

Preventive Actions of *N*-(3-Aminopropionyl)-L-Histidinato Zinc (Z-103) through Increases in the Activities of Oxygen-Derived Free Radical Scavenging Enzymes in the Gastric Mucosa on Ethanol-Induced Gastric Mucosal Damage in Rats

Mikio Ito, Daisuke Shii, Tetsuya Segami, Ryoji Kojima and Yoshio Suzuki

Department of Pharmacology, Faculty of Pharmacy, Meijo University, 150 Yagotoyama, Tenpaku-ku, Nagoya 468, Japan

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ABSTRACT—Z-103 at 1 to 25 mg/kg, p.o. prevented 100% ethanol-induced gastric mucosal lesions in a dose-dependent manner. Z-103 at 3 to 25 mg/kg, p.o. significantly elevated gastric mucosal superoxide dismutase (SOD)-like activity 1 hr after its administration to normal rats. In addition, Z-103 at doses (10 and 25 mg/kg, p.o.) which prevented 100% ethanol-induced gastric lesion further increased gastric mucosal SOD-like and glutathione peroxidase (GSH-px) activities elevated by 60% ethanol. Z-103 (10 and 25 mg/kg) significantly inhibited the increase in thiobarbituric acid-reactive substances in gastric mucosa injured by 60% ethanol. The combination with cycloheximide, a protein synthesis inhibitor, completely abolished the prevention of 60% ethanol-induced gastric mucosal lesions and the elevation of both free radical scavenging enzyme activities in the mucosa by Z-103 (10 mg/kg, p.o.). These results suggest that Z-103 may partly protect rat gastric mucosa against ethanol-induced damage by scavenging oxygen-derived free radicals via increases in the synthesis of SOD-like and GSH-px enzymes in the mucosa.

Keywords: Z-103, Ethanol, Gastric lesion, Superoxide dismutase, Glutathione peroxidase

N-(3-Aminopropionyl)-L-histidinato zinc (Z-103) is a chelate compound that consists of L-carnosine and zinc. Z-103 has been reported to have potent protective actions against several acute experimental gastric and duodenal lesions in rats without reducing gastric secretion (1). We have demonstrated that Z-103 markedly promotes the healing of acetic acid ulcers in rats with limited food-intake-time (2). However, the mechanisms of the anti-ulcer actions of this compound have not been well-defined. Arakawa et al. (3) reported that Z-103 markedly protected rat gastric mucosa against ethanol-induced damage without affecting the mucosal prostaglandin (PG) E₂ level. Their finding suggests that the preventive effects of Z-103 on acute experimental gastric or duodenal lesions may be partly due to the PG-independent cytoprotective action of this agent.

It has been recently demonstrated that oxygen-derived free radicals may play an important role in the pathogenesis of acute experimental gastric lesions induced by ischemia/reperfusion (4, 5), stress (6, 7), ethanol (8–11), and nonsteroidal anti-inflammatory

drugs (12, 13). Oxygen-derived free radicals cause tissue injury through lipid peroxidation of cellular membrane components (14) and by degradation of hyaluronic acid, the principal component of the epithelial basement membrane (15). On the other hand, mammalian cells have been indicated to possess free radical-scavenging enzymes such as superoxide dismutase (SOD), which dismutates superoxide radicals (O₂⁻), and glutathione peroxidase (GSH-px), which dismutates hydrogen peroxide (H₂O₂) formed from O₂⁻ by SOD. If the generation of oxygen-derived free radicals in the gastric mucosa exceeds the ability of free radical-scavenging enzymes to dismutate the radicals, the gastric mucosa may be injured by free radicals. Z-103 has been already demonstrated to inhibit superoxide generation by polymorphonuclear leukocytes and to have an antioxidative action in vitro (16). However, it still remains unclear if Z-103 affects the synthesis or activities of free radical scavenging enzymes in the gastric mucosa. Therefore, in the present study, we investigated if Z-103 protects rat gastric mucosa against ethanol-induced

damage via the increase in SOD and GSH-px activities in the gastric mucosa.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley strain SPF rats (Nihon SLC, Shizuoka) weighing 170 to 190 g were fasted for 24 hr prior to the experiments. For the last 17 hr, the animals were deprived of water and placed in individual cages to limit their movements in order to prevent ingestion of hair and faces.

Drugs

Drugs used were Z-103 (Zeria Pharmaceutical Co., Ltd., Tokyo), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (Kanto Chemicals Co., Ltd., Tokyo), L-carnosine (Wako Pure Chemical Industries, Ltd., Osaka), human Cu, Zn-SOD (Superoxide Dismutase, Ube Industries, Ltd., Tokyo) and allopurinol (Sigma Chemical Co., St. Louis, MO). Z-103, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, L-carnosine or allopurinol was suspended in 0.5% methylcellulose. Human Cu, Zn-SOD was dissolved in 0.9% NaCl.

