# Role of Histamine in Aggravation of Gastric Acid Back-Diffusion and Vascular Permeability in Septic Rats

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## Abstract

**The aim of the present study was to investigate the role of histamine in aggravation of gastric acid back-diffusion and vascular permeability in lipopolysaccharide (LPS)-induced septic rats. Male specific pyrogen-free Wistar rats were deprived food for 24 h before the experiment. Intravenous LPS (3 mg/kg dissolved in sterilized saline) was given to rats 12 h after food removal. Control rats received sterilized saline only. Under diethylether-anesthesia, the pylorus and esophageal sphincters of rats were ligated. Vagotomy also was performed. The stomachs were then irrigated for 3 h with physiological acid solutions containing 0-150 mM HCl plus adequate amount of NaCl. Increases in various ulcerogenic parameters, such as gastric acid back-diffusion, mucosal histamine concentration, luminal hemoglobin (Hb) content and stomach ulcer, were dependent on the concentration of acid solutions irrigated in stomachs of those LPS rats. Gastric vascular permeability also was increased in an acid concentration-related manner. In those LPS rats, high correlation was found between extents of acid back-diffusion and mucosal ulceration. Increased vascular permeability also closely related to the luminal Hb content. Moreover, these ulcerogenic parameters were dose-dependently ameliorated by intraperitoneal ketotifen and ranitidine. Diamine oxidase also was effective in inhibition, but exogenous histamine on the contrary, produced exacerbation of these ulcerogenic parameters. In conclusion, histamine plays a pivotal role in modulating gastric acid back-diffusion and vascular permeability that are greatly associated with hemorrhagic ulcer in septic rats.**

**Key Words: sepsis, gastric hemorrhage, ulceration, acid back-diffusion, vascular permeability, histamine**

## Introduction

Lipopolysaccharide (LPS) derived from cell membrane lysis of Gram negative bacteria may cause sepsis and multiple organ failures that are associated with a high mortality rate. In the clinic, septic shock and sequential multiple organ failure have a strong correlation with poor outcome (12, 22, 26, 31).

Histamine, one of autacoids, is widely distributed in biological tissues. Its biological effects are complex. Physiologically, histamine plays a pivotal role in the regulation of various gastric functions, such as acid secretion, motility and mucosal blood flow (2, 3, 9, 36). However, overproduction of histamine may associate allergy (7, 11, 36), inflammation and tissue injury (6, 10, 18). During septicemia, histamine may be augmented in various tissues (5, 18, 19). Our previous reports demonstrate that LPS produced by Escherichia coli can cause severe gastric hemorrhage and stomach ulcer in rats (19, 20).

In the stomach, an increase in gastric acid back-diffusion (mucosal permeability to free hydrogen ions) is closely related to ulcer formation (8, 15-17); whereas an increase in mucosal vascular permeability may

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greatly associate with gastric hemorrhage (26, 38). These pathological factors may be fatal (1). Whether the pathogenic effect of histamine on gastric mucosa is mediated by an aggravation of gastric acid back-diffusion and mucosal vascular permeability in septic rats, however, is unknown. The purpose was therefore to study the role of histamine in modulating gastric acid back-diffusion and microvascular permeability that intimately associated with gastric hemorrhagic ulcer in septic rats. Additionally, the effects of several drugs, including ketotifen (an antihistamine and mast cell stabilizer), ranitidine (a $H_2$-receptor antagonist), diamine oxidase (a histamine degradation-enzyme) and exogenous histamine, on indicated ulcerogenic parameters in septic rats were also investigated.

## Materials and Methods

### *Animals*

Male specific pyrogen-free Wistar rats, weighing 200-250 g, were obtained from and housed in The Laboratory Animal Center, National Cheng Kung University in Tainan, Taiwan. The rats were housed individually in a room with 12-h dark-light cycle and with central air conditioning (25℃, 70% humidity). Rats were allowed free access to tap water and pellets rodent diet (the Richmond standard, PMI Feeds, Inc. St. Louis, MO). The animal care and experimental protocols were in accord with the guidelines of the National Sciences Council of Taiwan (NSC 1994) and were approved by The Laboratory Animal Advisory Committee of National Chen-Kung University. Prior to performing the experiment, rats were moved to cages equipped with wire mesh to avoid coprophagy. All rats 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. Control (non-LPS) rats received sterilized normal saline only.

