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*BASIC RESEARCH*

# Role of gastric oxidative stress and nitric oxide in formation of hemorrhagic erosion in rats with ischemic brain

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## Abstract

**AIM:** To investigate the role of gastric oxidative stress and nitric oxide (NO) in the formation of gastric hemorrhagic erosion and their protection by drugs in rats with ischemic brain.

**METHODS:** Male Wistar rats were deprived of food for 24 h. Under chloral hydrate (300 mg/kg) anesthesia, bilateral carotid artery ligation was performed. The pylorus and carotid esophagus of the rats were also ligated. The stomachs were then irrigated for 3 h with either normal saline or simulated gastric juice containing 100 mmol/L HCl plus 17.4 mmol/L pepsin and 54 mmol/L NaCl. Rats were killed and stomachs were dissected. Gastric mucosa and gastric contents were harvested. The rat brain was dissected for the examination of ischemia by triphenyltetrazolium chloride staining method. Changes in gastric ulcerogenic parameters, such as decreased mucosal glutathione 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.

**RESULTS:** Bilateral carotid artery ligation produced severe brain ischemia (BI) in rats. An exacerbation of various ulcerogenic parameters and mucosal hemorrhagic erosions were observed in these rats. The exacerbated ulcerogenic parameters were significantly ($P < 0.05$) attenuated by antioxidants, such as exogenous glutathione and allopurinol. These gastric parameters were also improved by intraperitoneal aminoguanidine (100 mg/kg) but were aggravated by $N^G$-nitro-L-arginine-methyl ester (L-NAME: 25 mg/kg). Intraperitoneal L-arginine (0-500 mg/kg) dose-dependently attenuated BI-induced aggravation of ulcerogenic parameters and hemorrhagic erosions that were reversed by L-NAME.

**CONCLUSION:** BI could produce hemorrhagic erosions through gastric oxidative stress and activation of arginine-nitric oxide pathway.

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**Key words:** Lipid peroxide; Acid back-diffusion; Glutathione; Allopurinol; L-arginine; Nitric oxide synthase; Aminoguanidine

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## INTRODUCTION

Brain ischemia (BI) resulting from cardiovascular stenosis, hypercholesterolemia or intracranial hemorrhage is frequently found in the clinic. The development of this disease may lead to cerebral hypoxia, brain tissue damage, and stroke. The peripheral manifestations of BI may include seizures[12], neurological deficiency[5] and hemostatic disturbance[8]. However, whether gastric mucosal integrity is also impaired during the occurrence of BI is still unknown till now. In general, the disruption and/or degeneration of gastric mucosal cells may result in the decrease in gastric defensive factors, including mucosal reduced glutathione (GSH), and/or in increase of offensive factors, such as gastric acid back-diffusion and generation of oxyradicals. Our previous papers indicated that acid back-diffusion was a critical factor of acid-induced exacerbation of gastric hemorrhagic ulcer in diabetic or starved rats[19,20]. The back-diffused free acid may damage gastric mucosal cells by increasing oxyradicals and the release of histamine[15]. In fact, oxyradicals produced during oxidative stress play a pivotal role in the etiology of many diseases[26], including cancer[1] and sepsis[14,15]. However, effects of gastric oxidative stress and oxyradical scavengers, such as exogenous reduced GSH or allopurinol on the formation of gastric hemorrhage and mucosal erosion in gastric juice-irrigated stomachs of rats with ischemic brain, remain obscure.

Ample documents demonstrate that nitric oxide (NO) plays an important role in physiological vasodilatation, cytotoxicity and vascular diseases. NO and prostacyclin released from the endothelium may act synergistically to inhibit platelet aggregation and adhesion[36] that are

associated with pathophysiological mechanisms of many diseases[10]. L-arginine, an NO donor, is an important amino acid nutrient in living organs. Whether this amino acid can protect gastric mucosa against gastric hemorrhage and stomach erosion through the production of NO in rats with ischemic brain is also unknown. In the present study, the non-selective NO synthase inhibitor, $N^G$-nitro-L-arginine-methyl ester (L-NAME) or relatively selective inducible NO synthase (iNOS) inhibitor, aminoguanidine (AMG), were used to clarify the role of NO in the protection of gastric mucosa against damage in rats with ischemic brain. Taken together, the aim of the present study was to investigate whether or not oxidative stress-induced changes in acid back-diffusion, histamine release and mucosal oxyradical generation as well as in GSH level and NO liberation are important to modulate gastric hemorrhagic erosion in stomachs of rats with ischemic brain.

