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• *CLINICAL RESEARCH* •

Effect of lysozyme chloride on betel quid chewing aggravated gastric oxidative stress and hemorrhagic ulcer in diabetic rats

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Abstract

AIM: To evaluate the protective effect of lysozyme chloride on betel quid chewing (BQC) aggravated gastric oxidative stress and hemorrhagic ulcer in rats with diabetes mellitus (DM).

METHODS: Male Wistar rats were challenged intravenously with streptozotocin (65 mg/kg) to induce DM. Rats were fed with regular pellet food or BQC-containing diets. After 90 d, rats were deprived of food for 24 h. Rat stomachs were irrigated for 3 h with normal saline or simulated gastric juice. Rats were killed and gastric specimens were harvested.

RESULTS: An enhancement of various gastric ulcerogenic parameters, including acid back-diffusion, mucosal lipid peroxide generation, as well as decreased glutathione levels and mucus content, were observed in DM rats. After feeding DM rats with BQC, an exacerbation of these ulcerogenic parameters was achieved. Gastric juice caused a further aggravation of these ulcerogenic parameters. Daily intragastric lysozyme chloride dose-dependently inhibited exacerbation of various ulcerogenic parameters in those BQC-fed DM rats.

CONCLUSION: (1) Gastric juice could aggravate both DM and BQC-fed DM rat hemorrhagic ulcer; (2) BQC exacerbated gastric hemorrhagic ulcer in DM rats via enhancing oxidative stress and reducing defensive factors; (3) lysozyme chloride effectively protected BQC aggravated gastric damage in DM rats.

Key words: Glutathione; Lipid peroxide; Oxyradical; Prostaglandins; Gastric mucus; Mucosal damage; Acid back-diffusion

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INTRODUCTION

Betel quid chewing (BQC) is a habit of human beings in most parts of the world. It has been estimated that more than 10% of the world's population chew betel quid for its mild psychoactive effects[39]. Ample documents demonstrate that BQC is a carcinogenic agent either in animal[4] or in human beings[7]. BQC can result in cancer of buccal cavity[26], esophagus[31], stomach[8], lung, and liver[4]. Since the stomach is the organ that directly contacts BQC, it is likely that gastric mucosa can also be damaged after its long-term consumption.

On the other hand, diabetes mellitus (DM) is an important fatal disease that is frequently found in the clinic. Gastrointestinal disturbance, such as gastric hemorrhagic ulcer, is one of the main manifestations observed in poor controlled DM patients. Previous document demonstrates the importance of acid back-diffusion in the formation of hemorrhagic ulcer in DM rats[22]. Whether BQC can aggravate DM-induced gastric hemorrhagic ulcer is unknown.

During gastric oxidative stress, the imbalance of aggressive and defensive factors in the stomach plays a pivotal role in gastric hemorrhage and ulcer formation. The aggressive factors include gastric acid back-diffusion and oxyradical generation, while defensive factors involve glutathione (GSH) and mucus biosynthesis[17]. Aggravation of aggressive factors and/or attenuation of defensive substances may result in high risk of gastric hemorrhage and 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 damaged mucosal barriers and consequently causes exacerbation of hemorrhage and ulceration. This acid back-diffusion is considered as a sensitive marker of gastric mucosal integrity[10,21]. In fact, gastric acid back-diffusion is greatly associated with hemorrhage and ulcer formation in various inflammatory diseases[12,17]. In the disease state, oxidative stress of the stomach may occur and result in an elevation of mucosal lipid peroxide (LPO) that are generated from the reaction of oxyradicals and cellular polyunsaturated fatty acid. Whereas reduced GSH is a sulfhydryl compound, which may act to prevent lipid peroxidation produced by noxious oxyradicals that can damage gastric mucosal cells[3]. Various studies have demonstrated a decrease in GSH level in inflammatory and ulcerated gastric mucosa[14,19]. The mucosal protective effect

of GSH on gastric damage induced by nonsteroidal anti-inflammatory drugs[35], ethanol[36] or lipopolysaccharide[17] has been well documented. Nevertheless, the role of mucosal GSH and oxyradicals in BQC-induced hemorrhagic ulcers are unknown. Gastric mucus, which plays an important role in the mucosal protection, can be eliminated by acid or noxious agents[1]. Nevertheless, the influence of BQC on gastric mucus secretion in DM rats also remains obscure.

