2 ml/100 g body wt. The same volume of a solution containing 1% carboxymethyl cellulose was used as a vehicle (VEH).

Induction of Small Intestinal Lesions

The animals were not fasted in this study. DIC (1–30 mg/kg PO) was administered, and the animals were sacrificed by diethyl ether overdose 24 hours after DIC administration. The small intestines were spread out on paper, opened along the antimesenteric side, and the contents were removed. The length (in millimeters) of the individual lesions was measured under a dissecting microscope with a 1-mm square grid eyepiece ($\times 10$), and the sum of the lengths of all the lesions in each intestine was used as the lesion index (LI). The person measuring the lesions did not know the treatments given to the animals.

Experimental Design

Effects on Small Intestinal Lesions Induced by DIC Alone.

In this study, a high dose of DIC (10 mg/kg PO) was used, and MPAs or MUC were administered orally 30 minutes before DIC. The intestinal lesions were examined 24 hours after DIC treatment.

Effects on Exacerbation of DIC-Induced Intestinal Lesions by RAN or OPZ. RAN (30 mg/kg) or OPZ (100 mg/kg) was administered orally 30 minutes before DIC (3 or 6 mg/kg PO), and each of MPAs, MUC, and ATR was given orally 30 minutes before RAN. The intestinal lesions were examined 24 hours after DIC administration.

Measurement of Intestinal Motility

Intestinal motility was determined using a miniature balloon according to the method described in our previous article (Takeuchi et al., 2002). In brief, rats were anesthetized with urethane (1.25 g/kg i.p.) and a midline incision was performed to expose the small intestine. A thin, water-filled balloon, made from silicone rubber and attached to a polyethylene catheter, was then introduced into the ileum via a small incision made ~10 cm proximal to the ileocecal junction. The volume in the balloon was adjusted to give an initial resting pressure of ~10–15 cm H₂O, which was insufficient to cause active distension of the intestinal wall. After allowing the preparation to rest for 30 minutes, intestinal motility was monitored on a personal computer (Dynabook, Satellite A20; Toshiba, Tokyo, Japan) as intraluminal pressure changes using a pressure transducer and a DC Strain Amplifier (AS2101; NEC, Tokyo, Japan). After basal motor activity had stabilized (≥ 1 hour after the operation), the effects of the drugs were examined.

Histological Examinations

DIC (10 mg/kg) was administered orally in fed rats, and 3 hours later the lower part of the ileum (between 15 and 30 cm proximal to the ileocecal junction) was removed after bleeding of whole blood from the abdominal aorta under deep anesthesia with diethyl ether. The contents were washed out with physiological saline, the preparations were infused with Carnoy's solution, and the whole preparation was fixed in Carnoy's solution for 24 hours. MPAs were administered 30 minutes before DIC, and the lower ileum was removed 3 hours after DIC treatment. In another experiment, RAN (30 mg/kg) or OPZ (100 mg/kg) was administered orally in fed rats, and 3 hours later the ileal preparations were removed and fixed in Carnoy's solution.

Staining with PAS. Serial sections (4 μ m) of Carnoy's-fixed, paraffin-embedded samples of each tissue were mounted on slides. According to conventional methods, PAS staining was performed.

Measurement of PAS-Positive Area in the Mucosa. Photographs of the ileal mucosa stained with PAS in the rats given DIC with or without MPAs, or RAN and OPZ, were taken under a microscope ($\times 10$). PAS-positive area in the mucosa was measured using ImageJ 1.4 (National Institutes of Health, Bethesda, MD) according to the method described in our previous paper (Yoda et al., 2010).

Statistical Analysis. All data are expressed as the mean \pm S.E.M. Differences between groups were analyzed by Student's *t* test for

paired group comparisons, or analysis of variance (Dunnett's multiple range test) if more than two variables were considered, with the significance level set at $P < 0.05$. The ID_{50} values and their 95% confidence intervals (CIs) were calculated by logistic regression analysis.

Results

Ulcerogenic Effect of DIC on the Small Intestinal Mucosa

DIC was administered orally under fed conditions, and the intestinal lesions were examined 24 hours later. There are few reports that VEH alone causes lesions in the small intestine. However, in the present study, in the rats given VEH alone, small lesions were observed in the small intestine, and the LI was 8.1 ± 1.8 mm ($n = 8$). DIC (1–10 mg/kg) produced multiple lesions in the middle and lower parts of the small intestine in a dose-dependent manner, but the LIs in the groups given 1 and 3 mg/kg DIC were not significantly different from that in the group given VEH (Fig. 1). The LIs in the rats given 6 and 10 mg/kg DIC were 42.6 ± 6.5 mm ($n = 8$, $P < 0.001$ vs. VEH) and 101.5 ± 11.0 mm ($n = 8$, $P < 0.001$ vs. VEH), respectively.

Effect of MPAs and Mucin on Intestinal Lesions Induced by DIC Alone

Effect of MPAs. As shown in Table 1 (Exp. 1–3), the formation of the intestinal lesions caused by a high dose of DIC (10 mg/kg) was prevented by MIS (0.01–0.3 mg/kg), IRS (1–30 mg/kg), and REB (10–300 mg/kg) in a dose-dependent manner. The ID_{50} values (CI) were 0.08 (0.05–0.14) mg/kg ($n = 7$), 12.4 (6.8–33.6) mg/kg ($n = 7$), and 283.4 (128.2–5436.3) mg/kg ($n = 7$), respectively. The effect of high doses of each drug was significant (Table 1).

