JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 1999, 50, 2, 183—195

H. NISHIWAKI, S. KATO, S. SUGAMOTO, M. UMEDA, H. MORITA, T. YONETA, K. TAKEUCHI

ULCEROGENIC AND HEALING IMPAIRING ACTIONS OF MONOCHLORAMINE IN RAT STOMACHS: EFFECTS OF ZINC L-CARNOSINE, POLAPREZINC

Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, * Department of Pharmacology, Central Researh Institute, Zeria Pharmaceutical Co. Ltd., Saitama, Japan

Effects of a novel zinc compound (polaprezinc), N-(3-aminopropionyl)-L-histidinato zinc, on the mucosal ulcerogenic and healing impairing responses induced by monochloramine (NH_2Cl) were examined in rat stomach. Oral administration of NH_2Cl (> 60 mM) produced severe hemorrhagic lesions in unanesthetized rat stomachs with a marked increase of thiobarbituric acid reactants (TBAR). Pretreatment of the animals with polaprezinc (3 ~ 30 mg/kg, p.o.) showed a dose-dependent inhibition against gastric ulcerogenic and TBAR responses induced by NH_2Cl (120 mM). Likewise, mucosal exposure to NH_4OH (60 mM) in urethane aneshetized stomachs made ischemic by bleeding from the carotid artery (1 ml per 100 g body w.t.) resulted in severe gastric lesions. This ulcerogenic response caused NH_4OH plus ischemia was also attenuated by prior application of polaprezinc as well as taurine (25 mg/ml, 1 ml). On the other hand, the healing of gastric mucosal lesions induced by NH_2Cl occurred more slowly than of ethanol-induced lesions, and the latter was significantly delayed by the repeated administration of NH_2Cl. Polaprezinc (> 10 mg/kg, p.o.) given twice daily for 7 days not only accelerated the healing of NH_2Cl-induced gastric lesions but also antagonized the delayed healing of ethanol-induced lesions in the presence of NH_2Cl as well. Polaprezinc showed a scavenging action against NH_2Cl *in vitro*. These results suggest that NH_2Cl caused deleterious action on the healing of pre-existing acute lesions as well as irritating action to the mucosa in the rat stomach. Polaprezinc not only protects the stomach against injury caused by NH_2Cl but also promotes healing of NH_2Cl-induced gastric lesions as well as the delayed healing of ethanol-induced lesions caused by NH_2Cl. Although the detailed mechanisms underlying these actions of polaprezinc remain unknown, they may be partly attributable to a scavenging action of this agent against NH_2Cl.

Key words: *ammonia, monochloramine, polaprezinc, gastric lesion healing, Helicobacter pylori, rat*

INTRODUCTION

Helicobacter pylori (*H. Pylori*) has been recognized as the major cause of gastritis and peptic ulcer diseases (1, 2). This bacteria has a high activity of urease enzyme, resulting in an abnormally high concentration of ammonia (NH_4OH) in the stomach of infected patients (3). On the other hand, *H. pylori*-associated chronic active gastritis is characterized by an invasion of neutrophils in the gastric mucosa (1, 2, 4). Since neutrophils utilizes the H_2O_2-myeloperoxidase (MPO)-halide system to generate an oxidant capable of destroying a variety of microorganisms and mammalian cell targets (5, 6), it is assumed that neutrophil-derived hypochlorous acid (HClO) interacts with NH_4OH to generate cytotoxic monochloramine (NH_2Cl) (7—9). Although several papers showed a irritating action of NH_2Cl on the gastric mucosa (9—11), the influence of this substance on the healing of gastric lesions remains to be still unclear.

On the other hand, a novel zinc compound (polaprezinc), N-(3-aminopropio-nyl)-L-histidinato zinc, is a chelate compound consisting of zinc ion and L-carnosine (*Fig. 1*). This agent not only prevents gastric mucosal lesions in a wide variety of experimental models but shows the healing promoting action of gastric ulcers as well (12, 13). This action of polaprezinc may be accounted for by cytoprotective and antioxidative activities (12, 14, 15), although the detailed mechanisms remain unknown. Thus, it is of interest to test wheter or not this agent has any prophylactic action against gastric ulcerogenic and healing responses induced by NH_2Cl.

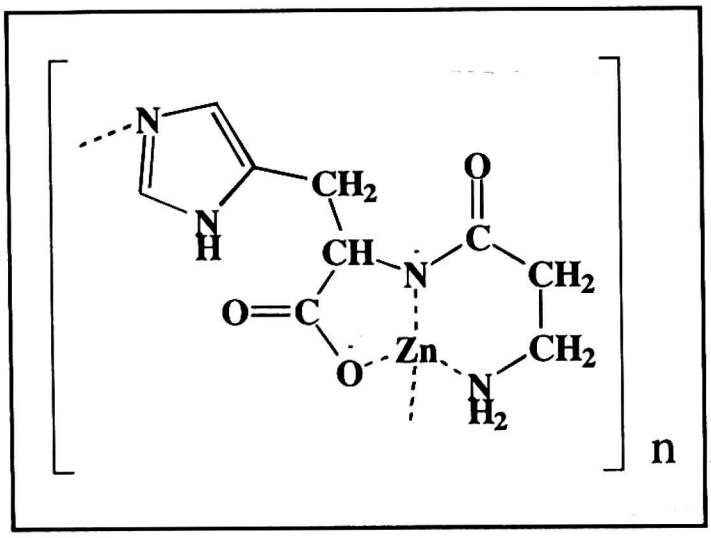

Fig. 1. Structure of Polaprezinc; N-(3-aminopropionyl)L-histidinato zinc; a chelate compound consisting of zinc ion and L-carnosine.