## MATERIALS AND METHODS

### 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 central air conditioning (25 ºC 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 accordance with the guidelines of the National Science Council of Taiwan (NSC 1994). Before the experiment, rats were deprived of food for 24 h. BI induced by bilateral carotid artery ligation (BCAL) for 3 h was carried out under potent anesthesia of intraperitoneal chloral hydrate (300 mg/kg). Adequate amount of chloral hydrate was injected to rats for the maintenance of anesthesia. To maintain spontaneous respiration, a polypropylene tube (3.0 mm in diameter and 50 mm in length) was intubated in the rat trachea. Control rats received sham BCAL. At the end of the experiment, rat brains were dissected and sliced for 2-mm thick at a distance of 4, 6, and 8 mm from the forebrain. Brain slices were soaked in triphenyltetrazolium chloride solution for 30 min at 37 ℃ as described by Isayama *et al*[18]

### Chemicals

The following reagent-grade chemicals were used. Acivicin, allopurinol, AMG, L-arginine, chloral hydrate, *n*-butanol, 2,2'-dinitro-5,5'-dithio-dibenzoic acid, pyridine, L-NAME, *o*-phthaldialdehyde (OPT), rat hemoglobin, reduced GSH, sodium lauryl sulfate, 1,1,3,3-tetramethox ypropane, trichloroacetic acid and 2-thiobarbiturate were purchased from Sigma, St. Louis, MO, USA. The purity of all drugs was over 98%. All chemical solutions were freshly prepared before use.

### Drug administration

Intraperitoneal L-arginine (0-500 mg/kg), AMG (100 mg/kg), L-NAME (25 mg/kg, i.p.), GSH (800 mg/kg) or allopurinol (100 mg/kg) were administered to rats 30 min before gastric juice irrigation.

### Gastric surgical procedures

Rat stomachs were surgically exposed for the ligation of pylorus and carotid esophagus. A small incision was made in the forestomach of rats. The stomach contents were gently expelled from the incision. A polypropylene tube (1.0 mm internal diameter×20 mm long) was inserted through the same incision and secured with a ligature. Subsequently, the stomach was rinsed meticulously with warm saline (37 ℃). 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 as previously described[14]. Briefly, 7-mL of either normal saline or simulated gastric juice containing 100 mmol/L HCl, 17.4 mmol/L pepsin and 54 mmol/L NaCl[25] 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 for 20 min at 3 000 r/min

### Quantitation of gastric sample

The volumes of the initial and final samples were measured. Gastric acidity of the samples was assessed by titrating 1.0 mL of sample gastric contents with 0.1 mol/L NaOH to pH 7.0 on an autoburette titrator (Radiometer, Copenhagen, Denmark). The net flux of ions through gastric mucosa was calculated as follows: Net flux = Fv× Fc-(7-Iv)×Ic, where Fv and Iv are the volumes (mL) of final sample and initial sample, respectively, while Fc and Ic are the ionic concentrations (mmol/L) 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 and histological studies of gastric mucosa

As soon as the final sample was collected, the stomach was filled with 10 mL/L 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 mm×1 mm) under a dissecting microscope (×0.7-×3.0; American Optical Scientific Instrument 569, Buffalo, NY, USA). The erosion areas were determined as previously described[14]: Erosion area=length×width×π/4. The total erosion area ($mm^2$) of each stomach was recorded. Histological studies of the stomach were also conducted by methods as previously described[6]. Briefly, after gross examination, the specimens taken from the stomachs were blocked and immersed into 100 mL/L neutral formalin for 2 d. Blocks were then dehydrated in series of alcohol, cleared in xylene and embedded in paraffin. Sections (7 μm thickness) were cut and stained with hematoxylin and eosin as routine histological procedures. Each section was examined under a microscope (Nikon HF, X-IIA, Tokyo, Japan), and the tissue damage was quantified. Sections were scored as 0-5, in which 0 indicated a normal

appearance, 1 indicated mild injury in the epithelial cells, 2 indicated mild injury in the upper part of mucosal cells, 3 indicated hemorrhage or edema in the mid or lower part of mucosal cells, 4 indicated degranulation or necrosis of the epithelial cells and 5 indicated serious cell disruption of lower part of the mucosa. The index score of each section was evaluated on a cumulated basis to give a maximal score of 15.