### *Surgical Procedures*

After 24 h of food-deprivation, the stomachs of rats under ether anesthesia were surgically exposed for the ligation of pylorus and lower esophagus. To prevent the spontaneous gastric secretion, bilateral diaphragmatic vagotomy was performed in both LPS and non-LPS rats as described by Shay et al. (32). A small incision was made in the forestomach. The stomach contents were gently expelled from the incision. A polypropylene tube (1.0 mm in internal diameter and 20 mm in length) 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 Acid Back-Diffusion*

Gastric acid back-diffusion (the luminal $H^+$ loss) was quantified by the method previously described (16). Namely, isotonic solutions (7-ml) containing 0, 50, 100 and 150 mM HCl plus adequate amount of NaCl for physiological isotonicity 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 under deep diethylether-anesthesia. The gastric sample (final sample) was collected and centrifuged at 3000 r.p.m. for 20 min.

The volumes of the initial and final samples were measured. The acidity of gastric sample was assessed by titrating 1.0 ml of gastric contents with 0.1 M NaOH to pH 7.0 on an autoburette titrator (Radiometer, Copenhagen, Denmark). The net flux of 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 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 stomach was opened along the greater curvature and the mucosa was exposed. The length (mm) and the width (mm) of ulcer on the gastric mucosa were measured with a planimeter (1 x 1 mm) under a dissecting microscope (x 0.7 - x 3.0; American Optical Scientific Instrument 569, Buffalo, NY). The ulcer areas were determined as previously described (15); ulcer area = length x width x $\pi/4$. The total ulcer area ($mm^2$) of each

stomach was recorded. Gastric mucosal damage was determined by a person unaware of experimental procedures.

*Determination of Luminal Hemoglobin (Hb) Content*

The cleansed rat stomachs were perfused for 3 h with either normal saline or acid solutions (50, 100 or 150 mM HCl plus adequate amount of NaCl for physiological isotonicity). Gastric initial and final samples were collected by methods as demonstrated forehand. The blood attached on the gastric mucosa was carefully scrapped, 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 concentration of Hb in the samples was determined on a Hitachi spectrophotometer (model U-3210, Tokyo, Japan). The absorption maximum of Hb was measured at 376 nm (20). The appropriate irrigated solutions adjusted to pH 1.5 were used as blank. Absorbances of the samples was measured against a standard curve ($r^2 > 0.98$) contrasted with freshly prepared rat Hb (0.05- 1.00 mg/ml) treated in the same manner as the gastric samples. The net luminal Hb content was calculated as Fv x $F_{Hb}$ - (7-Iv) x $I_{Hb}$. 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 miligram Hb per stomach.

*Measurement of Mucosal Histamine*

Gastric mucosal histamine concentration was determined by the methods as described previously (29). 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 10 min. To 100 ml 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℃ for 10 h. To the frozen was added 200 ml 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℃) 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 mg/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.

*Determination of Mucosal Microvascular Permeability*

After collection of final samples, both LPS and non-LPS rats were injected with 1 ml of 1% Evans blue (EB) intravenously. Under diethylether-anesthesia, rats were killed by bleeding from the descending aorta 30 min after EB was injected. The stomachs were removed, and carefully lavaged with 5 ml of cold distilled water. The amount of dye diffused in gastric contents was measured. The extraction of dye was performed according to the modified method described previously (34). Namely, 2 ml of gastric contents were soaked overnight in stoppered glass tubes containing 2 ml of 3.5 M KOH at 37°C. Then, 18 ml of a mix solution of 4N $H_3PO_4$ and acetone (1.75:16.25) was added to each tube to make up a total volume of 25 ml. The tube was shaken vigorously for a few seconds and centrifuged at 3000 r.p.m. for 15 min. Absorbance of the supernatant was measured at 620 nm on a Hitachi spectrophotometer (model U-3210, Tokyo, Japan). In a preliminary study, the recovery rate of dye was about 95% under the present conditions. The amount of dye recovered from the gastric content was expressed as microgram of EB per stomach.

*Drug Administration*

Ketotifen (0.2-5.0 mg/kg), ranitidine (10-250 mg/kg), diamine oxidase (20 mg/kg) and histamine HCl (100 mg/kg) were dissolved in normal saline (154 mM NaCl solution) and challenged intraperitoneally to rats immediately after gastric irrigation.

