Comparison of the Effects of Pantoprazole and Its Enantiomers on Gastric Acid, Mucus, and Mucosa as Well as Endocrine Cells

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Pantoprazole is an excellent proton pump inhibitor for the treatment of acid-related lesions. In this study we compared the pharmacodynamic effects of (±)-pantoprazole sodium [(±)-PAN·Na] and its enantiomers on gastric acid secretion and their possible side effects on gastric mucus, mucosa, and gastric endocrine cells. (±)-PAN·Na, (−)-PAN·Na, and (+)-PAN·Na dose-dependently inhibited the secretion of basal gastric acid and histamine-induced gastric acid, and (–)-PAN·Na showed the most potent effect, which was confirmed in an *in vivo* experiment in rabbits and in an *in vitro* experiment using the stomachs of juvenile rats. On the other hand, (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na did not influence significantly the free and barrier mucus content in rats with short-term administration for 3 days. No obvious differences were observed in serum gastrin levels, volume densities of parietal cells, G cells and D cells, mucosal thickness, or relative stomach weight and body weight among the rats administered (–)-PAN·Na, (+)-PAN·Na, or (±)-PAN·Na for 40 days. Direct administration of (–)-PAN·Na can inhibit gastric acid secretion more effectively with no increase in side effects.

Key words —— proton pump inhibitor, pantoprazole, enantiomer, gastric acid, gastric mucus and mucosa, gastric endocrine cell

INTRODUCTION

Proton pump inhibitors are excellent pharmacologic agents for the treatment of acid-related lesions, including peptic ulceration, gastroesophageal reflux disease, and Zollinger-Ellison syndrome.[1] As a proton pump inhibitor, (±)-pantoprazole [(±)-5-(difluoromethoxy)-2-[[(3, 4-dimethoxy-2-pyridyl)-methyl] sulfinyl]-1H-benzimidazole] has been used clinically,[2] and its effectiveness and safety have been confirmed in the long-term treatment of patients with severe peptic ulcer and reflux disease.[3]

Molecular chirality is of great concern in terms of drug metabolism and pharmacologic mechanism.[4–6] Esomeprazole was the first proton pump inhibitor developed as an optical isomer of omeprazole and shows an improved pharmacokinetic profile, such as less interindividual variability and stronger activity to suppress gastric acid secretion than racemic omeprazole.[7–10] There are some reports on pharmacokinetic differences between pantoprazole and its enantiomers,[11–13] however, to date little information can be found on the pharmacodynamics of pantoprazole enantiomers.

We have compared the pharmacodynamic differences between pantoprazole sodium (PAN·Na) and its enantiomers in various models of gastric ulcers in rats and guinea pigs. (–)-PAN·Na shows stronger efficacy than (+)-PAN·Na and (±)-PAN·Na in preventing gastric mucosal lesions induced by water-immersion stress, aspirin, ethanol, reserpine, histamine, and pyloric ligation.[14, 15] We have also observed that (–)-PAN·Na is more potent than (+)-PAN·Na and (±)-PAN·Na in inhibiting acid secre-

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tion in adult rats with acute fistula.[15)]

Here, we present an extension of this work to compare the pharmacodynamics of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na on gastric acid secretion in an *in vivo* experiment in rabbits. In addition, the side effects of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na on gastric mucus and mucosa, serum gastrin levels, and volume density of gastric endocrine cells were also compared in rats.

MATERIALS AND METHODS

Reagents and Solutions —— Histamine was purchased from the Shanghai Biochemistry Institute (Shanghai, China). (±)-PAN·Na, (–)-PAN·Na, and (+)-PAN·Na were provided by Dr. Qing-He Wang, Department of Pharmaceutical Engineering, Shenyang Pharmaceutical University (Shenyang, China). The purity of (±)-PAN·Na was greater than 98%. The optical purity of (–)-PAN·Na and (+)-PAN·Na was 91.9% [$[\alpha]_D^{20} = -122°$ (c = 0.5, acetonitrile/methanol, 1:1)] and 91.3% [$[\alpha]_D^{20} = -120°$ (c = 0.5, acetonitrile/methanol, 1:1)], respectively. PAN·Na was dissolved in 0.9% saline before oral or intraperitoneal administration. In *in vitro* experiments, PAN·Na was initially dissolved in dimethyl sulfoxide (DMSO), and then diluted with a serosal solution to 1 mg/ml as a stock solution. The stock solutions were further diluted with the serosal solution to appropriate concentrations before use. The final concentration of DMSO on serosal side was less than 0.1%, which was confirmed to have no effect on acid secretion *per se*.

