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Protective Effects of Several Amino Acid-Nutrients on Gastric Hemorrhagic Erosions in Acid-Irrigated Stomachs of Septic Rats

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Abstract

Our previous report demonstrates that severe gastric mucosal damage is produced in lipopolysaccharide (LPS)-intoxicated rats. In the present study, we examined protective effects of several amino acids including taurine, phenylalanine and L-Arginine on gastric hemorrhagic erosions in acid-irrigated stomachs of LPS rats. The animals were deprived of food for 24 hr. Intravenous LPS (3 mg/kg) was challenged 12 hr after withdrawal of food. Gastric vagotomy was performed, followed by irrigation the stomachs for 3 hr with a physiological acid solution containing 100 mM HCl and 54 mM NaCl. The ulcerogenic parameters including increased gastric acid back-diffusion, mucosal histamine concentrations, lipid peroxide productions, luminal hemoglobin contents, stomach erosions and the lowered glutathione levels were markedly enhanced in LPS rat stomachs irrigated with acid solution. Both phenylalanine and taurine caused dose-dependent attenuations of these ulcerogenic parameters in LPS rats. L-arginine also was effective in inhibition. The inhibitory effect was restored by pretreatment of nitric oxide synthase inhibitors, such as N^G-nitro-L-arginine-methyl ester or L-N^G-(1-iminoethyl)-lysine. Furthermore, marked amelioration of hemorrhagic erosions in LPS rats was observed when a combination of these amino acid nutrients was used. The results provide evidence that these amino acid nutrients may ameliorate gastric hemorrhagic erosion via GSH synthesis stimulation, histamine cell membrane stabilization and antioxidant actions in LPS rat stomachs.

Key Words: Amino acids, gastric hemorrhagic erosion, sepsis, nitric oxide

Introduction

Lipopolysaccharide (LPS) liberated during Gram's negative bacterial cell wall lysis may produce sepsis in either man or animals. This disease may cause dysfunction of various organs including lungs, heart, blood vessels, kidneys and liver (16, 26, 34). Previous reports demonstrate that gastric hemorrhage and stomach erosions also occur in septic rats (5, 18), although low doses (at microgram level) of LPS on the contrary may possess gastric mucosal cytoprotective effects (41). The production of gastric hemorrhagic erosions in septic states may associate with the deterioration of ulcerogenic factors, such as the enhanced gastric acid back-diffusion (GABD), histamine liberation and lipid peroxide (LPO) production as well as the decreased glutathione (GSH) biosynthesis (18). Amino acid (AA) nutrients are essential to protein biosynthesis, cell growth and energy supply, and the deficiency of specific AA is considered as an etiology of organ dysfunction. The supplementation of proper nutrients may be beneficial to the healing of various disease (12). Therefore, nutrition therapy is commonly accepted in the clinic. Documents indicate that oxyradicals are closely associated with septic shock (18), diabetes mellitus (28) and starvation (21). The AAs such as taurine (TA), phenylalanine (Phe) and L-Arginine (L-Arg)

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may also possess potent antioxidant or oxyradical scavenging properties (11, 28, 39). L-Arginine also is a donor of nitric oxide (NO), which is associated with pathophysiological mechanisms of many diseases (14). Whether these AAs can protect gastric mucosa against gastric hemorrhage and stomach erosion in LPS rats is unknown. Moreover, whether NO produced by L-Arg via Arg-NO-pathway contributes to the protection of gastric mucosal damage in LPS rats also remains obscure. The aim of the present study was to investigate the protective effects of the indicated AAs on gastric hemorrhagic erosions by evaluating changes in various ulcerogenic parameters in acid irrigated stomachs of LPS rats. Additionally, the non-selective NO synthase inhibitor as N^G-nitro-L-arginine-methyl ester (L-NAME) or selective NO synthase inhibitor, L-N^G-(1-iminoethyl)-lysine (L-NIL) was used to clarify the role of L-Arg produced NO in the protection of gastric damage in LPS rats.