### Determination of hemoglobin

The cleansed rat stomachs were irrigated for 3 h with either saline or simulated gastric juice. Initial and final gastric samples were collected by aforementioned methods. The blood attached on the gastric mucosa was carefully scraped and added into the final sample. Subsequently, both initial and final samples were adjusted to pH 1.5 with 0.1 mol/L HCl. The concentrations of hemoglobin in samples were measured spectrophotometrically[15]. 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 ($r > 0.90$) contrasted with freshly prepared rat Hb (0.05-1.00 g/L) 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 the final sample and initial sample, respectively, while $F_{Hb}$ and $I_{Hb}$ are the luminal Hb concentrations (g/L) 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[19]. 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 mol/L $NaH_2PO_4$ plus 0.25 mol/L sucrose, pH 7.4). Acivicin (250 μmol/L), an irreversible inhibitor of γ-glutamyltransferase, was added to the homogenate to inhibit the catabolism of GSH. The samples were then centrifuged at 4000 r/min for 15 min at 4℃. To determine the recovery of reduced thiol, the supernatant was added with or without GSH (200 μmol of reduced GSH contained in phosphate buffer solution, pH 7.0). Subsequently, 0.5 mL of 0.25 mol/L trichloroacetic acid was added to 1.0 mL of the supernatant of each sample and kept for 30 min at 4℃. After centrifugation for 15 min at 3 000 r/min, the supernatant was used to determine GSH using 2,2'-dinitro-5,5'-dithio-dibenzoic 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 mmol/L), 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

The concentrations of gastric mucosal lipid peroxides (LPO) were determined by estimating malondialdehyde (MDA) using thiobarbituric acid test[32]. Briefly, 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 of distilled water. The mixture was incubated at 98℃ for 1 h. Upon cooling, 5 mL of n-butanol:pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged for 10 min at 4000 r/min. 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 nanomole MDA per gram wet tissue.

### Measurement of mucosal histamine

Gastric mucosal histamine concentration was determined by the methods as described previously[33]. Briefly, gastric mucosa was scraped and homogenized with trichloroacetic acid (90 mmol/L) in a final concentration of 100 g tissue/L. The homogenate was further centrifuged at 54000 r/min for 10 min. The OPT-NaOH solution was prepared by dissolving 10 mg of OPT in 1.0 mL methanol (10 g/L) and 4 mL of NaOH (0.06 mol/L). This mixed solution was then gassed with nitrogen for 10 min. Then, 2.2 mL of a 20 g/L OPT-NaOH solution was added to 100 μL of a 1/10-fold diluted sample of supernatant or histamine test solution. The mixture was placed at -20 ℃ for 10 h. Then 200 μL of 0.35 mol/L $H_2SO_4$ (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℃) 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 mg/L) that were treated in the same manner as the tissue samples. The results obtained from tissue samples were expressed as microgram histamine per gram wet tissue.

### Statistical analysis

The data obtained from the experiments were expressed as mean±SE. Differences in the data of experiments were analyzed statistically using ANOVA[28]. A simple regression analysis was used to determine the correlation between two different variances. $P < 0.05$ was considered statistically significant.

