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• BASIC RESEARCH •

Adaptive cytoprotection through modulation of nitric oxide in ethanol-evoked gastritis

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Abstract

AIM: To assess the mechanisms of protective action by different mild irritants through maintenance of gastric mucosal integrity and modulation of mucosal nitric oxide (NO) in experimental gastritis rats.

METHODS: Either 200 mL/L ethanol, 50 g/L NaCl or 0.3 mol/L HCl was pretreated to normal or 800 mL/L ethanol-induced acute gastritis Sprague-Dawley rats before a subsequent challenge with 500 mL/L ethanol. Both macroscopic lesion areas and histological damage scores were determined in the gastric mucosa of each group of animals. Besides, gastric mucosal activities of NO synthase isoforms and of superoxide dismutase, along with mucosal level of leukotriene $(LT)C_4$ were measured.

RESULTS: Macroscopic mucosal damages were protected by 200 mL/L ethanol and 50 g/L NaCl in gastritis rats. However, although 200 mL/L ethanol could protect the surface layers of mucosal cells in normal animals (protection attenuated by N^G-nitro-L-arginine methyl ester), no cytoprotection against deeper histological damages was found in gastritis rats. Besides, inducible NO synthase activity was increased in the mucosa of gastritis animals and unaltered by mild irritants. Nevertheless, the elevation in mucosal LTC_4 level following 500 mL/L ethanol administration and under gastritis condition was significantly reduced by pretreatment of all three mild irritants in both normal and gastritis animals.

CONCLUSION: These findings suggest that the aggravated 500 mL/L ethanol-evoked mucosal damages under gastritis condition could be due to increased inducible NO and LTC_4 production in the gastric mucosa. Only 200 mL/L ethanol is truly "cytoprotective" at the surface glandular level of non-gastritis mucosa. Furthermore, the macroscopic protection of the three mild irritants involves reduction of LTC_4 level in both normal and gastritis mucosa, implicating preservation of the vasculature.

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INTRODUCTION

Excessive ethanol ingestion can result in gastritis characterized by mucosal edema, subepithelial hemorrhages, cellular exfoliation and inflammatory cell infiltration[1]. Alcohol has been shown to affect the mucosal barrier and histology[2]. Morphologically, alcohol-induced gastric superficial injury involves mostly the inter-foveolar epithelium and gastric pits, and heals rapidly by restitution[3]. On the other hand, the deeper lesions involve intramucosal hemorrhage and vascular engorgement[4]. As a consequence of damage to microvessels, leakage of inflammatory mediators occurs, and vasoconstriction of submucosal arteries would result in ischemia. Eventually, these events would enhance the formation of more severe necrotic mucosal injury. Several products of arachidonate metabolism have been implicated to participate in the pathogenesis of ethanol-induced gastric mucosal damage[5].

It is known that neuronal modulating processes such as the release of vasoactive mediators are crucial for the gastric mucosa to resist the continual onslaught of aggressive agents[6]. Previous findings have suggested that there are interactions between the endothelium-derived vasodilator mediators, including that prostaglandins (PG), can regulate gastric mucosal microcirculation and integrity[7]. Endothelial cells also release a highly labile humoral vasodilator substance, now known to be nitric oxide (NO), that mediates the vascular relaxation induced by vagal stimulation[8]. Nonetheless, it should be noted that the production of NO from a calcium-independent (inducible) form of the enzyme could lead to cell injury in the endothelium[9]. Thus, the induction of NO synthesis may not always be beneficial. For instance, formation and interaction between superoxide and NO radicals are the key elements of oxidative challenges in the gastric mucosa.

There are some endogenous proinflammatory mediators that could be activated during the aggressive attack of noxious agents or severe tissue trauma. Leukotriene $(LT)C_4$ is one of these substances which would lead to microcirculatory disturbances and severe mucosal tissue injury[10]. Such a detrimental action may somehow involve the generation of reactive oxygen free radicals. In other words, these effects could be modulated by enzyme systems of the oxygen-handling cells, such as superoxide dismutase (SOD), which are able to protect cells against the toxic effects caused by oxygen species[11].

Acute hemorrhagic gastritis patients have underlying predisposing conditions such as alcohol abuse or use of NSAID. Besides endoscopic and surgical therapy, the focus on pharmacotherapy would be the enhancement of mucosal defense mechanisms so as to accelerate healing and prevent relapses[12]. The present study attempted to illustrate that different mild irritants had differential modes of action in the adaptive cytoprotection against ethanol-induced gastric mucosal damage in animals under gastritis condition, whereas some of these involved modulation of eicosanoids and NO biosynthesis in the gastric mucosa. These data may also provide the explanation why gastritis can predispose the stomach to ulceration.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (240-260 g) were used after

acclimatization for at least three days in a controlled room with constant temperature (22±1 °C) and humidity (65-70%). They were fed a standard diet of laboratory chow (Ralston Purina, USA) and had free access to tap water *ad libitum*. All experimental animals were deprived of food in individual wired cages 24 h beforehand.