Effects of Z-103 and other compounds on 100% ethanol-induced gastric mucosal injury

Experiments were carried out following a 24-hr fast. Z-103 (1, 3, 10 and 25 mg/kg), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (10 and 25 mg/kg/day) or L-carnosine (7.7 and 19 mg/kg) was given p.o. in a volume of 1 ml/100 g of body weight 1 hr prior to ethanol instillation. Human Cu, Zn-SOD (15,000, 30,000 and 45,000 U/kg) was given i.v. in a volume of 0.5 ml/100 g of body weight 10 min prior to ethanol administration. Allopurinol (30 and 100 mg/kg, 1 ml/100 g of body weight) was given i.p. 1 hr prior to treatment with the necrotizing agent. Furthermore, as the respective control, the vehicle (0.5% methylcellulose or 0.9% NaCl) was given instead of test compounds. Following the administration of test compounds, 1 ml of 100% ethanol was instilled intragastrically. One hour after ethanol instillation, the animals were sacrificed under ether anesthesia, and the stomachs were removed and then opened along the greater curvature. In order to evaluate the degree of gastric mucosal injury, the length (mm) and width (mm) of red streaks in the gastric mucosa were measured under observation with a stereoscopic microscope, and the area of the streaks (mm^2) was calculated by both products. The sum of the area of each streak was expressed in terms of the lesion index.

Effects of Z-103 on SOD-like activity in the gastric mucosa of normal rats

At 0, 0.5, 1, 2, 3 and 5 hr after the administration of Z-103 (10 mg/kg, p.o.) to normal rats, they were sacrificed and their stomachs were removed. The stomachs were opened along the greater curvature, and the corpus mucosa was removed by scraping. The mucosa was homogenized in 0.1 M Tris-HCl buffer (pH 7.4) and centrifuged at $9,000 \times g$ for 30 min at 4°C. The SOD-like activity in the supernatant was then determined by the nitrite method of Ohyanagi (17). Furthermore, the dose-related effects of Z-103 on SOD-like activity in the gastric mucosa of normal rats were evaluated at 1 hr after administration.

Effects of Z-103, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and L-carnosine on SOD-like and GSH-px activities in gastric mucosa injured by 60% ethanol

Z-103 (3, 10 and 25 mg/kg), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (10 and 25 mg/kg) or L-carnosine (7.7 and 19 mg/kg) was given p.o. to rats. The vehicle (0.5% methylcellulose) was given p.o. to the control animals. One hour later, 60% ethanol (1 ml) was instilled intragastrically. At 60 min after ethanol instillation, the animals were sacrificed and their stomachs were removed. The SOD-like and GSH-px activities in the corpus mucosa were determined by the nitrite method and the method of Paglia and Valentine (18), respectively.

Effect of Z-103 in combination with cycloheximide on 60% ethanol-induced gastric mucosal injury and on SOD-like and GSH-px activities in the injured mucosa

The gastric injury and SOD-like and GSH-px activities in the corpus mucosa were evaluated after the following treatment: 1) vehicle, 2) cycloheximide, 3) ethanol, 4) cycloheximide + ethanol, 5) ethanol + Z-103, 6) cycloheximide + ethanol + Z-103. Z-103 (10 mg/kg) was given p.o. 1 hr prior to ethanol. Cycloheximide (6 mg/kg) was given s.c. 30 min prior to Z-103 administration (10 mg/kg p.o.) and immediately before ethanol (60%, 1 ml) instillation. As the vehicle, 0.5% methylcellulose (1 ml/100 g of body weight) was given p.o. Ethanol (60%, 1 ml) was instilled intragastrically and 1 hr later, gastric mucosal injury and SOD-like and GSH-px activities in the corpus mucosa were evaluated.

Effect of Z-103 on thiobarbituric acid (TBA)-reactive substances in gastric mucosa injured by 60% ethanol

At 0, 5, 30 and 60 min after 1 ml of 60% ethanol was instilled intragastrically, the animals were sacrificed and their stomachs were removed. As an index of lipid peroxidation, TBA-reactive substances in the corpus mucosa were determined by the method of Ohkawa et al. (19). Furthermore, Z-103 (3, 10 and 25 mg/kg) was given p.o. The vehicle (0.5% methylcellulose) was

given p.o. to the control rats. One hour later, 60% ethanol (1 ml) was instilled intragastrically. At 60 min after the ethanol administration, TBA-reactive substances in the corpus were determined.

Statistical analyses

Results obtained were expressed as the mean \pm S.E. The data were analyzed by one-way analysis of ANOVA, and the statistical significance of the differences among groups was determined by Duncan's multiple range test.

RESULTS

Effects of Z-103, allopurinol and human Cu, Zn-SOD on 100% ethanol-induced gastric mucosal injury

Intragastric administration of each 1 ml of 100% ethanol to control rats produced large hemorrhagic, necrotic lesions in the gastric fundic area. Z-103 at 1 to 25 mg/kg, p.o. prevented the gastric lesions in a dose-dependent manner, when given 1 hr before the ethanol challenge (Fig. 1). The preventive action of Z-103 was significant at 10 and 25 mg/kg. Allopurinol (30 and 100 mg/kg, i.p.) and human Cu, Zn-SOD (30,000 and 45,000 U/kg, i.v.) were also effective in preventing the gastric lesion, when giving 1 hr and 10 min, respectively, before the necrotizing agent.

Effect of Z-103 on SOD-like activity in gastric mucosa of normal rats

When SOD-like activity in the corpus mucosa was determined at various times after Z-103 (10 mg/kg, p.o.) was given to normal rats, the activity had been significantly elevated by 0.5 hr, and the elevation then

continued up to 2 hr (Fig. 2A). In addition, Z-103 at 3 to 25 mg/kg, p.o. significantly elevated the SOD-like activity by 33 to 42% 1 hr after the administration (Fig. 2B).