Effect of MUC. MUC (10–300 mg/kg) inhibited the formation of intestinal lesions dose-dependently. The ID_{50} value (CI) was 200.4 (125.0–508.5) mg/kg ($n = 8$). The effects

TABLE 1

Effect of MPAs and MUC on small intestinal lesions induced by DIC in rats

DIC (10 mg/kg PO) was administered without fasting. MPAs and MUC were administered orally 30 minutes before DIC. Intestinal lesions were measured 24 hours after DIC administration. Data represent the mean \pm S.E.M. ($n = 7$ or 8).

Drug	Dose	No. of Rats	Lesion Index	Inhibition
	mg/kg PO		mm	%
Exp. 1 (MIS)	VEH	7	120.3 \pm 13.6	—
	0.01	7	93.6 \pm 8.5	22.2
	0.03	7	80.1 \pm 8.4*	35.4
	0.1	7	58.6 \pm 10.6***	51.3
	0.3	7	32.1 \pm 3.4***	73.3
Exp. 2 (IRS)	VEH	7	72.7 \pm 8.2	—
	1	7	59.4 \pm 6.0	18.3
	3	7	46.3 \pm 3.9*	36.3
	10	7	37.0 \pm 4.6**	49.1
	30	7	30.1 \pm 4.8***	58.6
Exp. 3 (REB)	VEH	7	100.6 \pm 10.8	—
	10	7	80.9 \pm 10.5	19.6
	30	7	86.6 \pm 6.1	13.9
	100	7	63.4 \pm 10.8	37.0
	300	7	48.7 \pm 6.5*	51.6
Exp. 4 (MUC)	VEH	8	131.6 \pm 6.4	—
	30	8	103.4 \pm 6.6*	21.4
	100	8	68.6 \pm 6.9***	47.9
	300	8	61.6 \pm 3.5***	53.2

Exp., experiment.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus VEH (Dunnett's test).

of doses of 30 mg/kg and higher were significant ($P < 0.05$ and 0.001 vs. VEH) (Table 1, Exp. 4).

Effect of RAN on DIC-Induced Intestinal Lesions

In this study, a low dose of DIC (3 mg/kg PO) was used. The LI in the group given VEH alone was 10.4 ± 6.0 mm ($n = 8$). Both DIC (3 mg/kg PO) and RAN (30 mg/kg PO) alone slightly increased the intestinal lesions, but the LIs were not significantly larger than that of the VEH group. However, the drugs administered together produced marked lesions in the intestine, and the LI was 82.0 ± 7.2 mm ($n = 8$, $P < 0.001$

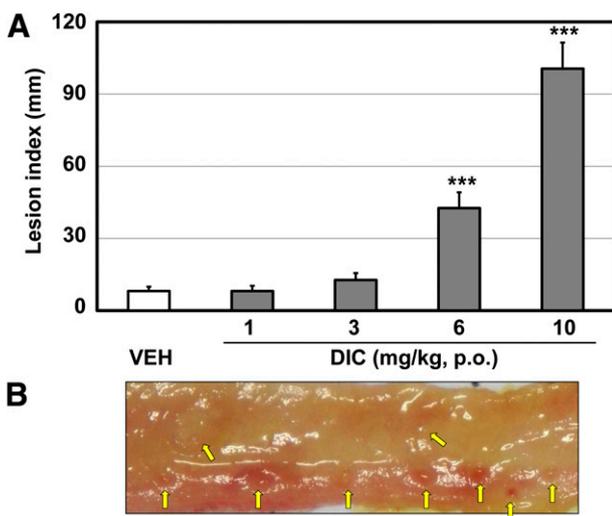


Fig. 1. Ulcerogenic effect of DIC on small intestinal lesions in rats. DIC (1–10 mg/kg) was administered orally without fasting, and intestinal lesions were measured 24 hours after DIC administration. (A) Dose-related response of ulcerogenic effect of DIC on the small intestine. Data represent the mean \pm S.E.M. ($n = 7$). *** $P < 0.001$ versus VEH (Dunnett's test). (B) Macroscopic observation of small intestinal lesions caused by 10 mg/kg DIC. Each arrow shows the mucosal lesions.

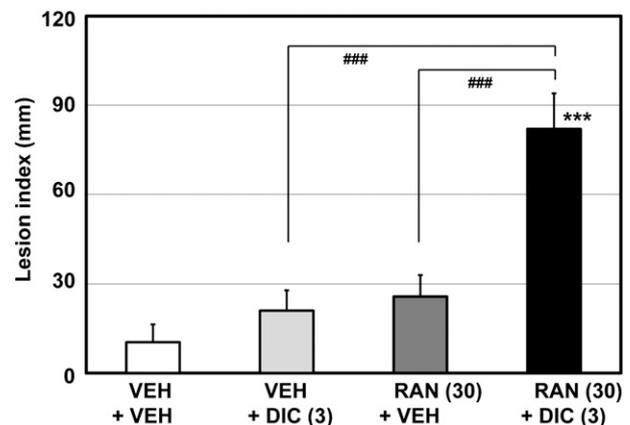


Fig. 2. Effect of RAN on small intestinal lesions induced by DIC in rats. DIC (3 mg/kg) was administered orally without fasting, and intestinal lesions were measured 24 hours after DIC administration. RAN (30 mg/kg) was given orally 30 minutes before DIC. Data represent the mean \pm S.E.M. ($n = 7$). *** $P < 0.001$ versus VEH (Dunnett's test); ### $P < 0.001$ (Student's t test). The dose (milligrams per kilogram) is listed in parentheses.

vs. VEH, DIC, and RAN alone) (Fig. 2). In another experiment, RAN (3–100 mg/kg PO) increased DIC-induced intestinal lesions from a dose of 3 mg/kg in a dose-dependent manner, but significant effects were only observed in the groups given 30 and 100 mg/kg RAN.

