Free Rad. Res. Comms., Vol. 14, No. 4, pp. 289–296
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EFFECT OF ZINC-CARNOSINE CHELATE COMPOUND (Z-103), A NOVEL ANTIOXIDANT, ON ACUTE GASTRIC MUCOSAL INJURY INDUCED BY ISCHEMIA-REPERFUSION IN RATS

T. YOSHIKAWA, Y. NAITO, T. TANIGAWA, T. YONETA, M. YASUDA, S. UEDA, H. OYAMADA and M. KONDO

First Department of Medicine, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602, Japan

(Received November, 14 1990; in revised form December 11, 1990)

The protective effect of a novel synthetic zinc-carnosine chelate compound, zinc N-(3-aminopropionyl)-L-histidine (Z-103), on the gastric mucosal injury induced by ischemia-reperfusion was studied in rats. Ischemia and reperfusion injury was produced on the rat stomach by applying a small clamp to the celiac artery for 30 min and by removal of the clamp for 30 min. The decrease in the gastric mucosal blood flow was not influenced by the treatment with Z-103. The increase in total area of the erosions on the stomach after ischemia-reperfusion and the increase in lipid peroxides in the gastric mucosa were significantly inhibited by the oral administration of Z-103. In addition, Z-103 inhibited lipid peroxidation of rat brain homogenate and liver microsome *in vitro*. These results suggest that the protective effect of Z-103 against the aggravation of gastric mucosal injury induced by ischemia-reperfusion may be due to its inhibitory effect on lipid peroxidation.

KEY WORDS: Gastric mucosal injury, lipid peroxidation, zinc, carnosine, ischemia-reperfusion.

INTRODUCTION

A novel synthesized agent, zinc N-(3-aminopropionyl)-L-histidine (Z-103), is a chelate compound consisting of zinc ion and L-carnosine (Figure 1). Carnosine was discovered in 1900 by Gulewitsch and Amiradzibi[1] in meat extracts, and is reportedly present at millimolar concentrations in several mammalian tissues, including skeletal muscle and brain.[2] Recently, it has been proposed that carnosine and related compounds such as anserine and homocarnosine act as antioxidants *in vivo*.[3,4] Kohen *et al.*[3] reported that carnosine decreased the rate of oxidation of linoleic acid by peroxyl radicals and inhibited hydroxylation of deoxyguanosine by an ascorbic acid/Cu^{2+} mixture, apparently by binding Cu^{2+}. Recent reports have suggested that carnosine efficiently scavenges singlet oxygen[5] as well as hydroxyl radicals.[6]

Zinc has also been shown to have an antioxidant effect, first by possibly decreasing the susceptibility of specific sulfhydryl groups to oxidation and second, by competing with prooxidant metals (i.e., Cu and Fe) for binding sites, thereby decreasing their ability of the latter to transfer electrons in a particular environment.[7] Zinc compounds have been reported to enhance the rate of healing in human gastric ulcers and to

Correspondence: Toshikazu Yoshikawa, MD, PhD, First Department of Medicine, Kyoto Prefectural University of Medicine, Kamigyo-Ku, Kyoto 602, Japan.

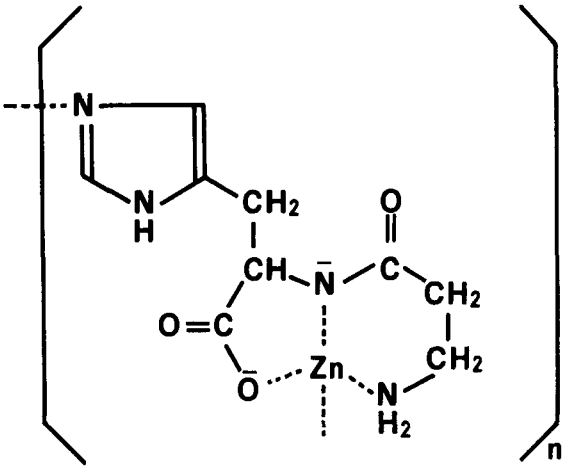

FIGURE 1 Chemical structure of zinc N-(3-aminopropionyl)-L-histidine (Z-103).

protect against various kinds of experimental ulcers.[8,9] Although it has been thought that such anti-ulcerative effects were due to the membrane-stabilizing activity of zinc ions (especially on mast cells and on lysosomes),[10,11] the relationship between the stabilizing and anti-oxidative effects of zinc is unclear. Because zinc and carnosine have anti-oxidative properties, some anti-oxidative actions are expected for Z-103.