*Chemicals*

The following chemicals in reagent grade were used. LPS (from *Escherichia coli,* Serotype 055:B5),

**Table 1. Intraluminal Acidity-Dependent Aggravated Ulcerogenic Parameters in Stomachs Irrigated with Normal Saline or Physiological Acid Solutions in LPS or Non-LPS Rats**

| | | Acid back-diffusion μmol/stomach | Histamine μg/g tissue | Vascular permeability μg EB/stomach | Hemoglobin mg/stomach | Ulcer area $mm^2$ |
|---|---|---|---|---|---|---|
| **Non-LPS** | | | | | | |
| **Normal saline** | | 16.2 ± 1.4 | 46.0 ± 4.0 | 0.3 ± 0.1 | 0.1 ± 0.1 | 0.0 ± 0.0 |
| **HCl (100mM HCl)** | | -213.4 ± 13.0# | 84.0 ± 6.2# | 0.8 ± 0.2# | 0.3 ± 0.1# | 0.3 ± 0.2# |
| **LPS** | | | | | | |
| **Normal saline** | | 10.4 ± 1.2# | 120.6 ± 0.8# | 1.3 ± 0.2# | 1.1 ± 0.2# | 12.6 ± 3.2# |
| **HCl** | **50 mM** | -128.4 ± 3.6* | 138.2 ± 5.6* | 2.0 ± 0.3* | 1.7 ± 0.3* | 26.4 ± 5.6* |
| | **100** | -311.8 ± 12.2* | 154.2 ± 11.5* | 4.8 ± 0.7* | 3.0 ± 0.4* | 59.2 ± 6.6* |
| | **150** | -358.7 ± 21.3* | 185.2 ± 23.8* | 6.6 ± 1.4* | 4.6 ± 0.8* | 93.5 ± 11.8* |

Data are means ± S.E.M (n= 7-8). Significant differences were analyzed by using ANOVA. #: $p< 0.05$ vs. LPS rat stomachs irrigating with normal saline; *$p< 0.05$ vs. LPS rat stomachs irrigating with normal saline. LPS= lipopolysaccharide; EB= Evans blue

**Table 2. Dose-Response Effects of Ketotifen and Ranitidine on Aggravated Ulcerogenic Parameters in Acid-Irrigated Stomachs of LPS Rats**

| | | Acid back- diffusion μmol/stomach | Histamine μg/g tissue | Vascular permeability μg EB/stomach | Hemoglobin mg/stomach | Ulcer area $mm^2$ |
|---|---|---|---|---|---|---|
| **Control** | | -301.8 ± 10.2 | 148.4 ± 10.0 | 5.3 ± 0.8 | 3.4 ± 0.5 | 56.0 ± 5.8* |
| **Ketotifen** | **0.2 mg/kg** | -288.4 ± 12.5 | 116.4 ± 7.8* | 4.5 ± 1.0 | 2.8 ± 0.3 | 40.6 ± 6.2* |
| | **1.0** | -212.8 ± 8.6* | 101.8 ±12.3* | 3.2 ± 0.6 | 1.4 ± 0.2* | 22.1 ± 5.0* |
| | **5.0** | -184.4 ± 5.8* | 80.4 ± 3.6* | 1.8 ± 0.3* | 0.6 ± 0.1* | 16.8 ± 3.6* |
| **Ranitidine** | **10 mg/kg** | -292.3 ± 13.7 | 152.6 ± 13.0 | 4.7 ± 0.7 | 3.0 ± 0.6 | 48.0 ± 6.4* |
| | **50** | -212.9 ± 11.2* | 132.2 ± 11.4 | 3.2 ± 0.4* | 1.8 ± 0.4* | 28.3 ± 7.1* |
| | **250** | -176.3 ± 5.4* | 118.5 ± 7.2* | 1.4 ± 0.3* | 1.0 ± 0.2* | 11.4 ± 2.2* |

Rat stomachs were irrigated for 3 h with a physiological acid solution containing 100 mM HCl and 54 mM NaCl. All values are means ± S.E.M. (n = 8). Significant differences were analyzed by using ANOVA. *$p< 0.05$ vs. control group. Abbreviations: LPS= lipopolysaccharide, EB = Evans blue

diamine oxidase, Hb (rat), histamine, ketotifen, trichloroacetic acid, acivicin, o-phthaldialdehyde, 1,1, 3,3-tetramethoxypropane, n-butanol pyridine, ranitidine, sodium laurylsulfate, and 2-thiobarbiturate were purchased from Sigma, St. Louis, Mo. U.S.A. 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 ± S.E.M. Significant differences in the data of experiments for single measurement traits were analyzed statistically by using ANOVA (24). Statistical significance was set at $p<0.05$. A simple regression analysis was used to determine the correlation between two different variances.

