Effect of Taurine on Gastric Oxidative Stress and Hemorrhagic Erosion in Brain Ischemic Rats

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Abstract

The effect of taurine on gastric hemorrhage and mucosal erosion in the brain ischemia (BI) is unknown. The aim of the research was to study the involvement of gastric oxidative stress in hemorrhagic erosion produced in BI rats. The protective effect of taurine on this erosion model was evaluated. Male Wistar rats were deprived of food for 24 h. Under chloral hydrate-anesthesia, bilateral carotid artery ligation (BCAL) was performed 12, 18 and 21 h after removal of food to obtain 12, 6 and 3 h of BI duration. The pylorus and carotid esophagus of rats also were ligated. The stomachs were then irrigated for 3 h with normal saline or simulated gastric juice containing 100 mM HCl plus 17.4 mM pepsin and 54 mM NaCl. The stomach was dissected. Gastric samples were harvested. The rat brain was dissected for examination of ischemia by using triphenyltetrazolium chloride staining method. Changes in gastric ulcerogenic parameters, such as decreased mucosal GSH level as well as enhanced gastric acid back-diffusion, mucosal lipid peroxide generation, histamine concentration, luminal hemoglobin content and mucosal erosion in gastric samples were measured. The results indicated that BCAL could produce severe BI in rats. Moreover, a BI-duration-dependent exacerbation of various ulcerogenic parameters also was observed in these rats. Intraperitoneal taurine (0-300 mg/kg) dose-dependently ameliorated gastric oxidative stress and hemorrhagic erosion in BI rats. Taken together, BI could produce gastric oxidative stress and hemorrhagic erosions that was ameliorated by taurine through stimulation of GSH biosynthesis and inhibition of oxidative stress.

Key Words: taurine, lipid peroxides, gastric damage, glutathione

Introduction

Brain ischemia (BI) resulted from intracranial hemorrhage, cardiovascular stenosis, or hypercholesterolemia is frequently found in the clinic. The development of this disease may lead to cerebral hypoxia, brain tissue damage and stroke (2, 4). The peripheral manifestations of BI include seizures (12), neurological deficiency (8) and hemostatic disturbance (9). In the clinic, gastric hemorrhage and ulceration have been reported in patients with stroke (37). Gastric mucosal damage also is demonstrated in spontaneous hypertensive rats with brain ischemia (20). However, the pathological mechanisms, including gastric oxidative stress and hemorrhagic erosion, and its taurine protection in rats with brain ischemic are unknown. In general, the disruption and/or degeneration of gastric mucosal cells may result in decrease of gastric defensive factors, including mucosal glutathione (GSH), and/or in increase of offensive factors, such as gastric acid back-diffusion and generation of oxyradicals. Our previous papers indicate that acid back-diffusion is a critical factor of acid-induced exacerbation of gastric hemorrhagic ulcer in diabetic or starved rats (17, 18). The back-diffused free acid may damage gastric mucosal cells by increasing oxyradicals and the release of histamine (16). In fact, oxyradicals produced during oxidative stress play a pivotal role in the etiology of many diseases (25), including cancer (6) and sepsis (15, 16). However,
the role of gastric oxidative stress and modulation of GSH in the formation of gastric hemorrhage and mucosal erosion in gastric juice-irrigated stomachs of BI rats remains obscure. Since bilateral carotid artery ligation (BCAL) can produce BI (20), therefore, this model was used in the present study.

Taurine (2-aminoethansulfonic acid) is a non-protein sulfur amino acid and is the most abundant free amino acid in the body. It plays an important role in several essential biological processes and acts as neuromodulator. It also has a function as osmoregulator and antioxidant in most body tissues (29). Whether or not taurine can protect gastric mucosal erosion produced by BI however is totally unknown. The aim of the study was thus to investigate effect of taurine on BI-induced gastric oxidative stress and hemorrhagic erosion in rats.

**Materials and Methods**

**Experimental Animals**

Male Wistar rats, weighing 200-250 g, were obtained from and housed in The Laboratory Animal Center, National Cheng Kung University, Tainan, Taiwan. Rats were housed individually in a room with 12-h dark-light cycle and with central air conditioning (25°C temperature, 70% humidity). Rats were allowed free access to water and pellet diets (the Richmond standard, PMI Feeds, Inc., St. Louis, MO, USA). The animal care and experimental protocols were in accord with the guidelines of The National Science Council of Taiwan (NSC 2004). Before the experiment, rats were deprived of food for 24 h. Under potent anesthesia of intraperitoneal chloral hydrate (300 mg/kg), BI was induced by BCAL 12, 18 or 21 h after removal of food to obtain 3, 6 and 12 h of BI duration. Adequate amount of chloral hydrate was injected to rats for maintenance of anesthesia. Care was taken to avoid gastric distension. The residues were gently removed.

