Comparison of the Effects of Pantoprazole Enantiomers on Gastric Mucosal Lesions and Gastric Epithelial Cells in Rats

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(±)-Pantoprazole sodium, [(±)-PAN•Na], is a proton pump inhibitor that is administered as a racemate. The protective effects of (–)-PAN•Na, (+)-PAN•Na and (±)-PAN•Na on various experimental ulcers in animals were compared. (–)-PAN•Na inhibited gastric lesions induced by water-immersion stress, aspirin, ethanol, and reserpine in a dose-dependent manner. The dose that inhibited 50% of lesions (ID_{50}) were 2.72 ± 1.03, 1.60 ± 0.64, 4.12 ± 2.18, and 2.77 ± 0.86 mg/kg, respectively. The ID_{50} values of (+)-PAN•Na and (±)-PAN•Na were 1.7, 2.6, 1.8, 2.7 and 1.5, 1.7, 1.6, 1.9 times higher, respectively, than that of (–)-PAN•Na. Primary culture of rat gastric epithelial cells was investigated as an *in vitro* model for comparing the cell protective effects among the three agents. Exposed to the three drugs at the concentrations of 2.5×10^{-1}–2.5×10^{-5} mg/ml, cultured cells were treated with either pH 3.5 medium or 3.5 mM indomethacin. Cytoprotection was evaluated by MTT. (–)-PAN•Na, (+)-PAN•Na and (±)-PAN•Na provided significant cytoprotective effects when the cells were pretreated with the drugs prior to exposure to 3.5 mM indomethacin, whereas, when they were treated concurrently, no significant cytoprotective effects were found. In pH 3.5 medium-induced damage, the three drugs had marked protective effects on gastric cells, however, when the concentration of drugs was high (0.25 mg/ml), only (+)-PAN•Na had significant cytoprotection. We concluded that the cytoprotective mechanisms of (–)-PAN•Na and (+)-PAN•Na are different. The results of *in vitro* experiments were not in complete accordance with the *in vivo* results, suggesting that the effects of the three drugs on ulcer inhibition are mainly due to the effects of acid inhibition rather than cytoprotection.

Key words ——— pantoprazole, cytoprotection, enantiomer, gastric epithelium cell culture, gastric ulcer

INTRODUCTION

Proton pump inhibitors (PPIs) are drugs that irreversibly inhibit proton pump (H^+/K^+ ATPase) function. They block the final step in acid production and, hence, are the most effective inhibitors of acid secretion.[1)] PPIs are the most potent gastric acid-suppressing agents in clinical use now.[2)] (±) Pantoprazole[(±)PAN],(±)-5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridyl)methyl]sulfinyl]-1h-benzimidazole is a substituted benzimidazole sulfoxide, which is a selective and long-acting PPI.[3)] In healthy Caucasian subjects, (±)-PAN was well tolerated after single and multiple intravenous or oral doses and produced a dose–dependent reduction in gastric acid output.[4–7)] Recent advances in analytical methods for the separation of enantiomers of PPIs have led to considerable interest in the stereoselective pharmacokinetics of PPIs enantiomers.[8–11)] It has been reported that chiral inversion of the sulfoxide drug flosequinan occurred in rats via formation of a sulfide metabolite. The sulfide metabolite is produced by reducing the sulfoxide group and its subsequent oxidation results in both flosequinan enantiomers.[12)] In rats, (±)-PAN undergoes extensive metabolism to form PAN-SO_2 as a major metabolite and PAN-S as a minor metabolite. It is, therefore, possible that re-oxidation of PAN-S to PAN, similar to flosequinan, occurs *in vivo*, resulting in the chiral inversion of PAN enantiomers. It had been reported that after either intravenous or oral administration, chiral inversion from (+)-PAN to (–)-PAN occurred,

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and this inversion is unidirectional.[13] However, there is no published report about the differences in (+)-PAN and (–)-PAN on gastric mucosal lesions in animals. In this study, the protective effects of (+)-PAN•Na and (–)-PAN•Na on the stomach were investigated in rats to compare the two agents.

