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Protective effect of cystathionine on acute gastric mucosal injury induced by ischemia-reperfusion in rats

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Abstract

We studied the protective effect of cystathionine on acute gastric mucosal injury induced by ischemia-reperfusion in rats. Under pentobarbital anesthesia, the celiac artery was clamped for 30 min and reperfused. Sixty minutes after the reperfusion, the total area of erosions and thiobarbituric acid-reactive substances in the stomach, as an index of lipid peroxidation, were measured and compared between control and cystathionine-treated groups. Intraperitoneal administration of cystathionine (1–20 mg/kg) 10 min before the ischemia significantly reduced both the total area of erosions and the level of thiobarbituric acid-reactive substances. When cystathionine (10 mg/kg) was administered orally, the significant reductions in the total area of erosions and level of thiobarbituric acid-reactive substances were also observed. There was a good correlation between the total area of erosions and the level of thiobarbituric acid-reactive substances. Cystathionine did not affect blood flow during ischemia-reperfusion. These results indicate that the protective effect of cystathionine on acute gastric mucosal injury induced by ischemia-reperfusion may be due to the scavenging action against superoxide radicals in vivo.

Keywords: Cystathionine; Ischemia-reperfusion; Superoxide radical; Gastric mucosal injury; (Rat)

1. Introduction

Many reports suggest a possible contribution of free radicals and reactive oxygen species in tissue injury which accompanies ischemia and reperfusion in several organs (Itoh and Guth, 1985; Perry et al., 1986; Hernandez et al., 1987; Suzuki et al., 1989; Yoshikawa et al., 1989, 1991). It has been indicated that superoxide radical or hydroxyl radical may be the major oxygen radical contributing to ischemia-reperfusion injury in the stomach (Itoh and Guth, 1985; Perry et al., 1986; Yoshikawa et al., 1989). These reactive oxygen species attack and damage many biological molecules, finally to increase lipid peroxides in membrane. These pathological changes may break down the cytoprotective system in the gastric mucosa, resulting in production of erosion or ulcer with attack by acid or pepsin (Grisham et al., 1987; Smith et al., 1987a; Szelenyi and Brune, 1988; Yoshikawa et al., 1989; Kvietys et al., 1990). Some reports indicate that the major sources of reactive oxygen species produced after ischemia-reperfusion are xanthine oxidase and activated polymorphonuclear leukocytes (Perry et al., 1986; Engerson et al., 1987; Smith et al., 1987b; Kvietys et al., 1990), and that acute gastric mucosal lesions induced by ischemia-reperfusion are decreased by removal of such reactive oxygen species (Mizui et al., 1987; Yoshikawa et al., 1989, 1991; Hirota et al., 1990; Salim, 1990).

Cystathionine is converted to cysteine by γ-cystathionase (homoserine dehydratase) under physiological conditions in liver. Our previous studies indicated a hepatoprotective effect of cystathionine against acetaminophen-induced necrosis (Kitamura et al., 1989) and the in vitro scavenging effect of superoxide radicals generated from human leukocytes. Therefore, it could be expected that cystathionine would be of help in various diseases induced by superoxide radicals. In this study, we have investigated the protective effect of

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cystathionine on acute gastric mucosal injury caused by ischemia-reperfusion in rats.

2. Materials and methods

2.1. Reagents

Cystathionine and superoxide dismutase (from human erythrocytes) were obtained from Sigma (St. Louis, MO, USA). Allopurinol, an inhibitor of xanthine oxidase was obtained from Wako Chemicals (Tokyo, Japan). All chemicals were of reagent grade.

2.2. Administration of drugs into rats

Cystathionine was dissolved in distilled water and administered intraperitoneally to the rats 10 min before the ischemia-reperfusion experiments. The controls were given only vehicle solution. Cysteine (15 mg/kg) was administered in the same manner as cystathionine. In some experiments, cystathionine (10 mg/kg) was given orally to the rats 30 min before the experiments.

Superoxide dismutase at a dose of 4000 units/kg was injected intravenously just before the reperfusion. Allopurinol (100 mg/kg) was dissolved in distilled water (pH 10.8) and administered orally to the rats twice, 48 and 24 h, before the experiments.