Materials and Methods

Animals

Male SPF Wistar rats, weighing 200-250 g, were obtained from and housed in The Laboratory Animal Center, National Cheng-Kung University in Taiwan. The rats were housed individually in a room with 12-h dark-light cycle and with central air conditioning (25°C, 70% humidity). They were allowed free access to tap water and pellet rodent diet (the Richmond standard, PMI Feeds, Inc. St. Louis, MO. U.S.A.). The animal care and experimental protocols were in accord with the guidelines of the National Science Council of Taiwan (NSC 1994), and were approved by The Laboratory Animal Advisory Committee of National Cheng-Kung University. Prior to performing the experiment, rats were moved to cages equipped with wire mesh to avoid coprophagy. They were deprived of food but allowed free access to tap water for 24 h. Intravenous LPS (3 mg/kg in 1.0 ml of sterilized normal saline) was given to rats 12 h after withdrawal of food. Non-septic rats received sterilized normal saline only. An augmented leukocyte numbers and blood coagulation time, the sensitive indicators of septic shock, in LPS rats is demonstrated in our previous paper (18). No death of the animals was observed through out the experiment. Therefore, LPS can satisfactorily produce sepsis at the indicated dose.

Surgical Procedures

After deprivation of food for 24 h, rats were anesthetized with diethylether and the stomachs were surgically exposed for the ligation of pylorus and lower esophagus. To prevent the spontaneous gastric secretion, bilateral diaphgramatic vagotomy was performed in both LPS and non-LPS rats as described by Shay et al. (38). A small incision was made in the forestomach. The stomach contents were gently expelled from the incision. A polypropylene tube (1.0 mm i.d. × 20 mm length) was inserted through the same incision and secured with a ligature. Subsequently, the stomach was rinsed meticulously with warm normal saline (37°C). Care was taken to avoid injury of blood vessels. The residues were gently removed.

Analysis of Luminal Electrolytes

Gastric acid back-diffusion (gastric luminal H^+ loss) was quantified by the method previously described (19). Namely, normal saline or isotonic acid solution (7 ml) containing 100 mM HCl and 54 mM NaCl was instilled into the cleansed stomach with a syringe. The luminal contents were mixed with the same syringe by three repeated aspirations and injections, and 3 ml of the fluid was taken as an initial sample. The incision of forestomach was tightly closed. The abdominal wound also was sutured. After 3 h, the rats were killed with an overdose of diethylether. The gastric sample (final sample) was collected. Both initial and final samples were centrifuged for 20 min at 3000 r.p.m..

Quantitation of Gastric Samples

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 of H^+ through gastric mucosa was calculated as follow: 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 (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 and Histological 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 erosions on the gastric mucosa were measured with a planimeter (1 × 1 mm) under a dissecting microscope (×0.7–×3.0; American Optical

Scientific Instrument 569, Buffalo, NY, U.S.A.). The erosion areas were determined as previously described (21); erosion area = length × width × π/4. The total erosion area (mm^2) of each stomach was recorded. Gastric mucosal damage was determined by a person unaware of experimental procedure. Histological studies of the stomach also were conducted by methods previously described (18). Briefly, after gross examining, the specimens taken from corpus were blocked and immersed into 10 % neutral formalin for two days. Blocks were then dehydrated in series of alcohol, cleared in xylene and embeded in paraffin. Sections (7 μm thickness) were cut and stained with hematoxylene 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. The section was scored with an index of 0-5 in which 0 indicated normal appearance; 1, mild injury in the epithelial cells; 2, mild injury in the upper part of mucosal cells; 3, hemorrhage or edema in the mid or lower part of mucosal cells; 4, degranulation or necrosis of the epithelial cells and 5, serious cell disruption of lower part of the mucosa. The index of each section was evaluated on a cumulated basis to give a maximal score of 15.

Determination of Hb

The cleansed rat stomachs were irrigated for 3 h with either normal saline or acid solutions (100 mM HCl plus 54 mM NaCl for isotonicity). Gastric initial and final samples were collected by the method as demonstrated above. 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 contents of Hb in the samples were measured spectrophotometrically (17). 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 the samples was measured against a standard curve (r^2>0.99) contrasted with freshly prepared rat Hb (0.05-1.00 mg/ml) treated in the same manner as the 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) in the final sample and initial sample, respectively. The results obtained from gastric samples were expressed in milligram Hb per stomach.