Recently, we and other investigators reported on the cytotoxic effects of reactive oxygens and lipid peroxidation in gastric mucosal injuries induced by ischemia,[12] ischemia-reperfusion,[13] and stresses including burn shock.[14] Using a burn shock model in rats, we found that a zinc-carnosine chelate compound (Z-103) inhibits the gastric mucosal lesions and the increase in tissue lipid peroxidation.[15] However, many cyto-protective factors in addition to free radical scavengers are intimately involved in the pathogenesis of burn shock-induced gastric injury. The aim of the present study was to determine whether Z-103 can ameliorate ischemia/reperfusion injury to the gastric mucosa, such injuries being thought to be produced by oxygen radicals and lipid peroxidation.

MATERIALS AND METHODS

Experimental model of gastric mucosal injury

Male Sprague Dawley rats weighing 180–220 g obtained from Keari Co., Ltd., Osaka, were used. The animals were not fed for the 18 hr prior to the experiments, but were allowed free access to water. Ischemia was induced under intraperitoneal pentobarbital anesthesia (25 mg/kg) by apply a small clamp to the celiac artery for 30 min followed by removal of the clamp for 30 min.[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 hr before ischemia. Control rats were given only 0.5% CMC solution.

Determination of gastric mucosal blood flow

The microcirculatory blood flow in the gastric mucosa was measured using a laser Doppler flowmeter (ALF 2100, Advance Co., Ltd., Tokyo). The effect of Z-103 at a dose of 30 mg/kg on the blood flow of the gastric mucosa was evaluated before, upon, and 30 min after ischemia, as well as upon and 30 min after reoxygenation.

Evaluation of gastric mucosal lesions

After ischemia-reperfusion, rats were killed by exsanguination via the abdominal aorta. The gastric mucosa was carefully examined macroscopically, and the extent of any gastric mucosal lesions was expressed in terms of the total area of the erosions.

Biochemical assay

Thiobarbituric acid (TBA)-reactive substances were measured in serum by the method of Yagi,[16] and in the gastric mucosal homogenate by the method of Ohkawa *et al.*[17] as an index of lipid peroxidation after ischemia-reperfusion. The level of TBA-reactive substances were expressed in terms of nmol of malondialdehyde. TBA (BDH Chemical, Poole, England) and 1,1,3,3-tetramethoxy propane (Tokyo Kasei, Co., Tokyo) were used for TBA assays and all other chemicals were of reagent grade. Total protein in the tissue homogenates was measured by the method of Lowry.[18] α-Tocopherol and total cholesterol were measured in serum by the HPLC fluorescence method of Abe.[19] In addition, effects of Z-103 on lipid peroxidation of rat brain homogenate and rat liver microsome were studied. Rat brain tissue obtained from Sprague-Dawley male rats weighing 200 g was homogenized in 35 mM phosphate buffer pH 7.4. To induce autoxidation, the homogenate was placed at 37°C in the presence or in the absence of the agent. Lipid peroxides produced by autoxidation was monitored by TBA-reactive substances determined by the method of Ohkawa *et al.*[17] The lipid peroxidation of rat liver microsome induced by ferrous sulfate (5 μM) and NADPH (0.1 mM) was also monitored by TBA-reactive substances in the presence and in the absence of the agent.

Statistics

Data was expressed as mean ± SEM and analyzed with Student's t-test by using a computer program. A p value of < 0.05 was regarded as significant.

RESULTS

Effect of Z-103 on gastric mucosal blood flow

Clamping of the celiac artery decreased gastric mucosal blood flow to 10% of that measured before clamping. Just after the removal of the clamp, the blood flow in the gastric mucosa recovered completely from its ischemic status. The decrease of blood flow during ischemia was not improved by treatment with Z-103 at a dose of 30 mg/kg (Figure 2).