2.3. Ischemia-reperfusion experiment

Male Wistar rats weighing 200–230 g from SLC (Shizuoka, Japan) were fasted for 18 h prior to the experiments, but were allowed free access to water. Gastric mucosal injury was produced by ischemia-reperfusion according to the method of Yoshikawa et al. (1989). Briefly, under pentobarbital anesthesia (50 mg/kg), the celiac artery was clamped with a small clamp (Sugita standard aneurysm clip, holding force 145 g, Mizuho Ikakogyo Co., Tokyo, Japan) for 30 min and reperfused by removal of the clamp to obtain the ischemia-reperfusion state. Sixty minutes after the reperfusion, the rats were killed by exsanguination via the abdominal aorta, and the stomach was removed. The picture of whole gastric mucosa was taken into the computer system of imaging analysis, and all parts of erosion were measured and calculated as the total area of erosions.

2.4. Preparation of histological specimens

After the ischemia-reperfusion experiment, the stomach was immediately removed and fixed with 3.7% formaldehyde-saline. Segments were stained with hematoxylin-eosin and processed further for light microscopic observation.

2.5. Determination of lipid peroxides in gastric mucosa

Thiobarbituric acid-reactive substances, an index of lipid peroxidation, were measured by the method of Buege and Aust (1978). 1,1,3,3-Tetraethoxypropane (Tokyo Kasei Co., Tokyo, Japan) was used as standard for malondialdehyde. The value for thiobarbituric acid-reactive substances was expressed as nmol/g wet weight of tissue.

2.6. Measurement of blood flow

Blood flow was measured using a laser Doppler flowmeter (BRL-100, Bioresearch Co., Nagoya, Japan). Under pentobarbital anesthesia, the probe of the laser Doppler flowmeter was attached to the serous membrane side of the stomach. The effect of cystathionine (20 mg/kg) on blood flow of the whole stomach was measured before, during and after the ischemic period of celiac arterial clamping. Mean blood flow was obtained from measurements for 5 min at each point.

2.7. Statistics

All results are expressed as means ± S.E.M. Statistical comparisons were done with Student's *t*-test, Scheffe's multiple comparison test or Kruskall-Wallis test. The results were considered significantly different when $P < 0.05$.

3. Results

3.1. Effect of cystathionine on gastric mucosal injury induced by ischemia-reperfusion

The total area of erosions, a morphological index of gastric injury induced by ischemia-reperfusion, was decreased by the intraperitoneal treatment with cystathionine in a dose-dependent manner (Fig. 1A). Cystathionine at more than 1 mg/kg caused a significant reduction in the total area of erosion. As shown in Fig. 1B, the protective effects of cystathionine were compared with the effects of other radical scavengers such as cysteine (15 mg/kg) or superoxide dismutase (4000 U/kg). Cystathionine (10 mg/kg) showed the most effective protection. Allopurinol (100 mg/kg), an inhibitor of xanthine oxidase, which is the enzyme of superoxide-radical generation, also showed the protective effect on the gastric mucosal injury. Therefore, it was suggested that the superoxide radicals derived from xanthine-xanthine oxidase may be involved in ischemia-reperfusion injury.

3.2. Histological observations

Light microscopic observations showed hemorrhage, erosion of mucosa and efflux of fibrin in the area of gastric injury of the ischemia-reperfusion control (Fig. 2, upper panel). Pretreatment with cystathionine (10 mg/kg i.p.) improved these pathological characteristics (Fig. 2, lower panel). No infiltrations of leukocytes were observed under any conditions of our study.

3.3. Effect of cystathionine on lipid peroxidation levels in gastric mucosa

The increase of the thiobarbituric acid-reactive substances value in the gastric mucosa after ischemia-reperfusion was significantly inhibited by the intraperitoneal treatment with cystathionine in a dose-dependent manner (Fig. 3A). Fig. 3B shows the comparison of the inhibitory effects of cystathionine and other scavengers against lipid peroxidation. The inhibition by cystathionine (10 mg/kg) was more effective than that by cysteine (15 mg/kg) or superoxide dismutase (4000 U/kg).