Lysozyme (muramidase), which widely distributes in various human tissues[27] is a stable polypeptide containing 18 amino acids. High content of lysozyme is found in mammalian milk, chicken eggshell membrane, and matrix[13]. This compound may possess multiple pharmacological actions, including analgesic[5], anti-inflammatory[33], hemostatic, anti-allergy[2], anticancer[25], and antibiotic[24] effects. The aim was to study the influence of chronic consumption of BQC on gastric oxidative stress, mucus production, and mucosal hemorrhagic ulcer in DM rat gastric mucosa. The mucosal protective effects of lysozyme chloride on this ulcer model were also evaluated.

MATERIALS AND METHODS

Chemicals used

The following chemicals in reagent grade were used: acivicin, alcian blue, lysozyme chloride, 1-butanol, *o*-phthaldialdehyde, pyridine, rat hemoglobin (Hb), reduced GSH, sodium laurylsulfate, trichloroacetic acid, 1,1,3,3-tetramethoxypropane, and 2-thiobarbiturate were purchased from Sigma (St. Louis, MO, USA). The purity of all drugs was over 98%. All chemical solutions were freshly prepared before use.

Drug administration

Intragastric lysozyme chloride (0-500 mg/kg) was challenged once daily to normal and those rats fed with BQC for 90 d. The control group received normal saline instead of chemicals.

Animals

Male specific pathogen-free Wistar rats, weighing 150±20 g, were housed individually in a room with a 12-h dark-light cycle and with central air conditioning (25 ℃, 70% humidity). Animal care and experimental protocols were in accordance with the guidelines of the National Sciences Council of Taiwan (NSC 2002). Prior to the experiments, rats were injected intravenously with streptozotocin (65 mg/kgt) to induce DM. Non-DM rats received vehicle only. Blood glucose level over 2 500 mg/L was considered as DM. All rats were divided into groups. Control rats received laboratory rodent diet (the Richmond standard, PMI feeds, St. Louis, MO, USA). Test groups were fed with the BQC diet prepared by mixing the laboratory rodent diet with chopped and crushed BQC (30%, w/w), which contained areca nuts (40%, w/w), gambir lime (30%, w/w) and betel stems (30%, w/w). The calories of BQC-diet were compensated near the control pellet diet (4.25 kcal/g) by adding adequate amounts of sugar and eggs. The BQC-diet was then dried in cold air and stored in a refrigerator (4 ℃). The rats were given fresh diet every day at 8 a.m. While chewing the test food, rats mimicked human beings in chewing and spitting the fiber contained in the BQC diet. The consumption of the diet, water, and the body weight of the animal were recorded every day before feeding, respectively.

Surgical procedures

After 90 d, rats were deprived of food for 24 h and anesthetized with diethylether. Rat stomachs were surgically exposed for the ligation of pylorus and lower esophagus. To prevent the spontaneous gastric secretion, a bilateral diaphragmatic vagotomy was performed in all rats. A small incision was then made in the forestomach. A polypropylene tube (3.5 mm internal diameter×20 mm long) was inserted through the same incision and secured with a ligature. The stomach was subsequently rinsed meticulously with warm normal saline (37 ℃). Care was taken to avoid injury to blood vessels. The residues were gently removed.