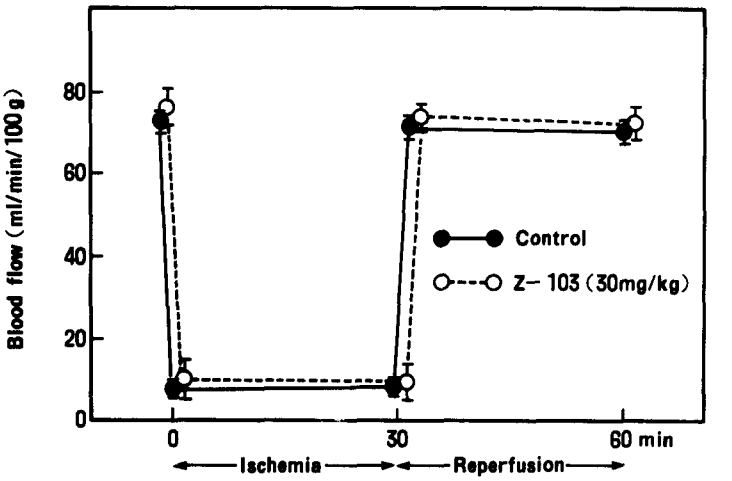

FIGURE 2 Effect of Z-103 on the gastric mucosal blood flow during ischemia-reperfusion. Each point indicates the mean ± SEM of 3 experiments.●—●, Group without administrationo f Z-103; ○—○, group treated with Z-103 at a dose of 30 mg/kg.

Effect of Z-103 on the gastric mucosal lesions

Multiple erosions and bleeding were revealed in the stomach after the reperfusion following 30 min of gastric mucosal ischemia. The total area of any gastric mucosal

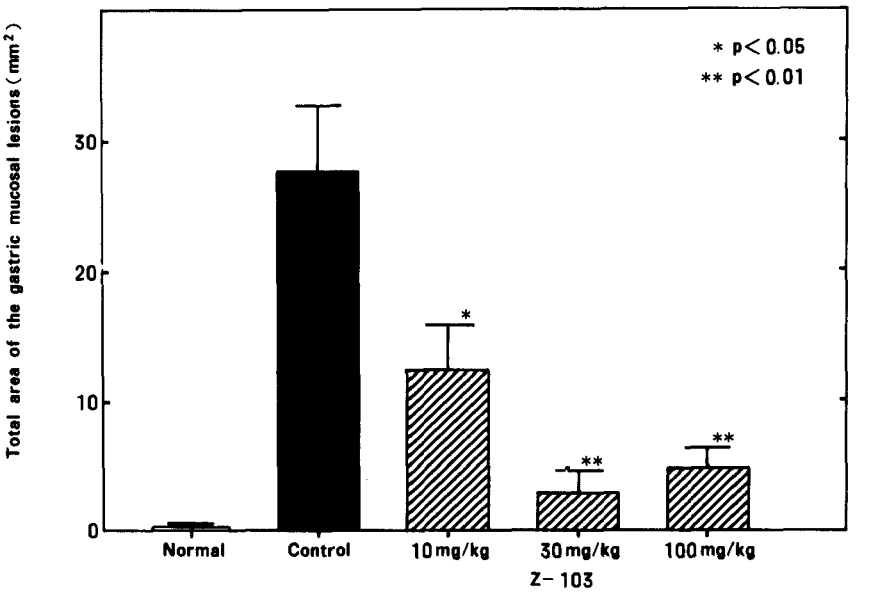

FIGURE 3 Effect of Z-103 on the total area of the gastric mucosal lesions induced by ischemia-reperfusion. Each value indicates the mean ± SEM of 7 experiments. $*p < 0.5$ and $**p < 0.01$ when compared with the control group without administration of Z-103.

