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European Journal of Pharmacology 491 (2004) 61–68
www.elsevier.com/locate/ejphar

Gastric oxidative stress and hemorrhagic ulcer in *Salmonella typhimurium*-infected rats

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Received 13 November 2003; received in revised form 21 January 2004; accepted 10 March 2004

Abstract

Infection of *Salmonella typhimurium* (*Salmonella typhi*) can lead to various organ diseases. This research first proposed that *Salmonella typhi*-infection could result in gastric oxidative stress and hemorrhagic ulcers that were ameliorated by ofloxacin, lysozyme chloride and several antioxidants, including exogenous glutathione (GSH), allopurinol and dimethylsulfoxide (DMSO). Male Wistar rats were given intrajejunally the live culture of *Salmonella typhi* [1×10^{10} colony-forming unit (CFU)/rat] and followed by deprivation of food for 36 h. Age-matched control rats received vehicle only. Rat stomachs were irrigated for 3 h with either normal saline or a simulated gastric juice containing 100 mM HCl, 17.4 mM pepsin and 54 mM NaCl. Infection of *Salmonella typhi* produced an aggravation of ulcerogenic factors, including enhancing gastric acid back-diffusion, mucosal lipid peroxide generation and hemorrhagic ulcer as well as an attenuation of mucosal GSH level. Intragastric irrigation of gastric juice caused further aggravation of these gastric biochemical parameters. This exacerbation of ulcerogenic factors was abolished by pretreatment of ofloxacin and lysozyme chloride. Antioxidants, such as reduced GSH, allopurinol and DMSO also produced significant ($P < 0.05$) amelioration of gastric damage in *Salmonella typhi*-infected rats. In conclusion, infection of *Salmonella typhi* substantially caused gastric oxidative stress and disruption of gastric mucosal barriers, consequently resulted in gastric hemorrhagic ulcerations that were effectively ameliorated by ofloxacin, lysozyme chloride and various antioxidants.

Keywords: *Salmonella typhi*; Gastric hemorrhage; Mucosal ulceration; Oxyradical; Glutathione; Ofloxacin

1. Introduction

The infection of *Salmonella typhimurium* (*Salmonella typhi*), the Gram-negative bacteria, may cause sepsis and multiple organ failures (Sprong et al., 1997). Patients with this bacterial infection may suffer from sepsis, fever, lethargy, seizure and respiratory disturbance. Symptoms of increasing intracranial pressure and change in mental status also were observed after this fatal infective disease (Lepage et al., 1984). Enteritis and diarrhea are reported being the most common clinical features of *Salmonella typhi* infection (Le Bacq et al., 1994). Hitherto, gastritis and mucosal hemorrhagic ulcer produced in this infective disease received little attention, and document concerning with the pathogenesis of gastric oxidative and hemorrhagic ulcers is lacking.

In general, the balance of offensive and defensive factors plays a pivotal role in gastric hemorrhage and ulcer formation in the stomach. The offensive factors include acid back-diffusion and oxyradical generation (oxidative stress) while defensive factors involve glutathione (GSH) and mucus secretion. Aggravation of offensive factors and/or attenuation of defensive substances may lead to ulcer formation.

When gastric mucosal barriers are inflamed or disrupted by acid, ethanol or aspirin, the intraluminal-free acid may diffuse back into gastric mucosa through injured mucosal barriers and leads to damage of cells and exacerbation of hemorrhagic ulcer. This acid back-diffusion is considered as a sensitive index of integrity of gastric mucosa (Davenport, 1969; Hung and Neu, 1997).

During gastric oxidative stress, reduced GSH acts to prevent the generation of lipid peroxides that resulted from the reaction of oxyradicals and polyunsaturated fatty acids of cells. Reduced GSH may mediate gastric cytoprotection by scavenge of free radicals (Szabo et al., 1981). The

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doi:10.1016/j.ejphar.2004.03.029

oxyradical generation, one of gastric offensive factors, is associated with the pathogenesis of many diseases (Marx, 1987). It can initiate tissue inflammation and cause cell damage (Bhatnagar, 1994). Whether infection of *Salmonella typhi* can produce gastric oxidative stress and result in the aggravation of gastric hemorrhage and mucosal ulceration, however, is totally unknown.