## RESULTS

### Morphological changes of gastric mucosa of rats with ischemic brain

Gastric mucosa was morphologically intact in sham

**Table 1 Influences of gastric juice on various gastric biomedical parameters in sham operation and BCAL rats**

| | Acid back-diffusion (μEq/ stomach) | Glutathione (μmol/g tissue) | Lipid peroxide (nmol MDA/g tissue) | Hemoglobin (mg/ stomach) | Erosion (area $mm^2$) | Histological score |
|---|---|---|---|---|---|---|
| Sham operation | | | | | | |
| Saline | 56.0±6.0 | 3.0±0.4 | 36.1±4.5 | 0.1±0.1 | 0.3±0.2 | 0.3±0.1 |
| Gastric juice | -135.6±4.4 | 2.6±0.3 | 50.1±6.4 | 0.3±0.1 | 0.4±0.8 | 0.7±0.3 |
| BCAL | | | | | | |
| Saline | 30.2±4.1[a] | 2.1±0.3[a] | 88.1±3.4[a] | 0.8±0.2[a] | 8.2±0.9[a] | 1.8±0.3[a] |
| Gastric juice | -250.2±10.4[c] | 1.4±0.2[c] | 137.6±10.4[c] | 2.0±0.4[c] | 40.2±7.1[c] | 4.5±0.7[c] |

Data are means±SE (N=8). Significant differences are analyzed by using ANOVA. [a]$P<0.05$ *vs* saline treated sham operation group; [c]$P<0.05$ *vs* gastric juice treated sham operation group, BCAL= bilateral carotid artery ligation, MDA = malonedialdehyde.

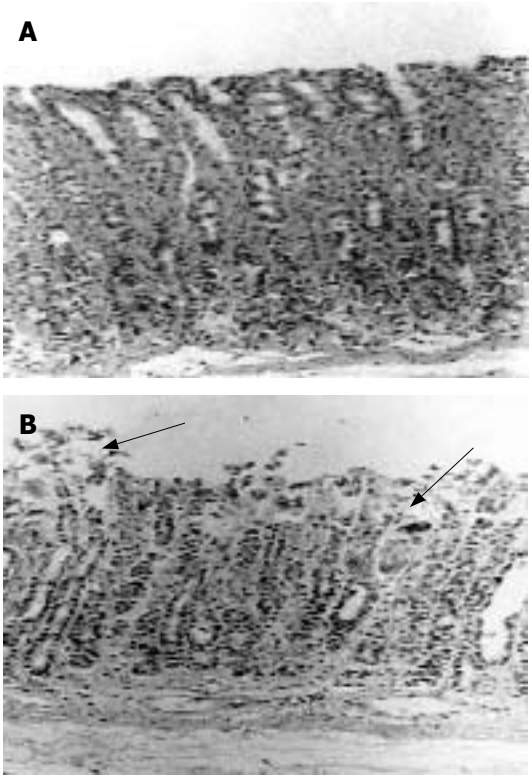


**Figure 1** Histological study of gastric mucosa in sham operative rats and rats with ischemic brain. **A:** In sham operative rat stomachs irrigated with gastric juice, gastric mucosal cells appear intact; **B:** Disruption of the upper mucosal cells and lamina propria (indicated by arrows) in rats with ischemic brain, the injured cells are characterized by karyorrhexis and dense homogenous acidophilic cytoplasm (HE×150)

operative rats. Nevertheless, gastric hemorrhagic erosions were observed in the stomachs of rats with ischemic brain (photo not shown). Histological studies showed that gastric mucosal cells appeared intact in sham operative rat stomachs irrigated with gastric juice (Figure 1A). However, necrotic cell-injury was found in both epithelial layers and lamina propria when gastric juice was present in the stomachs of rats with ischemic brain (Figure 1B). In sham operative rat stomachs irrigated with normal saline, no damage of gastric mucosal cells was observed. In gastric juice-irrigated stomachs of sham operative rats, gastric mucosal cells also appeared undamaged. In rats with ischemic brain, normal saline-irrigated stomachs produced a little 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 enhance mucosal cell damage in rats with ischemic brain (Table 1).