In the present study, we demonstrated the devastating effect of NH_2Cl on the gastric mucosal integrity as well as the healing of pre-existing gastric lesions in rat stomachs, and examined the prophylactic effects of polaprezinc on the mucosal ulcerogenic and healing responses induced by NH_2Cl.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, weighing 250 ~ 300 g (Charles River, Shizuoka, Japan). were used in all experiments. The animals were kept in individual cages with raised mesh bottoms and deprived of food but allowed free access to tap water for 18 hr prior to the experiments. Studies were carried out using 4 ~ 6 rats under both conscious and anesthetized conditions induced by urethane (1.25 g/kg, i.p.).

General procedures

The experiments were classified in roughly three sets of studies; one was to investigate the influences of NH_2Cl on the gastric mucosa in unanesthetized rats, the second to investigate the influence on NH_4OH on the gastric mucosa in anesthetized rats subjected to ischemia; under such situations it is assumed that NH_2Cl is generated endogenously from interaction of NH_4OH with neutrophil-derived HClO (9, 10), and the third is to examine the effect of NH_2Cl on the healing response of pre-existing gastric lesions induced by ethanol. In each study, the effect of polaprezinc on the mucosal ulcerogenic and healing responses induced by NH_2Cl were examined. In separate study, we also examined the scavenging action of polaprezinc against NH_2Cl as well as the effect of polaprezinc on the expression of insulin-like growth factor-1 (IGF-1) *in vitro* experiments.

Induction of gastric mucosal lesions

Study A: Irritant effects of NH_4OH, NaClO and NH_2Cl on the gastric mucosa were compared. The animals were administered 1 ml of NH_4OH (120, 600 and 1,800 mM), NaClO (120 mM), or NH_2Cl (20, 60, and 120 mM), orally by esophageal intubation. The solution of NH_2Cl was prepared by mixing the same concentration of NH_4OH and NaClO, immediately before the administration. The animals were killed 1 hr after the administration of each, agent, the stomachs removed, inflated by injecting 8 ml of 2% formalin and immersed in 2% formalin for 10 min to fix the gastric wall, and opened along the greater curvature. The area (mm^2) of hemorrhagic lesions was measured under a dissecting microscope with a square grid (× 10). The person measuring the lesions did not know the treatment given the animals. These procedures for evaluating macroscopical lesions were applied to all the subsequent studies. Polaprezinc (3 ~ 30 mg/kg) was administered p.o. 30 min before NH_2Cl treatment. Control animals received 0.5% carboxymethylcellulose solution (CMC) as the vehicle.

Study B: Under urethane anesthesia, the stomach was mounted on an *ex-vivo* chamber (the exposed area; 1.3 cm^2) (11, 16). The animals were subjected to ischemia by bleeding from the carotid artery (1 ml/100 g body w.t.), and then the mucosa was exposed to 1 ml of CMC, followed by 1 ml of NH_4OH (120 mM: a final concentration is 60 mM) for 1 hr. At the end of the experiment, the mucosa was dissected out, and the area (mm^2) of hemorrhagic lesions was measured as described above. Polaprezinc (2, 6 and 12 mg/ml, 1 ml) or taurine (25 mg/ml, 1 ml) was applied to the chamber 10 min before the onset of ischemia and NH_4OH treatment. Control animals received CMC as the vehicle.

Study C: The animals were given 1 ml of absolute ethanol or 120 mM NH_2Cl orally through esophageal intubation, then fed on normal chow from 1 hr later. On various days (1, 3, 5 and 7 days) after induction of lesions, the animals were killed, the stomachs removed, treated with 2%formalin, and the area (mm^2) of damage was measured as described above. In half the number of animals treated with ethanol was given NH_2Cl (20 mM) twice daily at 9:00 a.m. and 6:00 p.m. for 6 days. In the latter study, polaprezinc was administered p.o. in a dose of 30 mg/kg twice daily for 6 days, each 30 min before NH_2Cl treatment.

Determination of lipid peroxidation

The lipid peroxidation in the gastric mucosa was determined as thiobarbituric acid reactant (TBAR) at 1 hr after NH_2Cl treatment, according to the modified method of Ohkawa *et al* (17). Briefly, the animals were killed under deep ether anesthesia and the stomachs removed. After rinsing the stomach with cold saline, the musoca was scraped, weighed, and homogenized in 10 ml KCl. The homogenate was supplemented with the mixture of TBAR and boiled at 100° C for 1 hr. The TBAR were then supplemented with 5 ml of the mixture of n-butanol and pyridine, shaken vigorously for 1 min and centrifuged for 100 min at 4000 rpm. Absorbance was measured at 532 nm on Hitachi spectrophotometer and the results were expressed as mnole TBAR per mg protein.

Determination of NH Cl scavenging action

The NH_2Cl scavenging actions of polaprezinc as well as taurine were determined in an in vitro experiment, according to the method described by Lapenna *et al.* (18). It is known that HOCl interacts with β-carotene, inducing vitamine bleaching (19). This phenomenon is characteristic of the chemical reaction of chlorine species with carotene, and thus molecules capable of scavenging chlorine species can specifically antagonize β-carotene. In brief, the reaction mixtures contained 1.3 μmol/L β-carotene in 0.05 mol/L MES buffer, at pH 7.4, with or without polaprezinc or taurine (0.1 ~ 1 mM). NH_2Cl was synthesized by adding HClO to solution of ammonium chloride in 0.05 mol/L MES buffer, at pH 7.0, and added to the reaction mixture at the final concentration of 100 μmol, followed by 15 min incubation at 30°C to bleach β-carotene. β-carotene-related absorbance at 451 nm (A_{451}) was then spectrophotomerically recorded against appropriate drug-containing blanks to assess a specific effect. The concentration of NH_2Cl was calculated using a molar extinction coefficient of 429 M^{-1} cm^{-1}at 242 nm.