**Measurement of Gastric Acid Back-Diffusion**

Gastric acid back-diffusion (luminal H⁺ loss) was quantified by the method previously described (16). Namely, 7-ml of normal saline or simulated gastric juice containing 100 mM HCl, 17.4 mM pepsin and 54 mM NaCl (24) were instilled into the cleansed stomach with a syringe. The luminal contents were mixed with the same syringe by three repeated aspirations and injection, and 3 ml of the fluid was taken as an initial sample. The forestomach was tightly closed. The abdominal wound was sutured. After 3 h, rats were killed with an overdose of ether. The gastric sample (final sample) was collected and centrifuged under 4°C for 20 min at 3000 r.p.m.

**Quantitation of Gastric Sample**

The volumes of the initial and final samples were measured. Gastric acidity of samples was assessed by titrating 1.0 ml of sample gastric contents with 0.1 M NaOH to pH 7.0 on an autoburette titrator (Radiometer, Copenhagen, Denmark). The net flux or ions through gastric mucosa was calculated as follow: Net flux = Fv x Fc - (7-Iv) x Ic. Where Fv and Iv are the volumes (ml) of final sample and initial sample, respectively, while Fc and Ic are the ionic concentrations (mM) in the final sample and initial sample, respectively. The negative value means the luminal electrolyte loss and the positive value indicates the luminal electrolyte gain.

**Morphological Studies of Gastric Mucosa**

As soon as the final sample was collected, the stomach was filled with 1.0% formalin for 10 min. The mucosa was exposed by opening the stomach along the greater curvature. The length (mm) and the width (mm) of erosion on the gastric mucosa were measured with planimeter (1 x 1 mm) under a dissecting microscope (x 0.7 - x 3.0; American Optical Scientific Instrument 569, Buffalo, NY, USA). The erosion areas were determined as previously described (17); erosion area = length x width x π/4. The total erosion area (mm²) of each stomach was recorded. Gastric mucosal damage was determined by a person unaware of experimental procedure.

**Determination of Hemoglobin (Hb)**

The cleansed rat stomachs were irrigated for 3 h.
with either saline or simulated gastric juice. Gastric initial and final samples were collected by method as beforehand demonstrated. The blood attached on the gastric mucosa was carefully scraped, and added it to the final sample. Subsequently, both initial and final samples were adjusted to pH 1.5 with 0.1 M HCl. The concentrations of Hb in samples were measured spectrophotometrically (16). The absorption maximum of Hb was measured at 376 nm. The appropriate irrigated solutions adjusted to pH 1.5 were used as blank. Absorbances of samples were measured against a standard curve ($r^2 > 0.90$) contrasted with freshly prepared rat Hb (0.05 -1.00 mg/ml) treated in the same manner as gastric samples. The luminal Hb content was calculated as $Fv \times F_{Hb} - (7-Iv) \times I_{Hb}$. Where $Fv$ and $Iv$ are the volumes (ml) of final sample and initial sample, respectively, while $F_{Hb}$ and $I_{Hb}$ are the luminal Hb concentrations (mg/ml) in the final sample and initial sample, respectively. The results obtained from gastric samples were expressed as milligram Hb per stomach.

**Assay of Mucosal GSH**

The quantitation of gastric mucosal GSH was performed by methods as previously demonstrated (18). After the final sample was collected, the rat stomach was dissected. The corpus mucosa was scraped using two glass slides on ice, weighed and homogenized immediately in 2 ml of phosphate buffer (0.1 M NaH$_2$PO$_4$ plus 0.25 M sucrose, pH 7.4). Acivicin (250 µM), an irreversible inhibitor of γ-glutamyltransferase, was added to the homogenate to inhibit the catabolism of GSH. The samples were then centrifuged under 4°C at 4000 r.p.m. for 15 min. To determine the recovery of thiol, the supernatant was added with or without GSH (200 µmol of GSH contained in phosphate buffer solution, pH 7.0). Subsequently, 0.5 ml of 0.25 M trichloroacetic acid was added to 1.0 ml of the supernatant of each sample and kept for 30 min at 4°C. After centrifugation under 4°C for 15 min at 3000 r.p.m., the supernatant was used to determine GSH using 2, 2'-dinitro-5, 5'-dithio-dibenzaic acid. The optical density was measured at 412 nm on a Hitachi spectrophotometer (model U-3210, Tokyo, Japan). All samples were measured in duplicate. Recovery of added internal standard was greater than 90% in all experiments. Absorbances of the samples were measured against a standard curve constructed with freshly prepared GSH solutions (0.05-0.5 mM), which were treated in the same manner as the tissue samples. The results obtained from tissue samples were expressed as micromole per gram wet tissue.

