Dimethyl- β -Propiothetin, New Potent Resistive-Agent against Stress-Induced Gastric Ulcers in Rats

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Summary The preventive effect of dimethyl- β -propiothetin (DMPT) and methylmethionine at 5 mM each on stress-induced gastric ulcers in rats was compared. Gastric ulcer-diseased rats were induced by the water immersion method. The ulcer index of test rats in the DMPT group was noticeably lower, compared with those in the methylmethionine and the control groups. A comparison of the activities of serum transaminases and amylase, and the amounts of various blood components in test rats claimed that the DMPT group rats were most resistive to such strain compared with the control and the methylmethionine group rats. These results indicate that DMPT is a potent preventive-agent against the stress-induced gastric ulcers in rats without any side effects.

Key Words gastric ulcer, methylmethionine, dimethyl- β -propiothetin, sulfonium 3-amino-3-carboxypropyl dimethyl chloride, sulfonium carboxyethyl dimethyl bromide, rats

The majority of people today are exposed to various kinds of tension and emotional stress. Trends suggest these stresses will increase. These stresses can cause a number of diseases, especially those affecting the human brain and heart (1). Interestingly, a stomach disorder, gastric ulcer, is believed to be one of the most sensitive to mental stress (2,3). Thus, finding compounds which effectively prevent or heal ulcers may be important not only for humans but also for all living beings that suffer stress; fish, reptiles, amphibians, birds and animals. One of the sulfonium compounds, methylmethionine, has been proven to be a very effective natural healing compound for gastric ulcers in animals (4,5) and humans (6,7). A comparison of the preventive effect of another naturally occurring sulfonium compound, dimethyl- β -propiothetin, with that of methylmethionine on stress-induced gastric ulcers in rats is made here.

MATERIAL AND METHODS

Materials. Kits for determining the activities of transaminases and amylase, and the amounts of glucose, cholesterol, triacylglyceride and creatinine were purchased from Wako Pure Chemical Co., Ltd., Japan. Sulfonium 3-amino-3-carboxypropyl dimethyl chloride (methylmethionine) was also obtained from Wako Pure Chemicals Co., Ltd., Japan. Sulfonium 2-carboxyethyl dimethyl bromide (dimethyl- β -propiothetin (DMPT)) was synthesized by refluxing equimolar amounts of dimethylsulfide and bromopropionic acid at 40°C for 12 h. The crude crystals of DMPT thus obtained and the methylmethionine were washed separately with excess amounts of chilled diethyl ether, and were then recrystallized from their methanol solutions. These purified compounds were both employed at concentrations of 5 mM. Other chemicals used were of the best quality available.

Preparation of ulcer-diseased rats. Gastric ulcer was induced in rats by the water immersion method (8, 9). SD-Wistar male rats were purchased at 6 weeks of age (body weight, 171.1 ± 4.9 g (mean $\pm$ SD)) from Clea Japan Inc., Japan, and reared for 12 days on a freely fed commercial solid diet and either tap water or one of the test solutions. All rats were kept one per cage at a temperature of 25°C and relative humidity of 65%. The body weight of tested rats was measured twice on the 7th and the 12th day. The daily volume of tap water or test solution consumed by each rat was estimated for one day beginning from 2 or 8 days after the experiments started. After 12 days, the rats were deprived of food and drink for 24 h. Each starved rat was tightly fixed in an aluminum frame and wire net cage and was then vertically immersed to the height of the rat's xiphoid in a water bath at 23°C for 21 h in darkness.