Preparation of drugs

Drugs used in this study were urethane (Tokyo Kasei, Tokyo, Japan), taurine, β-carotene (Sigma Chemicals, St. Louis, Mo., USA) and polaprezinc (Zeria Pharmaceutical Co., Saitama, Japan). Other chemicals used were of reagent grade. Urethane was dissolved in saline. Tauurine or polaprezinc was dissolved or suspended with carboxymethylcellulose (CMC) solution, respectively. Each agent was prepared immediately before use. Drugs were administered i.p., p.o. or applied topically to the chamber, in a volume of 1 ml per rat. Control animals received CMC as the vehicle.

Statistics

Data are presented as the means ± SE from 4 ~ 6 rats per group. Statistical analyses were performed using a two-tailed Dunnett's multiple comparison test, and values of $P < 0.05$ were regarded as significant.

RESULTS

Mucosal Ulcerogenic Effect of NH_4OH, NaClO and NH_2Cl

Intragastric administration of NH_4OH at a low concentration (120 mM) did not produce any macroscopic damage in the stomach but produced severe

hemorhagic lesions in the gastric mucosa at the concentration of greater than 600 mM; the lesion score at 600 and 1,800 mM was 65.5 ± 11.0 mm^2 and 240.2 ± 23.6 mm^2, respectively. On the other hand, NaClO the neutrophil-derived oxidant, did not cause any damage in the gastric mucosa at the concentration of 120 mM. However, NH_2Cl generated from a reaction of NaClO with NH_4OH, produced severe hemorrhagic lesions in the rat stomach at the concentration of 60 mM or greater. The lesion score induced by NH_2Cl at 120 mM was 184.7 ± 14.5 mm^2, which is almost equivalent to that induced by NH_4OH at the concentration of 1,800 mM.

Effects of Polaprezinc on Mucosal Ulcerogenic and TBAR Responses Induced by NH_2Cl

Intragastric administration of NH_2Cl produced severe lesions in the stomach with a marked increase fo TBAR, an indicator of lipid peroxidation (*Fig. 2.*). These lesions induced by NH_2Cl were prevented by prior p.o. administration of polaprezine (3 ~ 30 mg/kg) in a dose-dependent manner, and a significant effect was observed at 10 mg/kg or greater, the inhibition at 30 mg/kg being 59.3%. In the stomach treated with NH_2Cl, the mucosal levels of lipid peroxidation as determined by TBAR was significantly increased, the values being 1.1 ± 0.12 nmol/mg protein, which is about 6 times greater than the control level. This increase in TBAR induced by NH_2Cl was also significantly reduced when the animals were pretreated with either polaprezinc (10 mg/kg) at the doses that significantly prevented the mucosal ulcerogenic response to NH_2Cl, although the values were still significantly higher than those in normal rats.

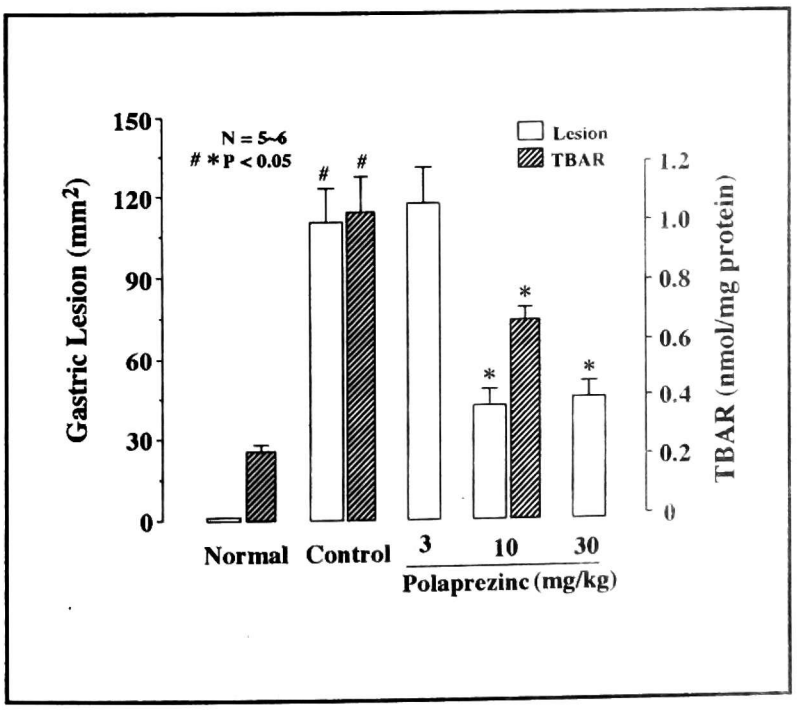

Fig. 2. Effect of polaprezinc on gastric lesions and changes in TBAR induced by NH_2Cl in rats. The animals were administered p.o. with 1 ml of NH_2Cl (120 mM), and killed 1 hr later. Polaprezinc (2 ~ 12 mg/ml) was administered p.o. in a volume of 1 ml/rat 30 min before NH_2Cl treatment. Data are presented as the means ± SE from 5 ~ 6 rats. Statistically significant difference at $P < 0.05$; * from normal; # from CMC.