We observed that back-diffused $H^+$ concentrations, luminal Hb contents and mucosal erosion in normal saline-irrigated stomachs of sham operative rats were negligible (Table 1). Gastric mucosal GSH concentrations and LPO generations in these rats were also at normal levels. Similar results were observed in gastric juice-irrigated stomachs of sham operative rats. In rats with ischemic brain, normal saline-irrigated stomachs produced significant increases in these gastric parameters compared with those found in sham operative rat stomachs irrigated with normal saline ($P<0.05$). When gastric juice was used instead of normal saline in rats with ischemic brain, remarkable exacerbations of gastric hemorrhagic erosions accompanied with great enhancement in acid back-diffusion and mucosal LPO as well as a lowering of mucosal GSH levels were observed. High correlations between exacerbated gastric mucosal damage and decreased GSH levels as well as between mucosal erosions and increased LPO were observed in those gastric juice-irrigated stomachs of rats with ischemic brain (Figure 2). Apparently, BI could produce gastric oxidative stress in rats. Furthermore, gastric juice was able to aggravate various ulcerogenic parameters in rats with ischemic brain.

### *Effect of allopurinol or exogenous GSH*

Intraperitoneal allopurinol (100 mg/kg) or reduced GSH (800 mg/kg) caused a significant decrease in acid back-diffusion and LPO generation as well as histamine concentration and hemorrhagic erosion in sham operative rats ($P<0.05$, Table 2). Gastric mucosal GSH levels were elevated in exogenous GSH-treated rats. In rats with ischemic brain, these two chemicals produced a pronounced inhibition in acid back-diffusion, LPO generation, histamine release and hemorrhagic erosions while GSH levels were greatly elevated.

### *Effect of L-arginine*

We observed that L-arginine (500 mg/kg) significantly increased gastric mucosal GSH levels but decreased acid back-diffusion, LPO and histamine concentrations in gastric juice-irrigated stomachs of sham operative rats

**Table 2 Effects of allopurinol or exogenous glutathione on various gastric parameters in sham operation and BCAL rats**

| | mg/kg | Acid back-diffusion (μEq/stomach) | Glutathione (μmol/g tissue) | Lipid peroxide (nmol MDA/g tissue) | Histamine (μg/g tissue) | Hemoglobin (mg/stomach) | Erosion area ($mm^2$) |
|---|---|---|---|---|---|---|---|
| Sham operation | | | | | | | |
| Vehicle | | -135.4±4.4 | 2.8±0.3 | 50.1±4.5 | 56.1±5.4 | 0.5±0.2 | 2.4±3.5 |
| Allopurinol | 100 | -112.0±3.8[a] | 3.0±0.3 | 40.2±4.7[a] | 40.5±5.1[a] | 0.2±0.1[a] | 0.2±0.2[a] |
| Glutathione | 800 | -98.0±2.4[a] | 3.4±0.2[a] | 36.1±2.5[a] | 38.1±4.6[a] | 0.1±0.1[a] | 0.4±0.2[a] |
| BCAL | | | | | | | |
| Vehicle | | -250.2±14.4 | 1.4±0.2 | 128.0±9.3 | 120.4±9.8 | 2.3±0.3 | 45.2±6.1 |
| Allopurinol | 100 | -181.5±5.8[c] | 2.2±0.2[c] | 68.4±5.1[c] | 77.3±6.9[c] | 1.2±0.2[c] | 20.3±2.9[c] |
| Glutathione | 800 | -146.0±3.4[c] | 2.7±0.2[c] | 56.1±4.5[c] | 80.1±4.6[c] | 0.1±0.1[c] | 12.4±3.5[c] |

Data are means±SE (N=6). [a]$P<0.05$ *vs* sham operative vehicle. [c]$P<0.05$ *vs* BCAL vehicle. MDA= malonedialdehyde, BCAL= bilateral carotid artery ligation.

**Table 3 Effect of L-NAME on L-arginine produced amelioration of various gastric biomedical parameters in sham operation and BCAL rats**

