Effect of Zinc-Carnosine Chelate Compound (Z-103) on Burn-Induced Gastric Mucosal Injury in Rats

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Summary The protective effect of a novel synthetic zinc-carnosine chelate compound, zinc N-(3-aminopropionyl)-L-histidine (Z-103), on the gastric injury induced by burn stress was studied in rats. Gastric lesions were produced by immersion of half of the animal's body into 80°C water for 10 s. The increase in total area of the erosions 3 h after the stress and the increase in thiobarbituric acid reactants in the gastric mucosa were significantly inhibited by the administration of Z-103 at doses of 30, 100, and 300 mg/kg. The decrease in blood flow in the gastric mucosa was not influenced by the treatment with Z-103. These results suggest that the protective effect of Z-103 against the aggravation of burn-induced gastric mucosal injury may be due to its inhibitory effect on lipid peroxidation.

Key Words: burn, gastric mucosal injury, zinc, carnosine

The novel synthetic agent zinc N-(3-aminopropionyl)-L-histidine (Z-103) is a chelate compound that consists of one zinc ion and L-carnosine. Carnosine was discovered in 1900 by Gulewitsch and Amiradzibi [1] from meat extract, and is reportedly present in the range of 1-20 mM in the skeletal muscle and brain of many animals and humans [2]. Recently, several reports have described the antioxidative actions of carnosine, such as efficient scavenging of singlet oxygen [3] and peroxyradicals [4], efficient chelation of copper and other transitional metals [4], and scavenging of superoxide radicals in the presence of zinc [5].

Zinc compounds have been reported to enhance the rate of healing of human
gastric ulcers and to protect against various kind of experimental ulcers [6, 7]. Willson [8] showed that zinc may play a vital role in masking labile sulfur sites and so reduce the damage from radical actions at critical times during the life of the cell. Girotti et al. [9] demonstrated that zinc strongly inhibited lipid peroxidation in a natural membrane exposed to xanthine-xanthine oxidase and iron, and concluded that zinc interfered with lipid peroxidation at the membrane level, possibly by altering or preventing iron binding.

Recently, much interest has been focused on the cytotoxic effects of reactive oxygen metabolites in gastro-intestinal diseases [10–12]. It has been suggested that lipid peroxidation and active oxygen species play an important role in the pathogenesis of acute gastric mucosal injury [12, 13] and multiple organ failure [14] induced by burn stress in rats. In this paper, we describe the influence of a novel synthetic zinc-carnosine chelate compound (Z-103) on gastric mucosal injury and its inhibitory effect on lipid peroxidation in gastric mucosa induced by burn stress.

**MATERIALS AND METHODS**

**Experimental model of gastric mucosal injury.** Male Wistar rats weighing 190–210 g obtained from Keari Co., Ltd., Osaka, were used. The animals were not fed for 18 h prior to experiments, but were allowed free access to water. Burn shock stress was induced by immersion of the posterior half of the rat’s body into 80°C water for 10 s [13]. The agent Z-103, which was a gift from Zeria Pharmaceutical Co., Ltd., Tokyo, was dissolved in 0.5% carboxymethyl cellulose sodium (CMC) solution, and given to the rats by gastric intubation 1 h before experiments. Control rats were given only 0.5% CMC solution.

**Determination of gastric mucosal blood flow.** The microcirculatory blood flow in the gastric wall was measured by laser doppler flowmetry (ALE 2100, Advance Co., Ltd., Tokyo). The effect of Z-103 (100 mg/kg) on the gastric mucosal blood flow was examined at 0, 10, 60, and 180 min after the burn stress.

**Evaluation of gastric mucosal lesions.** After stress loading, rats were killed by exsanguination via the abdominal aorta. The gastric mucosa was carefully examined macroscopically, and the extent of gastric mucosal lesions was expressed by the total area of the erosions.

**Biochemical assays.** Thiobarbituric acid (TBA)-reactive substances, an index of lipid peroxidation, were measured in serum by the method of Yagi [15], and in tissue by that of Ohkawa et al. [16]. The level of TBA reactants was expressed as nmol of malondialdehyde. TBA (BDH Chemicals, Poole, England) and 1,1,3,3-tetramethoxypropane (Tokyo Kasei Co., Tokyo) were used for the TBA assay. All other chemicals were of reagent grade.

RESULTS

Effect of Z-103 on gastric mucosal blood flow
After the burn stress the gastric mucosal blood flow decreased to 30% of that measured before the stress. The decrease in blood flow after the stress was not inhibited by the treatment with Z-103 at a dose of 100 mg/kg (Fig. 1).

Effect of Z-103 on gastric mucosal lesions
The total area of the gastric erosions increased gradually after the burn stress. Multiple erosions and severe bleeding were revealed in the stomach 3 h after the burn injury. The increase in total area of the erosions after the burn stress was significantly inhibited by the treatment with Z-103 at doses of 30, 100, and 300 mg/kg (Figs. 2, 3; Table 1).

Effect of Z-103 on TBA-reactive substances in serum and in gastric mucosa
Serum TBA-reactive substances were significantly increased in rats 10 min after the burn stress, and this increase was not inhibited by the treatment with Z-103 at any dose (Table 2).

TBA-reactive substances in the gastric mucosa were significantly increased 10 min after the burn stress, and then the values decreased gradually. The increase in TBA-reactive substances in the gastric mucosa was significantly inhibited by the treatment with Z-103 at doses of 30, 100, and 300 mg/kg (Fig. 4, Table 2).

Fig. 1. Effect of Z-103 on the gastric mucosal blood flow after burn stress. Each point indicates the mean ± SEM of 3 experiments. ○ ... ○, Group without administration of Z-103; ● —— ●, group treated with Z-103 at a dose of 100 mg/kg.
DISCUSSION

In the present study, Z-103 inhibited the increase in gastric mucosal lesions induced by the burn stress without reversing the reduced microcirculatory blood flow of the gastric mucosa. The increase in TBA-reactive substances in the gastric mucosa 10 min after the stress, at which time no macroscopic lesions were found there, was significantly inhibited by the treatment with Z-103. We earlier reported some evidence that tissue lipid peroxidation may play an important role in the

formation of gastric mucosal injury induced by burn shock [13]. Furthermore, superoxide dismutase, catalase, and allopurinol, the last being a competitive inhibitor of xanthine oxidase, can inhibit the aggravation of gastric mucosal injury [12]. Nishigaki et al. [14] reported that lipid peroxides produced by burn stress injure several organs including blood vessels. These results suggest that lipid
peroxidation may play some role in the gastric injury induced by burn stress. Z-103 shows anti-free radical actions in vitro (unpublished results), and the mechanism of its anti-ulcer action may be explained in part by these actions.

As biological roles of carnosine and its analogs, the following have been reported: wound healing [17], buffer action to neutralize lactic acid produced in skeletal muscle undergoing anaerobic glycolysis [18], and physiological activation of myosin ATPase [19]. However, no unified hypothesis that explain its role satisfactorily has appeared. Regarding the zinc component, the anti-ulcerative effect of Z-103 may in part be due to the membrane-stabilizing activity of this metal, which action is especially evident on mast cells and lysosomes [20, 21]. These reports suggest that Z-103 may have several effects besides its antioxidative effect. All these effects of Z-103 may contribute to the protection against gastric mucosal injury. Therefore, Z-103 may become an important therapeutic agent, especially as an anti-ulcer drug, in the future.

REFERENCES

ZINC-CARNOSINE COMPOUND AND GASTRIC INJURY