Primary gastric epithelial cell culture systems are used as cell models for studying stomach physiology and for the evaluation of anti-ulcer or anti-inflammatory agents. These cells have been derived from rat gastric mucosa.[14,15] Cultured gastric epithelial cells offer a promising tool for evaluating the cytoprotective effects of anti-ulcer agents in the absence of gastric and systemic factors and, thus, may provide a suitable model for studying the mechanisms by which these drugs influence gastrointestinal epithelial cell functions. In our study, primary cultured rat gastric mucosal cells were investigated as an *in vitro* model to measure the cytoprotective activities of (–)-PAN•Na, (+)-PAN•Na and (±)-PAN•Na. The purpose of this study was to compare the different cytoprotective effects of the three agents on cell damage brought about by either acid or indomethacin treatment *in vitro*.

MATERIALS AND METHODS

Agents —— (–)-PAN•Na, (+)-PAN•Na, and (±)-PAN•Na were provided by the Department of Pharmaceutical Engineering at Shenyang Pharmaceutical University. (–)-PAN and (+)-PAN were manufactured with (±)-PAN by the method of E. M. Larsson.[16] (±)-PAN, (–)-PAN and (+)-PAN were then reacted with sodium hydroxide to prepare their sodium salts. The purity of (±)-PAN•Na is above 98%. The optical purities of (–)-PAN•Na and (+)-PAN•Na are 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. The HPLC conditions for optical purity detection were as follows: Chiral-α_1-acid glycoprotein (AGP) column (150 × 4.0 mm, i.d. 5 μm) (Chrom Tech Sweden) used with acetonitrile: ammonium acetate (15 : 85) at a flow speed of 0.9 ml/min; UV wavelength: 290 nm.

3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-tetrazolium romide (MTT) was purchased from Amresco (Solon, Ohio, U.S.A.); Hydrochloric acid, ethanol, aspirin and reserpine were obtained from Huamei (Shenyang, China); All other chemicals and biochemicals were purchased from Sigma (U.S.A.).

Animals and Experimental Design —— Male Sprague-Dawley rats weighing 180–220 g and male Kunming mice weighing 18–22 g (Center of Animal Experiments, Shenyang Pharmaceutical University, China) were used. Prior to the experiments, the rats were housed under conditions consisting of controlled temperature, humidity, and illumination (12 : 12 light-dark cycles) in large cages with wire mesh bottoms to prevent coprophagia. They were fed laboratory chow and water but were deprived of food overnight before an experiment.

Water-Immersion Stress-Induced Gastric Lesions —— Water-immersion stress lesions were induced as described by Tadagi and Okabe.[17] The mice were given the drugs orally 30 min before being placed in a stress cage and being vertically immersed to the xiphoid process in a water bath maintained at 22 ± 1°C. Ten hr later, the animals were taken out of the cages and sacrificed by CO_2 asphyxiation. The stomach was removed, filled with 1 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 number of punctate ulcers in the corpus was determined under a dissecting microscope, and the sum of all punctations in each stomach was used as the lesion index.

Aspirin-Induced Gastric Lesions —— The experiments were performed as described by Mine *et al.*[18] Aspirin (150 mg/kg, *p.o.*) was orally administered 1 hr after oral administration of the test drugs, the rats were sacrificed 4 hr later, and then the stomach was excised and fixed with 1% formalin. The number of punctate ulcers was measured, and the sum of all punctations in each stomach was used as a lesion index for the individual rat.

Ethanol-Induced Gastric Lesions —— Drugs were administered orally 30 min before 1 ml of absolute ethanol was administered orally. The animals were sacrificed 1 hr after receiving the ethanol. The stomachs were excised and fixed with 1% formalin. The length (mm) of each lesion in the stomach was measured and used as a lesion index.