When bacteria died, the cell walls may be lysed to various serotypes of lipopolysaccharides that show different damaging effects and potencies on host living cells. Some kinds of lipopolysaccharides, such as those from *Helicobacter pylori* or *Escherichia coli* (*E coli*) (Brzozowski et al., 1998; Konturek et al., 1998) may even exert cytoprotective (adaptive) effects on living host cells particularly at low concentrations. To minimize this artifact and mimic the clinical infection, live culture of *Salmonella typhi* is used to induce gastric hemorrhage and ulceration in this research. Moreover, animal models with properly performed studies may provide important information for evaluating the bacterial infection and efficacy of therapeutic drugs in the treatment of human diseases (Andriole, 1989), rats were used as experimental animals. Therefore, the aim of the study was to clarify the role of acid back-diffusion, oxyradicals and GSH in modulating gastric hemorrhage and ulcer formation by measuring changes in various offensive and defensive factors in *Salmonella typhi*-infected rats. Additionally, protective effects of antibiotic, such as ofloxacin (a fluoroquinolone antibiotic) and lysozyme chloride (muramidase) as well as various antioxidants, including reduced GSH, allopurinol and dimethylsulfoxide (DMSO), on aggravation of gastric hemorrhage and mucosal ulcerations in *Salmonella typhi*-infected rats also were evaluated.

2. Materials and methods

2.1. Animals

Experimental protocols were conducted in accord with guidelines of the National Sciences Council of Taiwan and were approved by The Laboratory Animal Advisory Committee of National Cheng-Kung University. Before experiments started, laparotomy was performed in male specific pathogen-free Wistar rats (200–250 g, body weight). Single intrajejunal injection of live culture of *Salmonella typhi* [OU 5045, 1×10^{10} colony-forming unit (CFU) in 1.0 ml of sterilized phosphate buffer saline], which exerted a submaximal ulcer formation in our preliminary study, was given to rats. Control rats received sterilized vehicle only. After deprivation of food for 36 h, rats were anesthetized with diethylether and stomachs were surgically exposed for ligation of pylorus and lower esophagus. Gastric vagotomy also was performed to prevent spontaneous acid secretion. The stomach was rinsed meticulously with warm normal saline (37 °C) through an incision previously made in the forestomach. The residues were gently removed.

2.2. Determinations of gastric acid back-diffusion

Either normal saline or simulated gastric juice (7 ml) containing 100 mM HCl, 17.4 mM pepsin and 54 mM NaCl was instilled into the stomach with a 10-ml syringe. Then, 3 ml of fluid was taken as an initial sample. The forestomach and abdominal wound were tightly closed. After 3 h, rats were killed with an overdose of diethylether. Gastric contents (final sample) were collected and centrifuged for 20 min at 3000 r.p.m. The volumes of samples were measured. The acidity of samples was assessed by titrating 1.0 ml of gastric contents sample with 0.1 M NaOH to pH 7.0 on an autoburette titrator (Radiometer, Copenhagen, Denmark). The net flux of free hydrogen ions through gastric mucosa was calculated as follows:

$$\text{Net flux of free hydrogen ion} = \text{Fv} \times \text{Fc} - (7 - Iv) \times \text{Ic}.$$

where, Fv and Iv are the volume (ml) of the final sample and the initial sample, respectively, and Fc and Ic are the ionic concentrations (mM) of the final sample and the initial sample, respectively. A negative value for net flux indicates luminal electrolyte loss (Hung and Neu, 1997).

2.3. Determination of hemoglobin (Hb)

Both initial and final samples were adjusted to pH 1.5 with 0.1 M HCl solution. The Hb contents of the samples were measured spectrophotometrically (Holzer et al., 1989). The absorption maximum of Hb was measured at 376 nm. Appropriate irrigating solutions, adjusted to pH 1.5, were used as blank. Absorbances of the samples were measured against a standard curve (r^2>0.98) constructed with freshly prepared rat Hb (0.05–1.00 mg/ml) treated in the same manner as gastric samples. The luminal Hb content was calculated as:

$$\text{Luminal Hb content} = \text{Fv} \times F_{\text{Hb}} - (7 - \text{Iv}) \times I_{\text{Hb}}$$

where Fv and Iv are the volume (ml) of the final sample and the initial sample, respectively, while F_{Hb} and I_{Hb} are the luminal Hb concentrations (mg/ml) in final sample and initial samples, respectively. The results were expressed in mg Hb per stomach.