The serosal solution was composed of Na^+ (140.9 mM), K^+ (5.8 mM), Ca^{2+} (2.6 mM), Mg^{2+} (1.2 mM), Cl^- (125.8 mM), SO_4^- (1.2 mM), HCO_3^- (24.9 mM), $H_2PO_4^-$ (1.2 mM), and glucose (25 mM). A mucosal solution was prepared by modifying the serosal solution in which $NaHCO_3$ and KH_2PO_4were replaced with equimolar NaCl and KCl.

Animals —— Rabbits (1500–2500 g) and Sprague-Dawley (SD) rats (juvenile, 3–4 weeks, 30–40 g; adult, 160–220 g) of either sex were purchased from the Center of Animal Experiments, Shenyang Pharmaceutical University (Shenyang, China). The Animal Research Ethics Board of Shenyang Pharmaceutical University approved the experiments.

Collection of Gastric Juice of Rabbits and Determination of Gastric Acid Secretion —— Rabbits were fasted for 24 hr with free access to water before the experiments. Under anesthesia with urethane (0.5 mg/kg i.v.), the rabbits were fixed and a polyvinyl chloride catheter was inserted *via* the esophageal tract into the stomachs. Stomach contents were withdrawn and the gastric lumen was flushed with 0.9% saline at 36°C, and then 5 ml of 0.9% saline was injected into the gastric lumen. The perfusates were collected twice (as controls) at 30-min intervals preceding PAN·Na given intraperitoneally, and then the gastric juice was further collected 10 times at 30-min or 60-min intervals. Saline, equal to the volumes of gastric juice withdrawn (about 10 ml/hr), was administered following each collection.

Gastric acid was assayed with a pH meter (Hanna pH 312, Hanna, Italy) and a part of the specimens was titrated to pH 7 with 0.1 N NaOH. Gastric acid secretion was calculated by multiplying the H^+ concentration in a 1-ml sample by the collected volume of gastric juice per hour.

Determination of Histamine-Induced Gastric Acid Secretion in Isolated Stomachs of Juvenile Rats —— Gastric acid secretion in isolated stomachs was measured essentially as described by Black and Shankley.[16)] Juvenile rats were fasted for 24 hr with free access to water before the experiments. Under anesthesia with 2% pentobarbital sodium (0.1 g/kg i.p.), the stomach was exposed and the cardia ligated. One polyethylene catheter (diameter 2 mm) was inserted into the upper fundus for withdrawing the gastric contents and flushing the stomach lumen, and an additional catheter was inserted into the pylorus through the pyloric sphincter for collecting gastric juice. The stomachs were isolated and immersed in an organ bath containing 10 ml of serosal solution (36°C, pH 7.4) gassed with 95% O_2 and 5% CO_2. The stomachs were continuously perfused with unbuffered mucosal solution at 1 ml/min, which was gassed with 100% O_2 and maintained at an intragastric pressure of 12 cm H_2O. After 1 hr for stabilization of gastric acid secretion, PAN·Na and histamine were added into the organ bath (serosal side). The final concentration of histamine was gradually accumulated from 10^{-2} mM to 1 mM at 30-min intervals. The perfusate was continuously collected and kept on ice.

Determination of Free and Barrier Mucus Content in Adult Rats with Administration of PAN·Na for 3 Days —— After oral administration of PAN·Na for 2 days, the abdomen was opened under ether anesthesia, and the pylorus was ligated.

Before the abdomen was closed, PAN·Na was injected into duodenum for an additional administration. The rats were killed with an overdose of ether in 6 hr. The isolated stomach was opened along the greater curvature, and free mucus was collected.[17)]

The mucus content was estimated by measuring the amount of Alcian blue bound to a sample of the gastric contents.[18)] One milliliter of gastric juice was mixed with 3.3 ml of citric acid monohydrate phosphate buffer (pH 5.8) and 0.1 ml of 1% Alcian blue. After homogenization and adjustment to a total volume of 5 ml with distilled water, the mixture was incubated with shaking at 20°C for 24 hr and then centrifuged at 2500 rpm for 10 min. The Alcian blue concentration in the supernatant was estimated spectrophotometrically at 615 nm. The difference in the amounts of Alcian blue between the control and supernatant was the amount of Alcian blue bound to the insoluble gastric mucus.