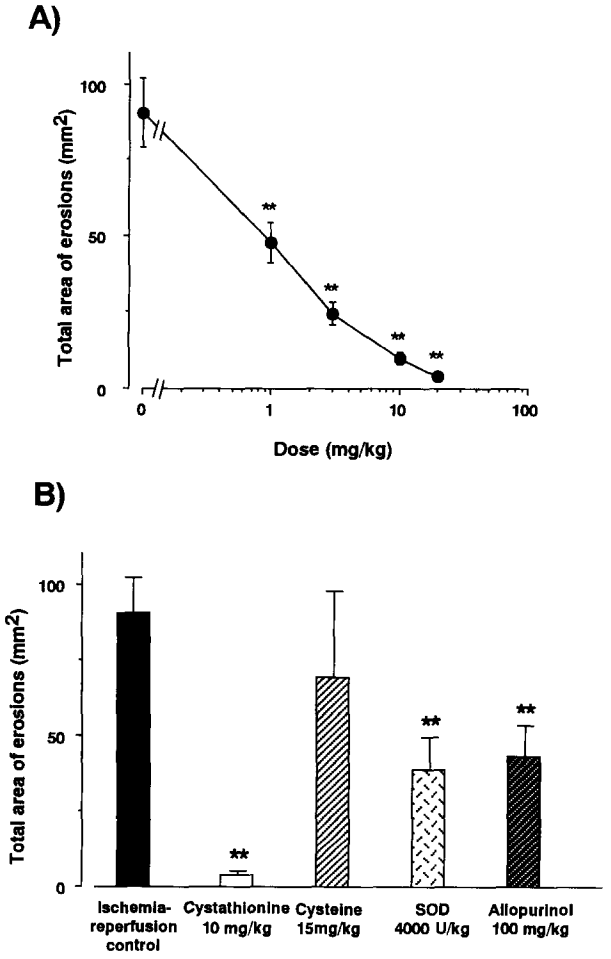

Fig. 1. (A) Dose-dependent effects of cystathionine (i.p.) on total area of erosions. Gastric mucosal injury was produced by ischemia-reperfusion of rat celiac artery by means of the clamping method. $^{**}P < 0.01$ each treatment vs. control (absence of cystathionine). Each point represents the mean ± S.E.M. from 6–8 observations. (B) Effects of cystathionine, cysteine, superoxide dismutase (SOD) and allopurinol on total area of erosions. $^{**}P < 0.01$ each treatment vs. ischemia-reperfusion control. Each column represents the mean ± S.E.M. from 6–8 observations.

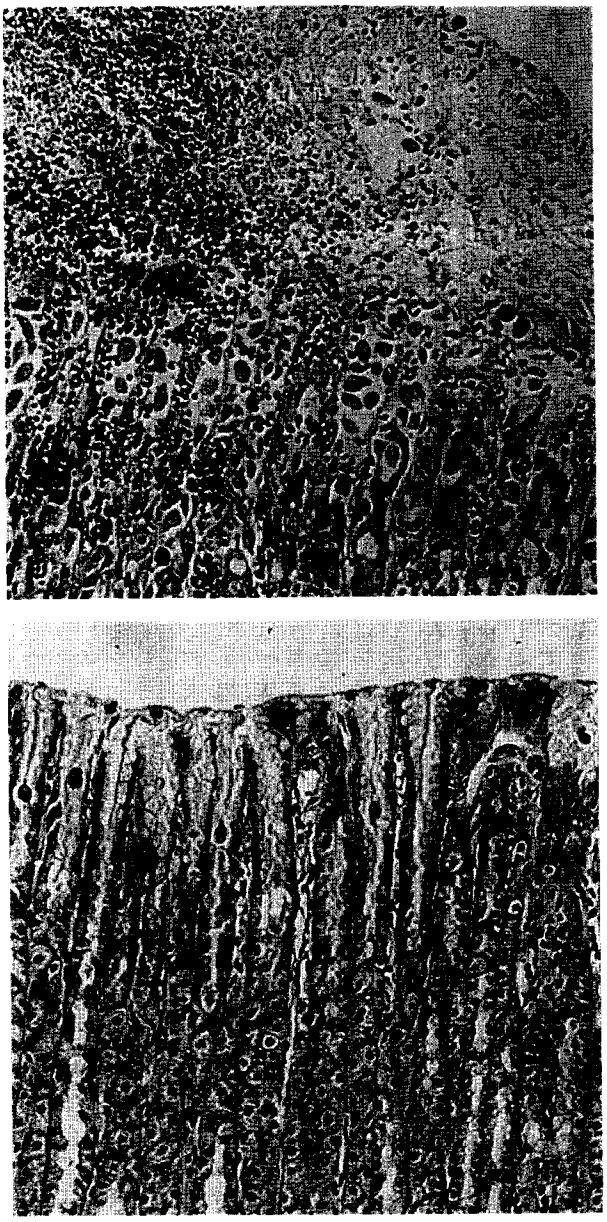

Fig. 2. Light microscopic view of the effect of: control (upper panel) or treatment with cystathionine (lower panel), on ischemia-reperfusion injury. Cystathionine (10 mg/kg) was given intraperitoneally 10 min before the experiment. Segments were stained with hematoxylin-eosin.