Effects of Z-103 on SOD-like and GSH-px activities in gastric mucosa injured by 60% ethanol

The SOD-like activity in the corpus of control rats 1 hr after 60% ethanol instillation (1 ml) was significantly higher (36.2%) than that of normal animals (Fig. 3A). Z-103 given 10 and 25 mg/kg, p.o. 1 hr before the ethanol markedly elevated the activity by 58% and 42%, respectively, in comparison with the control.

When GSH-px activity in the corpus was determined under the same condition as SOD-like activity, the GSH-px activity of control rats instilled with ethanol was also significantly higher (34.4%) than that of normal animals (Fig. 3B). Z-103 at 10 and 25 mg/kg, p.o. markedly elevated the activity by 39% and 40%, respectively, compared with the control.

Effect of Z-103 in combination with cycloheximide on 60% ethanol-induced gastric mucosal injury and on SOD-like and GSH-px activities in the injured mucosa

The gastric mucosal lesions induced by 60% ethanol (1 ml) were less severe than those induced by 100% ethanol (lesion index: 60% ethanol, $16.0 \pm 6.2 \text{ mm}^2$ vs. 100% ethanol, $97.1 \pm 11.7 \text{ mm}^2$) (Fig. 4). Z-103 (10 mg/kg, p.o.) markedly prevented 60% ethanol-induced mucosal lesions by 88%. In addition, Z-103 markedly elevated the SOD-like and GSH-px activities in the corpus by 58% and 39%, respectively. However, treatment with cycloheximide (6 mg/kg \times 2, s.c.) completely abolished the prevention of the mucosal lesion and the

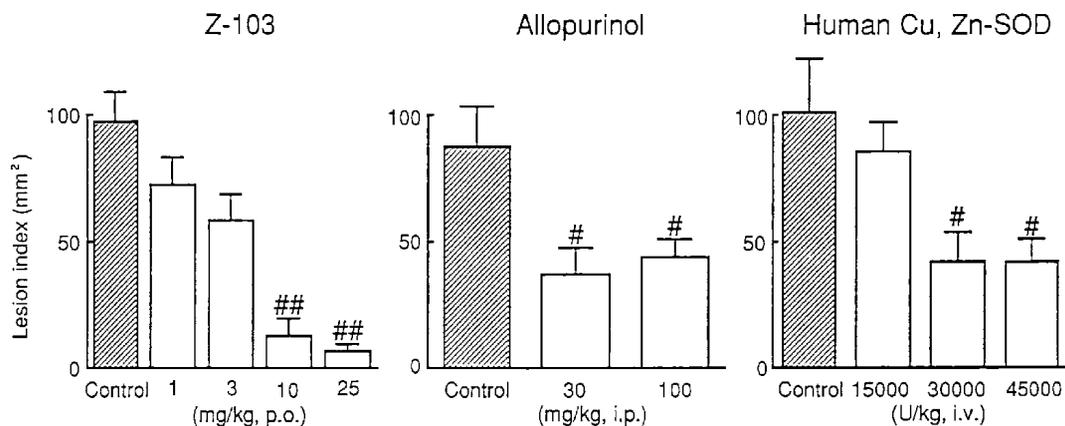


Fig. 1. Effects of Z-103, allopurinol and human Cu, Zn-SOD on 100% ethanol-induced gastric mucosal injury in rats. Rats were given Z-103, p.o. at 1 hr; allopurinol, i.p. at 1 hr; or human Cu, Zn-SOD, i.v. at 10 min prior to intragastric instillation of 100% ethanol (1 ml/rat). The lesions were evaluated 1 hr after the ethanol instillation. Each column denotes the mean \pm S.E. of 6 to 9 rats. #P < 0.05 and ##P < 0.01, compared with the respective control.

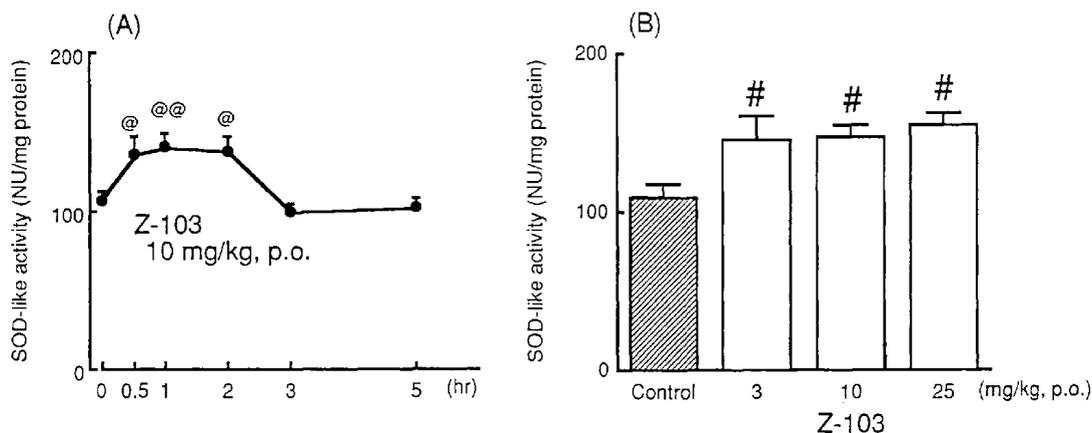


Fig. 2. Changes in SOD-like activity in the gastric mucosa after the administration of Z-103 to normal rats (A) and effects of Z-103 at various doses on the SOD-like activity (B). In the A experiment, SOD-like activity in the gastric mucosa was measured 0, 0.5, 1, 2, 3 and 5 hr after the administration of Z-103 (10 mg/kg, p.o.) to normal rats. In the B experiment, the SOD-like activities in the gastric mucosa were measured 1 hr after the administration of Z-103. Each plot or each column denotes the mean \pm S.E. of 5 to 6 rats. @ $P < 0.05$ and @@ $P < 0.01$, compared with 0 hr. # $P < 0.05$, compared with the control.