The barrier mucus was estimated by incubating the everted stomach in 20 ml of buffer containing Alcian blue (20 mg/ml) for 2 hr, then removing the stomach, centrifuging the reaction mixture, and measuring the concentration of remaining Alcian blue in the supernatant.

Determination of Serum Gastrin Level, Volume Density of Gastric Endocrine Cells, and Mucosal Thickness as well as Relative Gastric Weight in Rats after Administration of PAN·Na for 40 Days —— Male SD rats were fed with free access to commercial rat food pellets and water. The rats were administered either PAN·Na or solvent solution (0.5% carboxymethyl cellulose sodium) *via* a gastric tube, twice daily for 40 days. Blood specimens were obtained *via* eye vein 12 hr after the last administration of PAN·Na and sera were separated. Gastrin levels in the sera were measured in triplicate with a radioimmunoassay kit according to the manufacture's instructions (North Beijing Biology Technical Research Institute, Beijing, China).

The rats were starved for 24 hr before exsanguination from the abdominal aorta under pentobarbital anesthesia. The isolated stomachs were cut along the greater curvature, rinsed with ice-cold saline, dried with filter paper, and weighed. The relative gastric weight was calculated based on weight per kg body weight.

The volume densities of G cells and D cells in gastric mucosa were determined on paraformaldehyde-fixed, paraffin-embedded sections (4 μm) by immunohistochemical staining. Following incubation with the primary antibodies, a rabbit polycolonal antibody against gastrin (RAB-0080, Maixin-Bio, Fuzhou, China) or a rabbit polycolonal antibody against somatostatin (RAB-0153, Maixin-Bio), the biotinylated secondary antibody conjugated with streptavidin-peroxidase (UltraSensitive^{TM} S-P Kit, Maixin-Bio) was applied for visualization. The color was developed with the 3,3′diaminobenzidine (DAB) chromogen, and sections were counterstained with hematoxylin.

To detect mucosal thickness, the specimens from antrum and fundus were fixed in 10% formalin and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin. The volume density of cells and mucosal thickness were determined by examining three randomly selected fields of each specimen.

Statistical Analysis —— The data on gastric acid secretion are expressed as mean ± SEM, and statistically significant differences among multiple experimental groups were assessed using two-way analysis of variance, followed by Tukey's post-hoc test. The data on mucus, mucosa, endocrine cells, relative weight of the stomach, and body weight are expressed as mean ± S.D., and the data were assessed by one-way analysis of variance, followed by Dunnett's post-hoc test. p values less than 0.05 were considered to represent a statistically significant difference.

RESULTS

In the *in vivo* experiment in rabbits, (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na dose-dependently inhibited basal gastric acid secretion for more than 8 hr, and (–)-PAN·Na was more potent than (+)-PAN·Na and (±)-PAN·Na (Fig. 1). The time needed for increasing the pH value of gastric juice from 1.5–2.0 to 6.5–7.0 was 30, 120, and 90 min, respectively, after the administration of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na at the same dose of 6 mg/kg (Fig. 1 C). Similarly, in isolated stomachs of juvenile rats, (–)-PAN·Na inhibited histamine-induced acid secretion in a dose-dependent manner (Fig. 2 A) and was more potent than (+)-PAN·Na and (±)-PAN·Na (Fig. 2 B).

Free mucus content and barrier mucus content were determined in rats administered (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na for 3 days (Table 1). The free mucus content and barrier mucus content were not influenced significantly by the administration of PAN·Na, and there were no differences