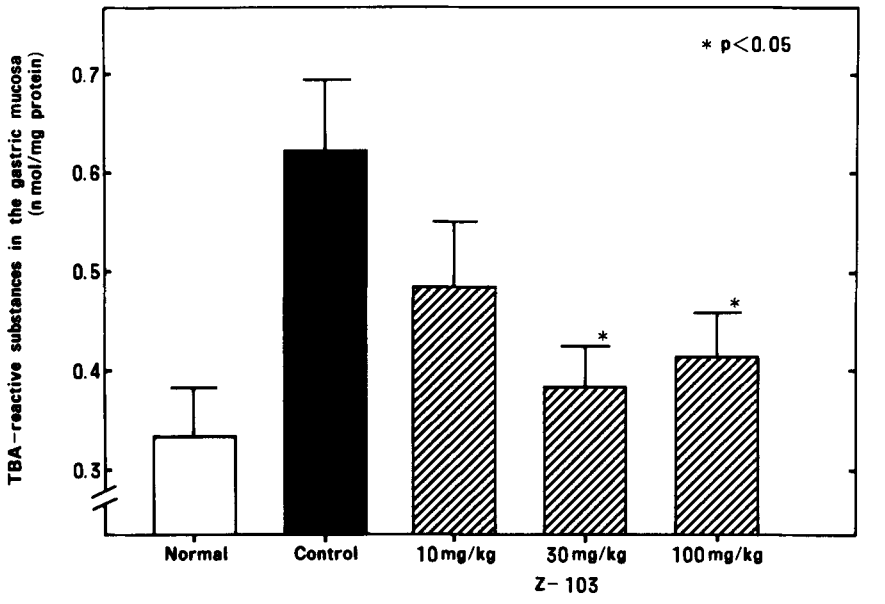

FIGURE 4 Effect of Z-103 on TBA-reactive substances in the gastric mucosa after ischemia-reperfusion. Each value indicates the mean ± SEM of 7 experiments. *$p < 0.05$ when compared with the control group without administration of Z-103.

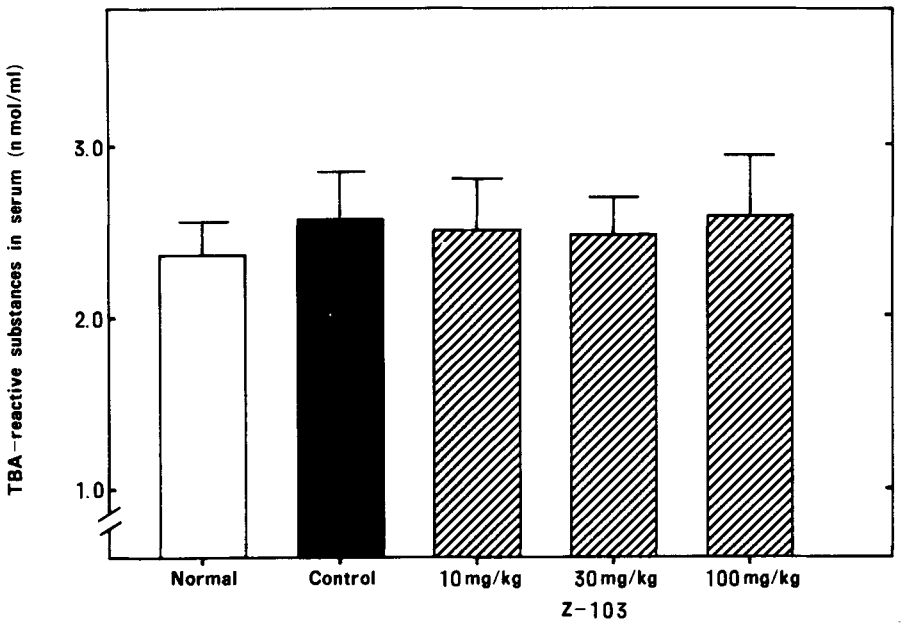

FIGURE 5 Effect of Z-103 on TBA-reactive substances in serum after ischemia-reperfusion. Each value indicates the mean ± SEM of 7 experiments.

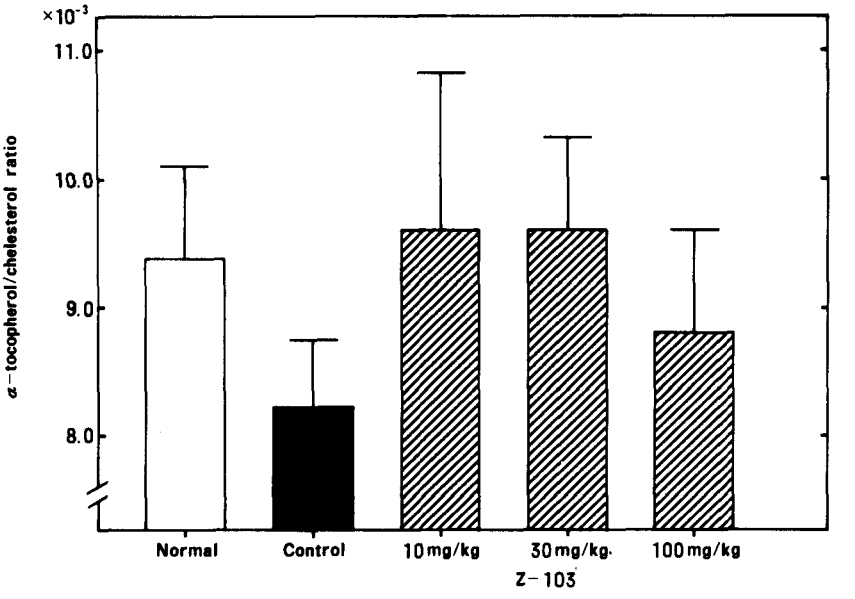

FIGURE 6 Effect of Z-103 on α-tocopherol/cholesterol ratio in serum after ischemia-reperfusion.

erosions induced by ischemia-reperfusion was significantly decreased by treatment with Z-103 at doses of 10, 30, and 100 mg/kg (Figure 3).