| | mg/kg | Acid back-diffusion (μEq/stomach) | Glutathione (μmol/g tissue) | Lipid peroxides (nmol MDA/g tissue) | Histamine (μg/g stomach) | Hemoglobin (mg/stomach) | Erosion area ($mm^2$) |
|---|---|---|---|---|---|---|---|
| Sham operation | | | | | | | |
| Vehicle | | -125.2±4.4 | 2.6±0.3 | 48.1±3.4 | 52.8±4.6 | 0.1±0.1 | 3.2±0.7 |
| L-arginine | 500 | -96.0±6.0[a] | 3.5±0.4[a] | 36.1±4.5[a] | 38.2±3.6[a] | 0±0[a] | 0.3±0.2[a] |
| L-NAME25 | | | | | | | |
| + L-arginine | 500 | -156.0±11.0[ac] | 2.3±0.2[c] | 60.1±4.5[a,c] | 72.4±6.3[a,c] | 0.7±0.1[a,c] | 12.0±3.5[a,c] |
| BCAL | | | | | | | |
| Vehicle | | -245.2±14.4 | 1.6±0.2 | 127.8±9.3 | 110.4±9.8 | 1.6±0.3 | 36.2±5.1 |
| L-arginine | | | | | | | |
| | 25 | -216.1±9.6[a] | 2.0±0.2 | 80.8±7.8[a] | 98.7±10.4 | 1.3±0.2 | 28.2±5.4 |
| | 250 | -189.4±3.6[a] | 2.3±0.3[a] | 66.7±4.4[a] | 84.3±7.4[a] | 1.0±0.1[a] | 20.0±4.8[a] |
| | 500 | -144.0±11.0[a] | 2.6±0.2[a] | 46.1±8.5[a] | 54.1±8.6[a] | 0.7±0.1[a] | 12.0±3.5[a] |
| L-NAME | 25 | | | | | | |
| + L-arginine | 500 | -287.6±16.4[ac] | 1.2±0.2[ac] | 157.6±11.3[ac] | 110.4±9.8[ac] | 2.8±0.3[ac] | 56.2±6.0[ac] |

Data are means±SE (N=6). [a]$P<0.05$ *vs* corresponding vehicle. [c]$P<0.05$ *vs* corresponding arginine treatment (500 mg/kg) of BCAL group. MDA= malonedialdehyde, BCAL= bilateral carotid artery ligation, L-NAME= NG-nitro-L-arginine-methyl ester.

($P<0.05$, Table 3). In stomachs of rats with ischemic brain, the ulcerogenic parameters, such as increased acid back-diffusion, LPO generation, hemorrhage and mucosal erosions, were dose-dependently attenuated by pretreatment with L-arginine, whereas the decreased mucosal GSH levels found in these rats with ischemic brain were effectively inhibited. These cytoprotective effects of L-arginine on gastric mucosa were reversed by concomitant administration of L-NAME (25 mg/kg).

### *Effects of L-NAME or AMG*

Intraperitoneal L-NAME caused significant aggravation of acid back-diffusion, LPO generation, histamine release and hemorrhagic erosions in rats with ischemic brain ($P<0.05$). Gastric mucosal GSH levels were attenuated (Figure 3). In AMG-treated rats with ischemic brain, a remarkable inhibition of acid back-diffusion, LPO generation and hemorrhagic erosion was achieved. Gastric mucosal GSH levels and histamine concentrations were significantly augmented ($P<0.05$).

## DISCUSSION

The pathological mechanisms underlying the aggravation of gastric hemorrhagic erosion in gastric juice-irrigated stomachs of rats with ischemic brain are complex. The present study demonstrated that the decreased GSH levels as well as increased mucosal LPO generation, histamine concentration and acid back-diffusion were exacerbated in rats with ischemic brain. We also observed the occurrence of gastric hemorrhage and erosion. Apparently, gastric oxidative stress was produced in rats with ischemic brain. During oxidative stress, gastric mucosal barriers were degenerated and disrupted. When gastric juice was irrigated in the stomachs of rats with ischemic brain, the intraluminal free $H^+$ back diffused through disrupted barriers to the gastric mucosa and thereby damaged the gastric cells. Consequently, aggravation of gastric hemorrhagic erosion and various ulcerogenic parameters occurred. The back-diffused gastric acid may also stimulate oxyradical release. The elimination of gastric mucosal GSH levels in gastric juice-irrigated stomachs of rats with ischemic brain 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 NO 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 has also been documented[37].