Effects of MPAs, MUC, and ATR on Exacerbation of DIC-Induced Intestinal Lesions by RAN

Effect of MPAs. RAN (30 mg/kg PO) markedly increased the lesions induced by DIC (3 mg/kg PO), and the increase was prevented by MIS (0.03–0.3 mg/kg), IRS (3–30 mg/kg), and

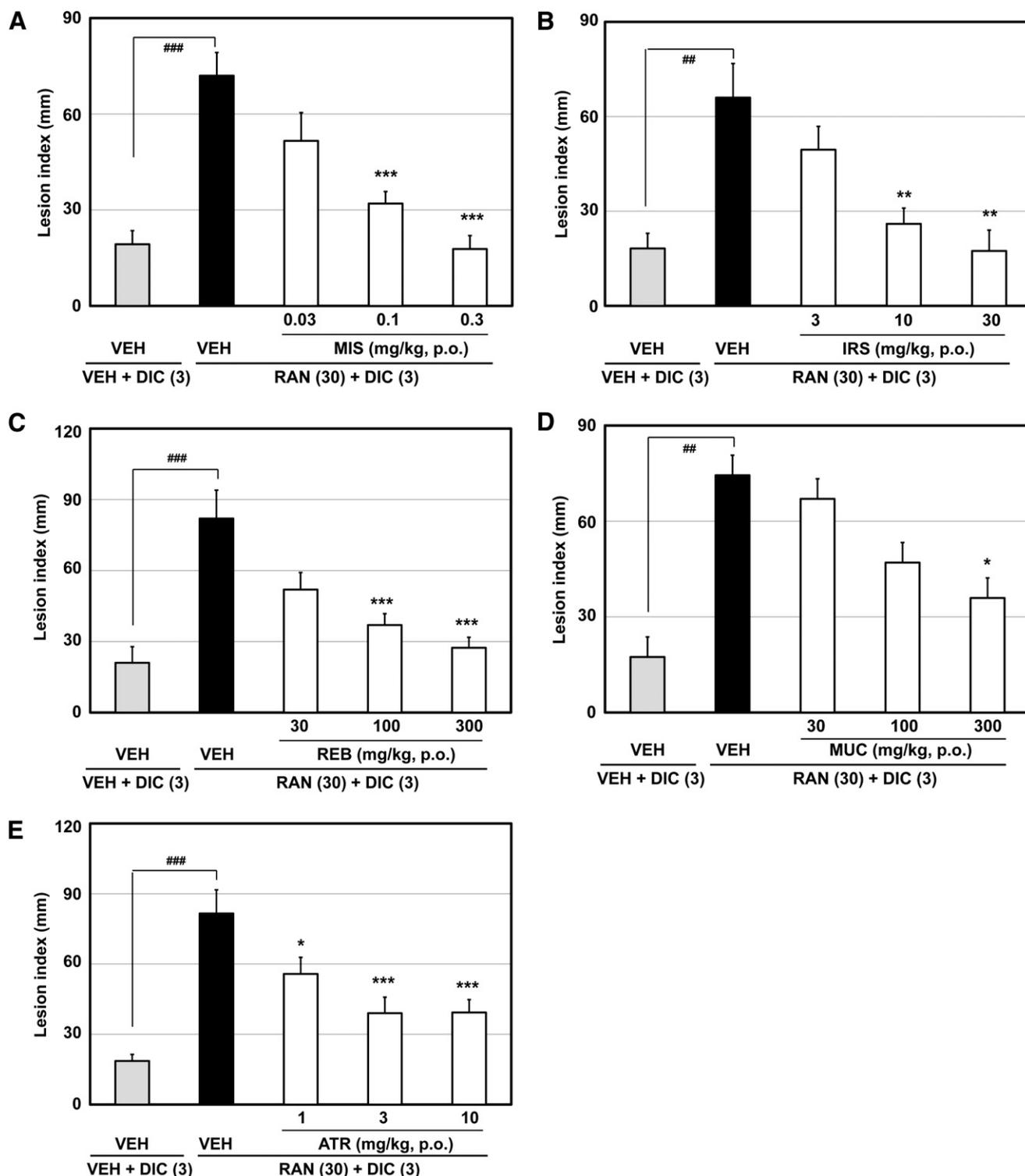


Fig. 3. Effect of drugs on the enhancing effect of RAN on DIC-induced small intestinal lesions. RAN (30 mg/kg PO) was administered 30 minutes before DIC (3 mg/kg PO), and intestinal lesions were measured 24 hours after DIC administration. Drugs were administered orally 30 minutes before RAN [MIS (A), IRS (B), REB (C), MUC (D), and ATR (E)]. Data represent the mean \pm S.E.M. ($n = 7$). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus VEH (Dunnett's test); # $P < 0.01$; ### $P < 0.001$ (Student's t test). The dose (milligrams per kilogram) is listed in parentheses.