Measurement of gastric acid back-diffusion

Gastric acid back-diffusion (the H^+ loss in the gastric lumen) was quantified by the method previously described[21]. Either normal saline or simulated gastric juice (7 mL) containing 100 mmol/L HCl, 17.4 mmol/L pepsin and 54 mmol/L NaCl was instilled into the cleansed stomach with a syringe. 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 forestomach was tightly closed. The abdominal wound was sutured. After 3 h, rats were killed with an overdose of diethylether. Gastric contents (final sample) were collected. Both initial and final samples were centrifuged at 3 000 r/min for 20 min. The volumes of the initial and final samples were measured. The acidity of samples was assessed by titrating 1.0 mL of gastric contents sample with 0.1 mol/L NaOH to pH 7.0 on an autoburette titrator (Radiometer, Copenhagen, Denmark). The net flux of ions through the gastric mucosa was calculated as follows:

$$\text{Ionic net flux} = F_v \times I_v - (7 - I_v) \times I_c$$

where F_v and I_v are the volume (mL) of the final sample and initial sample, respectively, and F_c and I_c are the ionic concentrations (in mmol/L) of the final sample and the initial sample, respectively. A negative value for net flux indicates luminal electrolyte loss and a positive value indicates luminal electrolyte gain.

Morphological and histological studies of gastric mucosa

As soon as the final sample was collected, the stomach was filled with 8 mL of 4 g/L formaldehyde for 10 min. The mucosa was exposed by opening the stomach along the greater curvature. The length (mm) and the width (mm) of ulcer on the mucosa were measured with a planimeter ($1\pi/4$).

The total ulcer area (mm^2) of each stomach was recorded. Histological studies of the stomach also were conducted by methods as previously described[21]. Sections were scored as 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 score of each section was evaluated on a cumulative basis to give a maximal score of 15.

Determination of hemoglobin (Hb)

Cleansed rat stomachs were irrigated for 3 h with either normal saline or gastric juice. Initial and final samples were collected by methods described above.

The blood attached to the gastric mucosa was carefully scraped and added to the final sample. Subsequently, both initial and final samples were adjusted to pH 1.5 with 0.1 mol/L HCl. The Hb content of the samples was measured spectrophotometrically[22]. 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:

$$\text{Hb content} = F_v \times F_{Hb} (7\text{-}I_v) \times I_{Hb},$$

where F_v and I_v are the volume (mL) of final sample and initial sample, respectively, and F_{Hb} and I_{Hb} are the luminal Hb concentrations (mg/mL) in the final and initial samples, respectively. The results obtained from gastric samples were expressed in mg Hb per stomach.

Mucosal GSH assay

The quantitation of gastric mucosal GSH was performed by following the methods that were described previously[19]. As soon as 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 phosphate buffer (0.1 mol/L NaH_2PO_4 plus 0.25 mol/L sucrose, pH 7.4). Acivicin (250 μmol/L), an irreversible inhibitor of γ-glutamyltransferase, was added to the homogenate to inhibit the catabolism of GSH. Samples were then centrifuged at 4 000 r/min for 15 min at 4 ℃.

To determine the recovery of reduced thiol, GSH (200 μmol reduced GSH contained in phosphate buffer solution, pH 7.0) was added to the supernatant. Subsequently, 0.5 mL, 0.25 mol/L trichloroacetic acid was added to 1.0 mL of the supernatant. The mixture was kept for 30 min at 4 ℃. After centrifugation for 15 min at 3 000 r/min, the supernatant was used to determine GSH using 2,2-dinitro-5,5-dithio-dibenzoic acid. The optical density was measured at 412 nm on a spectrophotometer (model U-3210, Hitachi, Tokyo, Japan). All samples were measured in duplicate. Recovery of added internal standard was greater than 90% in all experiments. Absorbances of the samples were measured against a standard curve constructed with freshly prepared GSH solutions (0.05-0.5 mmol/L) that were treated in the same manner as the tissue samples. The results obtained from tissue samples were expressed as μmol GSH/g wet tissue.

Determination of lipid peroxides (LPO)

The concentration of gastric mucosal LPO was determined by estimating malondialdehyde using the thiobarbituric acid test[30]. Briefly, 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 100 g/L KCl. The homogenate (0.5 mL) was added with a solution containing 0.2 mL 80 g/L sodium laurylsulfate, 1.5 mL 200 g/L acetic acid,1.5 mL 8 g/L 2-thiobarbiturate and 0.3 mL distilled water. The mixture was incubated at 98 ℃ for 1 h. Upon cooling, 5 mL of 1-butanol/pyridine (15:1) was added. The mixture was vortexed for 1 min and centrifuged for 10 min at 400 r/min. The supernatant was measured at 532 nm. The standard curve was obtained by using 1,1,3,3-tetramethoxypropane. The recovery was over 90%. All samples were measured in duplicate. The results were expressed as nmol malondialdehyde/g wet tissue.