2.4. Morphological and histological studies of gastric mucosa

The length (mm) and the width (mm) of ulceration on the gastric mucosa were measured with a planimeter (1 × 1 mm) under a dissecting microscope (× 0.7 to × 3.0; American Optical Scientific Instrument 569, Buffalo, NY). The total ulcer area (mm^2) of each stomach was recorded. Histological studies of stomachs were conducted by the routine hematoxylene and eosin staining methods. Each section

was examined under a microscope (Nikon HF, X-IIA, Tokyo, Japan), and the tissue damage was quantified as previously described (Hung, 2000).

2.5. Assay of mucosal GSH

The corpus mucosa was scraped using two glass slides on ice, weighed and homogenized immediately in 2 ml of phosphate buffer (0.1 M NaH_2PO_4 plus 0.25 M sucrose, pH 7.4). Acivicin (250 μM), an irreversible inhibitor of γ-glutamyltransferase, was added to the homogenate to inhibit the catabolism of GSH. Samples were then centrifuged at 4000 r.p.m. for 15 min at 4 °C. To determine the recovery of reduced thiol, GSH (200 μM of reduced GSH contained in phosphate buffer solution, pH 7.0) was added to the supernatant. Subsequently, 0.5 ml of 0.25 M trichloroacetic acid was added to 1.0 ml of the supernatant. The mixture was kept for 30 min at 4 °C. After centrifugation at 3000 r.p.m. for 15 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). 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) that were treated in the same manner as the tissue samples. The results were expressed as μmol GSH/g wet tissue (Hung and Wang, 2002).

2.6. Determination of lipid peroxide

The concentration of gastric mucosal lipid peroxide was determined by estimating malonedialdehyde using the thiobarbituric acid test (Ohkawa et al., 1979). Namely, rat stomachs 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-tetramethoxypropane. The recovery was over 90%. The results were expressed as nmol malonedialdehyde/g wet tissue.

2.7. Measurement of gastric mucus

Gastric mucus was assessed by the method described previously (Corn et al., 1974). Namely, the rat stomach was excised and washed with tap water. The sample was immersed in 10 ml of a solution containing alcian blue (1.0 mg/l), sucrose (0.16 M) and sod. acetate (0.05 M) for 2 h. To remove the unbound dye, the sample was washed for 15 and then 45 min in a 0.25 M sucrose solution. The mucus-bound dye was eluted by immersing gastric mucosa in 10 ml of 0.5 M $MgCl_2$ solution for 2 h. The obtained solution was mixed with 10 ml of diethylether. The absorbance of the color aqua solution was measured on a spectrophotometer (Hitachi, U3210) at 605 nm. The amount of alcian blue extracted from known graded concentrations (5–50 mg/l) of alcian blue solution. All samples were measured in duplicate. The results were expressed as μg alcian blue/g wet tissue.

2.8. Chemicals

Except ofloxacin was purchased from Daiichi Pharmaceutical, Tokyo, Japan, all agents were obtained from Sigma, St. Louis, Mo. USA. The purity of all agents was over 98%. All chemical solutions were freshly prepared before use.

2.9. Drug administration

Intragastric ofloxacin (100 mg/kg), which is highly effective in inhibition of *Salmonella typhi*-infection in mice (Fu et al., 1990), was challenged to rats 30 min before bacteria was given. Indomethacin (5 mg/kg) was administered intraperitoneally to rats just before gastric irrigation. Intragastric lysozyme chloride (0–500 mg/kg) and intraperitoneal reduced GSH (800 mg/kg), allopurinol (100 mg/kg) or DMSO (500 mg/kg) was given to rats 30 min before bacterial challenge and every 12 h thereafter. The doses of these drugs are effective in amelioration of gastric damage in various ulcer models in rats (Hung and Wang, 2002; Hung, 2000).

2.10. Statistical analysis

All data were expressed as means ± S.E.M. Significant differences were analyzed statistically by using analysis of variance (ANOVA) or the Tukey honestly significance test for pairwise comparison after ANOVA (Montgomery, 1984). Statistical significance was set at $P<0.05$. A simple regression analysis was used to determine the correlation between two variances.