REB (30–300 mg/kg) dose-dependently (Fig. 3, A–C). The high doses of each drug almost completely inhibited the increase of the lesions, and the effects were significant (Fig. 3, A–C).

Effect of MUC. MUC (30–300 mg/kg) prevented the increase of the lesions dose-dependently, and the LI in the group given 300 mg/kg MUC was significantly ($P < 0.05$) smaller than that of the VEH group (Fig. 3D). The effect of MUC seemed to be weaker than those of the MPAs.

Effect of ATR. ATR (1–10 mg/kg) prevented the increase of the lesions dose-dependently, and the LIs in the groups given 1, 3, and 10 mg/kg ATR were significantly ($P < 0.05$, 0.001, and 0.001) smaller than that of the VEH group (Fig. 3E). The increase of the lesions was not always completely prevented by ATR, even at a high dose of 10 mg/kg.

Effect of OPZ on DIC-Induced Intestinal Lesions

OPZ at 100 mg/kg was administered orally 30 minutes before DIC (3 and 6 mg/kg PO), and the intestinal lesions were examined 24 hours after DIC administration. As shown in Fig. 4, OPZ mildly increased the lesions induced by a low dose of DIC (3 mg/kg), but the effect was not significant. DIC (6 mg/kg) produced moderate lesions in the small intestine, and the lesions were significantly ($P < 0.001$ vs. DIC alone) increased by pretreatment with OPZ. In another experiment, OPZ (10–300 mg/kg PO) increased the lesions induced by DIC (6 mg/kg PO) in a dose-dependent manner, but significant effects were observed only in the groups given 100 and 300 mg/kg OPZ. On the basis of these results, in the following studies, both a moderate dose (6 mg/kg) of DIC and a high dose (100 mg/kg) of OPZ were used.

Effects of MPAs and MUC on Exacerbation of DIC-Induced Intestinal Lesions by OPZ

Effect of MPAs. OPZ (100 mg/kg PO) markedly increased the lesions induced by DIC (6 mg/kg PO), and the increase was prevented by MIS (0.03–0.3 mg/kg), IRS (3–30 mg/kg), and REB (30–300 mg/kg) dose-dependently (Fig. 5, A–C). The effects of high doses of each drug were significant (Fig. 5, A–C).

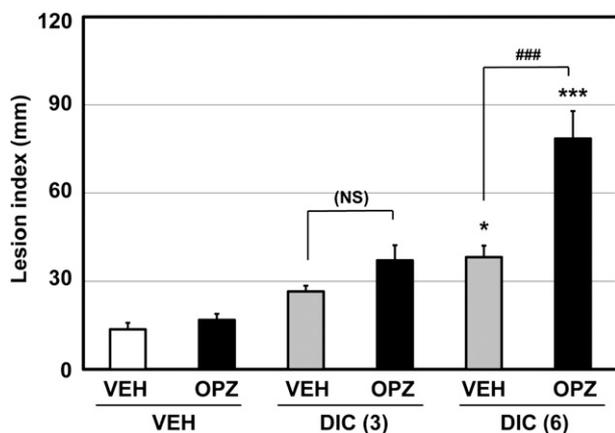


Fig. 4. Effect of OPZ on small intestinal lesions induced by DIC in rats. DIC (3 and 6 mg/kg) was administered orally without fasting, and intestinal lesions were measured 24 hours after DIC administration. OPZ (100 mg/kg) was given orally 30 minutes before DIC. Data represent the mean \pm S.E.M. ($n = 7$). * $P < 0.05$; *** $P < 0.001$ versus VEH (Dunnett's test); ### $P < 0.001$ (Student's t test). NS, not significant. The dose (milligrams per kilogram) is listed in parentheses.

Effect of MUC. MUC (30–300 mg/kg) prevented the increase of the lesions dose-dependently, and the LIs in the groups given 100 and 300 mg/kg MUC were significantly ($P < 0.01$ and 0.001) smaller than that of the VEH group (Fig. 5D). The effect of MUC seemed to be milder than those of the MPAs.

Effect of Drugs on Motility of the Small Intestine

In urethane-anesthetized rats, the ileum usually did not show spontaneous strong contractions. Each drug was administered subcutaneously every 2 hours. The effects on the motility were examined in three to rats for each drug, and similar results were obtained, although the pattern of the response (onset and amplitude of contractile response) for each drug was somewhat different among the rats. The typical responses to each drug are shown in Fig. 6. DIC (3 mg/kg s.c.) gradually increased motility, starting about 1 hour post-treatment (Fig. 6A). The motor-stimulating effect of DIC increased with the dose (10 and 30 mg/kg), and the onset of the effect seemed to be more rapid at higher doses (Fig. 6A). The motor-stimulating effect of DIC (10 mg/kg) was markedly enhanced by RAN (30 mg/kg) (Fig. 6, B and C). On the other hand, as shown in Fig. 6D, OPZ (100 mg/kg) did not affect the motility, although RAN markedly enhanced the motility in this rat. Both MIS (0.1 mg/kg) and ATR (10 mg/kg) decreased or abolished the increased motility caused by DIC (Fig. 6, E and H). Neither IRS (10 mg/kg) nor REB (100 mg/kg) affected the increased motility induced by DIC (10 mg/kg), although ATR almost abolished the motility in these rats (Fig. 6, F and G).