Determination of various viscera weight and blood components. These ulcerous rats were then anesthetized with diethyl ether and first the blood and then the viscera (stomach, heart, liver and kidney) were carefully removed. Fresh blood was immediately used to determine several factors. Hematocrit values (Packed cell volume) were measured by centrifugation (10,000 rpm, 5 min) of blood in a heparinized capillary glass tube (Drumond Scientific Co., Ltd., U.S.A.). The amount of hemoglobin (colorimetrically determined as hematin hydrochloride) was measured with a Sahli's Hemoglobinometer (Type M, Erma Optics Co., Ltd., Japan), and the amount of plasma proteins was measured with a Hitachi Protein Meter (Hitachi Co., Ltd., Japan). The residual blood was left at room temperature for 1 h in a plastic tube (a 9 ml-volume) (Insepack, Sekisui Chemical Co., Ltd., Japan) and then centrifuged. A portion of the supernatant (serum) was immediately tested to determine the activities of transaminases (glutamic acid-oxaloacetic acid transaminase (GOT) and glutamic acid-pyruvic acid transaminase (GPT)). The rest of the serum solution was stored in a freezer at -20°C. Several days after the rats were killed, it was tested for amylase activity, and the amounts of serum glucose, cholesterol, triacylglyceride and creatinine.

Calculation of ulcer index. The stomachs were carefully washed twice with distilled water and immersed in a 10% solution of formaldehyde for 1 h. They were then cut open along their greater curvatures and the diameters of ulcerous portions appearing in the glandular area were measured, after careful exclusion of excessive blood clots, under a microscope. Relative ulcer indexes were expressed as the ratio of the sum of diameters of the ulcerous parts of test rats against that of the control experiments.

RESULTS

Observation of the ulcerous stomachs of rats in the control and the methyl-

Fig. 1. Preventive effect of dimethyl- β -propiothetin (DMPT) and methylmethionine on stress induced gastric ulcers in rats. Each of five rats in the DMPT and the methylmethionine groups was orally fed 5mM solutions for 12 days, and the control group was given distilled water. All were then starved for 24 h, and subsequently immersed in a water bath at 23°C for 21 h. The diameters of the ulcerous areas appearing in the stomachs of these rats were measured and totaled (ulcer index), and then compared with the values (mean values as 100) of the control group. Values are shown as mean $\pm$ SD ($n=5$). Relative values in the DMPT and the methylmethionine groups are significantly different from those in the control group ($p<0.005$).

Table 1. General profiles of blood samples from rats that developed stress-induced gastric ulcers after previously being fed either dimethyl- β -propiothetin (DMPT) or methylmethionine.

Activity and component	Control	DMPT ^a	Methylmethionine ^a
Transaminases			
GOT (Karmen unit)	406.4 $\pm$ 132.7	310.4 $\pm$ 57.8 ^{cd}	460.0 $\pm$ 127.6
GPT (Karmen unit)	112.4 $\pm$ 34.7	75.4 $\pm$ 3.7 ^{cd}	121.4 $\pm$ 18.0
Amylase (unit ^b)	561.5 $\pm$ 79.5	505.8 $\pm$ 64.7 ^{cd}	640.0 $\pm$ 56.0 ^c
Plasma protein (%)	6.1 $\pm$ 0.2	6.3 $\pm$ 0.2 ^c	6.2 $\pm$ 0.2
Hematocrit values (%)	49.9 $\pm$ 5.9	48.7 $\pm$ 3.0	46.8 $\pm$ 1.6 ^c
Hemoglobin (%)	87.8 $\pm$ 3.8	92.6 $\pm$ 3.0 ^{cd}	102.8 $\pm$ 6.3 ^c
Cholesterol (mg/dl)	68.1 $\pm$ 8.7	50.6 $\pm$ 22.3	57.8 $\pm$ 4.3 ^c
Triglyceride (mg/dl)	87.5 $\pm$ 5.6	96.0 $\pm$ 9.1 ^{cd}	77.1 $\pm$ 6.2 ^c
Glucose (mg/dl)	78.1 $\pm$ 7.7	99.2 $\pm$ 9.7 ^c	105.5 $\pm$ 23.6 ^c
Creatinine (mg/dl)	1.5 $\pm$ 0.2	1.3 $\pm$ 0.2 ^c	1.2 $\pm$ 0.3 ^c

Values are shown as mean $\pm$ SD ($n=5$). ^a Used at a concentration of 5 mM.