3. Results

3.1. Morphological and histological alteration in Salmonella typhi-infected rat gastric mucosa

In the present study, gastric mucosa was morphologically intact in normal rats. Nevertheless, severe hemorrhagic ulcers were observed in *Salmonella typhi*-infected rats (photo not shown). Histological studies showed that in

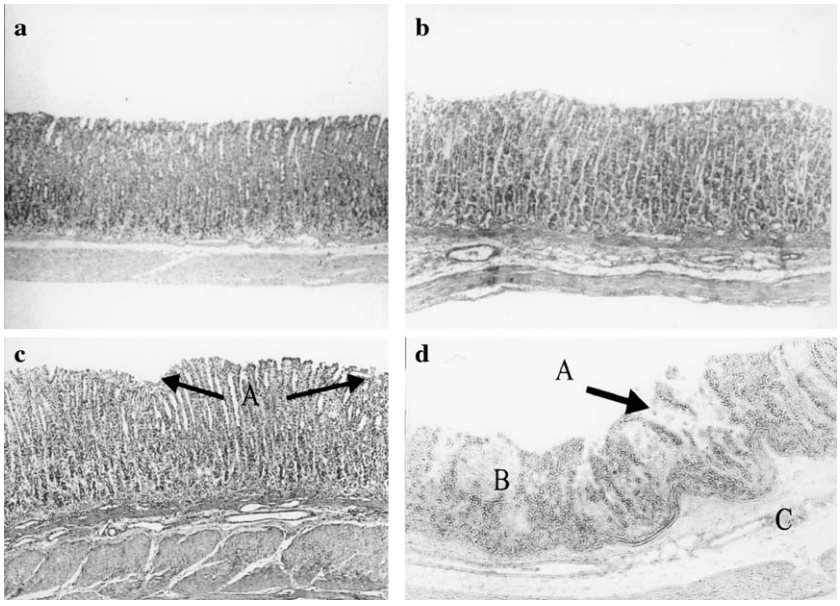

Fig. 1. Histological studies of gastric mucosa exposed for 3 h with normal saline or gastric juice in normal and *Salmonella typhi*-infected rats. Note that in normal rat stomachs irrigated with either normal saline (a) or gastric juice (b), gastric mucosal cells look intact. However, in normal saline-irrigated *Salmonella typhi*-infected rat mucosa (c), a disruption of gastric epithelial layer is observed. When stomachs of bacteria-infected rats are irrigated with gastric juice (d), a complete disruption of the upper mucosal cells (A) and lamina propria (B) is obtained (× 150). The injured cells are characterized by karyorrhexis and dense homogenous acidophilic cytoplasm. In most cases, gastric edema (C) also is observed (× 200).

normal rat stomachs irrigated with normal saline or gastric juice, gastric mucosal cells appeared intact (Fig. 1a, c). However, in *Salmonella typhi*-infected rat stomachs irrigated with normal saline, gastric cells were apparently damaged (Fig. 1b). Pronounced cell-injury was found in both epithelial layers and lamina propria when gastric juice was present in the stomach (Fig. 1d). The comparison of the degree of gastric histological damage produced by gastric juice in *Salmonella typhi*-infected rats to that in normal rats with the same treatments is demonstrated in Table 1. In normal rat stomachs irrigated with normal saline, no damage of gastric mucosal cells was observed. In gastric juice-irrigated stomachs of normal rats, gastric mucosal cells also appeared undamaged. In *Salmonella typhi*-infected rats, normal saline-irrigated stomachs produced more gastric mucosal cell damage than did those treatments in normal rats. Furthermore, a remarkable aggravation of mucosal cell damage was observed when gastric juice was used instead of normal saline. Apparently, intra-luminal gastric juice could enhance mucosal cell damage in *Salmonella typhi*-infected rats.

3.2. Changes in various gastric biochemical parameters in Salmonella typhi-infected rat stomachs irrigated with normal saline or gastric juice

In Table 1, it was shown that acid back-diffusion, mucosal GSH levels and lipid peroxide generations as well as luminal Hb contents and mucosal ulceration produced in normal saline- or gastric juice-irrigated stomachs of control rats were at normal levels. However,

Table 1
Changes in various gastric biochemical parameters in normal saline- or gastric juice-irrigated stomachs of *Salmonella typhi*-infected and non-infected rats

	Acid back-diffusion, μmol/stomach	Glutathione, μmol/g tissue	Lipid peroxide, nmol MDA/g tissue	Hemoglobin, mg/stomach	Ulcer area, mm^2/stomach	Histological score
Control						
Normal saline	13.4 ± 3.2^{a}	3.1 ± 0.3^{a}	34.6 ± 3.4^{c}	0.2 ± 0.1^{c}	0.2 ± 0.2^{c}	0.1 ± 0.1^{d}
Gastric juice	-130.6 ± 4.8^{b}	2.9 ± 0.2^{b}	48.7 ± 4.0^{c}	0.3 ± 0.1^{c}	1.2 ± 0.6^{c}	0.5 ± 0.3^{c}
Salmonella typhi						
Normal saline	18.5 ± 5.6^{a}	1.8 ± 0.2^{c}	88.4 ± 6.2^{b}	1.2 ± 0.3^{b}	45.5 ± 6.5^{b}	2.5 ± 0.4^{b}
Gastric juice	-328.6 ± 12.7^{c}	1.0 ± 0.1^{d}	140.4 ± 9.0^{a}	5.3 ± 0.8^{a}	144.6 ± 12.5^{a}	8.8 ± 1.6^{a}