Effect of Drugs on Mucus Distribution in the Mucosa

DIC (10 mg/kg) was administered orally in fed rats, and 3 hours later, the lower ileum was removed. The MPAs were administered 30 minutes before DIC. Figure 7 shows typical photos of the ileal mucosa stained with PAS from each group. In the group given VEH, PAS-positive substances were clearly observed in the goblet cells and over the surface and along the glands of the intestinal mucosa (Fig. 7A). DIC (10 mg/kg) apparently reduced the amount of PAS-positive substance in the goblet cells as well as in the glands, without causing any other obvious histological changes in the mucosa (Fig. 7B). The reduction in PAS staining caused by DIC was attenuated when MIS (0.1 mg/kg), IRS (10 mg/kg), and REB (100 mg/kg) were administered before DIC treatment (Fig. 7, C–E). Figure 8 shows the results of analysis using ImageJ 1.4 on the contents of PAS-positive substance in the mucosa. The PAS-positive area in the group given VEH was $3.7\% \pm 0.5\%$ ($n = 6$). DIC (10 mg/kg) significantly decreased the area to $1.7\% \pm 0.1\%$ ($n = 6$, $P < 0.01$ vs. VEH). MIS (0.1 mg/kg), IRS (10 mg/kg), and REB (100 mg/kg) all significantly ($P < 0.01$ and 0.05) prevented the decrease of the PAS-positive area by DIC.

In another experiment, either RAN (30 mg/kg) or OPZ (100 mg/kg) was administered orally to fed rats, and 3 hours later, the distribution of PAS-positive substance in the mucosa was examined. RAN, but not OPZ, mildly decreased the PAS-positive area in the mucosa.

Discussion

Although antisecretory drugs such as PPIs and H_2 -RAs are commonly used for the treatment of upper GI mucosal lesions