Our previous report demonstrated that increase in oxyradical generation as indicated by augmentation of

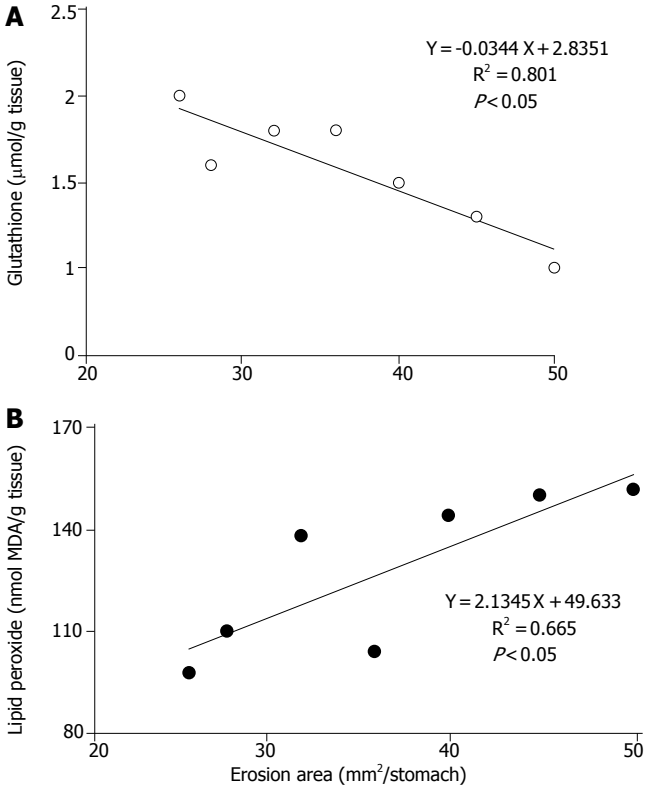


**Figure 2** Relationship between gastric mucosal ulceration and mucosal GSH levels as well as between gastric mucosal ulceration and lipid peroxide generation in gastric juice-irrigated stomachs of rats with ischemic brain. Rat stomachs were irrigated for 3 h with simulated gastric juice.

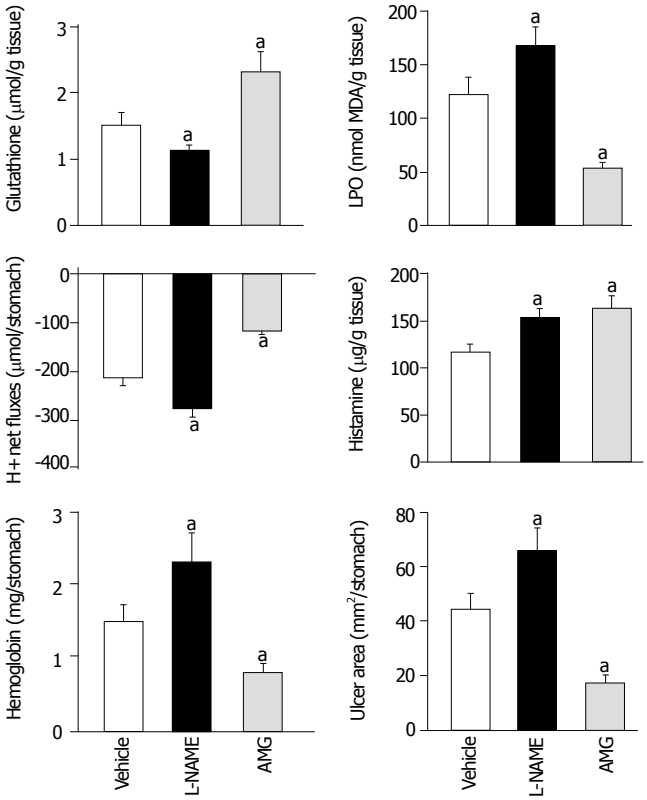


**Figure 3** Effects of L-NAME and AMG on various gastric parameters in gastric juice-irrigated stomachs of rats with ischemic brain. Data are expressed as mean± SE ($n$=8). $^aP<0.05$ *vs* vehicle.

concentration of mucosal LPO, the oxyradical metabolite, was parallel to the elevation of histamine concentration in endotoxemic rats[14]. In the present *in vivo* study, increased mucosal histamine release may not only be 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. The increased histamine found in rats with ischemic brain could be attenuated by allopurinol or exogenous GSH, implying that enzymology of oxyradical formation was involved in the metabolism of histamine. Taken together, increased histamine release and oxyradical generation in the gastric mucosa might result in hemorrhage and erosion in rats with ischemic brain. We have previously reported that activation of histamine $H_1$ and $H_2$ receptors is important in the formation of gastric hemorrhage and ulcer in septic rats[15].