^b Enzyme activity in which one unit is equal to the activity in 100 ml of serum which will destroy 10 mg of starch at 37°C for 30 min. ^{cd} Significantly different from controls ($p<0.05$) and vitamins ($p<0.05$).

methionine groups revealed a number of small superficial ulcerations covered with black-red blood clots. There were significantly fewer ulcers ($p<0.005$) in the DMPT group. The results of the relative ulcer index are given in Fig. 1, which shows values of 100 ± 6.13 , 76.8 ± 3.58 and 29.6 ± 7.12 in the control, the methylmethionine and the DMPT groups, respectively.

The general physiological blood profiles from ulcerous rats are given in Table 1. The control group rats gave the highest values of cholesterol, higher values of GOT, GPT, amylase and triglyceride, the lowest values of hemoglobin and glucose. The DMPT group rats showed the highest values of triglyceride, higher values of hemoglobin and glucose, and the lowest values of GOT, GPT, amylase and cholesterol. The methylmethionine group rats represented the highest values of GOT, GPT, amylase, hemoglobin and glucose, higher values of cholesterol, and the lowest values of triglyceride under the experimental conditions used. The values of hematocrit, plasma protein, creatinine and the ratio of the viscera (heart, liver and kidney) weight against body weight (data not shown) were almost similar in the three groups.

The results of the body weight of the group rats are given in Fig. 2. There was an increase (around 8 and 10%) of the body weight on the 7th and the 12th day in the DMPT group rats, respectively, compared to that of the control and the methylmethionine group rats, which had almost the same growth rate during the rearing period.

Daily volume of a 5 mM test solution consumed by each rat was measured at 2 or 8 days after the experiments and results are given in Fig. 3. The values were 37.0 ± 6.78 , 36.0 ± 2.00 (208.8 ± 11.6 mg vitamin/kg weight) and 40.0 ± 3.16

Fig. 2. Growth of rats fed on DMPT and methylmethionine solutions. The body weight was measured on the 7th and the 12th days, respectively. Experimental conditions were the same as in Fig. 1. Marks, $\circ$, $\triangle$ and $\square$, show the growth of the control, the methylmethionine and the DMPT group rats, respectively. Values are shown as mean $\pm$ SD ($n=5$). Symbols, a, b, represent the significant difference from controls ($p<0.025$) and vitamins ($p<0.025$), respectively.

(254.9 ± 20.2 mg DMPT/kg) ml/day ($n=5$) on the second day or 53.0 ± 7.48 , 54.0 ± 5.83 (230.9 ± 24.9 mg vitamin/kg) and 51.0 ± 8.60 (229.8 ± 38.8 mg DMPT/kg) ml/day ($n=5$) on the eighth day of the control, the methylmethionine and the DMPT groups in this order.

DISCUSSION

A sulfonium compound, DMPT, proves to be a precursor of dimethylsulfide (10, 11) which is the primary agent responsible for the sea-smell at the shore, but which is really produced by sea algae (12, 13). DMPT has been proven to occur world-wide in aquatic organisms, algae (10–12), krill (14), mollusks (15, 16), fish

Fig. 3. Daily volume of test solutions ingested on the 2nd and the 8th days. Experimental conditions were the same as in Fig. 1. Values are shown as mean $\pm$ SD ($n=5$). Symbols, a, b, show the significant difference from controls ($p<0.1$) and vitamins ($p<0.025$).

(17,18), and also in higher plants (19,20). However, the significance of the compound in these organisms had remained obscure, except for its role as an osmoprotectant in algae (21,22). We examined effects of the compound and related compounds on the feeding, growth and swimming power of freshwater and marine fish, and on the suspending and running power of rats. Thus, we found that the addition of DMPT, but not methylmethionine, to test diets or to drink markedly attracts the feeding of test fish (goldfish, carp, crucian carp, tilapia, red sea bream and yellowtail) (23,24), greatly stimulates the growth of fish (goldfish, red sea bream, yellowtail and flounder) (24,25), and significantly activates the swimming power of fish (goldfish, carp and red sea bream) (26), and the suspending and the running power of rats (27). These findings intrigued me to examine the preventive effects of previous oral ingestion of DMPT and a closely related compound,