Data are means ± S.E.M. ($n = 8$). Significant differences are analyzed by using the Tukey honestly significant difference tests for pairwise comparison after ANOVA. The differences between those treatments with different letters are statistically significant ($P < 0.05$). MDA = malonedialdehyde, *Salmonella typhi* = *Salmonella typhimurium*.

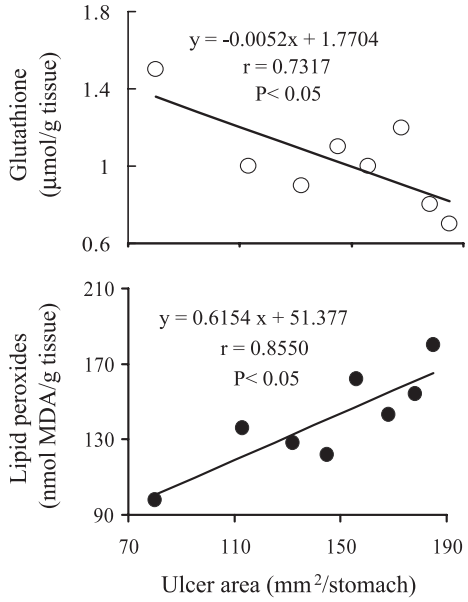

Fig. 2. Relationship between gastric mucosal ulceration and mucosal GSH levels as well as between gastric mucosal ulceration and lipid peroxide generation in gastric juice-irrigated stomachs of *Salmonella typhi*-infected rats.

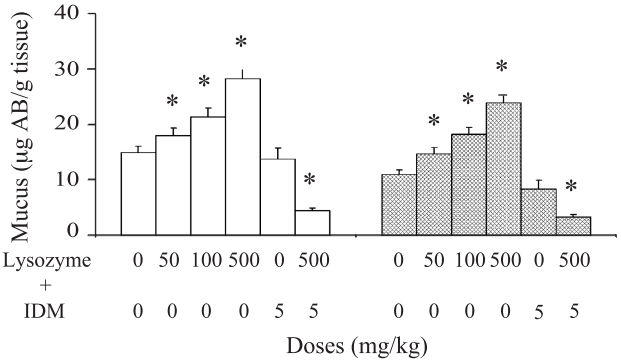

Fig. 3. Dose-response of lysozyme chloride and the effect of indomethacin on gastric mucus secretion in normal (▭) and *Salmonella typhi*-infected (▨) rats. Data are means ± S.E.M. $n = 6–8$. *$P < 0.05$ vs. respective vehicle.

greater acid back-diffusion, mucosal lipid peroxide generation, luminal Hb contents and mucosal ulceration as well as lower mucosal GSH levels were observed in normal saline-irrigated stomachs of *Salmonella typhi*-infected rats. Moreover, a remarkable exacerbation of these ulcerogenic factors was found in gastric juice-irrigated stomachs of those bacteria-infected rats. The increased lipid peroxide generation and decreased GSH were closely correlated with ulcer formation in those bacteria-infected rat stomachs irrigated with gastric juice (Fig. 2). These changes in biochemical parameters confirmed the histological findings that the presence of gastric juice in the lumen is important in enhancing gastric oxidative stress and hemorrhagic ulcer in *Salmonella typhi*-infected rats.

3.3. Protective effect of oflozacin or lysozyme chloride on gastric damage in Salmonella typhi-infected rats

As shown in Table 2, oral oflozacin (100 mg/kg) 30 min prior to bacterial challenge completely prevented gastric hemorrhage and mucosal ulceration in *Salmonella typhi*-infected rats. Increased gastric acid back-diffusion, lipid peroxide generation as well as decreased GSH concentration found in those bacteria-infected rats also were abolished. On the other hand, oral lysozyme chloride produced a dose-dependent amelioration of gastric hemorrhagic ulceration and various gastric aggressive parameters. Lysozyme chloride also exerted stimulation of gastric mucus secretion, which was significantly inhibited by indomethacin in both *Salmonella typhi*-infected and normal rats (Fig. 3).