In the present study, L-arginine produced a dose-dependent increase in mucosal GSH levels and an attenuation of mucosal histamine and LPO concentrations in rats with ischemic brain. Gastric hemorrhage and stomach erosions were also remarkably ameliorated. Apart from its roles in protein biosynthesis and as an intermediate in the urea cycle, L-arginine is a substrate for NO production and phosphocreatine synthesis as well as a precursor to proline, glutamate and putrescine via ornithine. NO is formed from L-arginine by NO synthases (NOS). Three isoforms of this enzyme have been identified; two are constitutively expressed and one is iNOS[7]. NO has dual effects on living cells. One is defensive and the other is injuring. Over-expression of NO may cause cell damage and dysfunction that plays a pivotal role in many diseases, including intestinal ischemia-reperfusion[20], hypertension[30], inflammation[6], cell death[2]. On the other hand, physiological NO can protect cells against oxidative damage[38]. It also mediates many of the manifestations of septic shock, including hemodynamic instability[9]. L-arginine has been successfully used to increase vascular dilation and basal NO synthesis, and thereby reducing ischemia/reperfusion-induced injury[31]. Oral supplementation of L-arginine in hypercholesterolemic patients with endothelial dysfunction increased endothelial-dependent dilation of the bronchial artery during increased cardiac output[4]. L-arginine also promotes mucosal repair after intestinal ischemia/reperfusion[35] and relieves mucosal damage during endothelin-induced ulceration[21]. These protective effects of L-arginine are abrogated by L-NAME, a non-selective inhibitor of NO synthase. It has been reported that L-arginine can enhance mucosal growth and repair by supporting polyamine synthesis[27]. Also, it can accelerate healing of gastric erosions in rats and this effect could be attributed to the stimulation of gastric mucosal blood

flow through increased NO synthesis and stimulation of mucosal growth[3]. Altogether, these beneficial effects of L-arginine may account for the protective effects of L-arginine on gastric hemorrhagic erosions in rats with ischemic brain. In the present study, the attenuation of hemorrhage and stomach erosion in rats with ischemic brain by parental challenge of L-arginine may be associated with NO formation via arginine-NO-pathway.

The present study also showed that AMG significantly reduced gastric hemorrhagic ulcer in rats with ischemic brain. We also observed inhibition of lipid peroxide generation and augmented GSH level. However, it augmented mucosal histamine concentration. AMG is a relatively selective inhibitor of iNOS, but also is a specific inhibitor of histaminase, an enzyme that breaks down histamine. The elevated histamine by AMG might be a net result of its inhibition on histaminase and iNOS. In fact, specific inhibition of iNOS that is up-regulated in many tissues during diseases has been explored as a therapy for diseases. AMG has been shown to prevent the endotoxin-induced impairment of vascular reactivity when administered *in vivo*[19]. It has also been reported to reduce streptozotocin-induced hyperglycemia[34] and brain damage produced by occlusion of the middle cerebral artery in rats[29]. Taken together, iNOS may play an important role in the formation of hemorrhagic erosion in rats with ischemic brain.

In the present study, L-NAME failed to protect gastric mucosal damage in rats with ischemic brain. On the contrary, it reversed mucosal protective effect of L-arginine. The result might be due to the inhibition of constitute NOS by L-NAME. In fact, NO synthesis may provide protection to tissues after ischemia/reperfusion-induced injury of the tissue[22]. Document indicates that inhibition of NO synthesis by L-NAME increases ischemia/reperfusion-induced injury, which can be reversed by perfusion with NO or its donors that can decrease injury and neutrophil accumulation[27].

In conclusion, gastric oxidative stress and excess iNOS production may play a pivotal role in modulation of hemorrhagic erosion through increased acid back-diffusion, histamine release and oxyradical generation as well as decreased GSH levels in rats with ischemic brain.

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