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1. **Title page.**

Title:

Exacerbation of NSAID-induced small intestinal lesions by anti-secretory drugs in rats: The role of intestinal motility

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2. Rnnning title page.

a) Ranning title:

Exacerbation of intestinal ulcer by anti-secretory drugs

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the Discussion: 1,335 words

d) A list of nonstandard abbreviations used in this paper:

ACh, acetylcholine; ATR, atropine; CAP, capsaicin; CIM, cimetidine; CSSN, capsaicin sensitive sensory neuron; DIC, diclofenac; FAM, famotidine; H_2-RA, histamine H_2-receptor antagonist; IND, indomethacin; LI, lesion index; L-NAME, N-nitro-L-arginine methyl ester; LPZ, lansoprazole; NSAID, nonsteroidal anti-inflammatory drug; $NO^{\bullet}$, nitric oxide; OPZ, omeprazole; PPI, proton pump inhibitor; RPZ, rabeprazole; RAN, ranitidine; SNP, sodium nitroprusside; VEH, vehicle

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ABSTRACT

Anti-secretory drugs such as histamine H_2-receptor antagonists (H_2-RAs) and proton pump inhibitors (PPIs) are commonly used for the treatment of gastric and duodenal ulcers induced by NSAIDs. However, the effects of these drugs on NSAID-induced small intestinal ulcers are not fully understood. The effects of H_2-RAs and PPIs on NSAID-induced gastrointestinal lesions and small intestinal motility were examined in rats. Male Wistar rats (180-220 g) were used. Indomethacin (10 mg/kg) was administered orally in fasted or fed rats, and gastrointestinal lesions were examined 24 h after indomethacin. Intestinal motility was measured using a balloon method under urethane anesthesia. Indomethacin produced multiple lesions in the gastric corpus in fasted rats and in the small intestine in fed rats. 1) H_2-RAs (cimetidine, ranitidine, and famotidine) and PPIs (omeprazole, lansoprazole and rabeprazole) markedly inhibited the formation of gastric lesions. 2) The drugs, except for lansoprazole, increased intestinal lesions. 3) H_2-RAs augmented the increase in intestinal motility caused by indomethacin, and the effects of H_2-RAs on motility and intestinal lesions were markedly inhibited by atropine. 4) Lansoprazole inhibited the formation of intestinal lesions, and the effect was prevented both by pharmacological ablation of capsaicin-sensitive sensory neurons and pretreatment with L-NAME, a selective inhibitor of NO synthesis. The results suggest that 1) inhibition of acid secretion by anti-secretory drugs may exacerbate NSAID-induced intestinal lesions, 2) H_2-RAs further

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aggravate lesions by increasing intestinal motility via activation of cholinergic pathways, and 3) lansoprazole protects the intestinal mucosa against NSAID-related ulcerative stimuli.

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Introduction

Recent progress in endoscopic techniques, such as capsule endoscopy 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 small intestine in humans (Graham et al.., 2005; Maiden et al., 2005; Goldstein et al., 2005; Matsumoto et al., 2008). Anti-secretory drugs such as histamine H_2-receptor antagonists (H_2-RAs) and proton pump inhibitors (PPIs) are commonly used for the treatment of upper gastrointestinal mucosal lesions induced by NSAIDs (Leandro et al., 2001; Peura 2004; Yeomans et al. 2006; Scarpignato and Hunt 2010; Sugano et al., 2011). However, the effects of the drugs on small intestinal ulcers are not fully understood. We previously reported that cimetidine (CIM) did not prevent but rather aggravated small intestinal lesions induced by indomethacin (IND) in cats despite a marked inhibition of the duodenal lesions (Satoh et al., 2009). Wallace et al. (2011) recently reported that repeated treatment with PPIs for 9 days exacerbated small intestinal lesions induced by NSAIDs in rats.

It is important to elucidate the effect of anti-secretory drugs on NSAID-induced intestinal lesions, because many patients take H_2-RAs or PPIs to prevent upper gastrointestinal side effects associated with NSAID use. Therefore, in the present study, we examined the effects of

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typical H_2-RAs [CIM, ranitidine (RAN) and famotidine (FAM)] and PPIs [omeprazole (OPZ), lansoprazole (LPZ) and rabeprazole (RPZ)] on NSAID-induced gastrointestinal lesions in rats, and found that the anti-secretory drugs, except for LPZ, exacerbated intestinal lesions, though all the drugs markedly inhibited the formation of gastric lesions. The possible mechanism of the exacerbation by anti-secretory drugs was examined in relation to the effect of the drugs on intestinal motility.

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Materials and Methods

Ethics approval. Experimental protocols were approved by the Animal Research Committees in Kyoto Pharmaceutical University, Kyoto, Japan.

Animals. Seven-week old male Wistar rats (Shimizu Laboratories, Shizuoka, Japan) weighing 180-220g were used. The animals were given rat Chow pellets (MF, 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: acetylcholine (ACh), aminophyline, atropine (ATR), capsaicin (CAP), carboxymethylcellulose, cimetidine, diclofenac (DIC), dimetylsulfoxide, famotidine, Freund's complete adjuvant (killed *M. tuberculosis,* H37Ra), indomethacin, lansoprazole (Wako, Osaka, Japan), N-nitro-L-arginine methyl ester (L-NAME, Sigma, St. Louis, Mo, USA), omeprazole (Wako), prostaglandin E_2 (PGE_2, Ono, Osaka, Japan), rabeprazole sodium (Bosche Scientific, New Brunswick, NJ, USA), ranitidine, $NaHCO_3$ (Wako), sodium nitroprusside (SNP, Sigma), terbutaline (Sigma), Tween 80, and urethane (Wako). Drugs for oral or subcutaneous administration were suspended in a 1% carboxymethylcellulose solution. PPIs were suspended in 1% carboxymethylcellulose containing 1% $NaHCO_3$ for oral administration. H_2-RAs for i.v. injection were dissolved in saline containing 10%

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dimethylsulfoxide. The drugs were prepared just prior to the experiments and administered in a volume of 0.2 ml/100 g body wt.

Induction of gastric lesions. IND (10 mg/kg) was administered orally after a 16h-fast in normal rats. Anti-secretory drugs were given orally; PPIs were administered once (30 min before IND) and H_2-RAs were administered twice (30 min before and 6 h after IND). The animals were sacrificed by ether overdose 24 h after IND administration. The stomachs were removed, filled with 10 ml of 1% formalin solution and then immersed in the same formalin solution for 15 min. The stomachs were opened along the greater curvature. The length (mm) of the individual lesions in the corpus was measured under a dissecting microscope with a 1-mm square grid eyepiece (x 10), and the sum of the lengths of all the lesions in each stomach was used as the lesion index (LI). The person measuring the lesions did not know the treatments given to the animals.

Induction of small intestinal lesions. Rats were given regular chow pellets (CE-2, Japan Clea, Osaka, Japan). IND (10 mg/kg) or DIC (10 mg/kg) was administered p.o. in unfasted rats. Anti-secretory drugs were administered and the animals sacrificed according to the same schedule as for induction of gastric lesions. The small intestines were spread out on paper, opened along the anti-mesenteric side, and the contents removed. The length (mm) of the individual lesions was measured under a dissecting microscope with a 1-mm square grid

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eyepiece (x 10), and the sum of the lengths of all the lesions in each intestine was used as the LI.

Induction of arthritis by adjuvant. Arthritis in rats was induced by the method described in our previous paper (Kato et al., 2007). Briefly, 50 μl of Freund's complete adjuvant (10 mg/ml of killed *M. tuberculosis,* suspended in paraffin oil) was injected into the plantar region of the right hindpaw under ether anesthesia, and 2 weeks later the animals were studied.

Pharmacological ablation of capsaicin-sensitive sensory neurons (CSSN). Pharmacological ablation of CSSN was performed according to the method described in our previous paper (Takeuchi et al., 1991). CAP was dissolved in saline containing 10% ethanol and 10% Tween 80. Rats received subcutaneous injections of CAP (20, 30 and 50 mg/kg, s.c.) under ether anesthesia for 3 consecutive days 2 weeks before the experiment. The animals were treated twice with terbutaline (0.1 mg/kg, i.m.) and aminophyllin (20 mg/kg, i.m.) 30 min before and just after CAP injection to prevent the respiratory impairment associated with CAP injection. The functional ablation of CSSN was confirmed 2 weeks after CAP treatment by the lack of protective wiping movements after the instillation of a drop of CAP (0.1 mg/ml) into 1 eye.

Determination of the role of endogenous $NO^{\bullet}$. To examine the possible involvement of endogenous $NO^{\bullet}$ in the intestinal mucosal protective action of LPZ, 20 mg/kg of L-NAME (a selective inhibitor of $NO^{\bullet}$ synthesis) was administered s.c. 30 min before oral administration of LPZ, CAP, or PGE_2.

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Measurement of intestinal motility. Intestinal motility was determined using a miniature balloon according to the method described in our previous paper (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 about 10 cm oral to the ileocaecal junction. The volume in the balloon was adjusted to give an initial resting pressure of about 10-15 cm H_2O, which was not sufficient to cause active distension of the intestinal wall. After allowing the preparation to rest for 30 min, intestinal motility was monitored on a personal computer (Dynabook, Satellite A20, Toshiba, Tokyo, Japan) as intra-luminal pressure changes using a pressure transducer and a DC Strain Amplifier (AS2101, NEC, Tokyo, Japan). After basal motor activity had stabilized (≥1 h after the operation), the effects of the drugs were examined. The area of contraction (area under the curve) of the small intestine was measured every 30 min using a Power Lab System 4/25 (AD Instruments, Nagoya, Japan) and a computer software program (Chart & Scope, AD Instruments), and was expressed as cm H_2O/30 min.

Statistics. All data are expressed as mean ± SEM. Differences between groups were analysed by Student's *t*-test for paired group comparisons, or analysis of variance (Dunnett's multiple range test) if more than 2 variables were considered, with the significance level set at

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$p<0.05$.