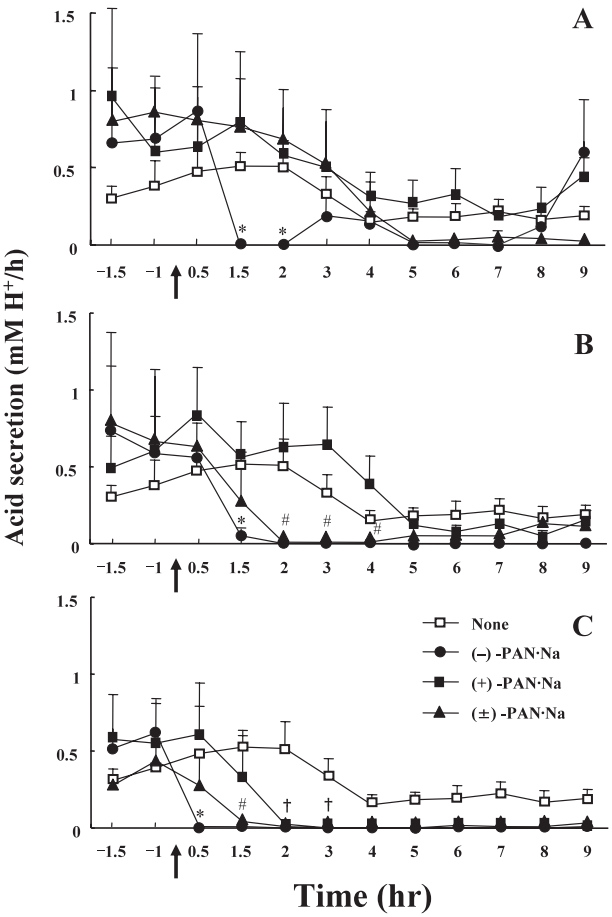

Fig. 1. Effects of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na on Gastric Acid Secretion in Rabbits

Rabbits were divided into four groups. One group as controls was given intraperitoneal 0.9% saline. The other three groups were given (–)-PAN·Na, (+)-PAN·Na, or (±)-PAN·Na. Each PAN·Na group was divided into three subgroups for administration of different doses of 1.5 mg/kg (A), 3 mg/kg (B) and 6 mg/kg (C). Data are mean ± SEM (n = 3). The arrows indicate the time when PAN·Na or 0.9% saline was injected intraperitoneally. $^{*}p < 0.05$ *vs.* corresponding values in other groups; $^{\#}p < 0.05$ *vs.* corresponding values in the control and (+)-PAN·Na groups; $^{\dagger}p < 0.05$ *vs.* corresponding values in the control group. Symbols in panel C apply to all panels.

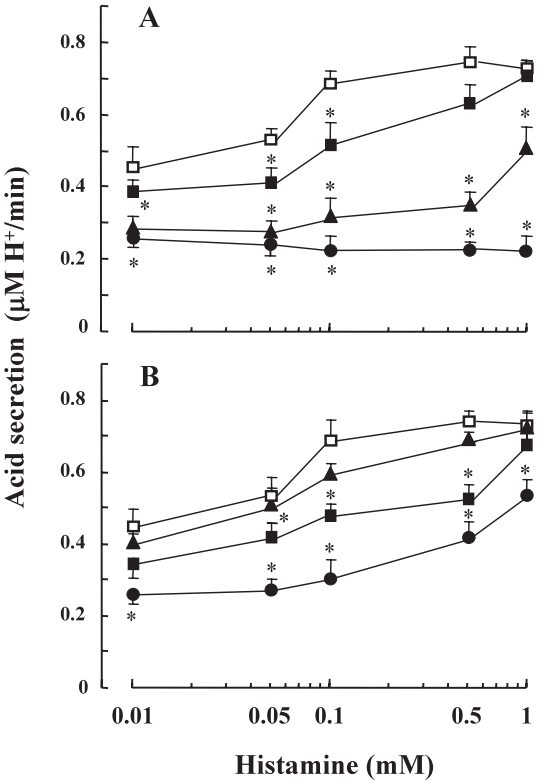

Fig. 2. Effects of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na on Histamine-Induced Acid Secretion in Isolated Stomachs of Juvenile Rats

Data are mean ± SEM (n = 5). A, (–)-PAN·Na at different concentrations: (□), control without (–)-PAN·Na; (■), 3 μM; (▲), 10 μM, and (●), 30 μM. $^{*}p < 0.05$ *vs.* corresponding values in the control group. B, (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na at the same concentration of 10 μM: (□), control without PAN·Na; (●), (–)-PAN·Na; (▲), (+)-PAN·Na, and (■), (±)-PAN·Na. $^{*}p < 0.05$ *vs.* corresponding values in other groups.

among rats administered (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na.