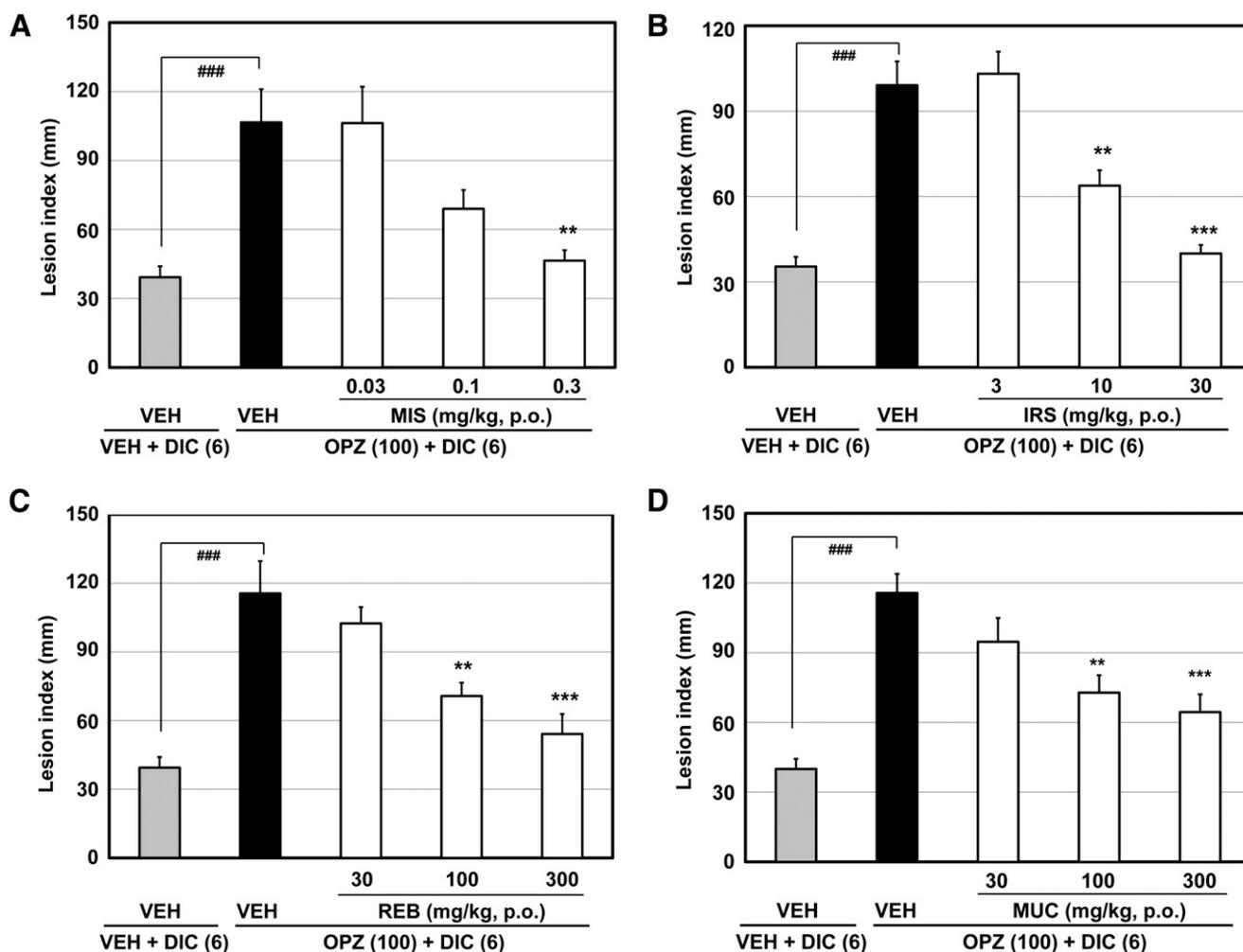


Fig. 5. Effect of drugs on the enhancing effect of OPZ on DIC-induced small intestinal lesions. OPZ (100 mg/kg PO) was administered 30 minutes before DIC (6 mg/kg PO), and intestinal lesions were measured 24 hours after DIC administration. Drugs were administered 30 minutes before OPZ [MIS (A), IRS (B), REB (C), and MUC (D)]. Data represent the mean \pm S.E.M. ($n = 7$). ** $P < 0.01$; *** $P < 0.001$ versus VEH (Dunnett's test); ### $P < 0.001$ (Student's t test). The dose (milligrams per kilogram) is listed in parentheses.

induced by NSAIDs, it has been reported that the antisecretory drugs exacerbated the small intestinal lesions induced by NSAIDs in rats (Wallace et al., 2011; Satoh et al., 2012). This was supported by the present study, that is, both RAN and OPZ markedly exacerbated the small intestinal lesions induced by DIC. However, at present, there are few effective agents for the treatment of small intestinal lesions induced by NSAIDs. In the present study, we examined the effects of the MPAs (MIS, IRS, and REB) on the small intestinal lesions induced by DIC in fed rats. The MPAs prevented the formation of intestinal lesions induced by high-dose DIC alone, and inhibited the exacerbation of lower-dose DIC-induced intestinal lesions by RAN or OPZ. These results strongly suggest the usefulness of the MPAs for the treatment of small intestinal lesions induced by NSAIDs with or without antisecretory drugs.

We examined the effects of RAN and OPZ on the formation of intestinal lesions caused by lower doses (3 and 6 mg/kg) of DIC because DIC is commonly used in patients at a lower dose, namely, 25 mg/person, three times daily (~ 1.5 mg/kg/day). In this study, 3 mg/kg DIC produced mild lesions in the small intestine in fed rats, although the LI was not significantly different from that of the VEH group. However,

when DIC was preceded by RAN, it produced marked lesions in the small intestine. The effect of RAN on lesion formation was observed dose-dependently from 3 mg/kg, and significant effects were observed at doses of 30 mg/kg and higher. On the other hand, RAN (30 mg/kg) did not show any significant ulcerogenic effect on the small intestinal mucosa by itself. These results indicate that H_2 -RAs aggravate mild lesions in the small intestine caused by NSAIDs, suggesting a possible risk of coadministration of NSAIDs and H_2 -RAs, even if the NSAID itself seems to be safe in terms of the GI mucosa.

In the present study, the intestinal motility was increased by DIC dose-dependently, and the motility was further enhanced by RAN. Furthermore, all of the increased motility by DIC, enhancement of the motility by RAN, and the exacerbation of DIC-induced intestinal lesions by RAN were inhibited by treatment with ATR. These results support the previous findings that H_2 -RAs exacerbated NSAID-induced intestinal lesions by increasing the intestinal motility via the cholinergic nervous system (Satoh et al., 2012). However, there are few reports of the effect of NSAIDs and RAN on small intestinal motility in humans, which will be useful for elucidating the pathophysiology of intestinal lesions induced by NSAIDs. This will be a subject for future research. In