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Results

Effect of drugs on IND-induced gastric lesions. In fasted rats, IND (10 mg/kg, p.o.) produced multiple linear lesions in the corpus mucosa at 24 h post-treatment.

Effect of H_2-RAs. Effect of CIM: The LI in the vehicle (VEH)-treated group was 25.6 ± 5.3 mm (n=7). The LIs were decreased by pretreatment with CIM (30 - 300 mg/kg, p.o., x2), dose-dependently (Table 1, Exp. 1). The effects at doses of 30 mg/kg or more were significant ($p<0.05$ or 0.01).

Effects of RAN and FAM: The LI in the VEH-treated group was 20.0 ± 3.0 mm (n=7). Both RAN (3 - 30 mg/kg, p.o., x2) and FAM (1 - 10 mg/kg, p.o., x2) inhibited the formation of gastric lesions dose-dependently (Table 1, Exp. 2). The effects at doses greater than 10 mg/kg (RAN) and 3 mg/kg (FAM) were significant ($p<0.05$ or 0.01).

Effect of PPIs. Effect of LPZ: The LI in the VEH-treated group was 25.9 ± 5.9 mm (n=7). LPZ (3 - 30 mg/kg, p.o.) decreased the LI in a dose-dependent manner (Table 2, Exp. 1). The effects were significant at doses of 10 mg/kg or higher ($p< 0.01$).

Effects of OPZ and RPZ: The LI in the VEH-treated group was 17.3 ± 2.3 mm (n=7). Both OPZ (10 - 100 mg/kg, p.o.) and RPZ (10 - 100 mg/kg, p.o.) inhibited the formation of gastric lesions, and the maximum effects were observed at 30 mg/kg with each drug. The effects at

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doses of 30 mg/kg and 100 mg/kg were significant ($p<0.05$ or 0.01) (Table 2, Exp. 2).

Effect of drugs on IND-induced intestinal lesions. In fed rats, IND (10 mg/kg, p.o.) produced multiple lesions in the middle and lower parts of the small intestine at 24 h post-treatment (Fig. 1A).

Effect of H_2-RAs. Effect of CIM: The LI in the VEH-treated group was 91.7 ± 14.3 mm (n=7). The LIs were increased by pretreatment with CIM (30 - 300 mg/kg, p.o., x2), dose-dependently (Fig. 2, Exp. 1). The effects at doses of 100 mg/kg and 300 mg/kg were significant ($p<0.05$ and 0.01).

Effects of RAN and FAM: The LI in the VEH-treated group was 80.2 ± 12.1 mm (n=7). Both RAN (3 - 30 mg/kg, p.o., x2) and FAM (1 - 10 mg/kg, p.o., x2) increased the formation of intestinal lesions dose-dependently (Fig. 2, Exp. 2). The effects at doses of 10 mg/kg or more were significant ($p<0.05$ or 0.01).

Effect of PPIs. Effect of LPZ: The LI in the VEH-treated group was 110.0 ± 18.0 mm (n=7). LPZ (3 - 30 mg/kg, p.o.) decreased the LI in a dose-dependent manner, and the effect at 30 mg/kg was significant ($p<0.01$) (Fig. 3, Exp. 1). The effect of s.c. LPZ on intestinal lesions was examined. The LI in the VEH-treated group was 146.1 ± 16.3 mm (n=7). LPZ at doses of 3, 10 and 30 mg/kg significantly ($p<0.01$) decreased the LI by 40%, 65% and 57% (n=7), respectively.

Effects of OPZ and RPZ: The LI in the VEH-treated group was 123.6 ± 8.0 mm (n=7). Neither

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OPZ (10 and 30 mg/kg, p.o.) nor RPZ (10 and 30 mg/kg, p.o.) affected the formation of intestinal lesions, but the drugs at a dose of 100 mg/kg increased lesion formation significantly ($p<0.01$ and 0.05, respectively) (Fig. 3, Exp. 2).

Effect of drugs on DIC-induced intestinal lesions. In fed rats, DIC (10 mg/kg, p.o.) produced multiple lesions in the middle and lower parts of the small intestine at 24 h post-treatment (Fig. 1B).

Effect of H_2-RAs: The LI in the VEH-treated group was 195.7 ± 8.5 mm (n=7). The LIs were increased by pretreatment with CIM (100 mg/kg, p.o., x2), RAN (30 mg/kg, p.o., x2), and FAM (10 mg/kg, p.o., x2). The effects of RAN and FAM were significant ($p<0.01$ and 0.05, respectively) (Table 3, Exp. 1).

Effect of PPIs: The LI in the VEH-treated group was 232.3 ± 20.2 mm (n=7). LPZ (30 mg/kg, p.o.) decreased the LI significantly ($p<0.01$). Whereas OPZ and RPZ at a dose of 100 mg/kg, p.o. mildly increased lesion formation, and the effect of RPZ was significant ($p<0.05$) (Table 3, Exp. 2).

Effect of drugs on IND-induced intestinal lesions in arthritic rats. Arthritis markedly increased IND-induced intestinal lesions.

Effect of H_2-RAs: The LI in the VEH-treated group was 178.6 ± 17.9 mm (n=7). CIM (100 mg/kg, p.o., x2), RAN (30 mg/kg, p.o., x2), and FAM (10 mg/kg, p.o., x2) increased the LI by 63%

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(p<0.01), 54%**,** and 41%, respectively (Table 4, Exp. 1)**.**

Effect of PPIs: The LI in the VEH-treated group was 248.9 ± 18.5 mm (n=7). LPZ (30 mg/kg, p.o.) decreased the LI significantly (p<0.01) by 43%, but neither OPZ (100 mg/kg, p.o.) nor RPZ (100 mg/kg, p.o.) affected the formation of lesions (Table 4, Exp. 2).

Effect of ATR on the RAN-induced increase in small intestinal lesion formation caused by IND. The effect of ATR on IND-induced intestinal lesions in RAN-treated and –untreated rats was examined to elucidate the mechanism of lesion enhancement by RAN. ATR was administered orally 30 min before RAN (30 mg/kg, p.o., x2) or VEH. The LI in the VEH-treated group was 115.5 ± 9.2 mm (n=7). ATR (1-10 mg/kg, p.o., x2) decreased the LI in a dose-dependent manner, and the effects at 3 mg/kg and 10 mg/kg were significant (p<0.01) (Fig. 3). RAN (30 mg/kg, p.o., x2) significantly (p<0.01) increased the formation of intestinal lesions; i.e., the LI was 256.6 ± 15.1 mm (n=7). The increase was markedly prevented by pretreatment with ATR (1-10 mg/kg, p.o. x2) (Fig. 4).

Effect of H_2-RAs on motility of the small intestine. In urethane-anesthetized rats, the ileum usually did not show spontaneous strong contractions. IND (10 mg/kg, s.c.) gradually increased motility starting at about 1 h after post-treatment (Fig. 5). A similar motility-enhancing effect was also observed after the administration of DIC (10 mg/kg, s.c.) (data not shown). Injection of CIM (10 and 30 mg/kg, i.v.), RAN (3 and 10 mg/kg, i.v.), and FAM (3 and 10 mg/kg,

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i.v.) mildly increased ileal motility. When the H_2-RAs were administered i.v. 2 h after IND, they produced marked contractions of the small intestine (Fig. 5). Among the 3 H_2-RAs tested, RAN showed the most prominent effect. Furthermore, when RAN (10 mg/kg, i.v.) was administered 30 min before IND (10 mg/kg, s.c.), it markedly increased the motor-stimulating effect of IND (Fig. 6A). The motor indexes at 30 and 60 min after IND in the group treated with RAN were significantly ($p<0.05$) larger than those of the VEH-treated group (Fig. 6B). The increases in motility caused by treatment with IND with or without RAN were abolished by administration of ATR (10 mg/kg, i.v.) (Fig. 6). Both CIM (30 mg/kg, i.v.) and FAM (10 mg/kg, i.v.) also increased IND-induced motility, and the effect of CIM was significant ($p<0.05$) (Fig. 7).

To elucidate the motility-enhancing effect of the H_2-RAs, the effects of H_2-RAs on motility caused by ACh were examined. ACh (1, 3 and 10 mg/kg, s.c.) increased intestinal motility in a dose-dependent manner. CIM (30 mg/kg, i.v.), RAN (10 mg/kg, i.v.) and FAM (10 mg/kg, i.v.) all enhanced the increase in intestinal motility caused by ACh (3 mg/kg, s.c.) (Fig. 8A), and the effects of CIM and RAN were significant ($p<0.05$ and 0.01, respectively) (Fig. 8B).

Effect of pharmacological ablation of CSSN and L-NAME on the mucosal protective action of LPZ. In VEH-treated rats, IND (10 mg/kg, p.o.) caused multiple lesions in the small intestine; the LI was 95.9 ± 11.7 mm (n=7). LPZ (10 mg/kg, p.o.), CAP (10 mg/kg, p.o.), and PGE_2 (0.1 mg/kg, p.o.) significantly ($p<0.01$) inhibited the formation of intestinal lesions by 50%,

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63% and 63% (n=7), respectively (Fig. 9, Exp. 1). Denervation of CSSN markedly increased the formation of intestinal lesions induced by IND (10 mg/kg, p.o.); i.e., the LI in the VEH-treated group was 197.7 ± 32.5 mm (n=7) ($p<0.05$ vs. VEH in Exp. 1). The denervation almost completely abolished the inhibitory effects of LPZ and CAP, but the inhibitory effect of PGE_2 was still observed (Fig. 9, Exp. 2).