**Determination of Mucosal Lipid Peroxides (LPO)**

The concentrations of gastric mucosal LPO were determined by estimating malondialdehyde (MDA) using the thiobarbituric acid test (28). Namely, the stomachs of rats were promptly excised and rinsed with cold saline. To minimize the possibility of interference of Hb with free radicals, any blood adhering to the mucosa was carefully removed. The corpus mucosa was scraped, weighed and homogenized in 10 ml of 100 g/l KCl. The homogenate (0.5 ml) was added with a solution containing 0.2 ml of 80 g/l sodium laurylsulfate, 1.5 ml of 200 g/l acetic acid, 1.5 ml of 8 g/l 2-thiobarbiturate and 0.3 ml distilled water. The mixture was incubated at 98°C for 1 h. Upon cooling, 5 ml of n-butanol: pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged under 4°C for 10 min at 4000 r.p.m. The supernatant was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane. The recovery was over 90%. All samples were measured in duplicate. The results were expressed as nanomol malondialdehyde per gram wet tissue.

**Measurement of Mucosal Histamine**

Gastric mucosal histamine concentration was determined by the methods as described previously (31). Namely, gastric mucosa was scraped and homogenized with trichloroacetic acid (90 mM) in a final concentration of 100 mg tissue/ml. The homogenate was further centrifuged under 4°C at 18000 r.p.m. for 10 min. The o-phthaldialdehyde (OPT)-NaOH solution was prepared by dissolving 10 mg OPT in 1.0 ml methanol (1% w/v) and 4 ml NaOH (0.06 mM). This mixed solution was then gassed with nitrogen for 10 min. Then, 2.2 ml of a 2% OPT-NaOH solution was added to 100 µl of a 1/10-fold diluted sample of supernatant or histamine test solution. The mixture was then placed at -20°C for 10 h. Two hundred µl of 0.35 mM H$_2$SO$_4$ (final pH 1.6-2.4) was added to this frozen mixture. After thawing, the sample was vortexed for 1 min. The fluorescence of the sample was read at room temperature (25°C) at 350 and 450 nm on a fluorescent spectrophotometer (Model 251-0030, Tokyo, Japan) using 1 cm$^2$ quartz cells. All samples were measured in duplicate. The fluorescence of the sample was calculated against a standard curve constructed with freshly prepared histamine solutions (0.78-25 µg/ml) that were treated in the same manner as the tissue samples. The results obtained from tissue samples were expressed as µg histamine/g wet tissue.

**Drug Administration**

Taurine (0-300 mg/kg) were given intraperitoneally to rats 30 min before normal saline or gastric juice irrigation.
Chemicals Used

The following chemicals in reagent grade were used. Acivicin, n-butanol, 2, 2'-dinitro-5, 5'-dithiodibenzoic acid, pyridine, o-phthalaldehyde, rat hemoglobin, GSH, sodium laurylsulfate, taurine, 1,1,3,3-tetramethoxypropane, 2-thiobarbiturate, trichloroacetic acid and triphenyltetrazolium chloride were purchased from Sigma, St. Louis, MO. USA. The purity of all drugs was over 98%. All chemical solutions were freshly prepared before use.

Statistical Analysis

The data obtained from the experiments were expressed as means ± SEM. Significant differences in the data of experiments for single measurement traits were analyzed statistically by using ANOVA or by using Tukey honestly significant difference tests for pairwise comparison after ANOVA (27). Statistical significance was set at \( P < 0.05 \).

Results

Brain Ischemic Duration-Dependent Aggravation of Various Ulcerogenic Parameters in Gastric Juice-Irrigated Rat Stomachs

In sham operative rats, the brain slice looks normal. However, the severity of BI in rats as indicated by intensity of pale white color was in a BCAL-time-related manner (photo not shown). The augmentation in luminal H\(^+\) loss (acid back diffusion), LPO generation, luminal Hb contents and erosion formation as well as the lowering of mucosal GSH concentration were dependent on the BI duration in rat stomachs irrigated for 3 h with gastric juice (Fig. 1).