Assay of Mucosal GSH

The quantitation of gastric mucosal GSH was performed by a method as previously demonstrated (21). 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 NaH2PO4 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 at 4000 r.p.m. for 15 min at 4 °C. 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 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 for 15 min at 3000 r.p.m., 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 mM) which were treated in the same manner as the tissue samples. The results obtained from tissue samples were expressed as micromol per gram wet tissue.

Determination of Mucosal LPO

The concentrations of gastric mucosal LPO were determined by estimating malonedialdehyde (MDA) using the thiobarbituric acid test (33). Namely, the stomachs of rats were promptly excised and rinsed with cold normal 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 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-tetrametho-xypropane. The recovery was over 90%. All samples were measured in duplicate. The results were expressed as nanomole malonedialdehyde per gram wet tissue.

Measurement of Histamine

Gastric mucosal histamine concentration was

Table 1. Changes in Various Ulcerogenic Parameters in non-LPS Rats and LPS Rat Stomachs Irrigated with Normal Saline or Acid Solution

	GABD μmol /stomach	GSH μmol /g tissue	LPO nmol MDA /g tissue	Hb mg /stomach	histamine μg/g tissue	ulcer area mm^2	histological scores
Non-LPS							
saline-irrigation	4.5 ± 1.8^{d}	3.1 ± 0.4^{a}	38.6 ± 4.3^{c}	0.1 ± 0.1^{d}	78.8 ± 3.1^{c}	0.0 ± 0.0^{d}	0.0 ± 0.0^{c}
acid-irrigation	-105.2 ± 5.2^{b}	2.9 ± 0.4^{a}	45.6 ± 3.5^{c}	0.2 ± 0.1^{c}	88.6 ± 3.4^{b}	0.3 ± 0.2^{c}	0.6 ± 0.3^{c}
LPS							
saline-irrigation	15.7 ± 2.8^{c}	2.5 ± 0.3^{a}	63.3 ± 4.3^{b}	0.8 ± 0.2^{b}	96.4 ± 4.2^{b}	7.3 ± 0.4^{b}	1.5 ± 0.5^{b}
acid-irrigation	-302.5 ± 8.8^{a}	1.2 ± 0.2^{b}	141.2 ± 8.2^{a}	4.1 ± 0.5^{a}	137.2 ± 8.1^{a}	25.4 ± 9.2^{a}	10.5 ± 3.6^{a}

Rats are deprived food for 24h. Intravenous sterilized normal saline or LPS (3 mg/kg) is challenged to rats 12h after withdrawal of food. The stomachs of rats are vagotomized and followed by irrigation for 3h with either normal saline or an acid solution containing 100 mM HCl and 54 mM NaCl. Data are means ± SEM (n=8). Significant differences in a column are analyzed by Tukey honestly significant after ANOVA. a>b>c>d. Abbreviations: LPS=lipopolysaccharide; GABD=gastric acid back-diffusion; GSH=glutathione; LPO=lipid peroxides; Hb=hemoglobin; MDA=malonedialdehyde.

determined by the methods as described previously (17). In brief, the corpus of gastric mucosa was scraped and homogenized with 1.5 % trichloroacetic acid in a final concentration of 100 mg tissue/ml. The homogenate was centrifuged 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 M). This mixed solution was then gassed with nitrogen for 1 0 min. To 100 μl of 1/10 fold diluted sample supernatant or histamine test solution was added 2.2 ml of 0.2% OPT-NaOH solution. The mixture was then placed at −20°C for 10 h. To the frozen was added 200 μl of 0.35 M H_2SO_4 (final pH 1.6-2.4). 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. Fluorescence of the sample was calculated against a standard curve constructed with freshly prepared histamine solutions (0.78-25 μg/ml) which were treated in the same manner as the tissue samples. The results obtained from tissue samples were expressed as microgram per gram wet tissue.