To examine the possible involvement of endogenous $NO^{\bullet}$ in the protective action of LPZ in the small intestine, 20 mg/kg of L-NAME (a selective inhibitor of $NO^{\bullet}$ synthesis) was administered s.c. 30 min before oral administration of LPZ, CAP, or PGE_2. Administration of L-NAME (20 mg/kg, s.c.) produced a marked increase in the formation of intestinal lesions induced by IND (10 mg/kg, p.o.); i.e., the LI in VEH-treated rats was 194.4 ± 14.8 mm (n=7) ($p<0.01$ vs. VEH in Exp. 1). The administration of L-NAME almost completely abolished the inhibitory effects of LPZ and capsaicin, but a significant effect of PGE_2 was still observed ($p<0.05$) (Fig. 9, Exp. 3). The effect of L-NAME on SNP ($NO^{\bullet}$-donor) induced inhibition of intestinal lesions caused by IND was examined. SNP (10 mg/kg, s.c.) significantly ($p<0.01$) inhibited the formation of intestinal lesions by 50% (n=7), and also decreased the lesions significantly ($p<0.05$) by 40.2% (n=7) after L-NAME treatment.

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DISCUSSION

In the present study we examined the effects of H_2-RAs (CIM, RAN and FAM) and PPIs (OPZ, LPZ and RPZ) on NSAID-induced gastrointestinal lesions in rats. Although all the drugs inhibited the formation of gastric lesions induced by IND in fasted rats, they showed differing effects on IND-induced small intestinal lesions in fed rats. That is, all of the H_2-RAs increased lesion formation in a dose-dependent manner at doses which inhibited gastric lesions, but LPZ decreased intestinal lesions dose dependently. OPZ and RPZ both increased lesion formation significantly at the high dose of 100 mg/kg. Similar effects of the drugs on the small intestine were observed when another NSAID, DIC, was used instead of IND. As DIC is commonly used in patients with arthritis and other diseases, the present results suggest a possibility that anti-secretory drugs may aggravate NSAID-induced intestinal lesions in humans. The effects of the drugs on intestinal lesions were further examined in rats with adjuvant-induced arthritis, because NSAIDs are often used in arthritic patients. Arthritis aggravated IND-induced small intestinal lesions as previously reported by us (Kato et al., 2007). In rats with adjuvant-induced arthritis, lesion formation was still increased by H_2-RAs. Together the results suggest that both H_2-RAs and high doses of OPZ and RPZ can exacerbate NSAID-induced intestinal lesions.

The question remains as to how anti-secretory drugs exacerbate NSAID-induced small

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intestinal lesions, in light of the fact that gastric acid does not seem to play any role in lesion formation. Though several different factors related to the pathogenesis of NSAID-induced intestinal ulcers have been suggested, we have proposed the importance of hyper motility of the small intestine and indigestive solid components of food such as cellulose in the formation of intestinal lesions induced by NSAIDs (Takeuchi et al., 2010; Satoh, 2010). We reported that IND increased intestinal motility in rats (Takeuchi et al., 2002) and in cats (Satoh et al., 2009), and decreased the mucus content of the small intestine in rats (Kunikata et al., 2002). Under conditions where the amount of surface mucus is decreased by NSAIDs, it is possible that indigestive 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, it is conceivable that strong inhibition of gastric acid secretion by drugs may exacerbate NSAID-induced intestinal lesions via increasing the amount of undigested solid components of food in the small intestine by dyspepsia in the stomach. Although there are no data that directly support this possibility in the present study, such a mechanism may explain why anti-secretory drugs exacerbate intestinal lesions induced by NSAIDs, especially at high doses; this in turn suggests that strong and sustained inhibition of gastric acid secretion may have a deleterious effect on NSAID-induced intestinal lesion. Though LPZ has strong anti-secretory activity (Satoh et al., 1989), it did not increase, but rather

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decreased, intestinal lesion formation. This finding may be explained by the protective action of LPZ on the intestinal mucosa, as we discuss in detail below.

The second possibility is that the drugs may affect intestinal motility and thereby exacerbate intestinal lesion formation. In the present study, we found that both IND and DIC increased intestinal motility in anesthetized rats. We examined the effects of H_2-RAs on intestinal motility and found that H_2-RAs markedly enhanced the motor-stimulating effect of IND. It has been reported that some H_2-RAs such as RAN and CIM have an inhibitory effect on acetylcholinesterase (Cheah et al., 1985; Aono et al., 1986) and stimulate intestinal motility (Ueki et al., 1993; Bortolotti et al., 1995). In the present study, H_2-RAs increased intestinal motility caused by exogenous ACh as well as by IND, and RAN showed the most prominent effect among the 3 H_2-RAs in accordance with their acetylcholinesterase-inhibitory activities (Cheah et al., 1985; Aono et al., 1986). As mentioned above, RAN increased IND-induced intestinal lesions, and the effects of RAN on motility and the formation of intestinal lesions were markedly prevented by treatment with ATR. These results indicate that H_2-RAs increase NSAID-induced intestinal lesions, at least in part, by increasing intestinal motility via activation of cholinergic pathways due to the inhibition of acetylcholinesterase.

It has been reported that Gram-negative enterobacteria such as *E. coli* play an important role in the formation of intestinal lesions induced by NSAIDs (Uejima et al., 1996; Reuter et a., 1997).

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Wallace et al. (2011) recently reported that PPIs (OPZ and LPZ) given to rats s.c. twice a day for 9 days increased the formation of small intestinal lesions induced by the NSAID naproxen given twice daily over the final 4 days of the experiment. They found that OPZ caused a significant reduction of Actinobacteria and *Bifidobacteria spp*. in the jejunum, and suggested that the PPIs exacerbate NSAID-induced intestinal injury through dysbiosis. Therefore, the third possibility is that H_2-RAs as well as PPIs may affect the growth of enterobacteria by inhibiting gastric acid secretion and thereby exacerbate small intestinal lesions. Although we did not examine the effects of anti-secretory drugs on the growth of intestinal microflora in the present study, it is questionable whether administration of PPIs and H_2-RAs once or twice would cause significant changes in the intestinal microflora within 24 h. Therefore, at present, it is difficult to draw conclusions on the role of enterobacteria in the effects of H_2-RAs and PPIs on NSAID-induced intestinal lesions observed in this study.

In contrast to the findings in sub-acute studies by Wallace et al. (2011), in the present studies LPZ did not increase, but rather decreased the formation of intestinal lesions induced by IND in rats. The difference in the results of the present study and those of Wallace et al. may be explained by the differences in experimental methods in both studies as described in the previous section; especially, the first administration of NSAID (naproxen) occurred after marked suppression of acid secretion had been achieved by repeated administration of the PPIs in the

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latter study. Kuroda et al. (2006) first reported that LPZ had an inhibitory effect on IND-induced small intestinal lesions in rats, and suggested that LPZ prevented intestinal lesions by anti-inflammatory and antioxidant mechanisms, because LPZ inhibited IND-induced increase of myeloperoxidase activity and thiobarbituric acid-reactive substance in the intestinal mucosa. Higuchi et al. (2009) and Yoda et al. (2010) reported that LPZ, but not OPZ, prevented IND-induced intestinal lesions in rats, and suggested that LPZ protected the intestinal mucosa by up-regulation of hemoxygenase-1 production in the mucosa. We previously reported that LPZ protects the gastric mucosa by a mechanism independent from its anti-secretory action, and that both CSSN and $NO^{\bullet}$ are involved in the protective mechanism of LPZ on the gastric mucosa (Murakami et al., 1996). We have also reported that both CSSN and $NO^{\bullet}$ play an important role in protecting the small intestinal mucosa against ulcerative stimuli triggered by NSAIDs (Mizoguchi et al., 2001; Kato et al., 2000, Tanaka et al., 2001). In the present study, the inhibitory effects of LPZ and CAP on IND-induced intestinal lesions were markedly abolished by pharmacological ablation of CSSN and pretreatment with L-NAME. On the other hand, the inhibitory effect of PGE_2 was still observed under these conditions, and SNP, an $NO^{\bullet}$ donor, decreased intestinal lesion formation significantly even after treatment with L-NAME. These results suggest that LPZ protect the intestinal mucosa against NSAID-induced ulcerative stimuli via mechanisms involving CSSN and $NO^{\bullet}$, as seen in the stomach.

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The present results suggest that 1) strong and sustained inhibition of gastric acid secretion by anti-secretory drugs may exacerbate the formation of NSAID-induced small intestinal lesions, 2) in addition to possessing anti-secretory activity, H_2-RAs may aggravate intestinal lesions by increasing intestinal motility via activation of cholinergic pathways, 3) LPZ can protect the intestinal mucosa, and both CSSN and $NO^{\bullet}$ may be involved in the protective mechanism. Lastly, it is concluded that anti-secretory drugs, with the exception of LPZ, are essentially inappropriate for the treatment of intestinal damage caused by NSAIDs.

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Authoship Contributions

Participated in research design: Satoh, Takeuchi

Conducted experiments: Satoh, Amagase

Performed data analysis: Satoh, Amagase

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

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Legends for Figures

Fig. 1. Macroscopic observation of small intestinal lesions caused by IND (A) and DIC (B) in rats. IND or DIC was administered orally at a dose of 10 mg/kg without fasting and intestinal lesions were observed 24 h later.

Fig. 2. Effect of H_2-RAs on small intestinal lesions induced by IND in rats. IND (10 mg/kg) was administered orally without fasting, and intestinal lesions were measured 24 h after IND administration. H_2-RAs were given orally twice, i.e., 30 min before and 6 h after IND. The lesion index was expressed as a percentage of the mean LI (mm) in VEH-treated group. Data represent mean ± SEM (n=7). *: $p<0.05$, **:$p<0.01$ vs. VEH (Dunnett's test)

Fig. 3. Effect of PPIs on small intestinal lesions induced by IND in rats. IND (10 mg/kg) was administered orally without fasting, and intestinal lesions were measured 24 h after IND administration. PPIs were given orally 30 min before IND. The lesion index was expressed as a percentage of the mean LI (mm) in the VEH-treated group. Data represent mean ± SEM (n=7). *: $p<0.05$, **:$p<0.01$ vs. VEH (Dunnett's test)

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Fig. 4. Effect of ATR on the enhancing effect of RAN on IND-induced small intestinal lesions. RAN (30 mg/kg, p.o.) was administered twice, i.e., 30 min before and 6 h after IND (10 mg/kg, p.o.). ATR (1-10 mg/kg, p.o.) was administered twice 30 min before RAN. Data represent mean ± SEM (n=7). *: $p<0.05$, **:$p<0.01$ vs. corresponding vehicle (Dunnett's test), $^{\#\#}$: $p<0.01$ (Student's *t*-test), N.S.: not significant, □: VEH,■: RAN.