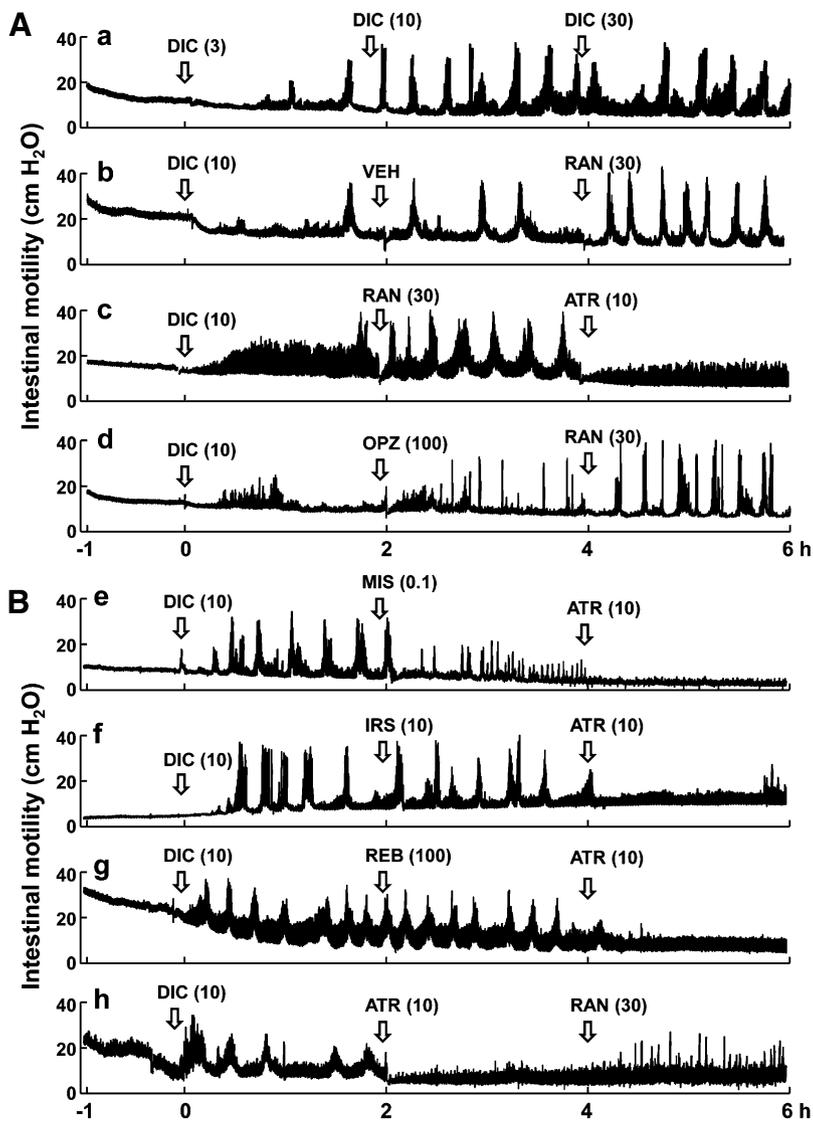


Fig. 6. Effect of drugs on DIC-induced intestinal motility in anesthetized rats. Each drug was administered subcutaneously every 2 hours. DIC (a, 3, 10, and 30 mg/kg) was administered every 2 hours. Each of VEH (b), RAN (c, 30 mg/kg), OPZ (d, 100 mg/kg), MIS (e, 0.1 mg/kg), IRS (f, 10 mg/kg), and ATR (g, 10 mg/kg) was administered 2 hours after DIC (10 mg/kg s.c.). The dose (milligrams per kilogram) is listed in parentheses.

addition, in the present study, RAN mildly decreased the PAS-positive area in the mucosa. Therefore, it is possible that the observation that RAN-depleted mucus distribution/content may explain, at least in part, the exacerbation of intestinal lesions by RAN.

On the other hand, even at a high dose of 100 mg/kg, OPZ did not affect the intestinal lesions induced by a low dose (3 mg/kg) of DIC, but markedly increased the lesions induced by a moderate dose (6 mg/kg) of DIC. The effect of OPZ was significant at higher doses of 100 and 300 mg/kg. In addition, OPZ neither affected the intestinal hypermotility induced by DIC nor decreased the PAS-positive area in the mucosa. These results suggest that OPZ exacerbates DIC-induced intestinal lesions via a different mode of action from that of RAN.

Wallace et al. (2011) reported that 9 days of treatment with OPZ caused a significant reduction of actinobacteria and *Bifidobacteria* spp. in the jejunum in rats, and suggested that PPIs exacerbated NSAID-induced intestinal injury through dysbiosis. As discussed later, we suggested that undigested solid components of food play an important role in NSAID-induced intestinal lesions (Satoh et al., 2009, 2010; Satoh, 2010). It is conceivable that strong and sustained inhibition of

gastric acid secretion by antisecretory drugs may exacerbate NSAID-induced intestinal lesions via dysbiosis and/or increasing the amount of undigested solid components of food in the small intestine due to indigestion in the stomach.

Although a deficiency of endogenous PGs due to cyclooxygenase inhibition is the most important base for the ulcerogenic responses to NSAIDs, several different factors related to the pathogenesis of NSAID-induced intestinal ulcers have been suggested (Takeuchi et al., 2010; Wallace, 2013). Among them, we have indicated the importance of hypermotility of the small intestine (Takeuchi et al., 2002), decrease of mucus secretion (Kunikata et al., 2002), and undigested solid components of food (Satoh et al., 2009, 2010), especially in the early stage of lesion formation in the small intestine induced by NSAIDs. Under conditions where the amount of surface mucus is decreased by NSAIDs, it is possible that undigested solid components of food may cause physical damage to the mucosal surface when the intestine is strongly contracted by NSAIDs, and accelerate the invasion of intestinal bacteria and irritants (bile acids, etc.) into the mucosa. Therefore, in the present study, we examined the effects of MPAs and antisecretory drugs on the intestinal