Morphological and Histological Alteration in Sham Operative and BI Rat Gastric Mucosa

Gastric mucosa was morphologically intact in sham operative rats. Nevertheless, numerous hemorrhagic erosions were observed in BI rats. Histological studies showed that in sham operative rat stomachs irrigated with gastric juice, gastric mucosal cells appeared intact (Fig. 2A). However, pronounced cell-injury was found in both epithelial layers and lamina propria when gastric juice was present in stomachs of BI rats (Fig. 2B). The comparison of the degree of gastric histological damage produced by gastric juice in BI rats to that in sham operative rats with the same treatments is demonstrated in Fig. 3. In sham operative rat stomachs irrigated with normal saline, no damage of gastric

\[ \text{Glutathione (\text{\textmu}mol/g tissue)} \]

\[ \text{Lipid Peroxides (\text{\textmu}mol MDA/g tissue)} \]

\[ \text{H\textsuperscript{+} Net Fluxes (\text{\textmu}mol/stomach)} \]

\[ \text{Hemoglobin (\text{\textmu}g/stomach)} \]

\[ \text{Histamine (\text{\textmu}g/g tissue)} \]

\[ \text{Erosion Areas (\text{mm}^2/stomach)} \]
Fig. 2. Histological study of gastric mucosa in sham operative and BI rats. Rats received sham operation or BCAL for 3 h. Rats stomachs were irrigated for 3 h with a simulated gastric juice containing 100 mM HCl, 17.4 mM pepsin and 54 mM NaCl. Gastric mucosa in sham operative rat stomach looks normal (A). However, in BI rat mucosa, a pronounced disruption of the upper cell layer and lamina propria is observed (indicated by arrows). The injured cells are characterized by karyorrhexis and dense homogenous acidophilic cytoplasm. (magnification ×100)

Fig. 3. Influence of gastric juice on various ulcerogenic parameters in sham operative and BI rats. Rat stomachs were irrigated for 3 h with normal saline (□) or gastric juice (■). Data are means ± SEM. n = 6-8. Significant differences are analyzed by using Tukey honestly significant difference tests for pairwise comparison after ANOVA. The difference between those treatments with different letters are statistically significant (P < 0.05).
mucosal cells was observed. In gastric juice-irrigated stomachs of sham operative rats, gastric mucosal cells also appeared undamaged. In BI rats, normal saline-irrigated stomachs produced more gastric mucosal cell damage than did those treatments in sham operative rats. Furthermore, a pronounced aggravation of mucosal cell damage was observed when gastric juice was used instead of normal saline in these rats. Apparently, intra-luminal gastric juice could aggravate mucosal cell damage in BI rats.

**Influences of Gastric Juice on Changes of Various Gastric Parameters in Sham Operative and BI Rats**

Fig. 3 also demonstrated that GSH level and LPO generation as well as histamine concentration and acid back-diffusion were normal in saline-irrigated stomachs of sham operative rats. Luminal Hb contents and mucosal erosion in these rats were negligible. Similar results were observed in gastric juice-irrigated stomachs of sham operative rats. In BI rats, normal saline-irrigated stomachs produced significant increases (P < 0.05) in these ulcerogenic parameters compared to those found in sham operative rat stomachs irrigated with normal saline. However, when gastric juice was used instead of normal saline in BI rats, a remarkable exacerbation of gastric hemorrhagic erosions accompanied with great enhancement in acid back-diffusion and mucosal LPO as well as in lowering of mucosal GSH levels were found. Furthermore, high correlation between exacerbated gastric mucosal damage and decreased GSH levels (r^2 = 0.801, P < 0.05) as well as between mucosal erosions and increased LPO (r^2 = 0.668, P < 0.05) were observed in those gastric juice-irrigated stomachs of BI rats. Apparently gastric juice could aggravate BI-induced gastric oxidative stress and hemorrhagic erosion in rats.

**Effect of Taurine on BI-Induced Gastric Parameters in Stomachs of Rats**

Table 1 showed that taurine (0-300 mg/kg) dose-dependently increased gastric mucosal GSH levels but decreased acid back-diffusion, LPO and histamine concentrations in gastric juice-irrigated stomachs of sham operative rats. In stomachs of BI rats, the ulcerogenic parameters such as increased acid back-diffusion, LPO generation, hemorrhage and mucosal erosions were dose-dependently attenuated by pretreatment of taurine. Whereas the decreased mucosal GSH levels found in these rats were effectively attenuated.