Fig. 5. Effect of H_2-RAs on IND-induced intestinal motility in anesthetized rats. H_2-RAs were administered i.v. 2 h after IND (10 mg/kg, s.c.). The dose (mg/kg) is listed in parenthesis.

Fig. 6. Effect of RAN and ATR on IND-induced intestinal motility. (A) RAN (10 mg/kg) was administered i.v. 30 min before IND, and ATR (10 mg/kg) was administered 2 h after IND. (B) Effects of RAN and ATR on the motor index caused by IND (time-dependent changes). Data represent mean ± SEM values (n=7). $^{\#}$: $p<0.05$ vs. VEH + IND (Student's *t*-test). The dose (mg/kg) is listed in parenthesis. ○: VEH + IND, ●: RAN + IND

Fig. 7. Effects of H_2-RAs on the intestinal motor index caused by IND. The motor index was measured before (left) and 30-60 min after (right) IND. Data represent mean ± SEM (n=7). *: $p<0.05$ vs. VEH (Dunnett's test).

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Fig. 8. Effect of H_2-RAs on ACh-induced intestinal motility in anesthetized rats. (A) ACh (3 mg/kg) was administered s.c. twice at 90 min intervals, and H_2-RAs were administered i.v. 30 min before the second ACh injection. The dose (mg/kg) is listed in parenthesis. (B) Effect of H_2-RAs on changes in the motor index caused by ACh. The post-treatment motor index measured 0-30 min after ACh was expressed as a percentage of the initial ACh response. Data represent mean ± SEM (n=7). *: $p<0.05$, **:<0.01 vs. VEH (Dunnett's test).

Fig. 9. Effect of pharmacological ablation of CSSN and L-NAME on the inhibitory action of drugs against IND-induced small intestinal lesions. LPZ (L), CAP (C), and PGE_2 (P) were administered p.o. 30 min before IND (10 mg/kg, p.o.). Denervation of CSSN was performed 2 weeks prior, and L-NAME (20 mg/kg) was administered s.c. 30 min before each drug. Intestinal lesions were examined 24 h after IND. Data represent mean ± SEM (n=7). *: $p<0.05$, **: $p<0.01$ vs. VEH (Dunnett's test). $^{\#}$ and $^{\#\#}$ in Exp. 2 and 3: $p<0.05$ and <0.01 vs. VEH in Exp.1 (Control) (Student's t-test), respectively.

Footnotes.

a) Financial support: We do not have any financial support on this study.

We also do not have any conflicts of interest.

b) Gastroenterology, 2010,138, (suppl 1), s-498. (Abstract for DDW 2010)

Satoh H, Amagase K, Takeuchi K,: Effects of antisecretory drugs on indomethacin-induced small intestinal lesions in rats

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Table 1. Effect of H_2-RAs on gastric lesions induced by IND in rats. IND (10 mg/kg, p.o.) was administered after a 16 h fast, and gastric lesions were measured 24 h after IND administration. H_2-RAs were given orally twice, i.e., 30 min before and 6 h after IND. Data represent mean ± SEM.

Drug	Dose (mg/kg, p.o.)	No. of rat	Lesion index (mm)	Lesion index (% of VEH)
Exp.1				
VEH		7	25.6 ± 5.3	100.0 ± 20.7
CIM	30 x 2	7	12.1 ± 1.3*	47.4 ± 5.2
CIM	100 x 2	7	4.3 ± 1.7**	16.8 ± 6.6
CIM	300 x 2	7	1.9 ± 1.2**	7.4 ± 4.7
Exp.2				
VEH		7	20.3 ± 3.0	100.0 ± 14.8
RAN	3 x 2	7	19.1 ± 1.5	93.9 ± 7.4
RAN	10 x 2	7	10.6 ± 2.2*	52.0 ± 11.1
RAN	30 x 2	7	2.8 ± 0.9**	13.8 ± 4.4

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FAM	1 x 2	7	13.6 ± 2.5	47.4 ± 5.2
FAM	3 x 2	7	9.0 ± 1.8*	16.8 ± 6.6
FAM	10 x 2	7	2.0 ± 10.3**	7.4 ± 4.7

**: p<0.05, **: p<0.01 vs. VEH (Dunnett's test)*

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Table 2. Effect of PPIs on gastric lesions induced by IND in rats. IND (10 mg/kg, p.o.) was administered after a 16 h fast, and gastric lesions were measured 24 h after IND administration. PPIs were given orally 30 min before IND. Data represent mean ± SEM.

Drug	Dose (mg/kg, p.o.)	No. of rat	Lesion index (mm)	Lesion index (% of VEH)
Exp.1				
VEH		7	25.9 ± 5.9	100.0 ± 20.7
LPZ	3	7	12.5 ± 5.6**	48.1 ± 21.7
LPZ	10	7	4.6 ± 2.2**	17.9 ± 8.5
LPZ	30	7	3.7 ± 3.2**	14.2 ± 12.3
Exp.2				
VEH		7	17.3 ± 2.3	100.0 ± 13.2
OPZ	10	7	11.1 ± 1.9	76.4 ± 11.0
OPZ	30	7	3.8 ± 1.7**	22.2 ± 9.6
OPZ	100	7	6.6 ± 0.8**	38.0 ± 4.7
RPZ	10	7	17.4 ± 3.0	100.7 ± 17.6

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RPZ	30	7	7.6 ± 1.9*	44.1 ± 11.0
RPZ	100	7	8.3 ± 3.0*	47.8 ± 17.4

**: p<0.05, **: p<0.01 vs. VEH (Dunnett's test)*

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Table 3. Effect of anti-secretory drugs on small intestinal lesions induced by DIC in rats. DIC (10 mg/kg, p.o.) was administered without fasting. H_2-RAs were administered orally twice, i.e., 30 min before and 6 h after DIC. PPIs were administered orally 30 min before DIC. Intestinal lesions were measured 24 h after DIC administration. Data represent mean ± SEM (n=7).

Drug	Dose (mg/kg, p.o.)	No. of rat	Lesion index (mm)	Lesion index (% of VEH)
Exp.1				
VEH		7	195.7 ± 8.5	100.0 ± 4.3
CIM	100 x 2	7	236.7 ± 17.8	120.7 ± 9.1
RAN	30 x 2	7	311.7 ± 20.3**	159.2 ± 10.4
FAM	10 x 2	7	272.9 ± 15.6*	139.4 ± 8.0
Exp.2				
VEH		7	232.3 ± 20.2	100.0 ± 8.7
LPZ	30	7	121.6 ± 12.8**	52.4 ± 5.5
OPZ	100	7	279.7 ± 13.9	120.6 ± 6.0
RPZ	100	7	310.0 ± 27.9*	134.0 ± 12.0

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*: p<0.05, **: p<0.01 vs. VEH (Dunnett's test)

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Table 4. Effect of anti-secretory drugs on small intestinal lesions induced by IND in adjuvant-induced arthritic rats. Arthritis was induced by injection of 50 μl of Freund's complete adjuvant (killed *M. tuberculosis*) into the plantar region of the right hindpaw under ether anesthesia, and 2 weeks later the animals were studied. IND (10 mg/kg, p.o.) was administered without fasting. H_2-RAs were administered orally twice, i.e., 30 min before and 6 h after DIC. PPIs were administered orally 30 min before DIC. Intestinal lesions were measured 24 h after the administration of DIC. Data represent the mean ± SEM (n=7).

Drug	Dose (mg/kg, p.o.)	No. of rat	Lesion index (mm)	Lesion index (% of VEH)
Exp. 1				
VEH		7	178.6 ± 17.9	100.0 ± 10.0
CIM	100 x 2	7	291.6 ± 31.8*	163.2 ± 17.8
RAN	30 x 2	7	275.2 ± 29.3	154.1 ± 25.5
FAM	10 x 2	7	251.7 ± 25.5	140.9 ± 14.3
Exp. 2				
VEH		7	248.9 ± 18.5	100.0 ± 7.4