Reserpine-Induced Gastric Lesions —— Reserpine (10 mg/kg, s.c.) was administered immediately after oral administration of the test drugs. Six hr later the mice were sacrificed, and the stomach was excised and fixed with 1% formalin. The number of punctate ulcers was determined and the sum of all punctations in each stomach was used as a lesion index.

Cell Culture —— Gastric mucosa from 1 to 2-week-old Sprague-Dawley rats was isolated as described

by Terano *et al.*[19] In brief, the gastric mucosal surface was washed thoroughly with sterile cotton and Hanks balanced salt solution (HBSS), and then rinsed with HBSS before being minced into approximately 1 mm^3 pieces. The minced tissues were incubated in HBSS containing 0.1% collagenase and 0.05% hyaluronidase at 37°C in a shaking water bath for 1 hr. The mixture was then pipeted several times and filtered through a sterile nylon mesh. The filtrate was washed twice with HBSS by centrifugation (200 g for 5 min) and resuspended in Coon's modified Ham's F-12 culture medium containing 100 μg/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamycin, 15 mM HEPES, 2 μg/ml fibronectin, and 10% fetal bovine serum. Cells were seeded at a density of $1.5–2 \times 10^5$ cells/cm^3 directly onto 96-well plates, and maintained in a Steri-Cult incubator at 37°C in a humidified atmosphere with 5% CO_2. The medium was changed daily. The confluent monolayers were formed after 4–5 days in 96-well plates.

Cell Growth and Cytoprotective Assays —— To measure the cytotoxicity of indomethacin on gastric mucosal cells, cells were incubated in serum-free medium containing 0.3125–10 mM indomethacin for 1 hr. Indomethacin was prepared as a 50 mM solution in $NaHCO_3$ (0.2 M) and then diluted in serum-free culture medium to the desired concentrations.

To study the effects of (–)-PAN•Na, (+)-PAN•Na, and (±)-PAN•Na on indomethacin-induced damage, cells were incubated in culture medium containing these drugs for 1 hr. The drug suspensions were then aspirated away, followed by another hr of treatment with 3.5 mM indomethacin. Alternatively, the cells were treated with drugs and 3.5 mM indomethacin concurrently for 1 hr. To study the effects of (–)-PAN•Na, (+)-PAN•Na, and (±)-PAN•Na on acid-induced damage, the cells were incubated with the agents for 2 hr and then exposed to pH 3.5 medium for 30 min.

MTT Colorimetric Assay —— Cells in 96-well plates were treated as above with the different drugs and then incubated in 100 μl of culture medium containing 10 μl of an MTT stock solution (5 mg/ml in PBS) for 4 hr at 37°C according to the method of Mosmann.[20] Following the incubation, 150 μl of DMSO was added to wells and the wells were shaken for 10 min. The color changes were recorded at 540 nm on a microplate reader (Spectra Classic, TECAN, Austria).

Statistics —— The doses of (–)-PAN•Na, (+)-PAN•Na, and (±)-PAN•Na that inhibit ulcer formation by 50% were determined (ID_{50} values). Data values are expressed as the mean ± S.E. and each subgroup was analyzed using the *t*-test (two-tailed). *p*-Values less than 0.05 were considered statistically significant.

Table 1. Effects of (−)-PAN·Na, (+)-PAN·Na and (±)-PAN·Na on Water-Immersion Stress-Induced Gastric Lesions in Mice

Drug	Dose (mg/kg)	Ulcer index	% inhibition	ID_{50} (mg/kg)
Control	—	61.4 ± 9.80		
(−)-PAN	2.5	29.8 ± 9.52*	51.5	2.72 ± 1.03
	5	22.9 ± 4.55**	62.7	
	10	5.10 ± 1.39**	91.7	
(+)-PAN	2.5	43.3 ± 13.0	29.5	4.60 ± 1.05
	5	35.4 ± 7.18	42.3	
	10	9.90 ± 2.18**	83.9	
(±)-PAN	2.5	46.2 ± 11.0	24.8	3.95 ± 1.80
	5	21.0 ± 4.33**	65.8	
	10	6.5 ± 1.35**	89.4	

*$p < 0.05$; **$p < 0.01$ *vs* control $n = 10$; $\overline{X} \pm$ S.E.