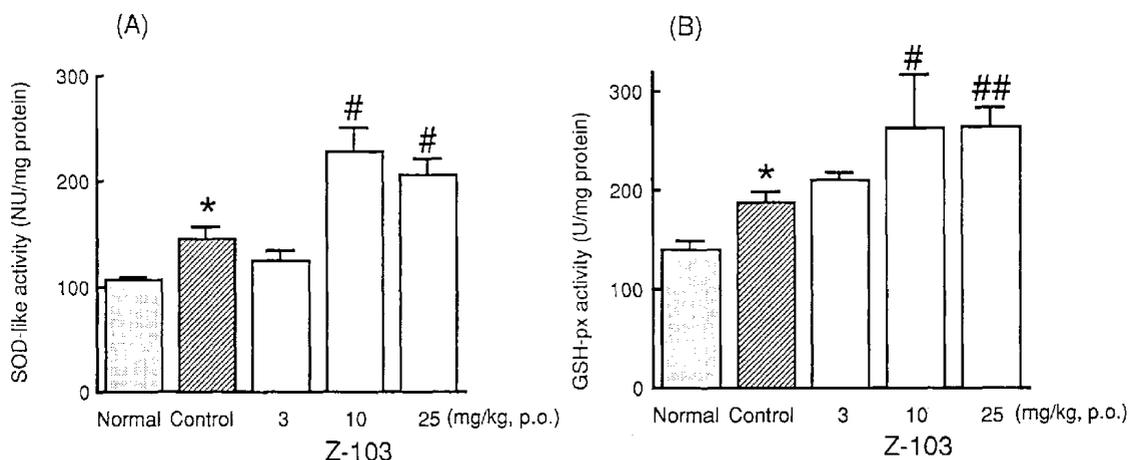


Fig. 3. Effects of Z-103 on SOD-like (A) and GSH-px (B) activities in the gastric mucosa injured by 60% ethanol in rats. Rats were given Z-103, p.o. at 1 hr prior to intragastric instillation of 60% ethanol (1 ml/rat). The SOD-like and GSH-px activities in the gastric mucosa were measured at 1 hr after the ethanol instillation. Each column denotes the mean \pm S.E. of 6 to 7 rats. * $P < 0.05$, compared with the respective normal rats. # $P < 0.05$ and ## $P < 0.01$, compared with the respective control.

elevation of SOD-like and GSH-px activities by Z-103 (10 mg/kg, p.o.). Treatment with cycloheximide abolished the elevation of both scavenging enzyme activities in the mucosa injured by 60% ethanol, but did not affect the ethanol-induced gastric mucosal lesions. In addition, cycloheximide did not induce any mucosal lesions and did not affect these scavenging enzyme activities in the mucosa when it was given to normal animals.

Effect of Z-103 on TBA-reactive substances in gastric mucosa injured by 60% ethanol

TBA-reactive substances were significantly increased 5 min after 60% ethanol instillation (1 ml) and the value at 60 min was 80% higher compared with that at 0 min (Fig. 5). The increase in TBA-reactive substances 60 min after the ethanol instillation was significantly inhibited approx. 50% by pretreatment with 10 and 25 mg/kg, p.o. of Z-103.