The effects of 40-day administration of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na on volume densities of G cells, D cells, and parietal cells and serum gastrin level were determined (Table 2). The long-term administration of PAN·Na showed a tendency to increase G cell volume density, although a significant increase was observed only in the (–)-PAN·Na 1 mg/kg, (+)-PAN·Na 9 mg/kg, and (±)-PAN·Na 1 mg/kg groups. Serum gastrin levels were significantly elevated only in the (–)-PAN·Na 3 mg/kg group. Conversely, D cell volume density in the antrum significantly decreased in all rats fed

Table 1. Free and Barrier Mucus Content in Rats after Administration of PAN·Na for 3 Days

Group (mg/kg)		Free mucus (mg)	Barrier mucus (mg)
Control		1.15 ± 0.48	1.22 ± 0.56
(–)-PAN·Na	1.5	1.74 ± 1.20	0.96 ± 0.10
	3	2.47 ± 1.85	1.02 ± 0.20
	6	1.66 ± 1.04	1.01 ± 0.26
(+)-PAN·Na	3	1.93 ± 1.22	1.11 ± 0.13
(±)-PAN·Na	3	1.48 ± 0.63	1.37 ± 0.12

Data are mean ± S.D. (n = 10).

PAN·Na. Similarly, D cell volume density in the corpus significantly decreased in rats fed PAN·Na, except in the(+)-PAN·Na 1 mg/kg group. A tendency for the parietal cell volume density to increase was observed, but a significant increase was observed only in rats fed (–)-PAN·Na 1 mg/kg and 3 mg/kg, and (±)-PAN·Na 1 mg/kg. No significant differences in the volume density of G cells, D cells,

and parietal cells were observed among rats given (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na.

The effects of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na on gastric mucosal thickness, relative gastric weight, and body weight gain are summarized in Table 3. Antral mucosal thickness increased significantly in rats fed PAN·Na in a dose-dependent manner, except for (–)-PAN·Na 1 mg/kg. However, the thickness of the corpus mucosa did not increase in any group. The relative stomach weight showed a tendency to increase, but significant increase was observed only in rats given (±)-PAN·Na 3 mg/kg. No significant differences were observed in body weight gain among rats administered solvent solution, (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na.

DISCUSSION

Pantoprazole is an irreversible proton pump inhibitor administered clinically as a racemic mixture. In the present study, we compared the *in vivo* effects of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na on gastric acid secretion in rabbits. (–)-PAN·Na showed more potent inhibition of basal gastric acid secretion. It is known that basal and stimulated gastric acid secretion is three- to four-fold greater in juvenile than in adult rats.[19] Therefore we compared the effects of (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na on gastric acid secretion in juvenile rats. (–)-PAN·Na was also more potent than (+)-PAN·Na and (±)-PAN·Na in inhibiting histamine-induced acid secretion in the isolated stomachs of juvenile rats. Unidirectional chiral inversion from (+)-PAN·Na to (–)-PAN·Na occurs after intravenous and oral administration of (+)-PAN·Na at inversion ratios of 36.3% and 28.1%, respectively.[20] The chiral inversion may be responsible for the weak activity of (+)-PAN·Na, although the direct effect of (+)-PAN·Na on inhibition of acid secretion cannot be excluded.

The gastric mucus is an important factor in mucosal protection from acid, pepsin, and various reagents such as alcohol and aspirin. Gastric mucus exists in two forms, free mucus soluble in the gastric juice and barrier mucus adherent to the mucosa. We observed that (–)-PAN·Na, (+)-PAN·Na, and (±)-PAN·Na even at 6 mg/kg, the maximum dose used in this study, did not reduce either free or barrier mucus content, indicating a low possibility of injury to the gastric mucosa.

It is reported that G cell volume density increases but D cell volume density decreases after the long-term administration of omeprazole. The increase in G cell volume density is explained by feedback regulation and the decreased intragastric acidity results in a decrease in D cell volume density, since D cell growth is dependent on the stimulation of gastric acidity.[21,22] In this study, long-term administration of PAN·Na did show a tendency to increase G cell volume density, serum gastrin levels, parietal cell volume density, or mucosal thickness in the antrum, or a tendency to decrease D cell volume density. However, the increases in G cell and parietal cell volume densities in rats fed lower concentrations of (–)-PAN·Na and (±)-PAN·Na were more obvious than in rats fed higher concentrations of (–)-PAN·Na and (±)-PAN·Na. This phenomenon might be explained by a reduced reaction following an overreaction due to the long-term and high-dose administration.