Measurement of gastric mucus

Gastric mucus was assessed by the method as described by Corne *et al*.[9]. Briefly, 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 mol/L) and sodium acetate (0.05 mol/L) for 2 h. To remove the unbound dye, the sample was washed for 15 min and then for 45 min in a 0.25 mol/L sucrose solution. The mucus-bound dye was eluted by immersing gastric mucosa in 10 mL of 0.5 mol/L $MgCl_2$ solution for 2 h. The obtained solution was mixed with 10 mL diethylether. The absorbance of the color aqua solution was measured on a spectrophotometer (U3210, Hitachi) at 605 nm. The amount of alcian blue was 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.

Statistical analysis

The data were expressed as mean±SE. Significant differences in single measurement traits were analyzed statistically by using the Tukey truly significant difference test for pairwise comparison after ANOVA or by using the Student's *t*-test[28]. Statistical significance was set at P<0.05.

RESULTS

Changes in gastric parameters in normal, DM, and BQC-fed DM rat stomachs irrigated with either normal saline or gastric juice

Table 1 indicated that in normal diet rat stomachs irrigated with normal saline no gastric acid back-diffusion but only little gastric acid secretion was achieved. Mucosal GSH and LPO generation were at normal levels. No mucosal ulceration and hemorrhage was observed in these rats (photo not shown). In saline-irrigated stomachs of DM rats, an enhancement of LPO generation as well as an attenuation of GSH and mucus secretion was obtained. After receiving the BQC-diet, marked necrotic ulcers accompanied by a further decrease in mucosal GSH and mucus were achieved in DM rats. The generation of mucosal LPO also was further aggravated in those rats, despite that no acid back-diffusion occurred.

When gastric juice was present in the stomachs of normal diet rats, the mucosa looked normal. Except when a normal level of acid back-diffusion occurred, various gastric parameters were not significantly different from those in saline-irrigated stomachs of normal rats. When DM rat stomachs were irrigated with gastric juice, a significant (P<0.05) increased acid back-diffusion and LPO generation accompanied with an attenuation of mucosal GSH and mucus

Table 1 Gastric juice-aggravated various gastric biochemical parameters in stomachs of normal, DM and BQC fed DM rats

	Acid back-diffusion μmol /stomach	Glutathione μmol/g tissue	Lipid peroxide nmol MDA/g tissue	Mucus μg/g tissue	Hemoglobin mg/stomach	Ulcer area mm^2 /stomach
Normal saline						
Control	8.6±1.2	3.8±0.4	45.6±4.2	230.2±18.4	0.2±0.2	1.4±0.6
DM	5.0±0.8[a]	2.4±0.3[a]	95.4±5.2[a]	186.1±10.2[a]	2.2±0.5[a]	64.6±8.5[a]
BQC+DM	3.2±0.2[a]	1.6±0.2[a]	132±11.4[a]	155.0±14.8[a]	4.6±0.6[a]	154.8±18.4[a]
Gastric juice						
Control	-164.2±5.4	3.4±0.2	54.0±7.8	220.0±14.5	0.6±0.2b	2.2±1.2
DM	-235.4±10.6[ac]	2.8±0.1[ac]	120.0±10.2[ac]	140.0±12.6[ac]	5.3±0.7[ac]	264.8±27.1[ac]
BQC+DM	-384.2±18.6[ac]	0.8±0.1[ac]	184.0±16.3[ac]	75.0±6.8[ac]	9.0±1.2[ac]	378.6±45.6[ac]

Data are mean±SE (n = 8). [a]P<0.05 *vs* corresponding control group. [c]P<0.05 *vs* corresponding group in saline-treatment. MDA = malondialdehyde, BQC = betel quid chewing, DM = diabetes mellitus.

secretion was observed and an exacerbated hemorrhagic ulcer also was produced. Further aggravation of gastric acid back-diffusion and LPO generation as well as decreased GSH and mucus production was found in those BQC-fed DM rats. More severe gastric hemorrhage and ulcer also was observed in these rats.