Chemicals and Reagents

The following chemicals in reagent grade were used. acivicin, L-arginine, n-butanol, 2, 2'-Dinitro-5, 5'-dithio-dibenzoic acid, L-NAME, L-NIL, LPS (from *Escherichia coli*, Serotype 055:B5), phenylalanine, pyridine, o-phthaldialdehyde, rat hemoglobin, reduced glutathione, sodium laurylsulfate, taurine, 1,1,3,3-tetramethoxypropane, trichloroacetic acid, and 2-thiobarbiturate were purchased from Sigma, St. Louis, Mo. U.S.A. The purity of all substances was over 98%. All chemical solutions were freshly prepared before use.

Drug Administration

Intravenous LPS (3 mg/kg in 1.0 ml sterilized normal saline) or sterilized normal saline was challenged to rats 12 h after withdrawal of food. Amino acids including TA (0- 250 mg/kg), Phe (0- 100 mg/kg), L-Arg (500 mg/kg) or a mixture of TA (250 mg/kg), Phe (100 mg/kg) and L-Arg (500 mg/kg) was given intraperitoneally to rats 1h before irrigating the stomach with acid solution containing 100 mM HCl and 54 mM NaCl. Both L-NAME (25 mg/kg) and L-NIL (30 mg/kg) were given intraperitoneally to rats 10 min before L-Arg was challenged.

Statistical Analysis

The data obtained from the experiments were expressed as mean ± SEM. Significant differences in the data of experiments for single measurement traits were analyzed statistically by using ANOVA or the Turkey honestly significant difference (HSD) test for pairwise comparison after ANOVA (29). Statistical significance was set at $p<0.05$.

Results

Alteration of Various Ulcerogenic Parameters in Normal Saline- or Acid Solution-Irrigated Stomachs of LPS and Non-LPS Rats

In this study, rats were deprived of food for 24

Table 2. Dose-Response Effect of TA on Various Ulcerogenic Parameters in Acid-Irrigated Stomachs of LPS Rats

	GABD μmol /stomach	GSH μmol /g tissue	LPO nmol MDA/g tissue	histological μg/g tissue	Hb mg/stomach	ulcer area mm^2
Non-LPS						
saline	−110.4±6.3	3.2±0.3	48.7±4.5	88.6±3.4	0.2±0.2	0.3±0.3
TA, 100 mg/kg	−98.6±5.2	3.3±0.2	43.3±3.8	82.8±5.7	0.2±0.1	0.0±0.0#
LPS						
saline	−322.7±7.8	1.1±0.2	138.4±11.2	143.2±12.1	3.9±0.5	28.3±6.2
TA, 50 mg/kg	−286.3±8.7*	1.7±0.3	116.7±12.5	98.6±11.3*	2.9±0.3*	25.6±4.5
100	−223.1±12.8*	2.4±0.3*	84.7±7.7*	58.7±7.3*	2.5±0.5*	14.7±4.3*
250	−173.0±10.9*	2.8±0.4*	71.2±7.2*	46.9±5.5*	2.0±0.6*	10.0±3.4*

Rats are deprived food for 24 hr. Intravenous LPS (3 mg/kg) is challenged to rats 12 hr after withdrawal of food. Non-LPS rats received normal saline only. The stomachs of rats are vagotomized and followed by irrigation for 3 hr with an acid solution containing 100 mM HCl and 54 mM NaCl. Saline or TA is given intraperitoneally to rats just before gastric irrigation. All values are means ± SEM (n=8). Significantly different vs. saline group are analyzed by using ANOVA. #p <0.05 vs. non-LPS saline; p<0.05 vs. LPS saline. Abbreviations: TA=taurine; LPS=lipopolysaccharide; GABD=gastric acid back-diffusion; GSH=glutathione; LPO=lipid peroxides; Hb=hemoglobin; MDA=malonedialdehyde.