## Results

**Table 3. Effects of Diamine Oxidase or Exogenous Histamine on Aggravated Ulcerogenic Parameters in Acid-Irrigated Stomachs of LPS Rats**

| | | Acid back-diffusion μmol/stomach | Histamine μg/g tissue | Vascular permeability μg EB/stomach | Hemoglobin mg/stomach | Ulcer area $mm^2$ |
|---|---|---|---|---|---|---|
| **Normal saline** | | -298.6 ± 9.4 | 151.6 ± 6.2 | 4.7 ± 0.4 | 4.0 ± 0.6 | 51.4 ± 7.2 |
| **Diamine oxidase** | **20 mg/kg** | -224.6 ±7.2* | 83.4 ± 9.2* | 2.6 ± 0.2* | 1.1 ± 0.2* | 36.3 ± 1.8* |
| **Histamine** | **100** | -354.6 ± 12.2* | 182.9 ± 8.6* | 6.6 ± 0.7* | 5.3 ± 0.4* | 73.0 ± 8.2* |

Rat stomachs were irrigated for 3 h with a physiological acid solution containing 100 mM HCl and 54 mM NaCl. All values are means ± S.E.M. (n = 8). Significant differences vs. control group were analyzed by using ANOVA. *$p< 0.05$ vs. normal saline. Abbreviations: LPS= lipopolysaccharide, EB= Evans blue.

*Luminal Acidity-Dependent Aggravation of Various Ulcerogenic Parameters in Normal Saline- or Acid-Irrigated Rat Stomachs*

As shown in Table 1, in normal saline-irrigated stomachs of non-LPS rats, neither gastric acid back-diffusion nor hemorrhagic ulcer was found. Due to gastric vagotomy, only very little gastric acid secretion in these animals was observed. Gastric vascular permeability and histamine concentrations also were at normal levels. When LPS (3 mg/kg) was challenged, an increase in acid back-diffusion, vascular permeability and mucosal histamine concentrations was achieved. Gastric hemorrhage and mucosal ulceration also were aggravated.

In a physiological acid (100 mM HCl plus 54 mM NaCl)-irrigated stomachs of non-LPS rats, a slight increase of mucosal histamine concentration and luminal EB was obtained. Nevertheless, gastric hemorrhage and ulceration were negligible.

In acid (50-150 mM HCl plus adequate amount of NaCl for isotonicity)-irrigated stomachs of LPS rats, a luminal acidity-dependent enhancement of gastric acid back-diffusion, histamine concentrations, luminal Hb content, vascular permeability and stomach ulcers was observed. The extent of aggravation of various ulcerogenic parameters in stomachs irrigated with acid solutions was greater than that irrigated with normal saline.

*Relationship Between Acid Back-Diffusion and Mucosal Ulceration as well as between Microvascular Permeability and Hemorrhage in Acid-Irrigated Stomachs of LPS Rats*

Figure 1 demonstrated that high correlation between gastric acid back-diffusion and mucosal ulceration as well as between microvascular permeability and hemorrhage was observed in the stomachs of LPS rats. Apparently, increased acid back-diffusion and vascular permeability in stomachs of LPS rats may exacerbate gastric damage and hemorrhage, respectively.

*Effects of Ketotifen and Ranitidine on the Aggravation of Acid-Induced Hemorrhagic Ulcers in LPS Rats*

As shown in Table 2, rats were challenged intravenously with LPS (3 mg/kg) for 12 h and followed by irrigating the stomachs for 3 h with an acid solution containing 100 mM HCl and 54 mM NaCl. The luminal Hb contents and stomach ulcers were severe. Gastric acid back-diffusion and microvascular permeability also were aggravated. Intraperitoneal ketotifen (0.2, 1.0, 5.0 mg/kg) produced a dose-dependent attenuation of gastric acid back-diffusion, luminal Hb content, vascular permeability and stomach ulcers in LPS rats. These ulcerogenic parameters produced in acid-perfused stomachs of LPS rats also were dose-relatedly ameliorated by ranitidine (10, 50 and 250 mg/kg). Apparently activation of $H_1$ and $H_2$ receptors was associated with aggravation of these ulcerogenic parameters.