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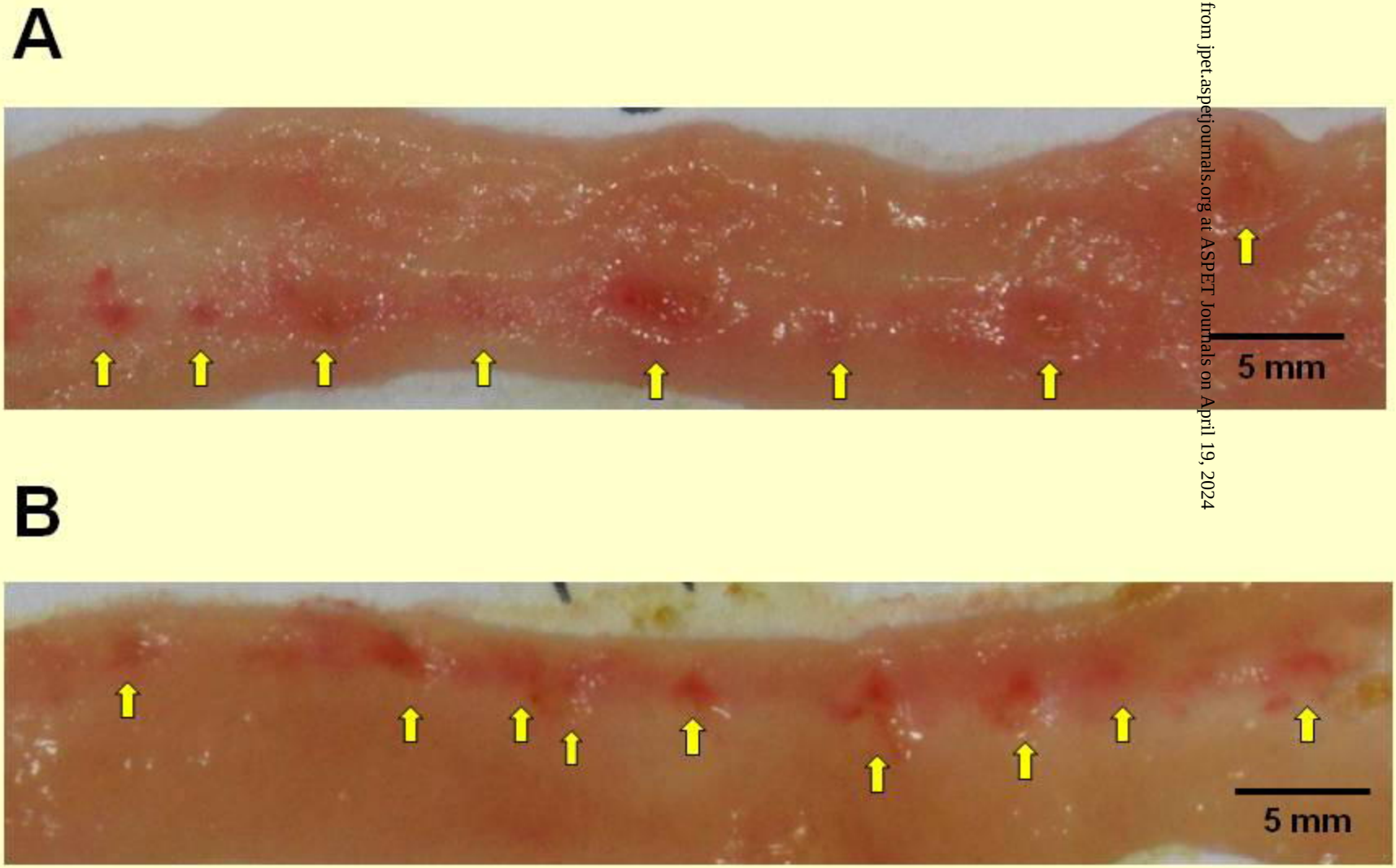
LPZ	30	7	140.7 ± 17.1**	56.5 ± 6.8
OPZ	100	7	222.1 ± 20.0	89.2 ± 8.0
RPZ	100	7	244.9 ± 28.7	98.3 ± 11.6

**: p<0.05, *:p<0.01 vs. VEH (Dunnett's test)*



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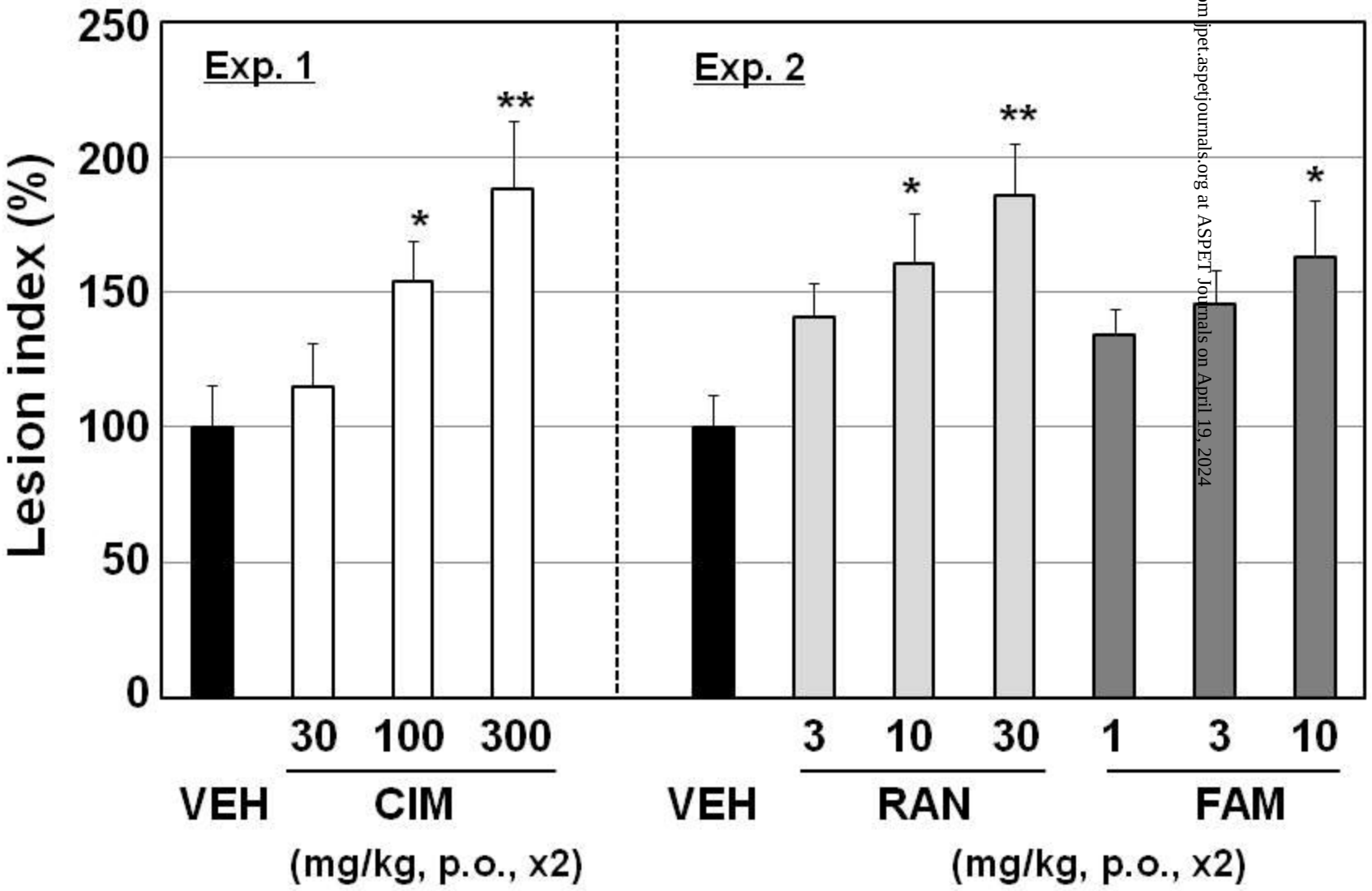

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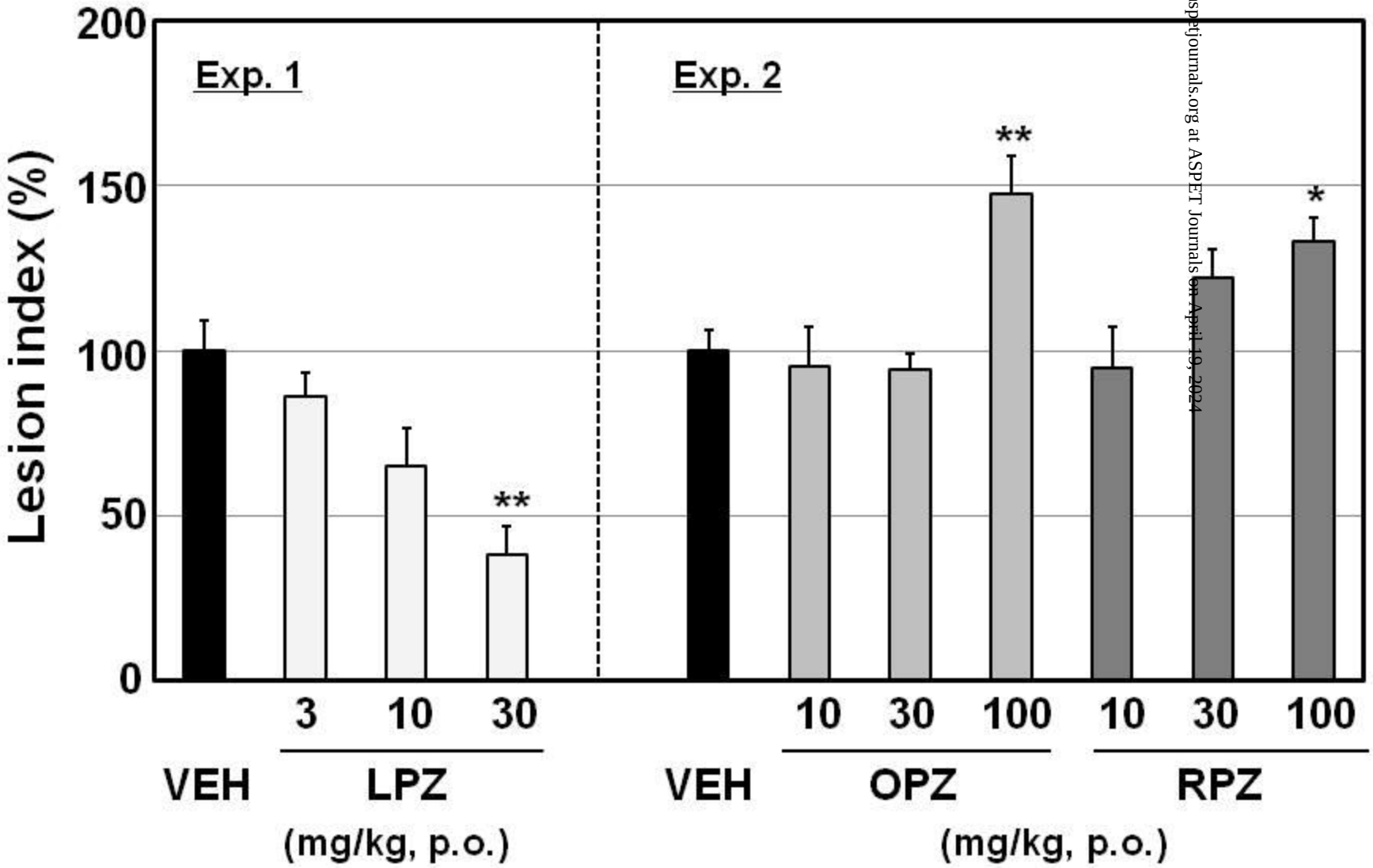