Table 3. Dose-Response Effect of Phe on Various Ulcerogenic Parameters in Acid-Irrigated Stomachs of LPS Rats

	GABD μmol /stomach	GSH μmol /g tissue	LPO nmol MDA/g tissue	histological μg/g tissue	Hb mg/stomach	ulcer area mm^2
Non-LPS						
saline	−125.6±3.8	3.2±0.3	58.7±4.8	84.2±6.7	0.1±0.1	0.3±0.3
Phe, 100 mg/kg	−101.4±3.2#	3.4±0.5	51.8±3.6	78.3±6.4	0.2±0.2	0.1±0.1
LPS						
saline	−321.4±16.4	1.1±0.1	160.9±20.5	135.3±13.3	3.1±0.2	24.3±5.4
Phe, 50 mg/kg	−201.2±14.2*	1.3±0.1	140.3±12.6	122.9±3.8*	2.6±0.4	20.0±5.0
100	−143.9±14.8*	2.1±0.2*	103.6±5.1*	71.0±1.1*	1.9±0.2*	5.8±1.5*
250	−125.4±13.0*	2.4±0.2*	64.6±7.0*	59.0±4.0*	1.3±0.3*	3.0±0.9*

Rats are deprived food for 24 hr. Intravenous LPS (3 mg/kg) is challenged to rats 12 hr after withdrawal of food. Non-LPS rats received normal saline only. The stomachs of rats are vagotomized and followed by irrigation for 3 hr with an acid solution containing 100 mM HCl and 54 mM NaCl. Intraperitoneal saline or Phe is challenged to rats 1 h before gastric irrigation. All values are means ± SEM (n = 8). Significantly different vs. saline group are analyzed by using ANOVA. # p< 0.05 vs. non-LPS saline;*p< 0.05 vs. LPS saline. Abbreviations: LPS = lipopolysaccharide; GABD = gastric acid back-diffusion; GSH = glutathione; Phe = phenylalanine; LPO = lipid peroxides; MDA = malonedialdehyde; Hb = hemoglobin. LPO = lipid peroxides; Hb = hemoglobin; MDA = malonedialdehyde.

hr, and LPS (3 mg/kg) was challenged intravenously 12 hr after removal of food. Non-LPS rats were given sterilized normal saline only. The stomachs of rats were vagotomized and followed by irrigation for 3 hr with either normal saline or acid solution containing 100 mM HCl and 54 mM NaCl. As shown in Table I, gastric hemorrhagic erosion was invisible in either normal saline- or acid-irrigated stomachs of non-LPS rats. Histological study also indicated that gastric epithelial cells and lamina propria were normal. The ulcerogenic parameters including GABD, mucosal histamine and LPO concentrations were at normal levels. In normal saline-perfused stomachs of LPS rats, a significant increase of various ulcerogenic parameters was obtained. In normal saline-irrigated stomachs of LPS rats, a remarkable exacerbation of gastric hemorrhage and erosions were achieved. The ulcerogenic parameters including GABD, mucosal histamine and LPO concentrations also were greatly deteriorated. The upper mucosal cells and to a lesser degree, the lamina propria cells were damaged. In most cases, severe gastric edema also was observed.

Table 4. Effects of Various NOS-Inhibitors on L-Arginine-Produced Attenuation of Uicerogenic Parametrs in Acid-Irrigated Stomachs of LPS Rats

	GABD μmol /stomach	GSH μmol /g tissue	LPO nmol MDA/g tissue	histological μg/g tissue	ulcer area mm^2
Non-LPS					
saline	−118.4±4.5	3.1±0.3	53.7±5.5	83.2±5.4	0.2±0.2
Arg, 500 mg/kg	−92.6±3.3#	3.5±0.2	47.6±4.2	78.4±3.8	0.0±0.0
LPS					
saline	−298.5±7.6	1.3±0.2	138.4±7.2	142.2±8.6	20.4±8.4
L-Arg, 500 mg/kg	−153.1±30.8*	2.4±0.1*	64.7±7.7*	58.7±7.3*	8.7±4.3*
L-Arg, 500 mg/kg+					
L-NAME, 25 mg/kg	−310.2±12.4*	1.0±0.1	150.6±10.3	134.0±7.4	32.3±5.6
L-Arg, 500 mg/kg+					
+L-NIL, 30 mg/kg	−284.6±6.8	1.2±0.3	127.5±7.8	129.4±6.2	28.0±7.