In conclusion, (–)-PAN·Na is more potent than (+)-PAN·Na and (±)-PAN·Na in inhibiting basal and histamine-induced gastric acid secretion without any increase in side effects. Therefore direct administration of (–)-PAN·Na can inhibit gastric acid secretion more effectively.

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Table 2. Volume Density (VD) of G Cells, D Cells and Parietal Cells and Serum Gastrin Level in Rats Administered PAN·Na for 40 Days

Group (mg/kg)		VD in antral mucosa (%)		G/D	VD in corpus mucosa (%)		Gastrin (pg ml^{-1})
		D cell	G cell		D cell	Parietal cell	
Control		0.57 ± 0.26	1.66 ± 0.41	2.9	0.65 ± 0.30	29.6 ± 3.02	45.8 ± 7.9
(–)-PAN·Na	1	0.20 ± 0.07*	2.99 ± 0.86*	15.0	0.39 ± 0.14*	39.77 ± 2.54*	49.8 ± 5.0
	3	0.26 ± 0.14*	1.97 ± 0.62	7.6	0.23 ± 0.15*	37.15 ± 1.82*	61.9 ± 8.3*,†
	9	0.16 ± 0.14*	2.05 ± 0.50	12.8	0.49 ± 0.20*	32.11 ± 3.74	48.0 ± 12
(+)-PAN·Na	1	0.38 ± 0.18*	1.72 ± 0.62	4.5	0.63 ± 0.33	31.91 ± 2.81	29.5 ± 7.7
	3	0.15 ± 0.11*	1.74 ± 0.30	11.6	0.26 ± 0.13*	34.77 ± 2.44	49.6 ± 1.4
	9	0.23 ± 0.19*	2.42 ± 0.43*	10.5	0.48 ± 0.25*	32.42 ± 2.37	49.3 ± 14
(±)-PAN·Na	1	0.32 ± 0.20*	2.54 ± 0.72*	7.9	0.33 ± 0.14*	39.94 ± 2.63*	39 ± 6.0
	3	0.11 ± 0.13*	2.13 ± 0.58	19.4	0.27 ± 0.21*	31.76 ± 4.04	43.5 ± 2.8
	9	0.08 ± 0.06*	1.84 ± 0.56	23.0	0.40 ± 0.23*	28.42 ± 2.28	59.6 ± 22.8

Data are mean ± S.D. (n = 5). *$p < 0.05$ *vs.* corresponding values in the control group; †$p < 0.05$ *vs.* corresponding values in the (+)-PAN·Na and (±)-PAN·Na groups at the same dose.

Table 3. Mucosal Thickness, Relative Weight of Stomach, and Body Weight Gain in Rats Administered PAN·Na for 40 Days

Group (mg/kg)		Mucosal thickness (μm)		Relative weight of stomach (g)	Body weight (g)	
		Antrum	Corpus		Day 0	Day 40
Control		95.4 ± 4.1	230 ± 24.2	6.0 ± 1.0	173 ± 16.8	285 ± 49.5
(–)-PAN·Na	1	105 ± 12.3	209 ± 13.6	6.4 ± 1.2	163 ± 5.4	269 ± 20.3
	3	161 ± 10.2*	228 ± 14.8	7.4 ± 1.4	168 ± 13.5	263 ± 31.7
	9	169 ± 18.3*	214 ± 9.45	7.9 ± 1.5	158 ± 13.3	236 ± 32.6
(+)-PAN·Na	1	135 ± 22.0*	218 ± 15.8	6.2 ± 0.48	165 ± 11.8	266 ± 31.8
	3	186 ± 21.0*	248 ± 18.1	7.6 ± 0.96	153 ± 9.3	215 ± 56.4
	9	240 ± 57.1*	192 ± 12.9	7.3 ± 1.0	161 ± 8.3	251 ± 39.0
(±)-PAN·Na	1	127 ± 17.0*	216 ± 19.9	7.5 ± 1.6	155 ± 17.1	282 ± 47.1
	3	129 ± 37.4*	219 ± 28.0	8.4 ± 0.18*	158 ± 12.4	232 ± 24.5
	9	208 ± 15.6*	200 ± 37.8	7.4 ± 0.23	157 ± 18.8	273 ± 29.4

Data are mean ± S.D. (n = 5). *$p < 0.05$ *vs.* corresponding values in the control group.

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