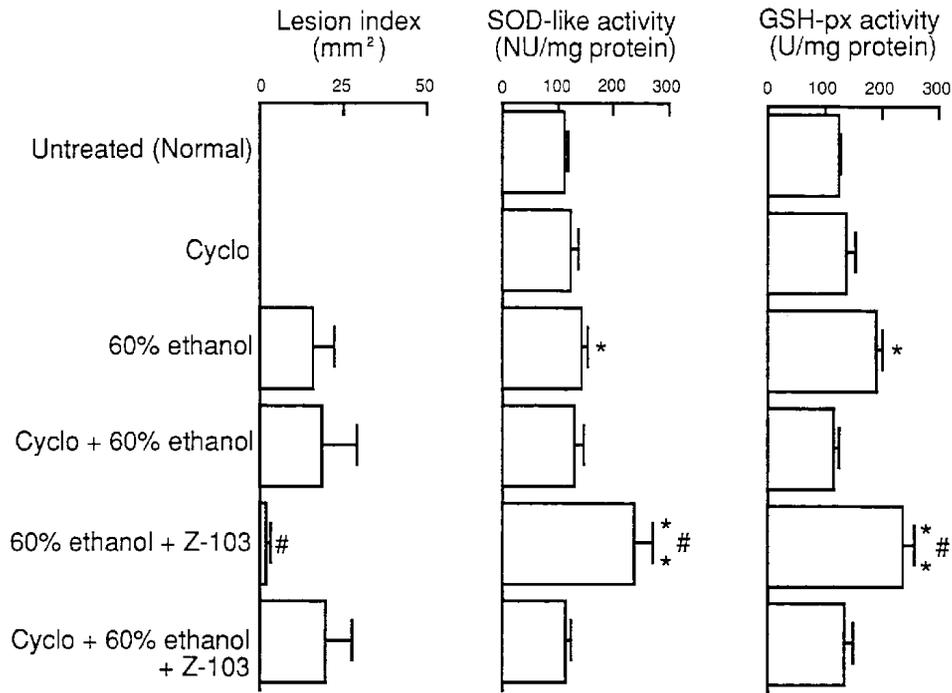


Fig. 4. Effects of Z-103 in combination with cycloheximide (Cyclo) on 60% ethanol-induced gastric mucosal injury in rats and on SOD-like and GSH-px activities in the injured mucosa. Rats were given Z-103 at 10 mg/kg, p.o. prior to intragastric instillation of 60% ethanol (1 ml/rat). Cyclo (6 mg/kg) was given s.c., twice, at 30 min prior to the administration of Z-103 and immediately before the ethanol instillation. Lesions were evaluated 1 hr after the ethanol instillation, and the SOD-like and GSH-px activities in the gastric mucosa were then measured. Each column denotes the mean \pm S.E. of 5 to 7 rats. * $P < 0.05$ and ** $P < 0.01$, compared with the respective untreated (normal) rats. # $P < 0.05$, compared with the respective "60% ethanol".

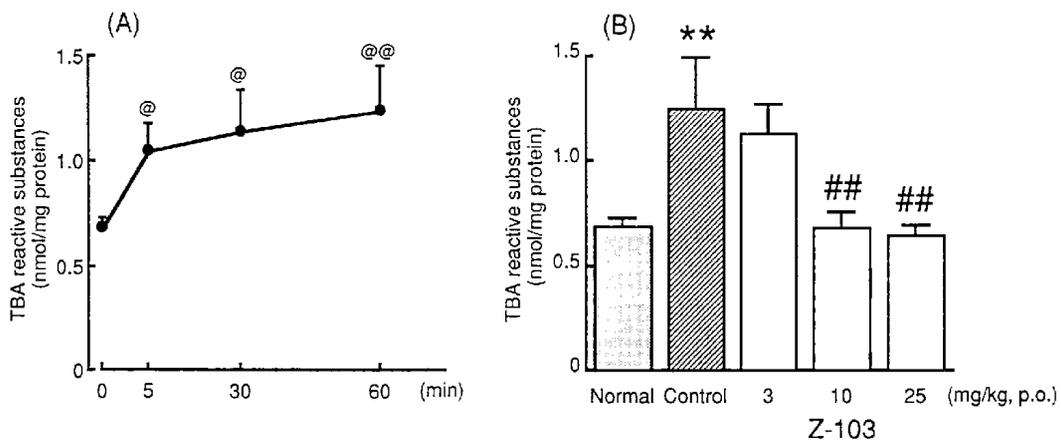


Fig. 5. Changes in TBA-reactive substances in the gastric mucosa after 60% ethanol instillation to rats (A) and effect of Z-103 on the TBA-reactive substances (B). In the A experiment, TBA-reactive substances in the gastric mucosa were measured at 0, 5, 30 and 60 min after intragastric instillation of 60% ethanol (1 ml/rat). In the B experiment, rats were given Z-103, p.o. at 1 hr prior to intragastric instillation of 60% ethanol (1 ml/rat), and TBA-reactive substances in gastric mucosa were measured at 60 min after the ethanol instillation. Each plot or each column denotes the mean \pm S.E. of 6 to 7 rats. @ $P < 0.05$ and @@ $P < 0.01$, compared with 0 min. ** $P < 0.01$, compared with the normal rats. ## $P < 0.01$, compared with the control.

Effects of ZnSO₄·7H₂O and L-carnosine on 100% ethanol-induced gastric mucosal injury and on SOD-like and GSH-px activities in gastric mucosa injured by 60% ethanol

When ZnSO₄·7H₂O and L-carnosine were given p.o. at 10 and 7.7 mg/kg, respectively, the doses that are contained in 10 mg/kg of Z-103 1 hr before 100% ethanol challenge, neither compound prevented the gastric lesions (Fig. 6). When the effects of higher doses of both compounds were examined, only ZnSO₄·7H₂O at 25 mg/kg prevented the lesions. In addition, ZnSO₄·7H₂O at 25 mg/kg significantly increased the SOD-like activity in the corpus injured by 60% ethanol and tended to increase GSH-px activity.

DISCUSSION

The present study indicates that Z-103 may partly protect rat gastric mucosa against ethanol-induced damage via the increase in oxygen-derived free radical scavenging enzyme activities in gastric mucosa.