Rats are deprived food for 24 hr. Intravenous LPS (3 mg/kg) is challenged to rats 12 hr after withdrawal of food. Non-LPS rats received normal saline only. The stomachs of rats are vagotomized and followed by irrigation for 3 hr with normal saline or the acid solution. All agents are challenged (i.p.) to rats 1 h before gastric irrigation. All values are means ± SE M (n = 6-8). Significantly different vs. saline group are analyzed by using ANOVA. #p< 0.05 vs. non-LPS saline; *p< 0.05 vs. LPS saline. Abbreviations: GSH = glutathione; LPO = lipid peroxides; MDA = malonedialdehyde; GABD = gastric acid back-diffusion; L-NAME = N^G-nitro-L-arginine-methyl ester; L-NIL = L-N^G-1-iminoethyl-lysine.

Effect of TA or Phe on Various Ulcerogenic Parameters in Acid-Irrigated Stomachs of Non-LPS and LPS Rats

In these experiments, rats were deprived of food for 24 hr, and LPS (3 mg/kg) was challenged intravenously 12 hr after removal of food. The stomachs of rats were vagotomized and followed by irrigation for 3 hr with acid solution containing 100 mM HCl and 54 mM NaCl. Intraperitoneal TA (0-250 mg/kg) or Phe (0-100 mg/kg) was challenged to rats 1 h before gastric irrigation As demonstrated in Table 2, in non-LPS rats, TA at the dose of 250 mg/kg produced a significant inhibition in GABD, although other ulcerogenic parameters were not affected. In acid-irrigated stomachs of LPS rats, a serious deterioration of various ulcerogenic parameters was observed. These ulcerogenic parameters were dose-dependently inhibited by TA (50-250 mg/kg). On the other hand, Phe at the dose of 100 mg/kg produced a significant inhibition in GABD (Table 3). Other ulcerogenic parameters were not affected. In LPS rats, intraperitoneal Phe (5- 100 mg/kg) produced a dose-dependent increase in gastric mucosal GSH levels in LPS rats. The amelioration of enhanced histamine and LPO generation also was in a dose-related manner.

Effect of L-NAME or L-NIL on L-Arg-Produced Amelioration of Various Ulcerogenic Parameters in Acid-Irrigated Stomachs of Non-LPS and LPS Rats

In this experiment, the vagotomized stomachs of rats were irrigated with acid solution as previously described. Intraperitoneal L-Arg (500 mg/kg, i.p.) was challenged to LPS rats 1 h before gastric irrigation. Either L-NAME or L-NIL was given intraperitoneally to rats 10 min before L-Arg was administered. Table 4 demonstrated that L-Arg did not affect gastric mucosal GSH, histamine and LPO concentrations in acid-irrigated stomachs of non-LPS rats, although GABD was significantly (p< 0.05) reduced. In the stomachs LPS rats, the ulcerogenic parameters such as increased histamine, LPO generation as well as gastric hemorrhage and mucosal erosions in LPS rats were significantly (p< 0.05) attenuated by pretreatment of L-Arg. The decreased mucosal GSH levels were effectively inhibited. In the L-NAME or L-NIL challenged LPS rat stomachs, L-Arg-induced attenuation of gastric hemorrhage and mucosal erosion was abolished. The decreased histamine and LPO production and the increased mucosal GSH levels by L-Arg in LPS rats also was restored by pretreatment of these NO synthase inhibitors.

Effect of AA-Mixture on Various Ulcerogenic Parameters in Acid-Irrigated Stomachs of Non-LPS and LPS Rats

Intraperitoneal AA mixture containing TA (250 mg/kg), Phe (100 mg/kg) and L-Arg (500 mg/kg) was used instead of individual AA in the present experiment. This AA mixture was challenged to rats 1 h before gastric irrigation. As indicated in Table 5, various ulcerogenic parameters and hemorrhagic erosions observed in acid-irrigated stomachs of non-

Table 5. Effect of AA-Mixture on Various Ulcerogenic Parameters in Acid-Irrigated Stomachs of LPS Rats