Table 2
Effects of ofloxacin and various oxyradical scavengers on various biochemical parameters in gastric juice-irrigated stomachs of *Salmonella typhi*-infected rats

Drugs	mg/kg	Acid back-diffusion, μmol/stomach	Glutathione, μmol/g tissue	Lipid peroxide, nmol MDA/g tissue	Hemoglobin, mg/stomach	Ulcer area, mm^2/stomach
Non-infection						
Vehicle		− 141.2 ± 4.0	3.3 ± 0.3	62.6 ± 3.8	0.4 ± 0.2	0.2 ± 0.2
Ofloxacin	100	− 148.2 ± 6.2	3.0 ± 0.2	48.2 ± 4.7	0.3 ± 0.3	0.4 ± 0.3
Lysozyme	50	− 128.8 ± 3.2[a]	3.4 ± 0.3	48.6 ± 3.1	0.1 ± 0.1	0 ± 0
	100	− 107.8 ± 3.6[a]	3.4 ± 0.1	42.0 ± 4.2[a]	0.2 ± 0.1	0 ± 0
	500	− 90.4 ± 4.2[a]	3.6 ± 0.3	36.0 ± 5.1[a]	0.2 ± 0.2	0 ± 0
Salmonella typhi-infection						
Vehicle		− 348.4 ± 17.3	1.1 ± 0.1[b]	141.2 ± 10.6	7.7 ± 0.6	154.0 ± 14.5
Ofloxacin	100	− 156.4 ± 5.0[a]	3.0 ± 0.2[a]	54.0 ± 6.0[a]	0.3 ± 0.2[a]	2.2 ± 0.2[a]
Lysozyme	50	− 276.0 ± 12.2[a,b]	2.0 ± 0.2[a,b]	78.0 ± 10.4[a,b]	3.0 ± 0.5[a,b]	20.0 ± 5.2[a,b]
	100	− 237.2 ± 11.3[a,b]	2.5 ± 0.3[a,b]	58.5 ± 11.4[a,b]	1.6 ± 0.3[a,b]	18.6 ± 3.4[a,b]
	500	− 202.8 ± 6.4[a,b]	2.8 ± 0.3[a,b]	44. 6 ± 0.5[a,b]	0.7 ± 0.4[a,b]	1.4 ± 0.6[a,b]

Data are means ± S.E.M. ($n = 6–8$). MDA = malonedialdehyde.
[a] $P < 0.05$ vs. respective vehicle-treated group.

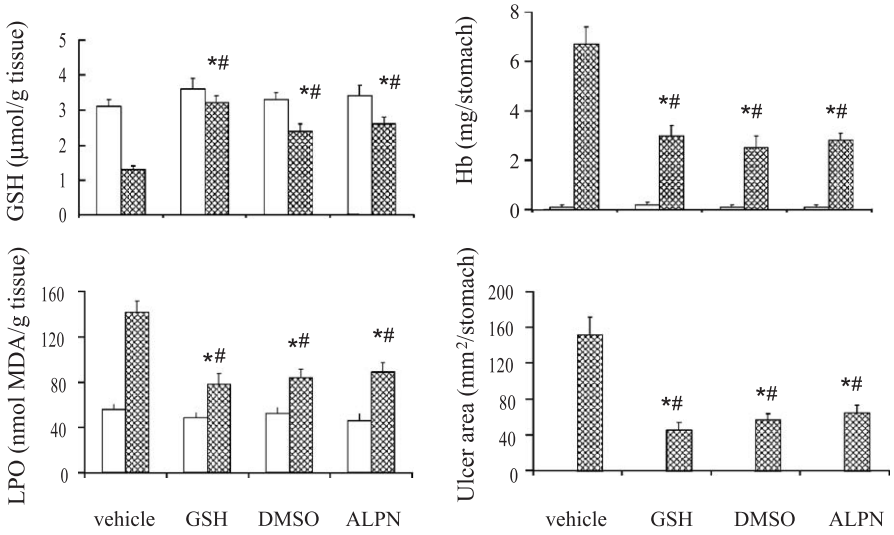

Fig. 4. Effects of several antioxidants on gastric parameters in gastric juice-irrigated stomachs of *Salmonella typhi*-infected rats. The stomachs of both normal (▭) and *Salmonella typhi*-infected (▩) rats were irrigated for 3 h with gastric juice. Data are means ± S.E. M. $n = 6–8$. $^{*}P < 0.05$ vs. respective vehicle, $^{\#}P < 0.05$ vs. respective drug treatment. GSH = glutathione, MDA = malonedialdehyde, ALPN = allopurinol. DMSO = dimethylsulfoxide.