methylmethionine, on stress-induced gastric ulcers in rats. The stress-induced gastric ulcers in rats were effectively made by the starvation-water immersion method, which brought about a number of ulcerous portions in the stomachs of the control group rats and, to a lesser extent, the vitamin U group rats. The relative ulcer index values of the ulcerous rats were the lowest in the DMPT group, when compared with those in the methylmethionine and the control groups. Therefore, the protective effects of DMPT on stress-induced gastric ulcers in rats proved to be much more significant than those of methylmethionine, in spite of almost the same intake of these compounds during the experimental period.

The comparison of enzyme activities and various component concentrations in blood of these group rats indicated that values for the activities of transaminase (GOT and GPT) and amylase (good measures for the function of liver and pancreas) were lowest in the DMPT group, and levels of creatinine (a measure for kidney function) were almost the same in all groups, although being a little higher than the normal values. This may provide evidence that the functions of the liver, pancreas and kidney of rats in the DMPT group were in the best condition under the ulceration treatment among these groups. This might also be supported by an around 10% increase of the body weight in the DMPT group rats during the short rearing period. The high values of hemoglobin and glucose in the DMPT group may reflect a sufficient source in oxygen carriers and energy. The latter may also be further augmented by excessive levels of triglyceride in the DMPT group. Total cholesterol was lowest, but normal, in the DMPT group although there were large individual differences. All of the above factors may contribute to the DMPT group's strong resistance to stress-induced gastric ulcers.

In the methylmethionine group hemoglobin and glucose levels were highest and triglyceride levels lowest, probably indicating a good supply of oxygen carriers and energy sources. However, transaminase and amylase activities in the methylmethionine group were too high. This indicates that the ulcer disease did great damage to the liver and pancreas of the rats in this group and may thus explain why the gastric ulceration in this group was less than in the control group, but much greater than in the DMPT group.

Our previous experiments showed that the oral administration of lower concentrations (0.1 and 0.5 mM) of DMPT elevates the levels of hematocrit, hemoglobin, triglyceride, glucose, and reduces the amounts of cholesterol with no effect on the activities of transaminases and amylase (28). The same trends were confirmed with higher concentrations (5 mM) of DMPT and methylmethionine solutions (27).

Therefore, gastric mucosae of rats fed on DMPT are believed to have taken marked resistance to stress, maintaining elevated energy levels (serum glucose, triglyceride amounts and probably oxygen carries) of test rats without any damage to their viscera. This hypothesis is also supported by the fact that the rats in the DMPT group required 2 to 3 times more of the gaseous anesthetic diethyl ether than those in the control and the methylmethionine groups during the removal

operation of their blood and viscera. The protective effect of DMPT against environmental stress was also confirmed in the experiments with tadpoles (brown frog) and fish (goldfish and red sea bream), showing a significantly increased survival rate of the DMPT group against those of the control and the methylmethionine groups (unpublished data).

Methylmethionine (methylmethionine sulfonium chloride) is evidenced to elicit, singly or along with antacids, a favorably healing effect on various experimental gastric ulcers: the stress-, the acetic acid-, the pyloric ligation-, the indomethacin- and the aspirin-induced gastric ulcers in rats to varying extents (4). Moreover, methylmethionine proves to exert an efficient cytoprotection action in rats (5), probably by enhancing the methylation reaction of mucosal chromatin protein and nucleic acids (29), while DMPT is proven to act as a methyl group donor more effectively than methylmethionine in the enzymatic synthesis of methionine from homocystein in animals (30,31). These findings and the results obtained suggest that DMPT is a new naturally occurring anti-ulcer agent, similar to, but much more effective than methylmethionine against various stress- and drug-induced gastric ulcers in animals and humans.

A further study to determine the physiological role of DMPT in fish and animals is under way.

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