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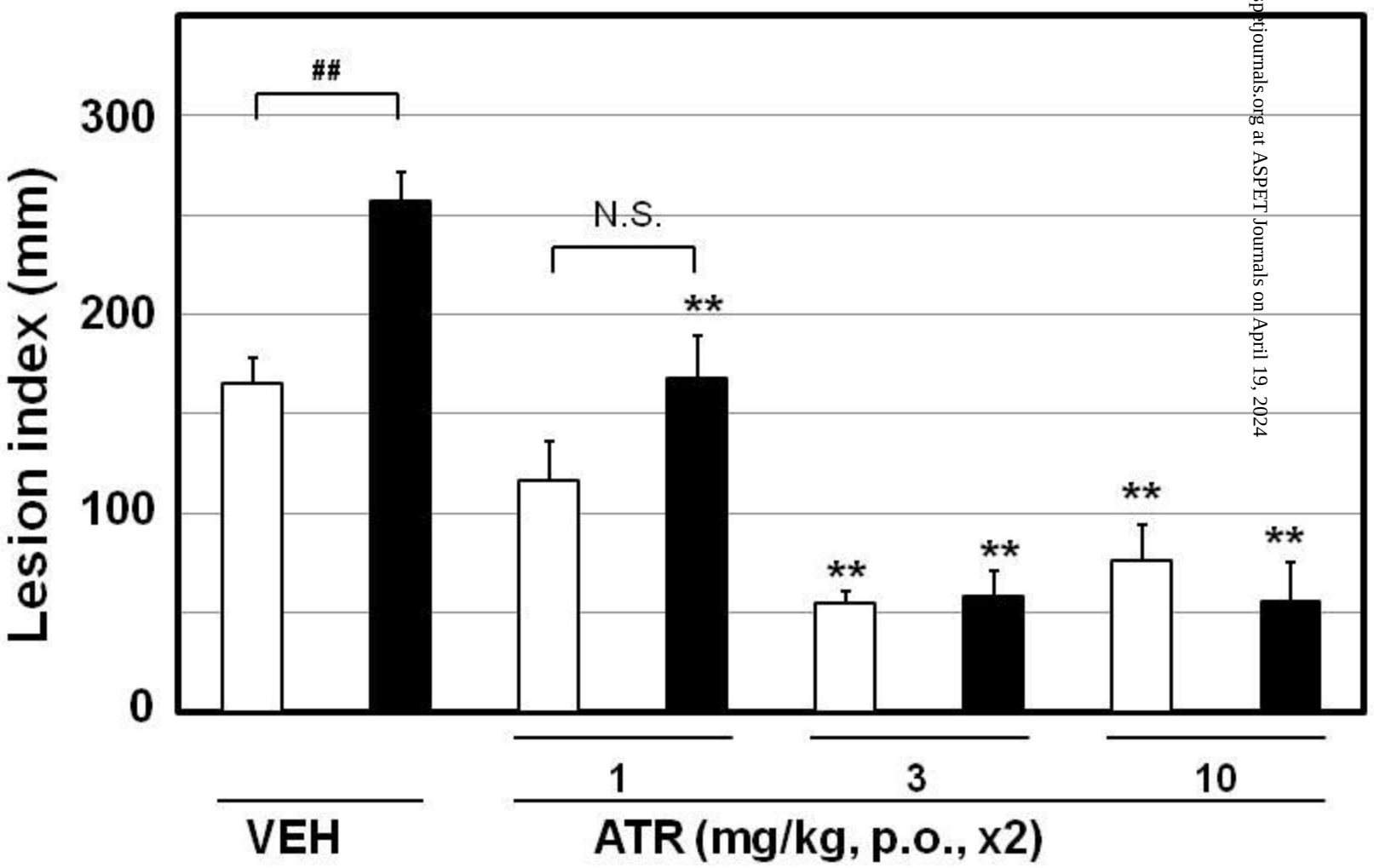

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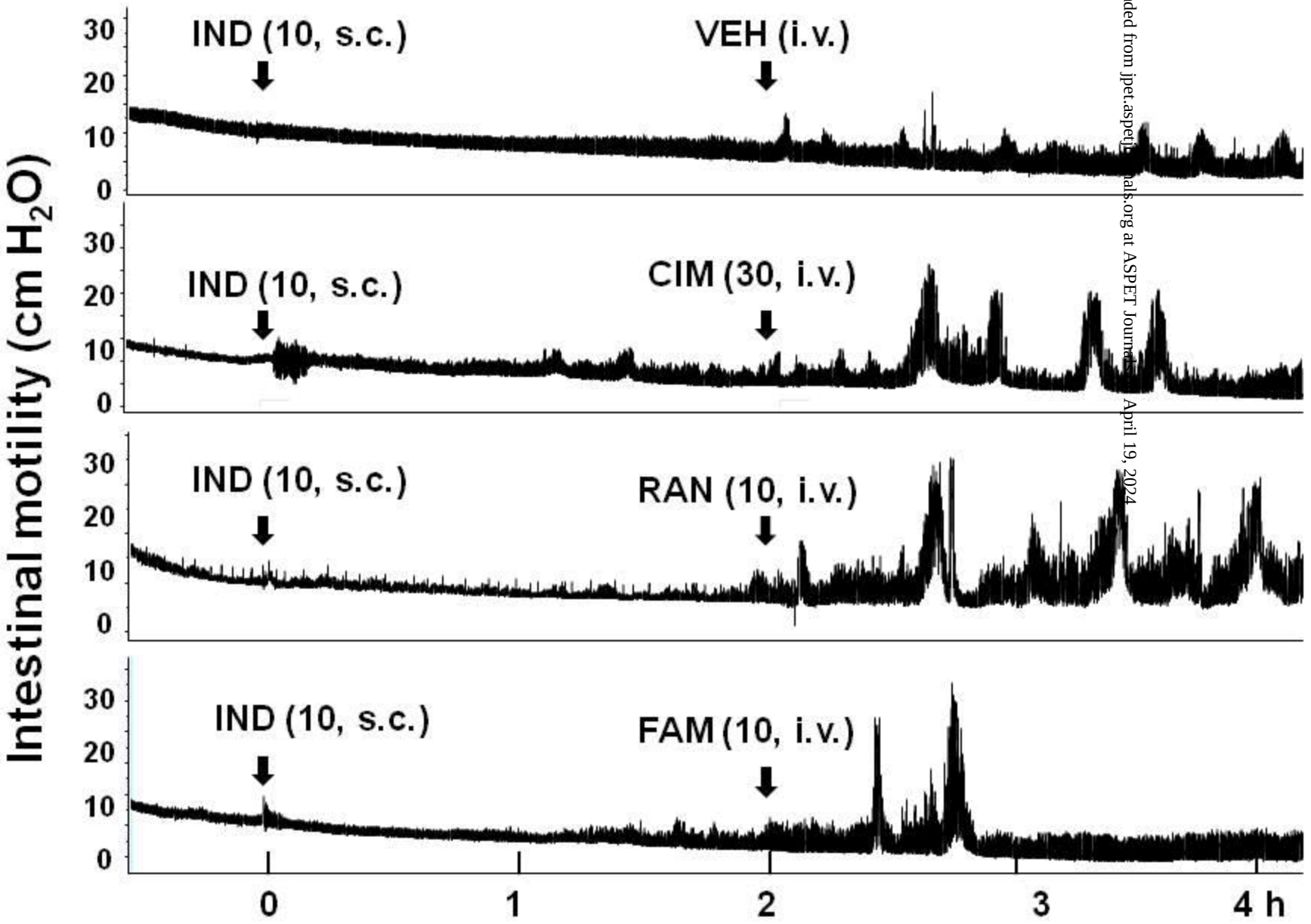
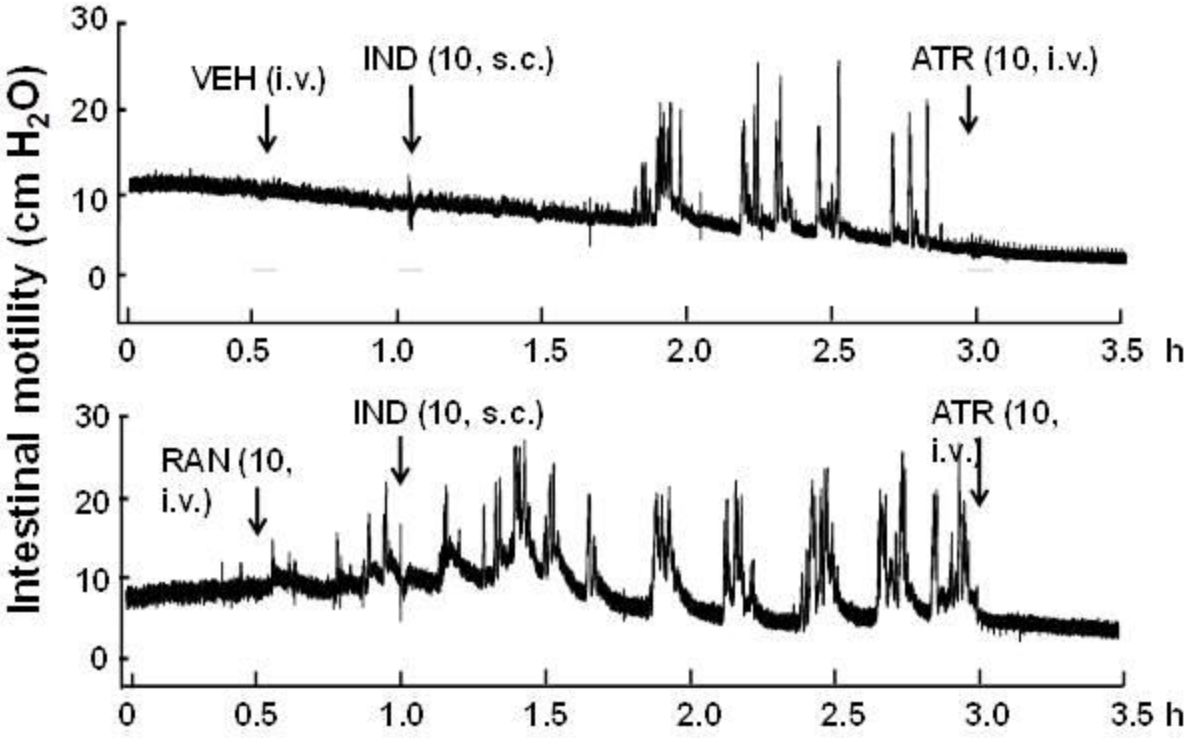
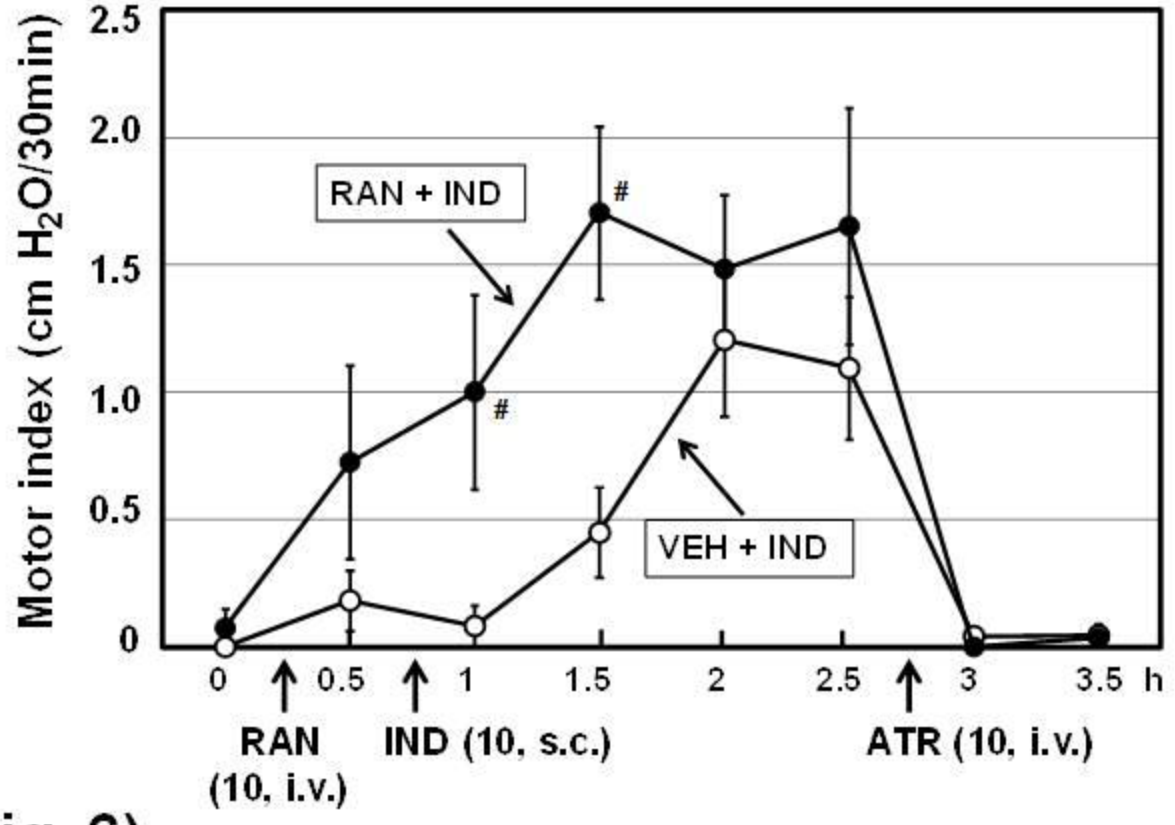