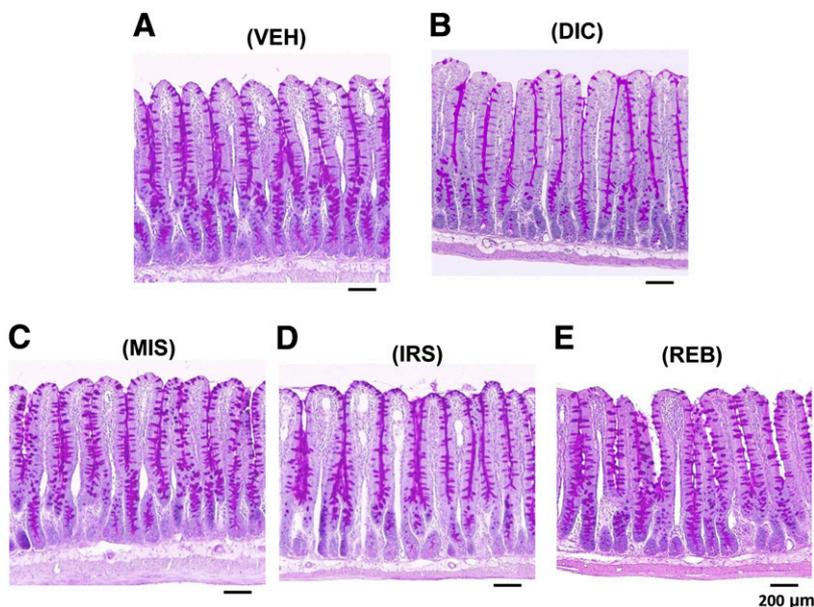


Fig. 7. Effect of MPAs on the distribution of PAS-positive substance in the ileal mucosa in rats (PAS staining). DIC (10 mg/kg) was administered orally without fasting. MIS (0.1 mg/kg), IRS (10 mg/kg), and REB (100 mg/kg) were administered orally 30 minutes before DIC. The lower ileum was removed 3 hours after DIC treatment. (A–E) Typical photographs of the mucosa in each group.

motility and distribution/content of PAS-positive substance (mucus) in the intestinal mucosa.

As mentioned earlier, DIC increased the intestinal motility dose-dependently in terms of both grade and duration. In addition, DIC markedly decreased PAS-positive substances in the goblet cells as well as in the glands of the intestinal mucosa without causing any other obvious histological changes in the mucosa at 3 hours after oral administration. MIS markedly inhibited the motility increased by DIC and significantly prevented the decrease of PAS-positive substance in the mucosa. The effects of MIS can be easily explained by the compensation of PGs reduced by DIC because MIS is a derivative of prostaglandin E₁. On the other hand, both IRS and REB, different from MIS, did not affect the intestinal hypermotility caused by DIC, but significantly reversed the decrease of PAS-positive substance in the mucosa. It has been reported that both IRS and REB increased mucus secretion in the GI tract in rats (Ishihara et al., 1992; Kamei et al., 2008) and in the stomach in

humans (Iijima et al., 2009), and the results of the present study are consistent with these findings. In the present study, we further examined the effects of MUC from porcine stomach on the intestinal lesions, and found that porcine MUC moderately prevented the formation of intestinal lesions caused by a high dose of DIC alone as well as the exacerbation of low-dose DIC-induced lesions by antisecretory drugs, probably by compensating for a decrease in barrier function caused by NSAIDs. From these results, it is suggested that mucus plays at least a partial role in the protective effects of MIS, IRS, and REB on the intestinal mucosa.

To reduce the adverse effects of NSAIDs on the GI tract without affecting their pharmacological activities, novel GI-sparing NSAIDs have been developed, that is, NSAIDs chemically coupled with mucosal protective moieties such as nitric oxide and hydrogen sulfide (Wallace and Del Soldato, 2003; Wallace et al., 2010), and formation of an NSAID mixed with phosphatidylcholine, a mucosal protective moiety (Lichtenberger et al., 2009; Cryer et al., 2011). In addition to these promising developments, in the present study, we found that both IRS and REB as well as MIS could prevent the intestinal lesions caused by DIC with or without antisecretory drugs. Furthermore, several recent pilot studies using CE have revealed that both IRS (Kuramoto et al., 2013) and REB (Niwa et al., 2008; Mizukami et al., 2012), as well as MIS (Watanabe et al., 2008; Fujimori et al., 2009) are effective in limiting the small intestinal side effects of aspirin and other NSAIDs, including DIC. Taking all the present findings and the clinical findings in healthy volunteers together, it is concluded that both IRS and REB could be used as new candidate drugs for the treatment of intestinal lesions induced by NSAIDs with and without antisecretory drugs.

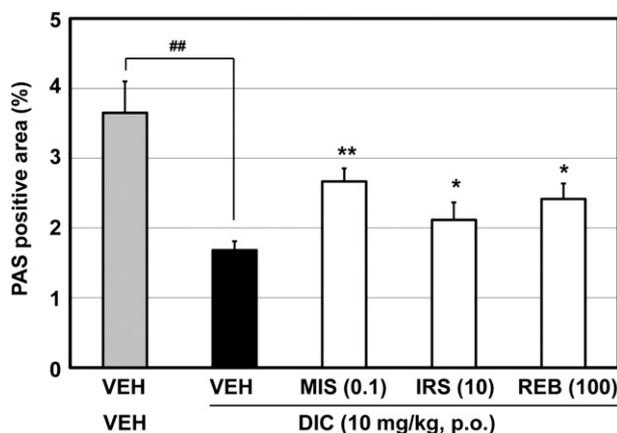


Fig. 8. Effect of MPAs on the distribution of PAS-positive substance in the ileal mucosa in rats (ImageJ analysis). Data represent the mean \pm S.E.M. values (percentage of PAS-positive area, $n = 6$). * $P < 0.05$; ** $P < 0.01$ versus VEH (Dunnett's test); ## $P < 0.01$ (Student's t test). The dose (milligrams per kilogram) is listed in parentheses.

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Authorship Contributions

Participated in research design: Satoh, Takeuchi.

Conducted experiments: Satoh, Amagase.

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Wrote or contributed to the writing of the manuscript: Satoh, Takeuchi.

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