Effects of Polaprezinc on Mucosal Ulcerogenic Responses Induced by NH_4OH in Rat Stomach under Ischemic Conditions

To confirm the protective action of polaprezinc on NH_2Cl-induced gastric toxicity, we tested the effect of polaprezinc on the mucosal ulcerogenic response induced by endogenously generated NH_2Cl by application of a low concentration of NH_4OH (60 mM) in the ischemic stomach. As shown in *Fig. 3*, topical application of NH_4OH in the stomach made ischemic by bleeding from the carotid artery (1 ml per 100 g body w.t.) resulted in severe hemorrhagic lesions within 1 hr, the lesion score being 53.6 ± 12.2 mm^2. The development of gastric lesions induced NH_4OH in ischemic stomach was totally inhibited when the mucosa was pre-exposed to taurine (25 mg/ml) before ischemia plus NH_4OH treatment. These lesions were also dose-dependently preventated by prior treatment with polaprezinc (2 ~ 12 mg/ml); a significant effect was observed at 6 mg/ml and 12 mg/ml, the inhibition being 87.7% and 91.4%, respectively.

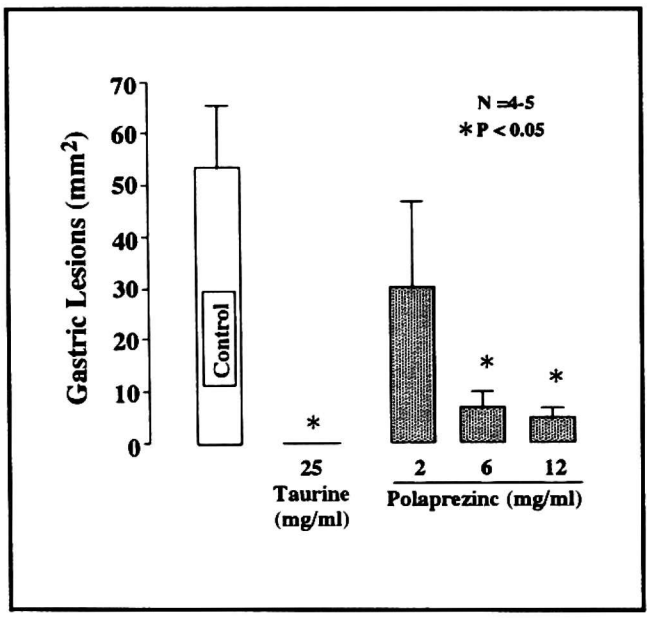

Fig. 3. Effect of polaprezinc on gastric lesions induced by NH_4OH in anesthetized rat stomachs under ischemic conditions. The stomach was mounted on an *ex-vivo* chamber, subjected to ischemia by bleeding from the carotid artery (1 ml/100 g body weight), and then exposed to NH_4OH (60 mM) for 1 hr thereafter. Polaprezinc (2 ~ 12 mg/ml) and taurine (25 ml/ml) were applied to the chamber in a volume of 1 ml, strating 10 min before the onset of ischemia and NH_4OH treatment. Data are presented as the means ± SE from 4 ~ 5 rats. * Statistically significant difference from control (CMC), at $P < 0.05$.

Effect of NH_2Cl on Healing of Gastric Mucosal Lesions

Oral administration of absolute ethanol or 120 mM NH_2Cl induced damage in the gastric mucosa, and the severity of lesions at 1 hr after treatment was almost similar, the lesion score being 173.3 ± 26.7 mm^2 or 156.4 ± 22.6 mm^2, respectively. The lesions induced by ethanol healed rapidly within 7 days, and the lesion score on day 7 was 7.0 ± 1.9 mm^2, which was only 4.0% of the initial damage score (*Fig. 4.*). On the other hand, the healing of NH_2Cl-induced gastric lesions occurred slowly as compared to those induced by ethanol at any time points, and the lesion score on day 7 was 55.0 ± 15.9 mm^2, which was still 35.2% of the initial damage score. In addition, the healing of ethanol-induced gastric lesions was signficantly delayed when the animals were treated with NH_2Cl given p.o. twice daily for 7 days. The lesion score in

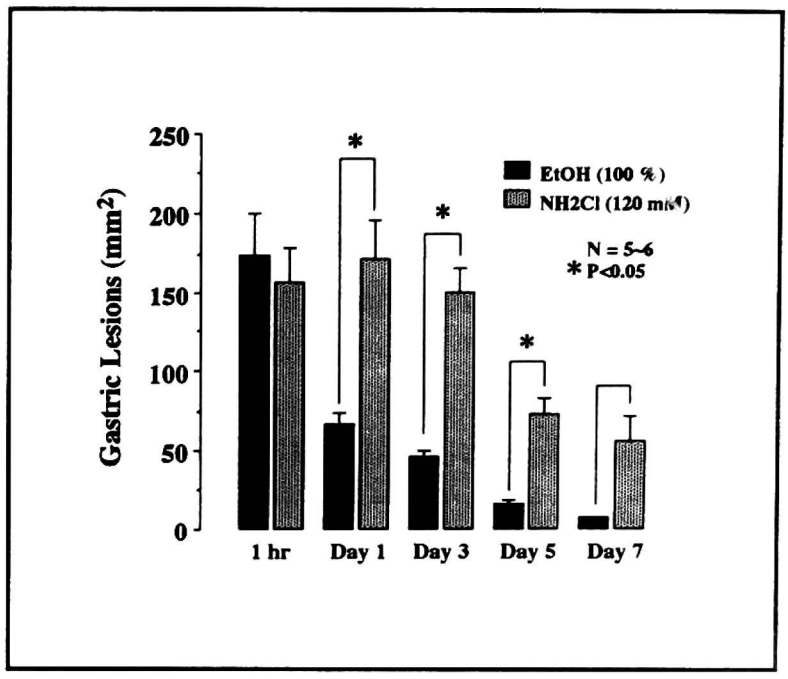

Fig. 4. The healing process of acute gastric lesions induced by absolute ethanol and 120 mM NH_2Cl in rats. Gastric lesions were induced by p.o. administration of 1 ml of absolute ethanol or 120 mM NH_2Cl, and the animals were killed 1 hr or various days after administration of these agents. Data are presented as the means ± SE from 5 ~ 6 rats. * Statistically significant difference from ethanol-treated group, at $P < 0.05$.