RESULTS

Effect on Water-Immersion Stress-Induced Gastric Lesions

Water immersion at 22 ± 1°C for 10 hr caused punctate lesions mainly in the glandular stomach and the mean lesion index in the control group was 61.4 ± 9.8. (–)-PAN•Na prevented the formation of these lesions in a dose-related manner, and the ID_{50} value was 2.72 mg/kg. (+)-PAN•Na and (±)-PAN•Na exerted significant inhibitory effects on the formation of lesions at high doses. The inhibitory effect of (–)-PAN•Na was about 1.7 and 1.5 times more potent than those of (+)-PAN•Na and (±)-PAN•Na, respectively (Table 1).

Effect on Aspirin-Induced Gastric Lesions

Aspirin produced punctate lesions in the glandular portion of the stomach and the lesion index of the control was 39.8 ± 9.98. (–)-PAN•Na dose–dependently inhibited the formation of aspirin-induced lesions. As shown in Table 2, (+)-PAN•Na and (±)-PAN•Na did not exert any inhibitory activity at 2.5 mg/kg.

Effect on Ethanol-Induced Gastric Lesions

Oral administration of absolute ethanol induced band-like congestive lesions in the glandular portion of the stomach. The lesion index in the control

Table 2. Effects of (−)-PAN·Na, (+)-PAN·Na and (±)-PAN·Na on Gastric Lesions Induced by Aspirin in Mice

Drug	Dose (mg/kg)	Ulcer index	% inhibition	ID_{50} (mg/kg)
Control	—	39.7 ± 9.98		
(−)-PAN	1.25	23.8 ± 4.59	40.1	1.60 ± 0.64
	2.5	12.9 ± 2.36*	67.5	
	5	8.35 ± 1.46**	79.0	
(+)-PAN	1.25	39.1 ± 9.61	1.61	4.18 ± 0.98
	2.5	27.8 ± 4.75	30.0	
	5	17.9 ± 2.72*	55.0	
(±)-PAN	1.25	32.5 ± 4.62	18.2	2.73 ± 0.60
	2.5	20.1 ± 3.57	49.4	
	5	10.3 ± 2.15**	74.1	

*$p < 0.05$; **$p < 0.01$ *vs* control $n = 10$; ($\overline{X}$± S.E.

Table 3. Effects of (−)-PAN·Na, (+)-PAN·Na and (±)-PAN·Na on Gastric Lesions Induced by Ethanol in Rats

Drug	Dose (mg/kg)	Ulcer index (mm)	% inhibition	ID_{50} (mg/kg)
Control	—	72.7 ± 11.9		
(−)-PAN	1.5	50.2 ± 5.7	31.0	4.12 ± 2.18
	3	41.2 ± 7.4*	43.3	
	6	30.4 ± 3.9**	58.2	
(+)-PAN	1.5	69.9 ± 12.1	3.86	7.63 ± 3.93
	3	58.6 ± 7.7	19.5	
	6	45.5 ± 5.0*	37.6	
(±)-PAN	1.5	56.2 ± 11.1	22.7	6.39 ± 5.23
	3	43.7 ± 6.1*	39.9	
	6	38.3 ± 6.3*	47.3	

*$p < 0.05$; **$p < 0.01$ *vs* control $n = 10$; $\overline{X}$ ± S.E.

group was 72.7 ± 11.9 mm, as shown in Table 3. (–)-PAN•Na exerted dose–dependent inhibition of the formation of ethanol-induced gastric lesions, however, at the same dose of 1.5 mg/kg, (+)-PAN•Na and (±)-PAN•Na did not exert any significant inhibitory effects.