Terano et al. (10) reported that SOD, a scavenger of

superoxide radicals, and allopurinol, an inhibitor of xanthine oxidase, significantly protected against the rat gastric mucosal lesions induced by 100% ethanol. We also demonstrated that allopurinol and human Cu, Zn-SOD significantly prevented the gastric mucosal lesions induced by 100% ethanol. In addition, the lipid peroxide content (TBA-reactive substances) in gastric mucosa was significantly increased 5 min after 60% ethanol instillation. These results suggest that oxygen-derived free radicals may be involved in the pathogenesis of ethanol-induced gastric mucosal lesions. The source of free radicals in ethanol-induced gastric lesions is yet not thoroughly elucidated. However, the beneficial effect of allopurinol on ethanol-induced gastric lesions obtained in this experiment suggests that xanthine oxidase, which has been considered a major source of free radicals in ischemia/reperfusion, may be one source of free radicals in ethanol-induced lesions. Because 50% ethanol instillation to the rat has been indicated to cause neutrophil infiltration into the gastric mucosa (20), neutrophils may be also considered as another source of free radicals in ethanol-induced lesions. Which free radical

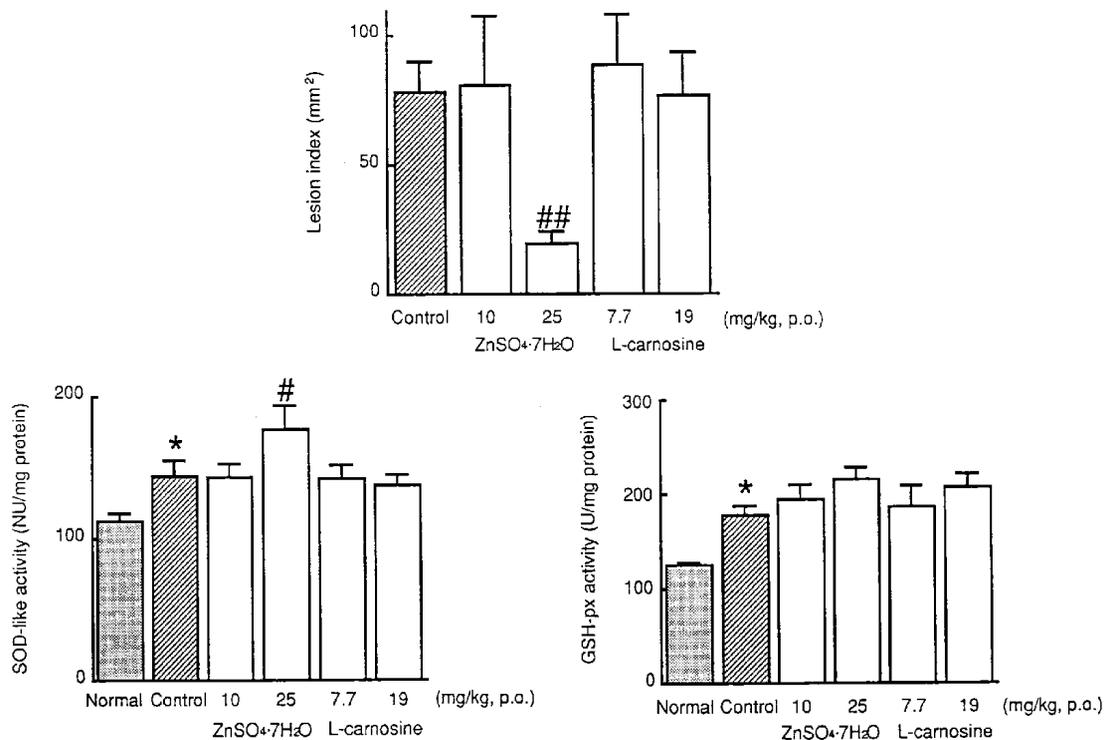


Fig. 6. Effects of ZnSO₄·7H₂O and L-carnosine on 100% ethanol-induced gastric mucosal lesion and on SOD-like and GSH-px activities in the gastric mucosa injured by 60% ethanol. Rats were given ZnSO₄·7H₂O or L-carnosine, p.o. at 1 hr prior to intragastric instillation of 100% ethanol (1 ml/rat), and the lesions were evaluated 1 hr after the ethanol instillation. Rats were given ZnSO₄·7H₂O or L-carnosine, p.o. at 1 hr prior to intragastric instillation of 60% ethanol (1 ml/rat), and SOD-like and GSH-px activities in the gastric mucosa were measured 1 hr after the ethanol instillation. Each column denotes the mean ± S.E. of 6 to 8 rats. *P < 0.05, compared with the respective normal rats. #P < 0.05 and ##P < 0.01, compared with the respective control.

plays the most important role in the pathogenesis of ethanol-induced gastric injury is not clear. It has been reported that dimethylsulfoxide as well as allopurinol and SOD has the ability to protect gastric mucosa against ethanol-induced injury (9, 10). Therefore, both superoxide anion and hydroxyl radical may be involved in the pathogenesis of ethanol-induced injury.

As oxygen-derived free radical scavenging enzymes in this experiment, we determined the activities of SOD (a scavenging enzyme of superoxide radicals) and GSH-px (a scavenging enzyme of hydrogen peroxide) in the gastric mucosa. In the determination of SOD-like and GSH-px activities in the corpus injured by ethanol, we used 60% ethanol instead of 100% ethanol to lessen the influence of bleeding in the mucosa, because in rats, SOD activity is much higher in the blood than in the stomach (21). The activities of both free radical scavenging enzymes in the corpus mucosa 1 hr after the ethanol instillation were significantly higher than those of normal rats. The exact reasons are not obvious why activities of both oxygen-derived free radical scavenging enzymes were elevated by the ethanol administration. However, it seems reasonable to consider that these oxygen-derived free radical scavenging enzyme activities are elevated to dismutate free radicals generated in the mucosa by the ethanol instillation. Furthermore, the development of gastric mucosal injury may occur when the generation of free radicals exceeds the ability of free radical scavenging enzymes to dismutate the radicals. In this study, Z-103 at doses of 10 and 25 mg/kg, p.o., which prevented the gastric lesions induced by 100% or 60% ethanol, further increased the mucosal SOD-like and GSH-px activities elevated by the application of 60% ethanol.