(Fig. 5.)

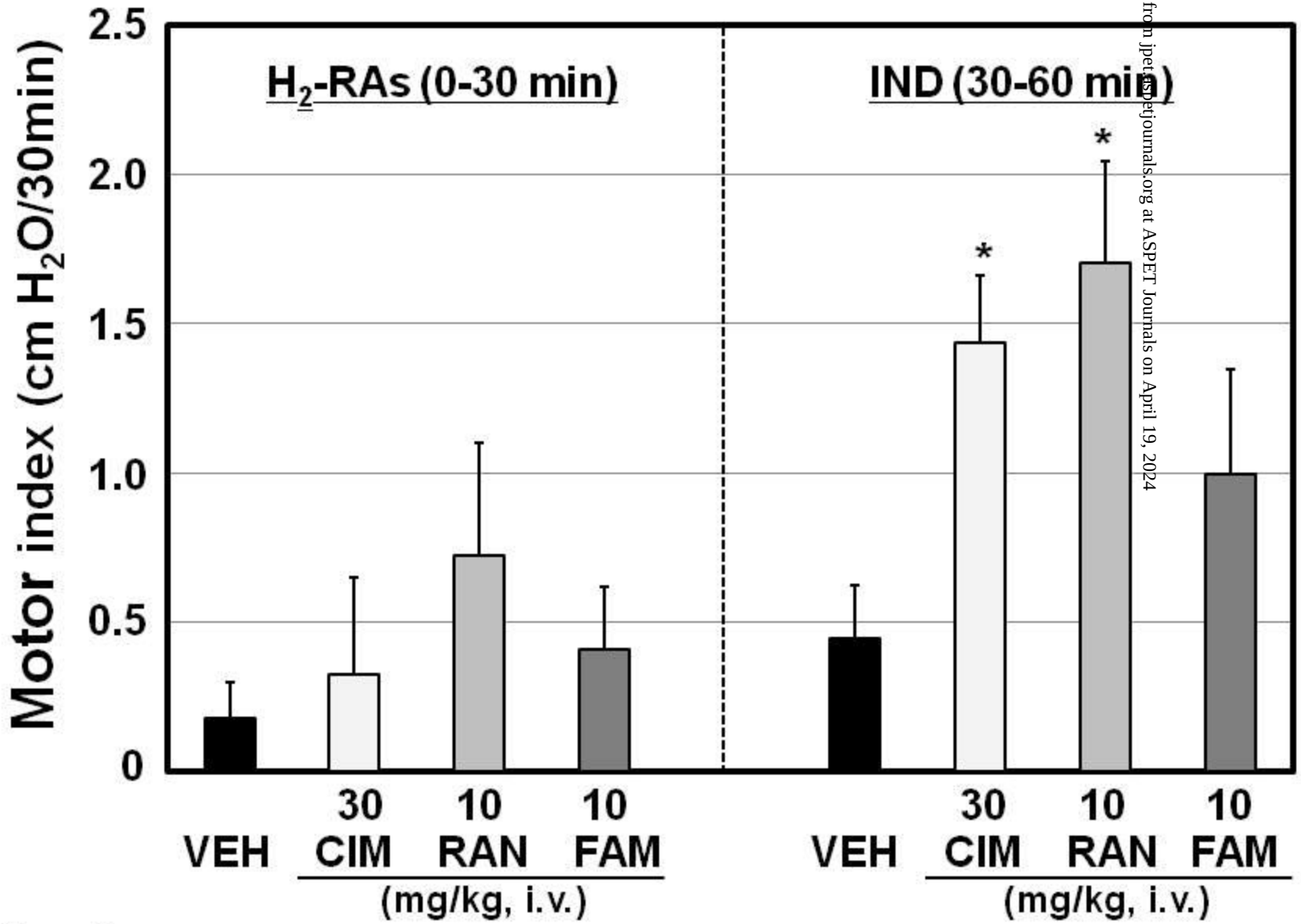
A.


Intestinal motility (cm H2O)
VEH (i.v.)
IND (10, s.c.)
ATR (10, i.v.)
RAN (10, i.v.)
IND (10, s.c.)
ATR (10, i.v.)
0
10
20
30
0
0.5
1.0
1.5
2.0
2.5
3.0
3.5 h