Effect on Reserpine-Induced Gastric Lesions

Reserpine caused punctate lesions mainly in the glandular stomach. The lesion index in the control group was 43.0 ± 4.4 (Table 4). (–)-PAN•Na, at doses of 5 and 2.5 mg/kg, significantly inhibited the formation of lesions, however, at the dose of 2.5 mg/kg, (+)-PAN•Na and (±)-PAN•Na did not exert any significant inhibitory effects.

Effect of Indomethacin on Cell Viability

Indomethacin exerted dose–dependent cytotoxicity in the gastric mucosal cells at concentrations from 0.3125 mM to 10 mM. (Fig. 1). The concentration which produced about a 50% decrease in viability was estimated as 1.25–2 mM.

Table 4. Effects of (−)-PAN·Na, (+)-PAN·Na and (±)-PAN·Na on Gastric Lesions Induced by Reserpine in Mice

Drug	Dose (mg/kg)	Ulcer index	% inhibition	ID_{50} (mg/kg)
Control	—	43.0 ± 4.4		
(−)-PAN	1.25	31.0 ± 5.2	27.9	2.77 ± 0.857
	2.5	23.9 ± 4.2**	44.5	
	5	13.4 ± 4.1***	68.9	
(+)-PAN	1.25	41.1 ± 6.3	3.81	7.44 ± 4.68
	2.5	37.7 ± 7.4	12.4	
	5	27.6 ± 4.4*	35.9	
(±)-PAN	1.25	39.9 ± 5.3	7.31	5.26 ± 2.10
	2.5	32.5 ± 4.7	24.5	
	5	21.2 ± 4.5**	50.7	

*$p < 0.05$; **$p < 0.01$ *vs* control $n = 10$; $\overline{X}$ ± S.E.

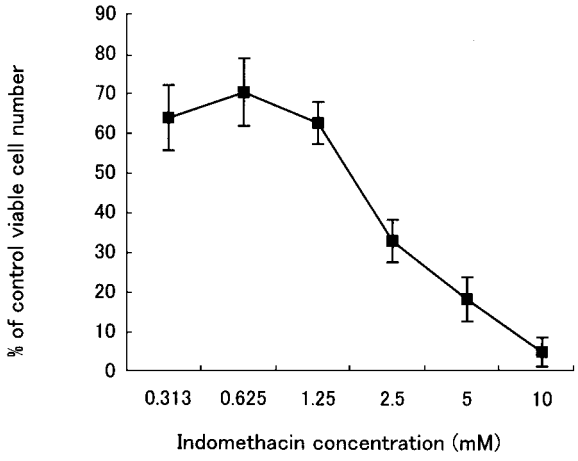

Fig. 1. Dose–Dependence of Indomethacin-Induced Cell Damage

Cultured gastric cells were exposed to indomethacin solutions for 1 hr before performing the MTT assay. Values are the mean ± S.E. for three cultures.

Protective Effects on Indomethacin-Induced Damage

When the gastric cells were treated with drugs and 3.5 mM indomethacin concurrently, only (+)-PAN•Na, as shown in Fig. 2, at the lower concentrations of 2.5×10^{-4} and 2.5×10^{-3} mg/ml, had a significant protective effect. As shown in Fig. 3, when cells were pretreated with 2.5×10^{-5}–2.5×10^{-1} mg/ml of the three drugs prior to exposure to 3.5 mM indomethacin, (–)-PAN•Na suspensions resulted in

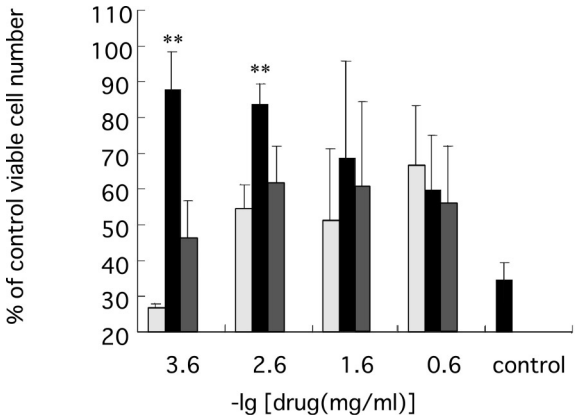

Fig. 2. Protective Effects of (–)-PAN•Na, (+)-PAN•Na and (±)-PAN•Na on Indomethacin-Induced Damage