Histological examination of gastric mucosa of DM and BQC-fed DM rats

Figure 1 shows the microscopic examination of normal, DM, and BQC-fed DM rat stomachs irrigated with gastric juice. Marked upper gastric mucosal damage was observed in DM rats. More severe mucosal damage in BQC-fed DM rat stomachs than those in DM rats was obtained. The comparisons of degrees of histological tissue damage in normal, DM and BQC-fed DM rat stomachs irrigated with saline or gastric juice are illustrated in Figure 2. When the stomachs of normal diet rats were irrigated with either saline or gastric juice, no appreciable damage of gastric mucosal cells was observed. In DM rats, normal saline-irrigation produced more mucosal cell damage than those were irrigated with normal saline or gastric juice in normal diet rats. A pronounced aggravation of mucosal cell damage was observed in gastric juice-irrigated stomach of DM rats. Further exacerbation of gastric damage was achieved in DM rats fed with BQC. In most cases, gastric edema also was observed in DM and BQC-fed DM rats.

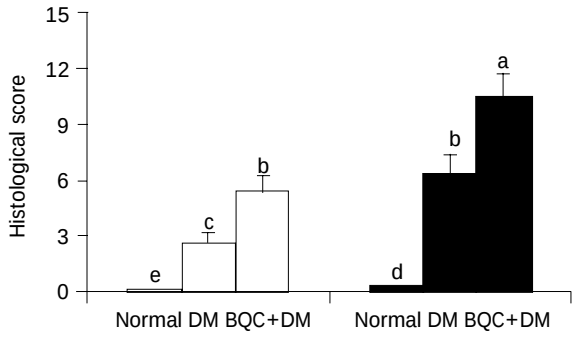

Figure 2 Histological scores of gastric mucosa in normal, DM, and BQC-fed DM rats. Rats were fed with normal diet or BQC diet for 90 d. Diabetes was induced by intravenous streptozotocin. Rat stomachs were irrigated for 3 h with normal saline (□) or gastric juice (■). Values are mean±SE, n = 6-8. Bars labeled with different letters are significantly different at P<0.05 based on Tukey statistic method.

Effects of lysozyme chloride on gastric parameters in gastric juice-irrigated stomachs of BQC-fed DM rats

As demonstrated in Table 2, low mucosal GSH levels and mucus secretion accompanied with high degree of acid back-diffusion and LPO generation were observed in 90-d BQC-fed DM rat stomachs irrigated with gastric juice. Severe gastric hemorrhage and mucosal ulceration also were achieved. Daily intragastric lysozyme chloride dose-dependently suppressed