3.4. Effectiveness of oral administration of cystathionine

Oral administration of cystathionine (10 mg/kg) also significantly reduced the total area of erosions (Fig. 4). However, the protective effect on oral administration

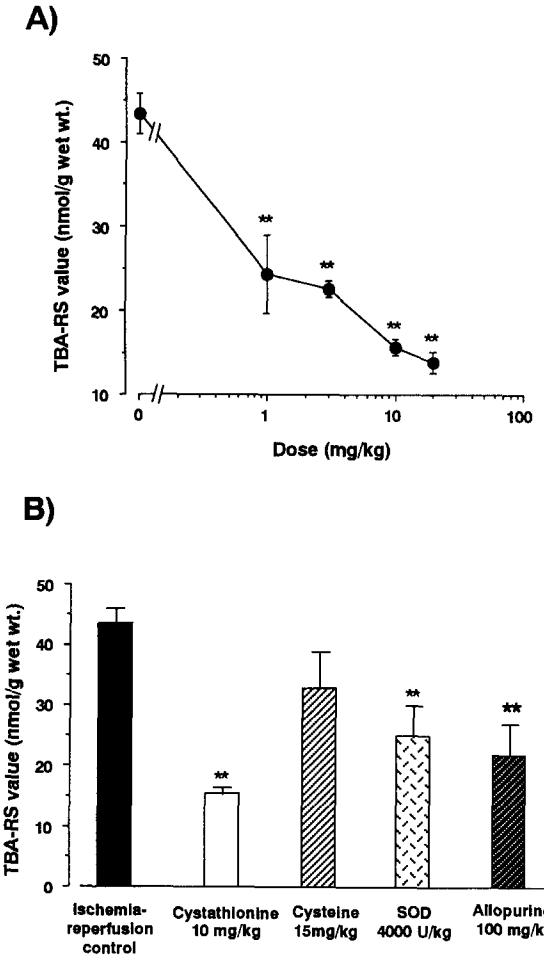

Fig. 3. (A) Dose-dependent effects of cystathionine (i.p.) on thiobarbituric acid-reactive substances (TBA-RS) value. $^{**}P < 0.01$ each treatment vs. control (absence of cystathionine). Each point represents the mean ± S.E.M. from 6–8 observations. (B) Effects of cystathionine, cysteine, superoxide dismutase (SOD) and allopurinol on thiobarbituric acid-reactive substances (TBA-RS) value. $^{**}P < 0.01$ each treatment vs. ischemia-reperfusion control. Each column represents the mean ± S.E.M. from 6–8 observations.

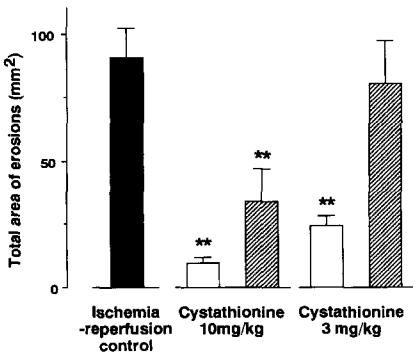

Fig. 4. Comparison between oral and intraperitoneal administration of cystathionine (shaded and open columns), respectively. $^{**}P < 0.01$ each treatment vs. ischemia-reperfusion control. Each column represents the mean ± S.E.M. from 6–8 observations.

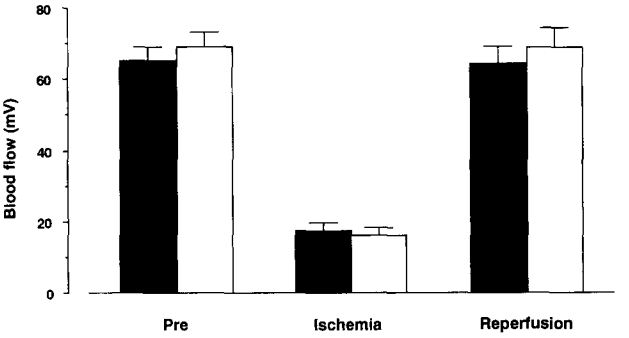

Fig. 5. Effect of cystathionine on blood flow. Mean gastric blood flow before clamping (Pre), during ischemia (Ischemia) or just after removal of the clamp (Reperfusion) was measured for 5 min. Blood flow of control and cystathionine (20 mg/kg)-treated rats is shown as solid and open columns (mean ± S.E.M. from 6–8 observations), respectively.

was weaker than that on intraperitoneal administration.