Gastric mucosal cells were incubated with these drugs and 3.5 mM indomethacin concurrently for 1 hr before performing the MTT assay. Values are the mean ± S.E. for three cultures. Significant differences compared with indomethacin treatment in the absence of drugs. $*p < 0.05$, $**p < 0.01$. [(–)-PAN_Na (□), (+)-PAN_Na (■)and (±)-PAN_Na(▩)].

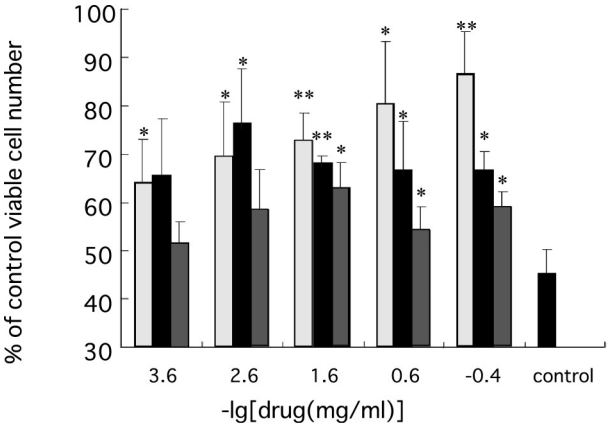

Fig. 3. Cytoprotective Effects of (–)-PAN•Na, (+)-PAN•Na and (±)-PAN•Na against Imdomethacin-Induced Damage

Cells were treated with drugs for 1 hr, exposed to 3.5 mM indomethacin for another 1 hr, and then an MTT assay was performed. Values are the mean ± S.E. for three cultures. The control contains indomethacin but no drugs. Significant differences compared with indomethacin treatment in the absence of drugs. $*p < 0.05$, $**p < 0.01$. [(–)-PAN_Na (□), (+)-PAN_Na (■) and (±)-PAN_Na (▩)].

cell survival rates up to 80.1% ($p < 0.05$), 83.1% ($p < 0.05$), 84.8% ($p < 0.01$), 89.2% ($p < 0.05$), and 92.6% ($p < 0.01$) of the control cell number, respectively. Thus, the protective effect was dose–dependent. However, (+)-PAN•Na and (±)-PAN•Na began to provide significant protection only at higher concentrations ($p < 0.05$ at 2.5×10^{-3} mg/ml; $p < 0.05$ at 2.5×10^{-2} mg/ml, respectively). (±)-PAN•Na showed less cytoprotective potency than (–)-PAN•Na and (+)-PAN•Na at the same dose.

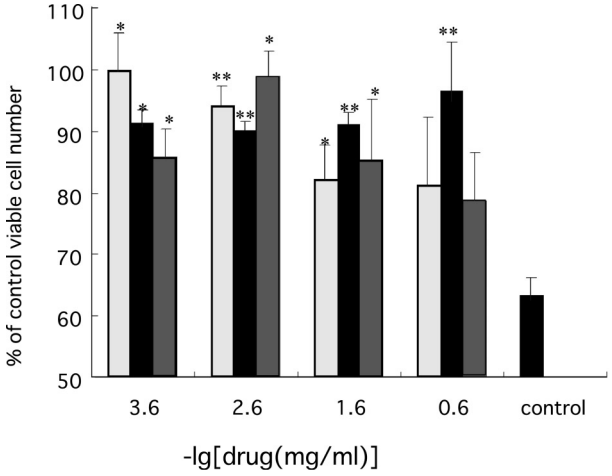

Fig. 4. Effects of (–)-PAN•Na, (+)-PAN•Na and (±)-PAN•Na on Gastric Mucosal Cells Injured by Acidified Medium

The cells were incubated with drugs for 2 hr and then with pH 3.5 medium for 30 min before performing the MTT assay. Values are the mean ± S.E. for three cultures. Significant differences compared with control (acid medium treatment in the absence of drugs), $*p < 0.05$, $**p < 0.01$. [(–)-PAN•Na (□), (+)-PAN•Na (■) and (±)-PAN•Na (▩)].