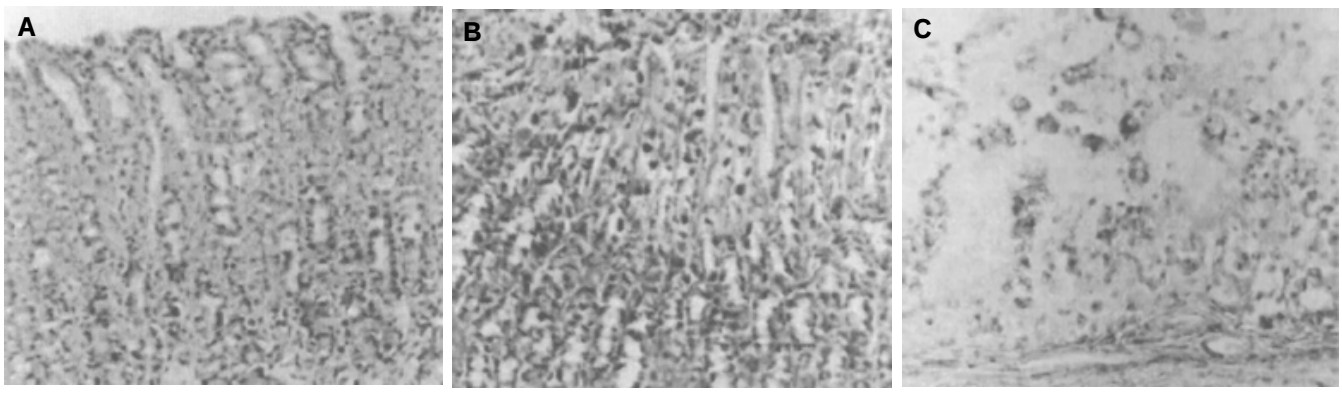

Figure 1 Histological studies of gastric mucosa exposed for 3 h gastric juice in normal, DM, and BQC-fed DM rats. Note that in normal rat stomachs irrigated with gastric juice (A), gastric mucosal cells look intact. However, gastric juice-irrigated DM rat mucosa (B), a disruption of gastric epithelial layer is observed. When stomachs of BQC-fed DM rats are irrigated with gastric juice (C), a complete disruption of the upper mucosal cells and lamina propria is obtained. The injured cells are characterized by karyorrhexis and dense homogenous acidophilic cytoplasm. In most cases, gastric edema also is observed (×150).

Table 2 Effects of lysozyme chloride on various biochemical parameters in gastric juice-irrigated stomachs of BQC-fed DM rats

	mg/kg	Acid back-diffusion μmol /stomach	Glutathione μmol/g tissue	Lipid peroxide nmol MD/g tissue	Mucus μg/g tissue	Hemoglobin mg /stomach	Ulcer area mm^2 /stomach
Control							
Saline		-132.0±3.4	3.6±0.3	62.6±7.0	206.4±14.6	0.4±0.2	3.2±0.5
Lysozyme chloride	500	-112.4±5.6[a]	4.6±0.5[a]	40.4±4.2[a]	245.4±16.5[a]	0.0±0.0[a]	0.0±0.0[a]
Indomethacin	5						
+ lysozyme chloride	500	-140.0±7.2	2.8±0.3[a]	74.5±6.8[a]	164.6±14.2	1.0±0.5	5.6±08
BQC+DM							
Saline		-365.2±20.4	0.8±0.2	160.4±11.6	83.2±10.2	8.0±0.8	396.8±34.2
Lysozyme chloride	50	-292.0±11.2[a]	1.7±0.3[a]	132.0±12.5[a]	126.6±7.4[a]	4.0±0.5[a]	306.0±25.2[a]
	100	-220.2±13.0[a]	2.4±0.3[a]	108.4±10.6[a]	182.8±9.2[a]	2.2±0.3[a]	168.6±12.4[a]
	500	-186.8±10.2[a]	3.4±0.3[a]	74.8±6.2[a]	216.3±16.4[a]	1.7±0.3[a]	44.5±6.4[a]
Indomethacin	5						
+ lysozyme chloride	500	-308.6±18.8[a]	1.5±0.2[a]	154.0±12.6[a]	140.8±11.5[a]	4.8±0.8[a]	320.4±30.4[a]

Data are mean±SE (n = 8). [a]P< 0.05 *vs* corresponding saline-group. MDA = malondialdehyde. MDA = malondialdehyde, DM = diabetes mellitus, BQC = betel quid chewing.

gastric hemorrhage and ulcer. This drug also inhibited the decreased mucosal GSH and mucus liberation as well as increased acid back-diffusion and mucosal LPO generations in BQC-fed DM rats in a dose-related manner. No visible side effects or toxicity of lysozyme chloride were observed in all experiments.