3.5. Effect of cystathionine on blood flow

In our present study, the variation of blood flow on whole stomach between each experimental animal was not large and stable blood flow data were obtained. Clamping of the celiac artery decreased gastric blood flow to 20–25% of that measured before the clamping. Just after the removal of the clamp, the blood flow recovered completely to its previous level. Treatment with cystathionine (20 mg/kg i.p.) affected neither the decreased blood flow during ischemia nor the recovered flow on reperfusion (Fig. 5).

4. Discussion

In the present study, cystathionine inhibited the increase in gastric mucosal lesions induced by ischemia-reperfusion in rats. These protective effects were observed at the dose of 1–20 mg/kg of cystathionine when administered intraperitoneally. Microscopic observation also showed the protective effect of cystathionine against the gastric mucosal lesions induced by ischemia-reperfusion.

The increase in the thiobarbituric acid-reactive substances value, the index of tissue lipid peroxidation, in the gastric mucosa after ischemia-reperfusion was also significantly inhibited by treatment with cystathionine. A good correlation ($r^2 = 0.897$) between total area of erosions and the level of thiobarbituric acid reactive substances was seen (Fig. 6). Many reports indicated that tissue lipid peroxidation plays an important role in the formation of gastric mucosal injury induced by reperfusion (Yoshikawa et al., 1986, 1989, 1991; Sakurai and Yamasaki, 1994). These results indicated that

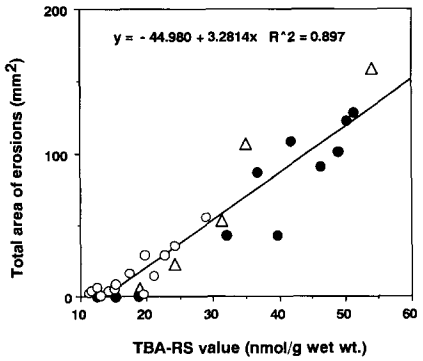

Fig. 6. Correlation between total area of erosions and thiobarbituric acid-reactive substances values. Solid circles, open circles and open triangles represent control, cystathionine and cysteine treatment, respectively. For each point the total area of erosions was measured, then the thiobarbituric acid-reactive substances value.

the protective effect of cystathionine against gastric mucosal lesions induced by ischemia-reperfusion is due to the inhibition of the increase in tissue lipid peroxidation. Superoxide and hydroxyl radicals are major reactive oxygen radicals contributing to ischemia-reperfusion injury in the stomach (Itoh and Guth, 1985; Perry et al., 1986; Yoshikawa et al., 1989). These reactive oxygen species attack and damage many biological molecules, finally to increase lipid peroxides in membrane. Hydroxyl radicals are derived from superoxide radicals via a Fenton reaction in the stomach (Grisham et al., 1986, 1987; Smith et al., 1987a).

On the other hand, the major source of superoxide radicals produced after ischemia-reperfusion is thought to be xanthine oxidase or activated polymorphonuclear leukocytes (Perry et al., 1986; Engerson et al., 1987; Smith et al., 1987b; Kvietys et al., 1990). It was reported that, in the present model, the xanthine-xanthine oxidase system in the gastric mucosal microcirculation was the main source of superoxide radicals (Yoshikawa et al., 1989, 1991). Our microscopic observations showed no leukocyte infiltration into the injured mucosa. Further, allopurinol, an inhibitor of xanthine oxidase in gastric mucosa, reduced the gastric erosions. These data support the conclusion that the major source of superoxide radicals in this model is the xanthine-xanthine oxidase system in the gastric mucosal microcirculation.

In our previous experiments, cystathionine also showed the scavenging effect on superoxide radicals derived from xanthine-xanthine oxidase in vitro (unpublished data). However, in the present study, the decrease in blood flow during ischemia and its recovery on reperfusion were not improved by the treatment with cystathionine. Further, in our preliminary experiment, cystathionine did not affect gastric acid secretion in ischemia-reperfusion. These results indicate that cystathionine may be able to scavenge the superoxide radicals and, therefore, may exert its protective effect against the gastric mucosal lesions induced by ischemia-reperfusion owing to the decrease in lipid peroxidation level in vivo.

Oral administration of cystathionine also led to the protective effect on gastric mucosal lesions induced by ischemia-reperfusion. Therefore, oral administration of cystathionine may be effective enough to use in vivo.

In conclusion, the protective effect of cystathionine on acute gastric mucosal injury induced by ischemia-reperfusion may be due to the scavenging effect against superoxide radicals.

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