The next experiment was carried out to clarify whether Z-103 causes the increase in the synthesis of free radical scavenging enzymes in the corpus mucosa in rats. Tsurufuji et al. (22) treated mice s.c. with cycloheximide at the dose of 6 mg/kg simultaneously with dexamethasone and again with dexamethasone 1.5 hr later to inhibit protein synthesis for 3 hr after the injection of the steroid. They demonstrated that the anti-inflammatory protein was newly synthesized within 3 hr after the application of the steroid. In the present study, the SOD-like activity in the corpus had been already significantly elevated by 0.5 hr after the administration of Z-103 (10 mg/kg, p.o.) to normal rats and the elevated activity then continued up to 2 hr. The experiment was done 60 min after 60% ethanol instillation to clarify if cycloheximide influences the actions of Z-103 on the ethanol-induced gastric mucosal lesions and free radical scavenging enzyme activities in the injured mucosa. We treated the animals with cycloheximide i.v.

twice, 30 min prior to Z-103 administration and immediately before the ethanol instillation, to inhibit the protein synthesis up to 1 hr after the ethanol instillation (up to 2 hr after treatment with Z-103). The combination with cycloheximide completely abolished the prevention of 60% ethanol-induced gastric lesions and the elevation of both oxygen-derived free radical scavenging enzyme activities in the mucosa by Z-103. Our results indicate that Z-103 directly promotes the synthesis of both scavenging enzyme proteins.

Generally, it has been believed that the elevation of oxygen-derived free radical scavenging enzymes is attributed to the increased oxygen-derived free radicals. However, the increase in TBA-reactive substances in the corpus mucosa 1 hr after 60% ethanol instillation was significantly inhibited by pretreatment with Z-103 (10 and 25 mg/kg). Namely, Z-103 at doses that increased free radical scavenging enzyme activities inhibited the lipid peroxidation. In addition, Z-103 significantly elevated the SOD-like activity in the gastric mucosa of normal rats. Consequently, it is unlikely that Z-103 secondarily elevates the SOD-like and GSH-px activities by increasing the production of free radicals. There are a few reports concerning compounds that elevate free radical scavenging enzymes activities (23–25). We have already demonstrated that TJ-8014, a new Japanese herbal medicine, containing *Bupleuni radix* mainly, promotes the synthesis of free radical scavenging enzymes in rat renal cortex (24). Furthermore, Kawamura et al. (5) reported that glucocorticoid activated glomerular free radical scavenging enzymes and protected glomeruli from oxidant injuries.

In summary, the present data suggest that Z-103 may partly protect rat gastric mucosa against ethanol-induced damage by scavenging oxygen-derived free radicals via the increase in the synthesis of these free radical scavenging enzymes.

Z-103 given, i.p., at 10 mg/kg did not elevate either free radical scavenging enzyme activity in the corpus mucosa of normal rats (unpublished data, M. Ito et al.). Therefore, the increasing action of this agent on free radical scavenging enzyme activities in the gastric mucosa may be mainly due to its local action. Z-103 is a chelate compound that consists of L-carnosine and zinc. In the present study, the amount of zinc present in Z-103 required for preventing 100% ethanol-induced gastric lesion was about one third the amount of $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$. However, L-carnosine was ineffective in preventing the ethanol-induced lesion even at a dose 2.5 times higher than the amount present in an effective dose of Z-103. Therefore, the L-carnosine present in Z-103 may potentiate the effect of zinc. Likewise, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ at the dose which prevented the gastric

lesions significantly increased the SOD-like activity in the corpus mucosa of the rats administered 60% ethanol and tended to increase the GSH-px activity, although L-carnosine was ineffective. Our results suggest that zinc ions present in Z-103 may enhance the synthesis of oxygen-derived free radical scavenging enzymes, while L-carnosine may help the effect of zinc ion.

As mentioned in the introduction, Z-103 has been already demonstrated to inhibit superoxide generation by polymorphonuclear leukocytes and to have anti-oxidative action in vitro (16). Therefore, Z-103 may protect rat gastric mucosa against ethanol-induced damage by its ability to increase the synthesis of free radical scavenging enzymes in the mucosa in addition to the actions mentioned above.