DISCUSSION

The pathological mechanisms underlying BQC-fed DM ulcer may be complex. Our previous paper demonstrates that chronic consumption of BQC can result in severe gastric hemorrhagic ulcer in rats[20]. In the present study, a remarkably increased acid back-diffusion was observed in those DM rat stomachs irrigated with gastric juice. Further aggravation of these ulcerogenic parameters was obtained in those DM rats fed with BQC. Obviously, chronic consumption of BQC diet could enhance various ulcerogenic parameters and result in greater gastric inflammation and breaking effects of gastric mucosal barriers in DM rats. Mechanisms of barrier-disruptive effect of BQC in DM rats are totally unknown. However, since BQC contained large amounts of gambir lime (which contains about 7-30% catechol, 20-50% catechu tannic acid and small amounts of quercetin waxes and oils; Merck Index, 1983) and calcium, the damage of mucosal barrier probably results from caustic injury from this component of BQC and DM-induced gastric cell degeneration. In clinical studies, it is reported that calcium polysulfite ingestion produces hemorrhagic necrosis of gastric mucosa[16]. BQC also contained arecoline, the main ingredient of areca fruit. Arecoline is a cholinergic agent that can cause gastric contraction and induce gastric secretion. Our previous paper demonstrates that arecoline causes seizure and results in gastric mucosal damage[17]. Therefore, after chronic consumption of BQC, gastric mucosal barriers might be severely disrupted and thus allowed copious free acid to diffuse back into the gastric mucosa during ulcer development.

Aggravation in LPO generation also was observed in gastric mucosa of BQC-fed DM rats. Apparently, the disruption of the mucosal barrier accompanied by gastric oxidative stress occurred in DM rats receiving BQC. In practice, oxyradicals can directly cause cell injury[3]. This cell damaging effect may associate with the occurrence of many diseases, including tissue inflammation and sepsis[17], hemorrhagic shock[11], and DM[29]. The initiation of gastric mucosal oxidative stress and inflammation might result from direct BQC-induced damage and degeneration of gastric mucosal cells induced by DM.

The lower mucosal GSH levels found in stomachs of DM or BQC-fed DM rats might be due to an increase in their consumption for scavenging oxyradicals and a decrease in cellular biosynthesis in damaged mucosal cells. In fact, depletion of neuronal GSH can cause an increase of neuronal nitric oxide synthase activity and cell death[6]. Moreover, GSH is used as a substrate or as a co-factor by several cytosolic and membrane-bound enzymes. A previous study demonstrated that exogenous GSH can produce a significant increase in mucosal GSH levels and a reduction in concentrations of mucosal LPO that associate with severe gastric hemorrhagic ulcers in acid-irrigated stomachs of septic rats[17]. Taken together, the imbalance between mucosal GSH biosynthesis and oxyradicals generation (gastric oxidative stress) was important in modulating BQC-induced ulcer in DM rats. A decreased gastric mucus also was found in DM or BQC-fed DM rats. Since BQC is an ulcerogenic substance, it might also damage gastric mucus cells and lead to attenuation of mucus biosynthesis. In fact, ulcerogens, such as aspirin and other non-steroidal anti-inflammatory drugs, have been reported to attenuate gastric mucus secretion[32]. Increase in mucus secretion can retard acid back-diffusion and is beneficial to the healing of mucosal damage[37].

In the present study, lysozyme chloride dose-dependently inhibited various aggressive parameters and elevated defensive factors in BQC-fed DM rats. It also ameliorated BQC-induced hemorrhagic ulcer in DM rats. This compound remarkably enhanced biosynthesis and secretion of gastric mucus, which is a potent gastroprotective substance. Lysozyme chloride also can enhance antral ulcer healing via stimulating mucus biosynthesis[18]. Indomethacin, a specific antagonist of prostaglandins, effectively reversed lysozyme chloride-induced cytoprotection of gastric mucosa. This implied that gastric mucosal protective effect of lysozyme chloride was partly mediated by stimulating biosynthesis and release of prostaglandins. Additionally, this drug has been reported to have analgesic and anti-inflammatory effects on pulpectomy and root canal filling[15]. It has also been shown to contribute

to the advantageous effects in the treatment of patients and experimental animals with cancer[34,38]. The attenuation of LPO generation produced by lysozyme chloride in BQC-fed DM rats explained that this compound might possess potent antioxidant effect, thus reduced consumption of GSH. Moreover, lysozyme chloride may stimulate GSH biosynthesis in mucosal cells. Considering the above-mentioned, this drug can increase the total levels of BQC-fed DM rat mucosal GSH and contribute to the ulcer healing.

In conclusion, long-term consumption of BQC produces aggravation of DM-induced hemorrhage and ulcer in rats via enhancing oxidative stress that can be ameliorated by lysozyme chloride.

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