Effect of Z-103 on TBA-reactive substances in serum and in gastric mucosa

TBA-reactive substances in the gastric mucosa significantly increased 30 min after reperfusion. The increase of TBA-reactive substances in the gastric mucosa after ischemia-reperfusion was significantly inhibited by treatment with Z-103 at doses of 30 and 100 mg/kg (Figure 4). Serum TBA-reactive substances in the control group and groups treated with Z-103 did not show any significant changes after ischemia-reperfusion when compared with normal rats undergoing sham operations (Figure 5).

Effect of Z-103 on α-tocopherol/total cholesterol ratio

The serum α-tocopherol/total cholesterol ratio of the control group and groups treated with Z-103 showed no significant changes after ischemia-reperfusion when compared with normal rats undergoing sham operations (Figure 6).

Effect of Z-103 on autoxidation of rat brain homogenates

Z-103 inhibited the increase of TBA-reactive substances in rat brain homogenates and rat liver microsomes in a dose dependent manner (Figure 7).

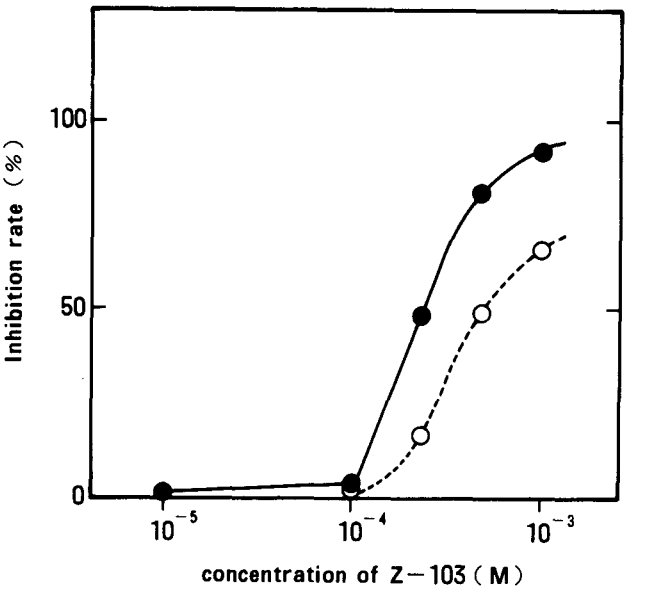

FIGURE 7 Effect of Z-103 on lipid peroxidation. ●—●, lipid peroxidation of rat brain homogenate; ○—○, lipid peroxidation of rat liver microsome.

DISCUSSION

In the present study, Z-103 inhibited increases in gastric mucosal lesions induced by ischemia-reperfusion without reversing the reduced microcirculatory blood flow of the gastric mucosa. Increases in TBA-reactive substances in the gastric mucosa after ischemia-reperfusion were also significantly inhibited by treatment with Z-103. We earlier reported on some evidence that tissue lipid peroxidation plays an important role in the formation of gastric mucosal injury produced by reperfusion, and superoxide dismutase, catalase, and allopurinol, a competitive inhibitor of xanthine oxidase, inhibit the aggravation of gastric injuries. The exact mechanism of the pharmacological effect of Z-103 *in vivo* is unknown. However, this agent inhibited the increase in TBA-reactive substances produced by autoxidation of rat brain homogenates and by NADPH-dependent lipid peroxidation of liver microsome in vitro as well as lipid peroxidation in ischemia-reperfusion gastric mucosal injury *in vivo*. In addition, the antioxidant ability of Z-103 has been demonstrated by recent in vitro studies. Z-103 inhibits superoxide generation by polymorphonuclear leukocytes, and hydroxyl radical production by the Fenton reaction.[20] Z-103 is also found to scavenge superoxide as well.[21] These data support the possibility that Z-103 reacts as an anti-oxidant *in vivo* and its anti-ulcer action may be explained in part by this mechanism.

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Accepted by Professor Trevor F. Slater