	GABD μmol /stomach	GSH μmol /g tissue	LPO nmol MDA/g tissue	histological μg/g tissue	Hb mg/stomach	ulcer area mm^2
Non-LPS						
saline	−138.6±4.5	2.7±0.2	61.7±4.8	82.3±5.7	0.1±0.1	0.3±0.3
AA-mixture	−83.4±5.2#	3.8±0.4#	31.8±3.6#	41.5±5.4#	0.0±0.0#	0.0±0.0#
LPS						
saline	−321.4±16.4	1.3±0.2	163.7±20.5	145.3±12.3	3.6±0.3	26.7±4.9
AA-mixture	−80.4±7.0*	3.6±0.2*	34.6±7.0*	50.0±3.0*	2.3±0.3*	2.0±1.0*

Rats are deprived food for 24 hr. Intravenous LPS (3 mg/kg) is challenged to rats 12 hr after withdrawal of food. Non-LPS rats received normal saline only. The stomachs of rats are vagotomized and followed by irrigation for 3 hr with an acid solution containing 100 mM HCl and 54 mM NaCl. Intraperitoneal saline or AA-mixture containing taurine (250 mg/kg), phenylalanine (100 mg/kg) and L-arginine (500 mg/kg) is challenged to rats 1 h before gastric irrigation. All values are means ± SEM (n = 8). Significantly different are analyzed by using ANOVA. #$p<0.05$ vs. non-LPS saline; $p<0.05$ vs. LPS saline. Abbreviations: AA = amino acid; GABD = gastric acid back-diffusion; GSH = glutathione; LPO = lipid peroxides; Hb = hemoglobin; MDA = malonedialdehyde.

LPS rats were significantly ($p< 0.05$) inhibited. In LPS rat stomachs, a remarkable improvement of ulcerogenic parameters, particularly the inhibition of LPO generation, was achieved. The increased mucosal GSH levels by AA-mixture may partly be due to a decrease in the consumption of GSH for scavenge of oxyradicals generated by LPS.

Discussion

The ulcerogenesis of gastric mucosal damage in septic disease may be complex. During septic shock, various ulcerogenic substances including tumor necrosis factor-alpha and other cytokines that are involved in the tissue damage may be released. In the present study, we found that severe gastric hemorrhage and stomach erosion as assessed by the histological and morphological examination, occurred in LPS rats, particularly when acid of high concentration was existed in the stomach. The deterioration of various ulcerogenic parameters such as enhanced GABD, and stimulated mucosal LPO and histamine generation also was observed in these LPS rats. Apparently, gastric mucosal barriers were disrupted in these septic animals.

As described above, AAs including TA, Phe and L-Arg possess potent antioxidant effects that may be beneficial to healing of the cell injury. Taurine can modulate many Ca^{++}-dependent processes and be involved in phospholipid/ Ca^{++} interaction (15). In the present study, TA caused a dose-dependent amelioration of gastric hemorrhagic erosions by attenuation of GABD, histamine release and oxyradicals generation in acid perfused stomachs of LPS rats. Our previous report also demonstrate that gastric histamine which closely associated with generation of oxyradicals in LPS rats can be attenuated by administration of mast cell stabilizing agents including ketotifen and zinc sulphate (20). Furthermore, TA may possess antioxidant effect, radioprotective function and cell membrane-stabilized actions (6). The antioxidant effect of TA may associate with facilitating removal of hypochloride , a strong oxidant generated from peroxide and chloride ions by myeloperoxidase. Taurine reacts with hypochloride and to form N-chlorotaurine , which can then be reduced to taurine and chloride. Additionally, N-chlorotaurine may itself have a regulatory role in the inflammatory process (37). Taken together, the reduction of mucosal histamine biosynthesis or release by TA may result from stabilization of histamine cell membranes. Taurine also is essential in the development of both nervous and visual systems, and can enhance insulin activity and cell growth and differentiation (36). In the hepatic dysfunction or cirrhosis, patients have low TA, cysteine and GSH concentrations, because liver is the major site for transfuration and synthesis of TA (3). The present study showed that mucosal GSH level was elevated while LPO was reduced by TA. These results may imply that TA can stimulate GSH biosynthesis and reduce oxyradical generation. In turn, gastric mucosal cells were protected. In fact, TA may protect against toxic effects of several compounds. For example, TA can protect against the neuroexitotoxic damage in cats fed with high concentration of cysteine that also is a sulfur-containing AA (36). In the rat, diet TA also has hypolipidemic and antiatherosclerotic effects (22, 30).