these animals was significantly greater at any time points than that in control animals treated with ethanol alone; the values on day 5 were 21.1 ± 1.6 mm^2 or 49.5 ± 4.8 mm^2 in the absence or presence of NH_2Cl treatment, respectively (*Fig. 5.*).

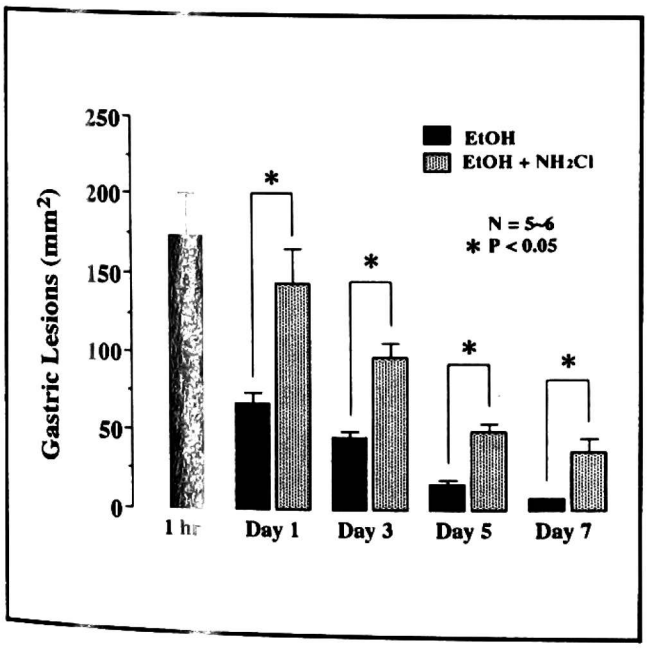

Fig. 5. Influence of NH_2Cl on the healing of ethanol-induced gastric lesions in rats. Gastric lesions were induced by p.o. administration of absolute ethanol. NH_2Cl (20 mM) was given p.o., twice daily for 7 days, starting from 12 hr after ethanol treatment. Data are presented as the means ± SE from 5 ~ 6 rats. * Statistically significant difference from ethanol-alone, at $P < 0.05$.

Effect of Polaprezinc on Healing Response of Gastric Mucosa

We examined the healing promoting effect of polaprezinc in two kinds of studies; the one was performed using NH_2Cl-induced gastric lesions, and the other using the delayed healing of ethanol-induced gastric lesions in the presence of NH_2Cl. Repeated p.o. administration of polaprezinc (30 and 60 mg/kg) for 5 days caused a dose-dependent healing promoting action on NH_2Cl-induced gastric lesions, and a significant effect was observed at 60

mg/kg, the healing rate being 43.6% (*Fig. 6.*). Likewise, polaprezinc showed a healing promoting action against the delayed healing of ethanol-induced gastric lesions in the presence of NH_2Cl. As shown in (*Fig. 7*), the healing of gastric lesions induced by ethanol was significantly delayed by daily administration of NH_2Cl for 5 days, the lesion score being 49.5 $\pm$ 4.8 mm^2, which was significantly greater than that (17.1 $\pm$ 2.3 mm^2) in control animals. Concurrent administration of polaprezinc (10 ~ 60 mg/kg) with NH_2Cl counteracted the delayed healing of these lesions in a dose-dependent manner, and a significant effect was observed at 30 mg/kg or greater.

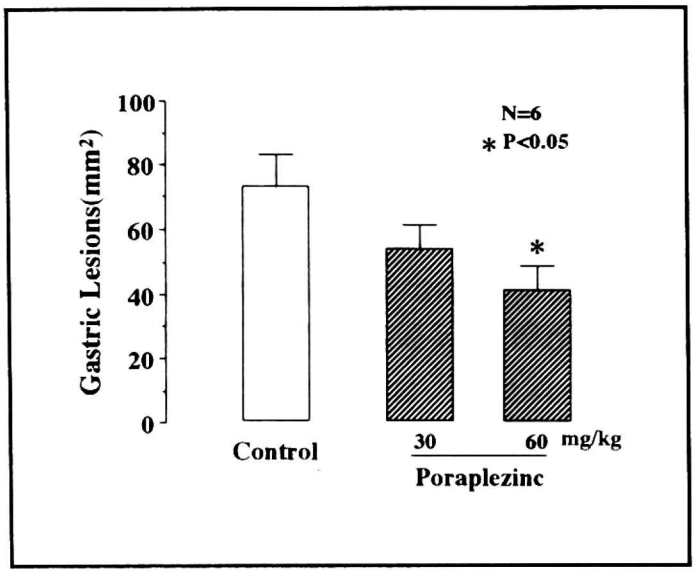

Fig. 6. Effect of polaprezinc on the healing of NH_2Cl-induced gastric lesions in rats. Gastric lesions were induced by p.o. administration of 120 mM NH_2Cl. Polaprezinc (30 and 60 mg/kg) was given p.o. twice daily for 5 days, strating from 12 hr after NH_2Cl treatment. Data are presented as the means $\pm$ SE from 6 rats. * Statistically significant difference from control, at $P < 0.05$.