*Effects of Diamine Oxidase and Exogenous Histamine on Various Ulcerogenic Parameters in Acid-Irrigated Stomachs of LPS Rats*

As shown in Table 3, high luminal Hb contents

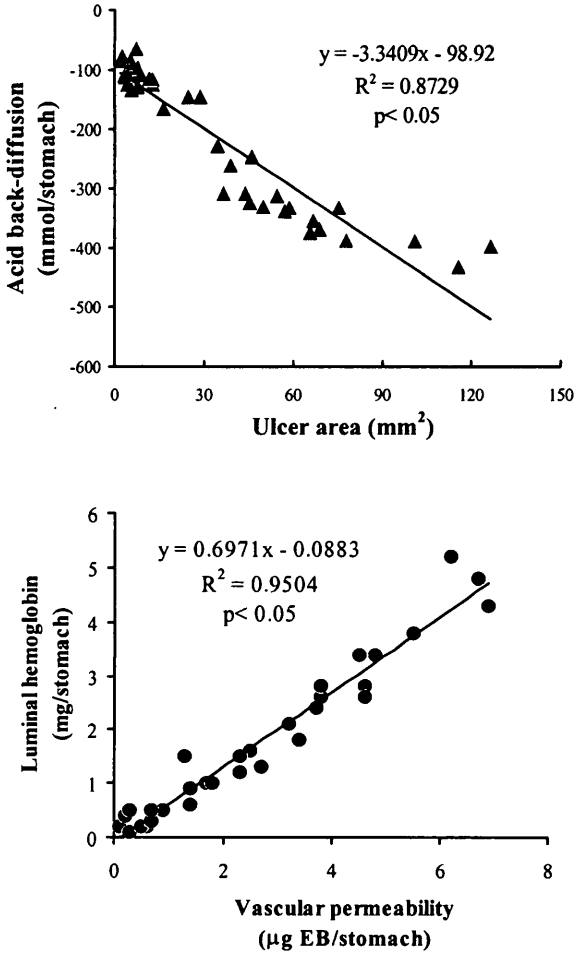


Fig. 1. Correlation between gastric acid back-diffusion and mucosal ulceration as well as between microvascular permeability and luminal Hb content in septic rats.

accompanied with great acid back-diffusion and severe mucosal ulceration were obtained in acid-irrigated stomachs of LPS rats. High concentration of histamine and microvascular permeability also were found in these animals. These ulcerogenic parameters were markedly attenuated by diamine oxidase (20 mg/kg) but was aggravated by exogenous histamine (100 mg/kg). Apparently, histamine is important in modulating both acid back-diffusion and microvascular permeability that associated hemorrhagic ulcer in septic rats.

## Discussion

The pathophysiological mechanisms of gastric hemorrhage and ulceration during sepsis may be complex. Previous report demonstrates that LPS at 3 mg/kg is able to produce severe sepsis indicated by increasing both leukocyte numbers and blood coagulation time in rats (19). In the present study, an increased acid back-diffusion and histamine concentration was found in rats with septicemia. In fact, other mediators, such as cytokines, nitric oxide, platelet-activating factor and eicosanoids, may also be responsible for most of the manifestations caused by LPS (4, 28, 30). Circulatory failure, leukocyte-induced tissue injury and coagulation disorder also appear to be critical determinants in the development of sequential organ failure [3]. It is suggested that neutrophil infiltration into gastric tissue may also play a crucial role in the formation of mucosal damage [3]. In the present study, an aggravation of ulcerogenic parameters, including gastric acid back-diffusion, microvascular permeability as well as luminal Hb content and mucosal ulceration was achieved in rats received LPS. This alteration of gastric mucosa was in an intraluminal acidity-dependent manner. The results implied that intraluminal free acid might diffuse back through the preexisted damaged gastric mucosal barriers that normally protect gastric mucosa against the invasion of free acid. In the present study, high correlation was found between gastric hemorrhage and microvascular permeability or between mucosal ulceration and acid back diffusion. The results implied that the exacerbation of gastric damage might greatly associate with an elevation of the permeability of both mucosa and microvascular vessels. Davenport reports that histamine may be released during the occurrence of acid back-diffusion that is responsible for gastric hemorrhage and mucosal ulceration induced by a variety of chemicals (8).