REFERENCES

- Seiki, M., Ueki, S., Tanaka, Y., Soeda, M., Hori, Y., Aita, H., Yoneta, T., Morita, H., Tagashira, E. and Okabe, S.: Studies on anti-ulcer effects of a new compound, zinc L-carnosine (Z-103). *Folia Pharmacol. Japon.* **95**, 257–269 (1990) (Abs. in English)
- Ito, M., Tanaka, T. and Suzuki, Y.: Effect of *N*-(3-aminopropionyl)-L-histidinato zinc (Z-103) on healing and hydrocortisone-induced relapse of acetic acid ulcers in rats with limited food-intake-time. *Japan. J. Pharmacol.* **52**, 513–521 (1990)
- Arakawa, T., Satoh, H., Nakamura, A., Nebiki, H., Fukuda, T., Sakuma, H., Nakamura, H., Ishikawa, M., Seiki, M. and Kobayashi, K.: Effects of zinc L-carnosine on gastric mucosal and cell damage caused by ethanol in rats. Correlation with endogenous prostaglandin E. *Dig. Dis. Sci.* **35**, 559–566 (1990)
- Itoh, M. and Guth, P.: Role of oxygen-derived free radicals in hemorrhagic shock-induced gastric lesions in the rat. *Gastroenterology* **88**, 1162–1167 (1985)
- Perry, M.A., Wadhwa, S., Parks, D.A. and Granger, D.N.: Role of oxygen radicals in ischemia-induced lesions in the rat stomach. *Gastroenterology* **90**, 362–367 (1986)
- Cochran, R.A., Stefanko, J., Moore, C. and Saik, R.: Cytotoxic-free radicals in stress ulcerations. *Surg. Forum* **28**, 159–161 (1982)
- Yoshikawa, T., Yoshida, N., Miyagawa, H., Takemura, T., Tanigawa, T., Sugino, S. and Kondo, M.: Role of lipid peroxidations in gastric mucosal lesion induced by burn shock in rats. *J. Clin. Biochem. Nutr.* **2**, 163–170 (1987)
- Mizui, T., Sato, H., Hirose, F. and Doteuchi, M.: Effect of antiperoxidative drugs on gastric damage induced by ethanol in rats. *Life Sci.* **41**, 755–763 (1987)
- Szelenyi, I. and Brune, K.: Possible role of oxygen free radicals in ethanol-induced gastric mucosal damage in rats. *Dig. Dis. Sci.* **33**, 865–871 (1988)
- Terano, A., Hiraishi, H., Ota, S., Shiga, J. and Sugimoto, T.: Role of superoxide and hydroxyl radicals in rat gastric mucosal injury induced by ethanol. *Gastroenterol. Japon.* **24**, 488–493 (1989)
- Shaw, S., Herbert, V., Colman, N. and Jayatilleke, E.: Effect of ethanol-generated free radicals on gastric intrinsic factor and glutathione. *Alcohol* **7**, 153–157 (1990)
- Pihan, G., Regillo, C. and Szabo, S.: Free radical and lipid peroxidation in ethanol- or aspirin-induced gastric mucosal injury. *Dig. Dis. Sci.* **32**, 1395–1401 (1987)
- Del Soldato, P., Foschi, D., Beboni, G. and Scarpignato, C.: Oxygen free radicals interact with indomethacin to cause gastrointestinal injury. *Agents Actions* **17**, 484–488 (1985)
- Fridovich, I.: The biology of oxygen radicals. *Science* **201**, 875–880 (1978)
- McCord, J.M.: Free radicals and inflammation: Protection of synovial fluid by superoxide dismutase. *Science* **185**, 529–531 (1974)
- Yoshikawa, T., Naito, Y., Tanigawa, T., Yoneta, T. and Kondo, M.: The antioxidant properties of a novel zinc-carnosine chelate compound, *N*-(3-aminopropionyl)-L-histidinato zinc. *Biochim. Biophys. Acta* **1115**, 15–21 (1991)
- Ohyanagi, Y.: Establishment of nitrite-kit for SOD activity determination. *Japan. J. Inflamm.* **4**, 63–73 (1984) (Abs. in English)
- Paglia, D.E. and Valentine, W.N.: Studies on the glutathione and glutathione characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **70**, 158–169 (1967)
- Ohkawa, H., Onishi, N. and Yagi, K.: Assay for lipid peroxide in animal tissue by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351–358 (1979)
- Tepperman, B.L. and Soper, B.D.: Effect of sialoadenectomy on ethanol-induced gastric mucosal damage in the rat: role of neutrophils. *Can. J. Physiol. Pharmacol.* **68**, 207–210 (1990)
- Peters-Joris, C., Vandevoorde, A.-M. and Baudhuin, P.: Subcellular localization of superoxide dismutase in rat liver. *Biochem. J.* **150**, 31–39 (1975)
- Tsurufuji, S., Sugio, K., Takemasa, F. and Yoshizawa, S.: Blockade by antigluco-corticoids, actinomycin D and cycloheximide of anti-inflammatory action of dexamethasone against bradykinin. *J. Pharmacol. Exp. Ther.* **212**, 225–231 (1980)
- Hattori, T., Ito, M. and Suzuki, Y.: Studies on antinephritic effect of TJ-8014, *Syo-Saiko-To-Kyo-Shokyo-Ka-Ouren-Bukuryou* (5): Effects on puromycin aminonucleoside nephrosis and its mechanisms. *Japan. J. Pharmacol.* **56**, 465–473 (1991)
- Hattori, T., Ito, M. and Suzuki, Y.: Studies on antinephritic effect of TJ-8014, a new Japanese herbal medicine (6). Effects on activities of reactive oxygen species-scavenging enzymes in original-type anti-GBM nephritis in rats. *J. Med. Pharm. Soc. for WAKAN-YAKU* **8**, 125–134 (1991)
- Kawamura, T., Yoshioka, T., Bills, T. and Fogo, A.: Glucocorticoid activates antioxidant enzymes and protects glomeruli from oxidant injuries. *Kidney Int.* **40**, 291–301 (1991)