Protective Effects on Acid-Induced Damage

As illustrated in Fig. 4, (–)-PAN•Na virtually completely blocked acid-induced cytotoxicity ($p < 0.05$) at the concentration of 2.5×10^{-4} mg/ml, and this cytoprotective effect decreased as the drug concentration increased. In contrast, a dose–dependent increase in the survival rate of cells was brought about by (+)-PAN•Na. The effect of (±)-PAN•Na was increased at lower concentrations and decreased at higher concentrations.

DISCUSSION

(–)-PAN•Na inhibited various gastric ulcers in rats in a dose–dependent manner. As the formation of most gastric lesions is inhibited by anti-secretory agents, acid secretion is considered to be an important factor in their pathogenesis.[21] In the present study, (–)-PAN•Na, (+)-PAN•Na, and (±)-PAN•Na inhibited water-immersion stress-induced gastric lesions in mice. (–)-PAN•Na was more potent than (+)-PAN•Na and (±)-PAN•Na when their ID_{50} values were compared, but when the doses were increased, the maximum inhibition tended to be similar (91.7, 83.9 and 89.4%, respectively). As proposed by other researchers,[13] there is a unidirectional inversion from (+)-PAN•Na to (–)-PAN•Na in rats. We considered that, only a part of (+)-PAN•Na in rat's body can be reversed to (–)-PAN•Na, thereby, if the

dose of (+)-PAN•Na administered to rats was so high, the reversed part of it was enough to inhibit the formation of ulcers. Water immersion restraint stress-induced gastric lesions are considered to be causally related to increased gastric secretion,[22] thereby, the inhibitory potencies of the three drugs from high to low were (–)-PAN•Na > (±)-PAN•Na > (+)-PAN•Na. These observations are in agreement with the studies on gastric acid secretion we have done before (unpublished).

As a type of non-steroidal anti-inflammatory drugs (NSAID), aspirin induced gastric lesions through blocking prostaglandin production.[23] Prostaglandins are critical factors in maintaining the defense and integrity of the gastric and intestinal wall through regulating mucosal blood flow, mucus and bicarbonate secretion, and epithelial cell proliferation and repair.[24] In our study, (–)-PAN•Na significantly inhibited the formation of lesions in a dose–dependent manner, however, (+)-PAN•Na and (±)-PAN•Na exerted marked inhibitory effects only at a high dose (5 mg/kg). This result was not in complete agreement with the result in the experiment of cell protection. In the cell injury experiments, (–)-PAN•Na, (+)-PAN•Na, and (±)-PAN•Na significantly protected the cells damaged by indomethacin, and the protective effect of (–)-PAN•Na was more potent than that of (+)-PAN•Na and (±)-PAN•Na. However, the potency of (±)-PAN•Na was lower than that of (+)-PAN•Na for cell protection. This result was contradictory with their anti-ulcer activity. We cannot offer any clear explanation for these discrepancies. However, our results do at least suggest that (+)-PAN•Na may have an anti-ulcer effect that is not dependent on an anti-secretory action but rather cytoprotection.

Acid is generally considered to play a minor role in the pathogenesis of ethanol-induced gastric lesions since the lesions are not inhibited by anti-secretory agents or antacids.[25] In the present study, the formation of ethanol-induced gastric lesions was not inhibited by 1.5 mg/kg of the three agents but was significantly inhibited by 6 mg/kg. These agents exerted inhibitory effects on ulcers at doses higher than their anti-secretory doses. It has been reported that oral administration of omeprazole inhibited the formation of ethanol-induced gastric lesions in rats, and the biosynthesis of prostaglandins is not involved in its protective effect.[26] Additionally, in the experiment of acid-induced damage, (+)-PAN•Na dose–dependently increased the survival rate of cells, but (–)-PAN•Na did not. This suggests that (+)-PAN•Na has a special cytoprotective effect which (–)-PAN•Na does not have. When an anti-secretive effect is not considered to be the main reason for the anti-ulcer effect in ethanol-induced gastric lesions, this cytoprotective effect decreased the differences in inhibitory potencies on gastric ulcers among the three drugs.