**Discussion**

The pathological mechanisms underlying the aggravation of gastric hemorrhagic erosion in gastric juice-irrigated stomachs of BI disease is complex. The present study demonstrated that the decreased GSH levels as well as increased acid back-diffusion, mucosal LPO generation and histamine concentration were exacerbated in a BI duration-dependent manner. The occurrence of gastric hemorrhage and erosion also was related to the duration of BI. Apparently, gastric oxidative stress was produced during BI development. During oxidative stress, gastric mucosal barriers were degenerated and disrupted. When gastric juice was irrigated in the stomachs of BI rats, the intraluminal free H^+ back-diffused through disrupted barriers to the gastric mucosa thereby damaged gastric cells. Therefore, aggravation of gastric hemorrhagic erosion and various ulcerogenic parameters occurred. The back-diffusion of gastric acid may also stimulate oxyradical release. The elimination of gastric mucosal GSH levels in gastric juice-irrigated stomachs of BI rats might be resulted from an increase in its consumption for scavenging oxyradicals, and from a decrease in its cellular biosynthesis. It is proposed that depletion of neuronal GSH can result in increase of neuronal nitric oxide synthase activity and cell death (11). In clinics, patients with peptic ulcer show a decreased gastric GSH (13). The cytoprotection of GSH on gastric mucosal injury induced by ethanol in rats also is documented (35).

Our previous report demonstrates that increase in oxyradical generation as indicated by augmentation of concentration of mucosal LPO, the oxyradical metabolite, is parallel to the elevation of histamine concentration in endotoxemic rats (18). In the present *in vivo* study, increased mucosal histamine release may not only derived from gastric mast cell per se, but also may be from those of other organs, including liver, lung, kidney or peritoneal tissues via circulation. Oxyradicals can directly attack mast cells, the predominant storage site for histamine, and cause cell degranulation. In turn, gastric mucosal histamine concentration is elevated. Since inflammation is greatly associated with oxyradical formation (23), and increase in mucosal histamine concentrations may exacerbate tissue inflammation, it is likely that the aggravation of mucosal inflammation by increased histamine concentrations can produce more oxyradicals. Taken together, increased histamine release and oxyradical generation in the gastric mucosa may result in hemorrhage and erosion in BI rats. We reported previously that activation of histamine H1 and H2 receptors is important in the formation of gastric hemorrhagic ulcer in septic rats (16).

On the other hand, taurine has an important role in cell protection against ischemia-reperfusion (21) and hypoxia (7). It possesses multiple biological and pharmacological actions. It may function as an
These effects may also participate in taurine-induced and prevention of cellular calcium overload. Taurine may include regulation of cell volume, cyclooxygenase 2 expression. Other cytoprotection in taurine may down-regulate the generation of inflammatory mediators, including superoxide, nitric oxide, tumor necrosis factor, interleukin 6 and prostaglandin E2. This provided the evidence that in additional to its mast cell membrane stabilizing effect, taurine might possess potent antioxidant effect on the oxidative stress. Although it has been shown that taurine does not directly react with superoxide, \( \text{H}_2\text{O}_2 \) or hydroxyl radical (1) yet it can react with hypochlorous acid (HOCI) during development of oxidative stress and erosion. Taurine has tissue-protection in many models of oxidant-induced injury. One possibility is that taurine reacts with HOCI, which is produced by the myeloperoxidase pathway, to produce the more stable but less toxic taurine chloramine.

Taurine effectively lowered the LPO generation and mucosal hemorrhagic damage in BI rats. It is likely that the action of taurine in the cells was indirect and possibly acts through stimulation of GSH biosynthesis and release. Documents indicate that taurine can improve the status of antioxidant defenses by increasing GSH peroxidase activity (3, 36). Despite of these effects, it cannot be excluded that taurine may down-regulate the generation of inflammatory mediators, including superoxide, nitric oxide, tumor necrosis factor, interleukin 6 and prostaglandin \( \text{E}_2 \). Taurine also exerts cytoprotective effects on various diseases, such as cardiovascular diseases (5), retinal degeneration (26), hepatic disorders (34) and tissue inflammation (33). In addition, it improves the chances of success of fertilization and the early embryonic development (10). Taurine has been reported to inhibit proinflammatory substances, such as nitric oxide, prostaglandin and tumor necrosis factor alpha by activated macrophages (30). Furthermore, cytoprotective effects of taurine may be partly via inhibition of inducible nitric oxide and attenuation of cyclooxygenase 2 expression. Other cytoprotection of taurine may include regulation of cell volume, membrane stabilizing activity, growth-promoting factor (32) and prevention of cellular calcium overload (22). These effects may also participate in taurine-induced gastric mucosal protection in BI rats.

In summary, the pathogenesis of gastric hemorrhagic erosion in gastric juice-irrigated stomachs of BI rats may associate with decreased mucosal GSH levels as well as the increased mucosal histamine and oxyradical generation that can be ameliorated by taurine.

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