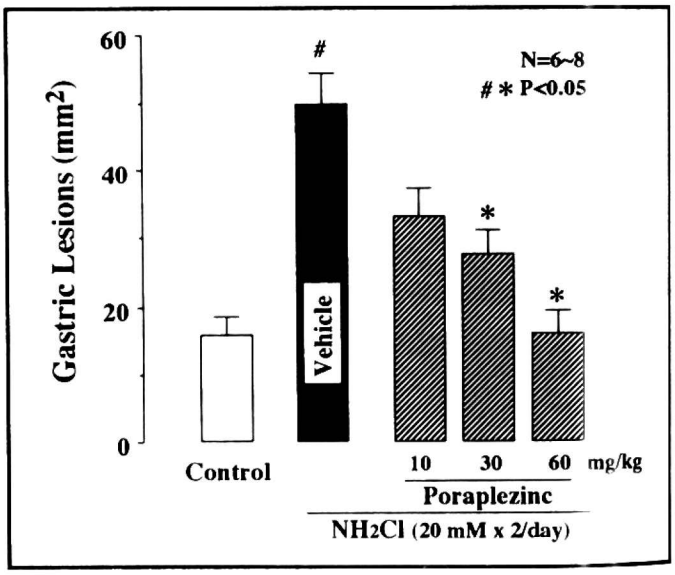

Fig. 7. Effect of polaprezinc on the delayed healing of ethanol-induced gastric lesions caused by NH_2Cl in rats. Gastric lesions were induced by p.o. administration of absolute ethanol. The healing was delayed by the repeated administration of NH_2Cl (20 mM), given p.o. twice daily for 5 days, strating from 12 hr after ethanol treatment. Polaprezinc (10 ~ 60 mg/kg) was given p.o. twice daily for 5 days, each 30 min before administration of NH_2Cl. Data are presented as the means $\pm$ SE from 6 ~ 8 rats. Statistically significant difference at $P < 0.05$; # from ethanol alone; * from control.

Scavening Action of Polaprezinc against NH_2Cl

Scavenging action of polaprezinc and taurine on NH_2Cl was examined in an *in vitro* study, using β-carotene bleaching test (18). Polaprezinc (0.1 ~ 1 mM) exhibited a concentration-dependent scavenging action against NH_2Cl as determined by inhibition rate of the NH_2Cl-induced β-carotene bleaching, the EC_{50} being about 0.03 mM (*Fig. 8*). The same effect was observed by taurine at similar concentration ranges, although the potency was less than that of polaprezinc, the EC_{50} being 0.25 mM.

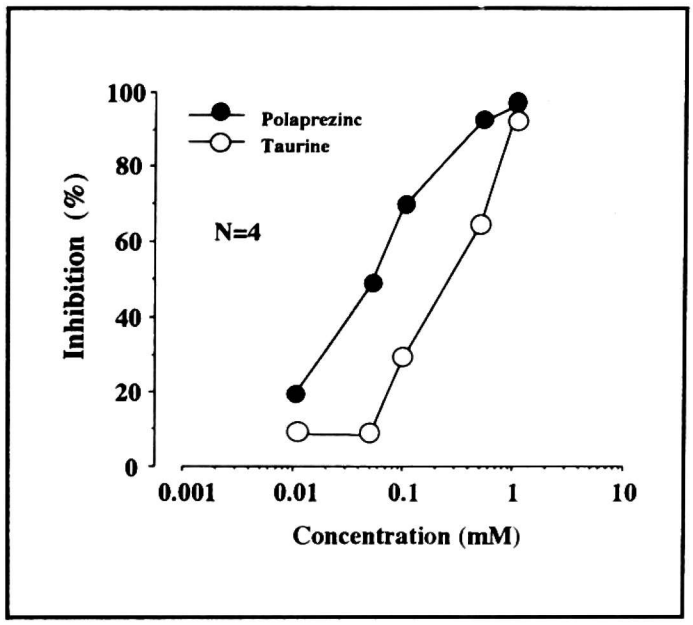

Fig. 8. Scavenging action of polaprezinc and taurine on NH_2Cl *in vitro*. The scavenging action was assessed by inhibition rate of the NH_2Cl-induced β-carotene bleaching. Data are presented as the means ± SE from 4 experiments.

DISCUSSION

The present study showed that NH_2Cl, either administered exogenously or generated endogenously through interaction of NH_4OH with neutrophil-derived HClO, exhibited a potent irritating action in rat stomachs and demonstrated that polaprezinc, a chelate compound of zinc ion and L-carnosine, affords a protection against such gastric damage induced by NH_2Cl. In addition, we also showed that NH_2Cl even at a low concentration impaired the healing of acute gastric lesions, and polaprezinc counteracted the deleterious effect of NH_2Cl on the healing response, exhibiting a healing promoting action.

H. pylori has a high activity of urease enzyme, resulting in an abnormally high concentration of ammonia (NH_4OH) in the stomach of infected patients (3). It is also known that NH_4OH interacts with neutrophil-derived HClO to generate cytotoxic NH_2Cl, a powerful oxidant capable of destroying a variety of microorganisms as well as mammalian cell targets (5—8). Murakami *et al.* (9) demontrated that NH_4OH-induced gastric mucosal lesions were significantly inhibited by taurine, a scavenger of HClO in rats, suggesting a pathogenic role of NH_2Cl in the development of these lesions. We also confirmed that even low concentration of NH_4OH damages the mucosa when the stomach was subjected to ischemia. It is generally accepted that ischemia activates xanthine oxidase, which is responsible to the production of reactive oxygen metabolites such as H_2O_2, and that the generation of HClO by neutrophils is dependent on the quantity of H_2O, and that the generation of HClO by neutrophils is dependent on the quantity of H_2O_2 produced (5, 6). It is assumed that NH_4OH even at low concentrations produces NH_2Cl by interaction with HClO in the ischemic stomach, resulting damage in the mucosa. Indeed, the mucosal lesions

induced by NH_4OH in the ischemic stomach were totally prevented in the presence of taurine.