Reserpine disturbed gastric secretion by depleting monoamine transmitters and then inducing gastric ulcers. In our experiments, all three agents (5 mg/kg) significantly inhibited gastric lesions induced by reserpine, however, their potencies were different. The ID_{50} values of (+)-PAN•Na and (±)-PAN•Na were 2.7 and 1.9 times higher than that of (–)-PAN•Na. This result was in agreement with those for other models, and suggests that this inhibitory effect is mainly due to acid inhibition rather than an anti-monoamine oxidation effect.

Collagenase-dissociated gastric mucosal cells are morphologically epithelial-like. They do not exhibit the acid-secreting properties of parietal cells, and do not require pentagastrin for growth.[19] In the present study, we examined the protective effects of (–)-PAN•Na, (+)-PAN•Na, and (±)-PAN•Na on cultured gastric mucosal cells damaged by acidified medium or indomethacin. Indomethacin is an inhibitor of the generation of prostaglandins, which are considered important in enhancing mucous and bicarbonate secretion. Romano *et al.*[27] reported that 10^{-6}–10^{-4} M indomethacin inhibited the secretion of prostaglandin E_2 significantly in a human gastric epithelial cell line. (–)-PAN•Na effectively prevented indomethacin-induced injury dose–dependently when it was applied to the cells prior to indomethacin treatment, however, it did not exert any protective effect when treated with indomethacin concurrently. This suggests that (–)-PAN•Na increased the resistance to indomethacin-induced cell damage by promoting prostaglandin E_2 release. (+)-PAN had significant cytoprotective effects regardless of whether it was applied to the cells concurrently or prior to indomethacin treatment. The 3.5 mM concentration of indomethacin used in our study would be more than sufficient to suppress any prostaglandin secretion. Thus, the cytoprotective role of (+)-PAN•Na has no relationship with the regulation of prostaglandin. However, it was reported that indomethacin appears to affect many more cellular systems than COX only, for example, mitochondrial energy metabolism, glycolysis, and the pentose phosphate pathways.[28] Understanding how (+)-PAN•Na influences these pathways and their contribution to

the cytoprotection is a challenge for the future. On the other hand, we were able to determine that the cytoprotective mechanisms of (–)-PAN•Na and (+)-PAN•Na were different. Furthermore, when applying (±)-PAN•Na in the same experiment, the potency of the cytoprotective effect was smaller than that of either (–)-PAN•Na or (+)-PAN•Na at the same dose. Further study is required to elucidate the reason for this.

Acidifying medium can decrease the viability of normal cells, for example, pepsinogen can be activated to pepsin at low pH, and then induce gastric mucosal cell damage. In the present study, all three drugs exerted inhibitory effects on acid-induced cell damage. We know that[1)] (±)-PAN•Na, in acid medium, can be stimulated and converted to sulphenamide that enables it to bind to the cysteine residues of enzymes. We believe (±)-PAN•Na and its enantiomers increased the viable cell numbers through inhibition of the activity of pepsin. However, if this reaction happened, where (intracellular or extracellular) and how it happens need to be studied.

In summary, the anti-ulcer effects of (–)-PAN•Na, (+)-PAN•Na, and (±)-PAN•Na were related to the doses administered to the animals. The ID_{50} values with the respect to gastric ulcer inhibition were (+)-PAN•Na > (±)-PAN•Na > (–)-PAN•Na. The three drugs had cytoprotective effects, however, their mechanisms are different.

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