Phenyalanine, one of the essential AA, is used

for tissue protein synthesis and hydroxylation to form tyrosine. Tyrosine is important to the synthesis of protein, catecholamines, melanin pigment, thyroid hormones and energy. Phenylalanine also can produce body energy, which is the first requirement of the body, and is important to the cell growth. In this study, Phe produced a dose-related elimination of various ulcerogenic parameters and resulted in an improvement of hemorrhagic erosions in LPS rats. The protective effect of Phe may associate with its oxyradical scavenging property and cytoprotective action. Document indicates that ferulic acid, the metabolite derived from Phe and tyrosine, possesses potent antioxidant effect against various inflammatory diseases (10).

In the present study, it also was found that L-Arg produced dose-dependent increase in mucosal GSH levels and elimination in mucosal histamine and LPO concentrations in LPS rats. Gastric hemorrhage and stomach erosions also were remarkably ameliorated. Aside from its roles in protein synthesis and as an intermediate in the urea cycle, L-Arg is a substrate for NO production and phosphocreatine synthesis as well as a precursor to proline, glutamate and putrescine via ornithine. Nitric oxide plays a pivotal role in many diseases, including hypertension (31), myocardial dysfunction (27), inflammation (7), cell death (1) and protecting against oxidative damage (42). It also may mediate many of the manifestations of septic shock including hemodynamic instability (13). Nitric oxide and citrulline are formed from L-Arg by NO synthetase (NOS). To date, three isoforms of this enzyme have been identified; two are constitutively expressed and one is inducible NOS (iNOS) (8). Although specific inhibition of iNOS that is upregulated in many tissues during sepsis has been explored as a therapy for sepsis shock, documents indicate that selective inhibition of the activity of iNOS prevents circulatory failure, but not the organ injury or dysfunction caused by endotoxin (43). Inhibition of iNOS also intensifies tissue injury and functional deterioration in autoimmune interstitial nephritis (9). In the present study, both L-NAME and L-NIL did not significantly protect gastric mucosal damage in LPS rats (data not shown). On the contrary, they reversed mucosal protective effect of L-Arg in LPS rats. In fact, NO synthesis may provide protection to tissues after ischemia and reperfusion (IR) injury of tissues (24). Inhibition of NO synthesis increased IR injury which can be reversed by perfusion with NO or its donors that can decrease injury and neutrophil accumulation (25). Since L-Arg is a donor of NO, the attenuation of hemorrhage and stomach erosion in LPS rats by parental challenge of L-Arg may be due to an increase in NO formation via Arg-NO-pathway. In fact, L-Arg has been successfully used to increase vascular dilation and basal NO synthesis, and thus reduce IR injury (32, 40). Oral supplementation of L-Arg 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 IR (35) and relieved mucosal damage during endothelin-induced ulceration (23). These protective effects of L-Arg are abrogated by inhibition of NO synthesis. L-Arg may also enhance mucosal growth and repair by supporting polyamine synthesis (27). Also, intragastric L-Arg can accelerate healing of gastric ulcers in rats and this effect could be attributed to stimulation of gastric mucosal blood flow through increased NO synthesis, stimulation of mucosal growth though polyamine synthesis, and stimulation of gastrin levels (2). Altogether, these beneficial effects of L-Arg may account for the protective effects of L-Arg on gastric hemorrhagic erosions in LPS rats.

The combination of TA, L-Arg and Phe produced remarkable inhibition of gastric hemorrhage and stomach erosions as well as of various ulcerogenic parameters in LPS rats. The results may result from the potent antioxidant effect and histamine cell membrane stabilizing property of the AA-mixture. It may be useful to the prescription of these AA nutrients for the patients with septic shock, particularly those who are suffering from gastrointestinal disturbances.

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