Conventionally, histamine receptors are defined as $H_1$, $H_2$ and $H_3$ subtypes (13). In the gastrointestine, histamine is able to produce contraction of smooth muscle cells but cause blood vessel dilation (9). These effects may be mediated by activation of both $H_1$ and $H_2$ receptors. On the basis of molecular study, histamine may cause the endothelial cells to contract and separate at their boundaries and thus to expose basement membrane, which is freely permeable to free acid, plasma protein and fluid. The gap in the endothelial cells also may permit passage of circulating cells that are recruited to the tissues during mast cell response. Activation of $H_1$ receptors usually elicits an increase in phosphoinositol hydrolysis and intracellular calcium. The intracellular phospholipase $A_2$ and endothelium-derived relaxing factor (nitric oxide) may be activated and followed by an increase in the formation of prostaglandin $I_2$. Nitric oxide may subsequently stimulate a cyclic guanyl monophosphate-dependent protein kinase

and cause a decrease in intracellular calcium. On the other hand, activation of $H_2$ receptors may increase intracellular cAMP and intracellular calcium levels (3). These functions of histamine $H_1$ and $H_2$ receptors may lead to increase in vascular dilatation and permeability (28). Gastric hemorrhage and edema may follow these biological responses. Moreover, histamine is able to increase gastric mucosal permeability to electrolyte (30) and render the stomach more susceptible to acid-induced damage. In the experimental animal, increased mucosal histamine has been reported to elicit gastric secretion and mucosal lesion (2). Both histamine $H_1$ and $H_2$ antagonists are able to protect gastric mucosa against damage (19, 27, 35). In LPS rats, either ketotifen or ranitidine produced a dose-dependent inhibition in acid-induced enhancement in acid back-diffusion, microvascular permeability, luminal Hb contents and ulcer formations. Our previous paper demonstrate that cimetidine, another histamine $H_2$ receptor antagonist, can effectively reduce gastric acid back-diffusion and hemorrhagic ulcers either in tannate-treated or in streptozotocin-induced diabetic rats (18, 19). Therefore, activation of both histamine $H_1$ and $H_2$ receptors might be important in the aggravation of acid back-diffusion and microvascular permeability that closely associated gastric hemorrhagic ulcers in LPS rats.

The aggravation of various ulcerogenic parameters also was significantly attenuated by ketotifen, which showed both antihistamine ($H_1$ receptor blocker) and mast cell membranes stabilizing effects (27). Therefore, mast cell histamine is important in the development of hemorrhagic ulcer in LPS rats. It also is reported that ketotifen can inhibit the mucosal ulceration induced by various ulcerogens (23). Ketotifen is beneficial to the prevention or protection against allergy, asthma and inflammation by stabilizing mastocyte membranes (3, 11). Additionally, ketotifen may possess gastric antisecretory and mucosal cytoprotective effects in rats (27). In the present in vivo study, increased mucosal histamine concentrations by LPS may not only be from gastric mast cells but also be partly from those in peritoneal and other tissues via blood circulation. In cellular studies, ketotifen is reported to inhibit $^{45}Ca$ uptake and reduce histamine release in mast cells stimulated by antigen (36). Thus, decreased mucosal histamine concentrations by ketotifen could be associated with the reduction of intracellular $Ca^{++}$ and histamine biosynthesis in the gastric mucosa.

On the other hand, ranitidine, a specific histamine $H_2$-receptor antagonist, produced a significant attenuation in acid back-diffusion and gastric microvascular permeability as well as hemorrhagic ulcer in LPS rats. Histamine $H_2$-receptors are located on vascular smooth muscle and parietal cell membranes. Increase in vascular permeability and vasodilator effects caused by activation of such receptors might be counteracted by administration of ranitidine. Ample documents demonstrate that cimetidine and ranitidine, the $H_2$ receptor antagonists, are effective in amelioration of gastric mucosal damage (14-16, 19, 35).

In biological tissues, histamine can be specifically degraded by histaminase (33). This diamine oxidase significantly inhibited various ulcerogenic parameters in acid-irrigated stomachs of LPS rats. Conversely, elevation of gastric histamine level by challenged LPS rats with exogenous histamine, produced an aggravation of various ulcerogenic parameters. These results confirmed the forehand illustration that mucosal histamine played a pivotal role in modulating gastric hemorrhagic ulcer in LPS rats.

The present study provided strong evidences that gastric histamine might be important in modulating gastric hemorrhage and mucosal ulceration by increasing both gastric acid back-diffusion and microvascular permeability in LPS rats.

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