3.4. Protective effects of various antioxidants on gastric hemorrhagic damage in Salmonella typhi-infected rats

Exogenous GSH significantly ($P < 0.05$) inhibited the elimination of GSH levels and the elevation of lipid peroxide generation as well as the exacerbation of hemorrhagic ulcers found in *Salmonella typhi*-infected rats (Fig. 4). Apparently, it could compensate decreased mucosal GSH level and provided gastric cell-repairing effect in *Salmonella typhi*-infected rats. In a similar manner, the oxidative stress-induced gastric damage and various ulcerogenic parameters in *Salmonella typhi*-infected rats was significantly ($P < 0.05$) inhibited by intraperitoneal allopurinol or DMSO. Thus, both enzymatic and non-enzymatic oxidative stress were involved in the formation of gastric hemorrhagic ulcer in *Salmonella typhi*-infected rats.

4. Discussion

The pathophysiological changes underlying *Salmonella typhi*-induced gastric hemorrhagic ulceration are complex. The present study showed that gastric oxidative stress and mucosal hemorrhagic ulcers substantially occurred in *Salmonella typhi*-infected rats. Close negative correlation ($r = -0.8477$, $P < 0.05$; $n = 8$) between great lipid peroxide generation and low GSH levels was found in *Salmonella typhi*-infected rat stomachs irrigated with gastric juice (figure not shown). The attenuation of GSH levels may reflect the increase in consumption of GSH for scavenging of oxyradicals. Since lipid peroxide is the metabolite of oxyradicals that may initiate cell injury (Bhatnagar, 1994), decreased GSH concentration in stomachs of *Salmonella typhi*-infected rats was likely be also associated with oxyradical-induced damages of GSH-containing cells and thus resulted in the reduction of GSH biosynthesis. Moreover, intimate relationships of ulcer formation to lipid peroxide generation and GSH levels were observed in *Salmonella typhi*-infected rats. Taken together, infection of *Salmonella typhi* can lead to mucosal hemorrhagic ulcer via gastric oxidative stress. In the intestine, reactive oxygen species is reported being involved in *Salmonella*-induced enterocyte damage (Mehta et al., 1998). It is proposed that lower mucosal GSH level is one of the etiologic factors of peptic ulcer formation (Hung, 2000; Hirokawa and Kawasaki, 1995). Decrease in GSH level has also been reported to aggravate lung inflammation that is closely associated with oxyradical generation (Rahman and MacNee, 2000). Thus, attenuation of mucosal GSH can render gastric mucosa more susceptible to gastric hemorrhage and ulceration in *Salmonella typhi*-infected rats.

In the present study, greater acid back-diffusion also was found in *Salmonella typhi*-infected rat stomachs. The results explained the fact that gastric mucosal barriers were substantially disrupted in infective rats. Mechanisms of this mucosal barrier injury are totally unknown. However, during infective inflammation and oxidative stress, many biochemical events, such as histamine, inducible nitric oxide and inflammatory cytokines are released (Strijbos et al., 2001). These cytotoxic substances other than oxyradicals may contribute to the damage of gastric mucosal barriers. The free acid in gastric juice thus diffused back through loosen tight junction into gastric mucosa thereby produced hemorrhagic necrotic ulceration. Our previous papers demonstrate that lipopolysaccharide of *E coli* can cause septicemia and gastric hemorrhagic damage via enhancement of acid back-diffusion in rats (Hung, 2000; Hung and Hsu, 1998).