Polaprezinc a chelate compound of zinc ion and L-carnosine significantly prevented the mucosal ulcerogenic responses induced by NH_2Cl in the normal stomach or by NH_4OH in the ischemic stomach, confirming our previous results (22). We also reported the protective action of polaprezinc against NH_2Cl-induced gastric injury was not affected by either sensory differentation, indomethacin or L-NAME, excluding the involvement of endogenous prostaglandins and nitric oxide as well as sensory neurons in its mechanism (23). This compound has been shown to exhibit various actions, including membrane stabilization (24) and anti-oxidative action (13). Since the generation of NH_2Cl in the ischemic stomach exposed to NH_4OH is a process depending on the presence of superoxide radicals, it is possible that the protective action of polaprezinc in such stomach may be accounted for by its anti-oxidative action. Indeed, the amount of TBAR was increased by NH_2Cl, suggesting an increase of lipid peroxidation in the gastric mucosa, and these changes were significantly prevented by either polaprezinc. Although a cause-effect relationship between these two events remains unclear, it was evident in the present study that polaprezinc has a scavenging property against NH_2Cl. Again it should be noted in this study that the mucosal lesions induced by NH_4OH in the ischemic stomach were totally prevented in the presence of taurine, the scavenger of NH_2Cl.

On the other hand, NH_2Cl significantly impaired the healing of pre-existing gastric lesions induced by ethanol. Indeed, gastric lesions induced by NH_2Cl healed slowly as compared to those induced by ethanol. These results strongly suggest that NH_2Cl has a devastating effect on the healing response of gastric lesions, in addition to a irritating action on the gastric mucosa. The healing of chronic ulcers is reportedly modified in the presence of superoxide radicals (25—27). Naito *et al* (27) showed that treatment with dimethylsulfoxide (DMSO) or rebamipide, a novel hydroxy radical scavenger, counteracted the exacerbation or relapse of acetic acid-induced gastric ulcers in rats.Thus, it is possible that a cytotoxic action of NH_2Cl as a radical species might contribute to its deleterious effect on the healing response. Certainly, the influence of NH_2Cl on other functions such as gastric mucosal blood flow should also be considered as well. As expected, polaprezinc exhibited a significant accelerating effect on the healing of NH_2Cl-induced gastric lesions as well as the delayed healing of ethanol-induced lesions in the presence of NH_2Cl. It is not unreasonable to speculate that the healing promoting effect of polaprezinc may also be due to its scavenging action against NH_2Cl. Alternatively, this action of polaprezinc might be accounted for by its stimulating effect on cell prolif-

eration. Zinc, an essential trace element in animals, is known to be involved in enzymes and transcription factors responsible for several biological functions such as nucleotide synthesis, protein synthesis, and gene expression in cellular proliferation and differentiation (28). Zinc deficiency retards growth in animals and the healing of several injuries such as gastric ulcers (29, 30). Polaprezinc, a compound containing zinc, has been shown to reverse the retardation of gastric ulcer healing caused by zinc deficiency in rats (30). Dorup *et al* (21) reported that zinc deficient rats showed lower values of serum concentration of insulin-like growth factor-1 (IGF-1), which are reversed by supplementation with zinc. Morita *et al.* (31) recently reported that polaprezinc increased the gene expression of IGF-1 mRNA in the stomachs of streptozotocin diabetic rats. Thus, it may be possible that polaprezinc exerts a healing promoting action through the expression of IGF-1.

The present results taken together suggest that NH_2Cl, either administered exogenously or generated endogenously, damages the gastric mucosa and impairs the healing of acute gastric lesions. Polaprezinc not only protects the stomach against injury caused by NH_2Cl but also promotes the delayed healing of acute gastric lesions caused by NH_2Cl as well. Although the detailed mechanisms underlying these actions of polaprezinc remain unknown, they are partly attributable to its scavenging action of NH_2Cl. Since an important feature of *H. pylori* infection in the stomach is infiltration of neutrophils in the gastric mucosa (1, 2, 4), it is highly possible that NH_2Cl is formed in the inflamed gastric mucosa, where neutrophil and *H. pylori* are located in juxtaposition. Thus, the present study also suggests that polaprezinc may have therapeutic potential in the prevention and/or treatment of gastric mucosal damage related with *H. pylori*.

REFERENCES

1. Marshall BJ, Warren JR. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; 1: 1273—1275.
2. Graham DY. Campylobacter pylori and peptic ulcer disease. *Gastroenterology* 1989; 96 (suppl.): 615—625.
3. Marshall BJ, Langton SR. Urea hydrolysis of patients with campylobacter pyloridis infection. *Lancet* 1983; 1: 965—966.
4. Whitehead R, Truelove SC, Hear MWL. The histological diagnosis of chronic gastritis in fiberoptic gastroscope biopsy specimens. *J Clin Pathol* 1972; 25: 1—11.
5. Badwey JA, Karnovsky ML. Active oxygen species and the functions of phagocytic leukocytes. *Annu Rev Biochem* 1980; 46: 695—726.
6. Klevanoff SJ. Oxygen metabolism and the toxic properties of phagocytes. *J Annu Intern Med* 180; 93: 480—489.