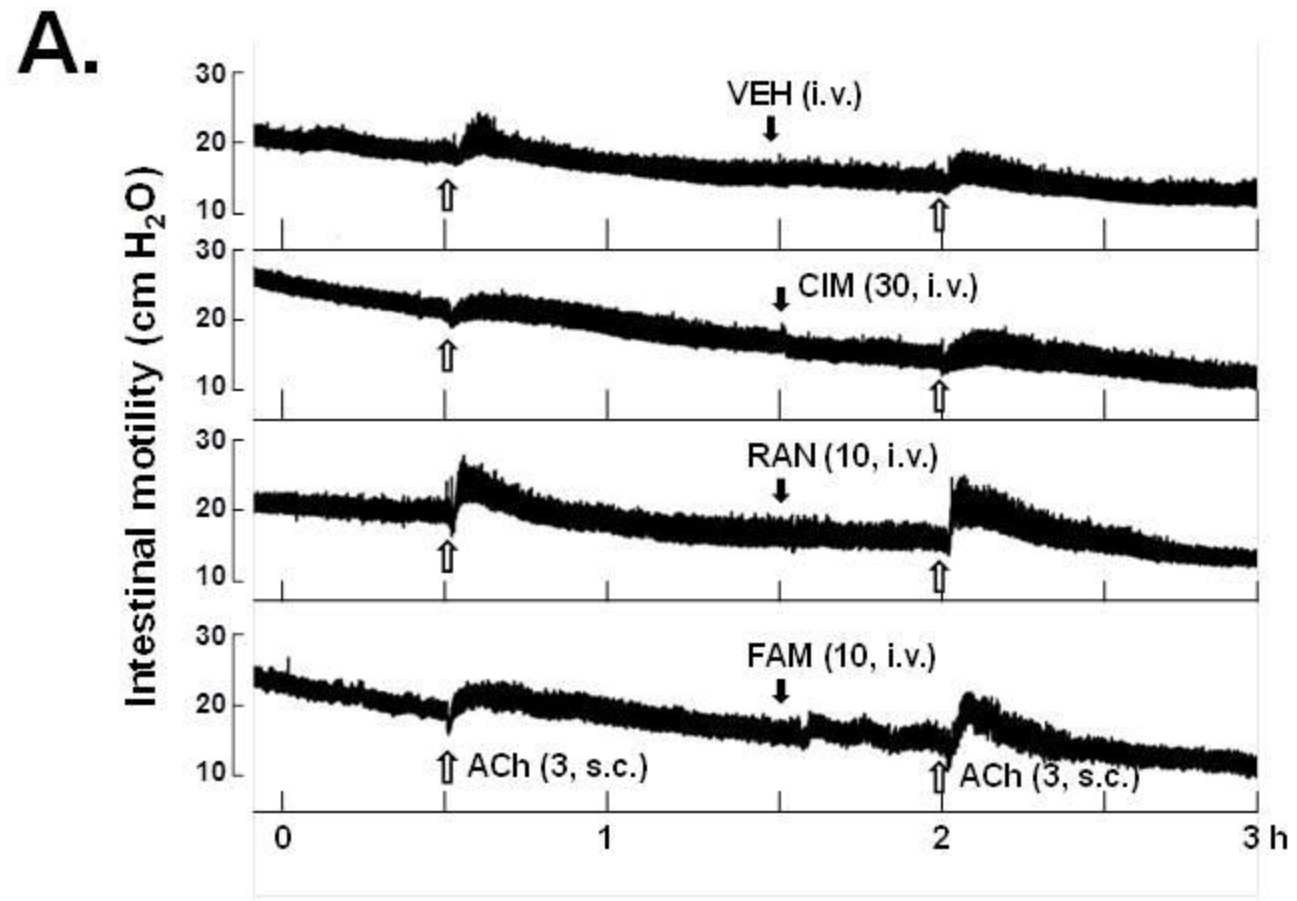
B.

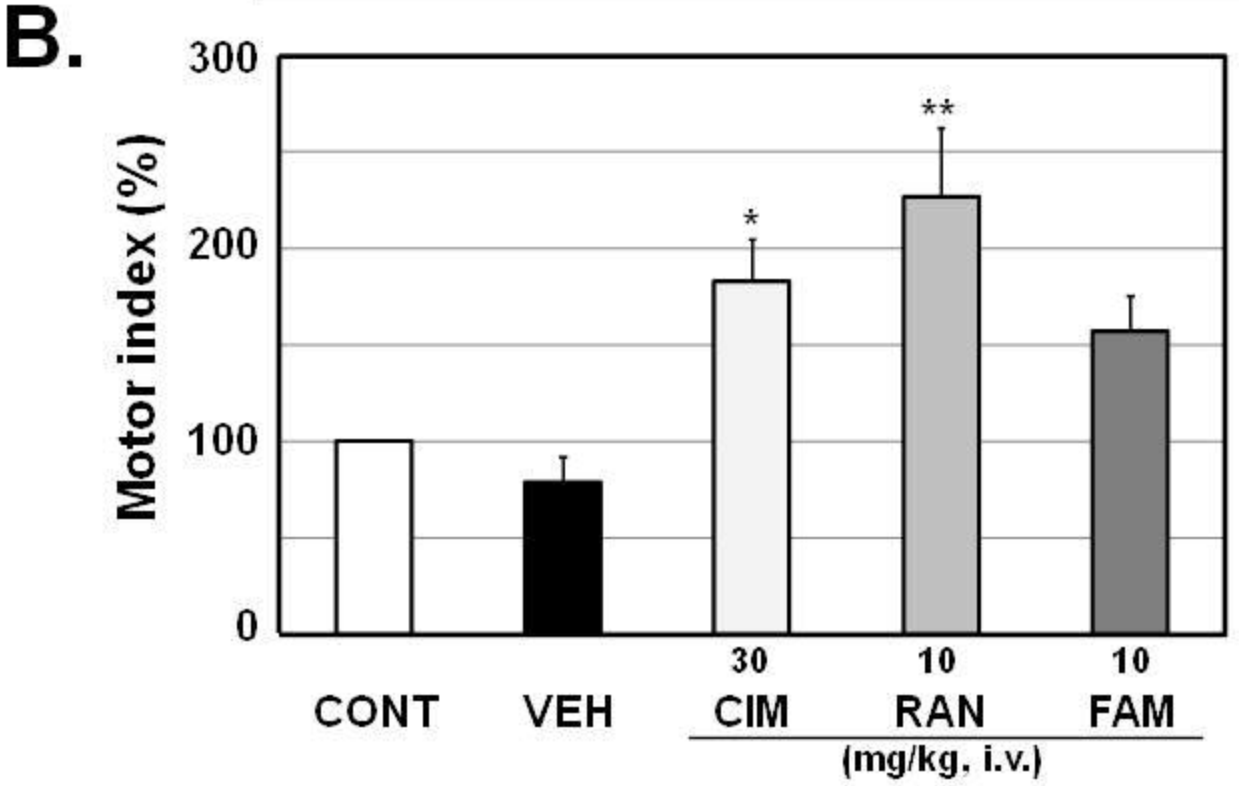

Motor index (cm H2O/30min)
RAN + IND
VEH + IND
#
#
0
0.5
1.0
1.5
2.0
2.5
0
0.5
1
1.5
2
2.5
3
3.5 h
RAN (10, i.v.)
IND (10, s.c.)
ATR (10, i.v.)

(Fig. 6)

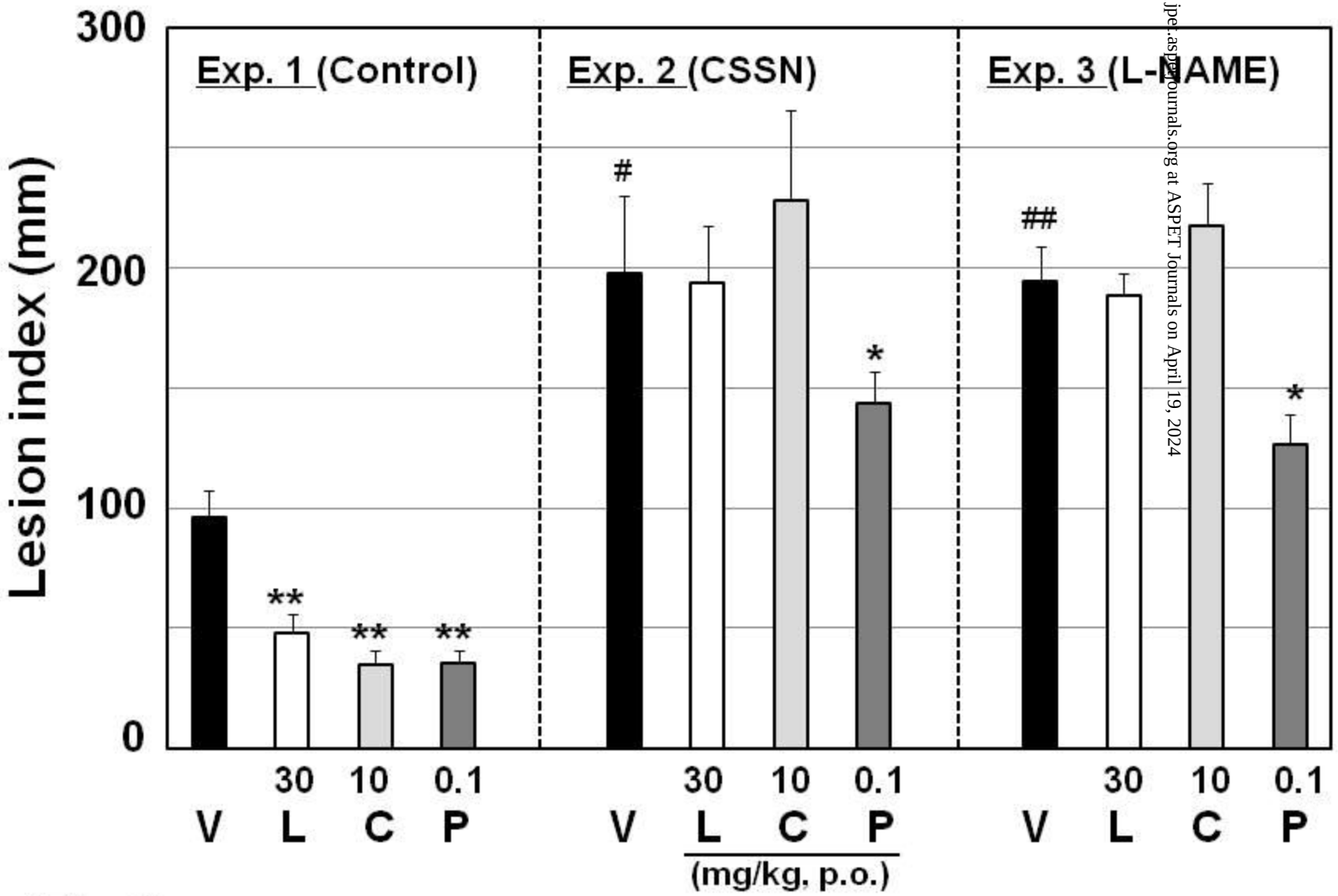


(Fig. 7)


A.
Intestinal motility (cm H_2O)
30
20
10
VEH (i.v.)
CIM (30, i.v.)
RAN (10, i.v.)
FAM (10, i.v.)
ACh (3, s.c.)
ACh (3, s.c.)
0
1
2
3 h


B.
Motor index (%)
300
200
100
0
*
**
CONT
VEH
30
CIM
10
RAN
10
FAM
(mg/kg, i.v.)

(Fig. 8)



(Fig. 9)