On the other hand, ofloxacin, a fluoroquinolone antibiotic, possesses an excellent activity against Gram-negative and Gram-positive bacteria. This bactericidal effect is due to its blockade of bacterial DNA synthesis by inhibiting bacte-

rial topoisomerase II and IV (Chambers, 2001). Inhibition of topoisomerase II leads to prevention of the relaxation of positively supercoiled DNA that required for normal transcription and replication. Whereas, inhibition of topoisomerase IV interferes with separation of replication chromosomal DNA into the respective daughter cells during cell division. In vivo study shows that oral ofloxacin can increase survival time in *Salmonella typhi*-infected mice. This effect was more potent than that of ampicillin, chloramphenicol or ciprofloxacin (Fu et al., 1990). Intragastric ofloxacin abolished the aggravation of increased lipid peroxide generation and decreased GSH in *Salmonella typhi*-infected rats. Therefore, gastric oxidative stress and hemorrhagic ulcer were predominantly resulted from the infection of these bacteria. Document demonstrates that ofloxacin may possess antioxidant effect (Ebringer et al., 1997), which might also contribute to amelioration of gastric oxidative stress and hemorrhagic ulcers produced in *Salmonella typhi*-infection.

The present study also showed that lysozyme chloride dose-dependently inhibited acid back-diffusion and lipid peroxide generation as well as elevated mucosal GSH and mucus contents in *Salmonella typhi*-infected rats. In fact, gastric mucus stimulated by prostaglandins plays a pivotal role in the protection of mucosal damage induced by various ulcerogens (Tsukimi and Okabe, 2001; Takeuchi et al., 2001). Increase in mucus secretion may greatly contribute to the amelioration of mucosal hemorrhagic ulcer occurred in this bacterial infection. The elevation of gastric mucus by lysozyme was effectively attenuated by indomethacin, a specific inhibitor of prostaglandin biosynthesis. The results indicated that lysozyme-stimulated gastric mucus secretion was partly mediated by the release of prostaglandins that possess potent gastric cytoprotective effects. Documents indicate that antigen–antibody complex which related to increased activity of lysozyme can stimulate prostaglandin biosynthesis and release in mouse peritoneal macrophages (Bonney et al., 1979). Lysozyme is shown to possess analgesic, anti-inflammatory and antibiotic effect on pulpectomy and root canal filling (Horiuchi et al., 1981). This compound also is reported being associated with immune response of patients with cancer. (Yamaoka and Yoshioka, 1983; Sava et al., 1993). The attenuation of lipid peroxide generation produced by lysozyme chloride in *Salmonella typhi*-infected rats explained that this compound possessed potent antioxidant effect, which resulted in attenuation of oxyradical generation and consumption of reduced GSH. Moreover, lysozyme chloride may stimulate GSH biosynthesis in mucosal cells. Taken together, this drug can increase total levels of gastric mucosal GSH and mucus secretion that contribute to the ulcer healing.

Although allopurinol does not affect the activity of superoxide dismutase, an endogenous oxyradical scavenger, in rat antral ulcerated mucosa (Chen et al., 1993), it is able to inhibit the enzymatic activity of xanthine oxidase, which can react with free ferrous ions and generate both hydrogen peroxide and hydroxyl radicals that directly attack gastric mucosal cells. Whereas DMSO is able to prevent the formation of hydrogen peroxide derived from oxyradicals in endotoxaemic rats (Hung, 2000). The DMSO-related compound, dimethylthiourea, the congener of DMSO has been shown to protect rats against Gram-negative sepsis and decreases proinflammatory cytokines (Sprong et al., 1997). Both allopurinol and DMSO produced significant inhibition in mucosal oxyradical generation and decreased GSH level in gastric juice-irrigated stomachs of *Salmonella typhi*-infected rats. The results confirmed forehand findings that gastric oxidative stress was involved in the formation of hemorrhagic ulcer in *Salmonella typhi*-infected rats. Importantly, increased oxyradical generation is parallel to the augmentation of mast cell histamine release in the damaged gastric mucosa (Hung, 2000; Hung and Hsu, 1998; Kimura et al., 1998). Allopurinol and DMSO are reported to attenuate histamine release by inhibiting mast cell degranulation, an important step for inflammatory cascade in rats (Hung, 2000; Nielsen and Johanson, 1986; Hung and Hsu, 1998). The mast cell stabilizing effect of these two oxyradical scavengers might also contribute to the amelioration of gastric hemorrhage and mucosal ulceration in *Salmonella typhi* infected rats.

In conclusion, infection of *Salmonella typhi* produced gastric oxidative stress and hemorrhagic ulcers that could be ameliorated by ofloxacin, lysozyme chloride or oxyradical scavengers, including GSH, allopurinol and DMSO.

Acknowledgements

The authors wish to thank professor L.I. Ho in Department of Microbiology, National Cheng-Kung University for her supply of live *Salmonella typhimurium.* This research was partly supported by a grant (NSC 92-2320-B-006-081) from National Science Council of Taiwan.

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