7. Grisham MB, Jefferson MM, Thomas EL. Chlorination of endogenous amines by isolated neutrophil. Ammonia-depended bactericidal, cytotoxic, and cytolytic activities of the chloramines. *J Biol Chem* 1984; 259: 1040—10413.
8. Grisham MB, Hernandez LA, Granger Dn. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *Am J Physiol* 1986; 251: G567—G574.
9. Murakami M, Asagoe K, Dekigai H, Kusaka S, Saita H, Kita T. Products of neutrophil metabolism increase ammonia-induced gastric mucosal damage. *Dig Dis Sci* 1995; 40: 268—273.
10. Dekigai H, Murakami M, Kita T. Mechanism of *Helicobacter* pylori-associated gastric mucosal injury. *Dig Dis Sci* 1995; 40: 1332—1339.
11. Takeuchi K, Ishihar Y, Okada M, Niida H, Okabe S. A continuous monitoring of mucosal integrity and secretory activity in rat stomach. A preparation using a lucide chamber. *Jpn J Pharmacol* 1989; 49: 235—244.
12. Seiki M, Ueki S, Tanaka Y, *et al.* Studies on anti-ulcer effects of a new compound zinc L-carnosine (Z-103). *Folia Pharmacologica Japonica* 1990; 95: 257—269.
13. Ito M, Tanaka T, Suzuki Y. Effect of N-(3-aminopropionyl)-L-histadinato zinc (Z-103) on healing and hydrocortisone-induced relapse of acetic acid ulcers in rats with limited food-intake-time. *Jpn J Pharmacol* 1990; 52: 513—521.
14. Arakawa T, Satoh H, Nakamura A *et al.*Effects of Zinc-L-carnosine on gastric mucosal and cell damage caused by ethanol in rats. *Dig Dis Sci* 1990; 35: 559—566.
15. Yoshikawa T, Naito Y, Tanigawa T, Yoneta T, Kondo M. The antioxidant properties of a novel zinc-carnosine chelate compound, N-(3-amino-propionyl)-L-histidinato zinc. *Biochim Biophys Acta* 1991; 1115: 15—22.
16. Takeuchi K, Ohno T, Okabe S. Irritative and protective activity of mild irritants in rat stomach. *Dig Dis Sci* 1987; 32: 889—896.
17. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thio-barbituric acid reaction. *Anal Biochem* 1989; 95: 351—358.
18. Lapenna D, De Gioia S, Ciofani G, Cuccurullo F. Ambropxolls a scavenger of hypochlorous acid and monochloramine. *Pharmacology* 1994; 49: 132—135.
19. Harrison JE, Watson BD, Schultz J. Myeloperoxidase and singlet oxygen: A reappraisal. *FEBS Lett* 1978; 92: 327—332.
20. Chomczynski P, Sacchi N. Single-step methods of isolation by acid guanidinium thiocyanatephenol-chloroform extraction. *Anal Biochem* 1987; 162: 156—159.
21. Dorup I, Flyvbjerg A, Everts M, Clausen T. Role of insulin-like growth factor-1 and growth hormone in growth inhibition induced by magnesium and zinc deficiencies. *Br J Nutr* 1991; 66: 505—521.
22. Nishiwaki H, Kato S, Takeuchi K. Irritant action of monochloramine in rat gastric mucosa. *Gen Pharmacol* 1997; 29: 713—718.
23. Kato S, Nishiwaki H, Konaka A, Takeuchi K. Mucosal ulcerogenic action of monochloramine in rat stomachs: Effects of polaprezinc and sucralfate. *Dig Dis Sci* 1997; 42: 2156—2163.
24. Cho CH, Luk CT, Ogle CW. The membrane-stabilizing action of zinc carnosine (Z-103) in stress-induced gastric ulceration in rats. *Life Sci* 1991; 49: PL189—PL194.
25. Salim AS. Oxygen-derived free radicals and the prevention of duodenal ulcer relapse: A new approach. *Am J Med Sci* 1990; 300: 1—6.
26. Naito Y, Yoshikawa T, Matsuya K *et al.* Effect of oxygen radical scavengers on the quality of gastric ulcer healing in rats. *J Clin Gastroenterol* 1995; 21 (Suppl.): S82—S86.
27. Naito Y, Yoshikawa T, Matsuyama K, Nishimura S, Yagi N, Kondo M. Effects of free radical scavengers on indomethacin-induced aggravation of gastric ulcers in rats. *Dig Dis Sci* 1995; 40: 2019—2021.
28. Vallee BL, Auld DS. New perspective on zinc biochemistry: Cocatalytic sites in multi-zinc enzymes. *Biochemistry* 1993; 32: 6493—6500.

29. Watanabe T, Arakawa T, Fukuda T, Higuchi K, Kobayashi K. Zinc deficiency delays gastric ulcer healing in rats. *Dig Dis Sci* 1995; 40: 1340—1344.
30. Kuwayama H, Matsuo Y. Effect of Z-103, a chelate compound of zinc and L-carnosine, on epithelial proliferation in intact and regenerated mucosa of rats. *Ther Res* 1992; 13: 1597—1603.
31. Morita H, Seto K, Hori H, Yoneta T. Effect of polaprezinc on gastric mucosal lesions induced in diabetic rats: Relation to insulin-like growth factor-1. *Ulcer Res* 1999; in press.

Received: September 11, 1998
Accepted: April 13, 1999

Author's address: Koji Takeuchi, Ph.D., Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Yamashina, Kyoto 607-8414, Japan. E-mail: takeuchi@mb.kyoto-phu.ac.jp.