Treatments and induction of gastritis

Animals in the "normal" (non-gastritis) groups received oral administration (10 mL/kg) of either distilled water or one of the three mild irritants, 200 mL/L ethanol, 50 g/L NaCl and 0.3 mol/L HCl via a stainless steel orogastric tube, 15 min before the administration of 500 mL/L ethanol (10 mL/kg). For animals that were used to demonstrate the action of mild irritants alone (basal), distilled water was given instead of 500 mL/L ethanol. All animals were sacrificed 30 min later.

To induce gastritis, 800 mL/L ethanol was given to the rats orally (10 mL/kg). The treated animals were returned to their home cages with provision of food and tap water. After 24 h, all the 800 mL/L ethanol-treated animals were deprived of food again, with tap water supply only. Similar experiments as those of the "normal" animals were performed using these gastritis rats 24 h following starvation (i.e. the experiments were carried out 48 h after the induction of gastritis). Our preliminary study showed that at this time point the gastritis animals were free from any gross macroscopic lesion or erosion, but the deeper mucosal cells comprising more than 800 mL/L of the total mucosal thickness were damaged or morphologically changed when observed microscopically.

In order to investigate the participation of the endogenous vasoactive mediator during the processes, N^G-nitro-L-arginine methyl ester (L-NAME, 12.5 mg/kg, i.v.) was pretreated 15 min before the administration of mild irritants to inhibit endogenous NO production[13].

Macroscopic evaluation of gastric mucosal damage

The animals were sacrificed by a sharp blow behind the head and followed by cervical dislocation. Their stomachs were removed and opened along the greater curvature. After thoroughly rinsed in ice-cold saline solution and blotted dry, the area of macroscopic lesions on the mucosa was traced onto a glass slide, and measured by transparent 1-mm^2 grids[14]. A section of each stomach tissue was removed and preserved for subsequent microscopic studies. Finally, the glandular mucosa of the rest of stomach tissue was scrapped by using a glass slide, weighed and immediately frozen in liquid nitrogen. The mucosal samples were stored at -70 °C until assayed for various endogenous mediators.

Histological evaluation of gastric mucosal damage

Within a week of formalin fixation, the gastric tissues were processed for paraffin embedding. A (1.0× 0.5×0.3) cm^3 block of gastric tissue was dehydrated by immersion in progressively increasing concentrations of ethanol. Slices of 6-μm thick sections were stained by the periodic acid-Schiff technique, and counterstained by Harris' hematoxylin solution. The stained sections were then left in the fume-cupboard overnight.

An Olympus microscope (200×) with a scaled eyepiece was used for the morphometric study. Any histological damage in a section was quantified according to the method from O'Brien and coworkers[15]. The criteria for damage were the absence of gastric mucosal cells or the presence of grossly disrupted cells. The standards for evaluation of the severity of microscopic mucosal damage were as follows: type I damage-length of luminal surface mucous cells damaged or vacuolated, type II damage-extensive luminal surface cell damage with disrupted and exfoliated cells lining the gastric pits, type III damage-disrupted cells of the gastric glands beneath the damaged surface and gastric pit cells. For each tissue sample, four measurements of the total mucosal length as well as the length with damaged or disrupted mucosal cells were examined (in mm) and averaged. The final index for the degree of histological damage was represented by the percentage of the damaged mucosal length in terms of the total length of the gastric mucosa.

Determination of NO synthase activity in gastric mucosa

A mucosal sample was placed in a buffer solution (pH 7.2) containing 10 mmol/L HEPES, 0.32 mol/L sucrose, 0.1 mmol/L EDTA, 1 mmol/L DL-dithiothreitol, 10 μg/mL of soybean trypsin inhibitor, 10 μg/mL of leupeptin, 2 μg/mL of aprotinin, and 1 mg/mL of phenyl-methanesulfonyl fluoride. The sample was homogenized for 20 s under ice-cold condition, and then centrifuged at 22 000 *g* for 30 min at 4 °C. An aliquot of 100 μL from the supernatant was withdrawn for protein assay[16].

The NO synthase activity was determined from the conversion of [^{3}H] L-arginine to the NO co-product citrulline[17]. The supernatant was passed over a 0.75-mL column containing Dowex AG50WX-8 resins to remove any endogenous arginine[18]. The reaction mixture comprised 100 μL of the supernatant and 150 μL of buffered solution (pH 7.2) containing 10 mmol/L HEPES, 0.7 mmol/L NADPH, 150 μmol/L $CaCl_2$, 7 mmol/L L-valine to inhibit any arginase[19], and 1 μCi of [^{3}H] L-arginine. The amount of [^{3}H] L-citrulline formed in this reaction mixture represented the total NO synthase activity. A similar reaction mixture was also prepared, with the addition of 1 mmol/L EGTA, which removed Ca^{2+} ions from the system[13]. Product formation that remained persistent in this system determined the inducible NO synthase activity. Incubation of the mixtures with or without EGTA was continued for 30 min at 37 °C. The reaction was terminated by adding 50 μL of 200 mL/L perchloric acid, and the solution mixture was neutralized by 160 μL of 1 mol/L NaOH[20]. This was followed by the dilution with 540 μL of deionized water containing 1 mmol/L L-arginine and 1 mmol/L citrulline. Subsequently, the resulting 1 mL mixture in each reaction tube was applied onto a chromatographic column containing 0.5 g of Dowex AG50WX-8 resins[21]. Following the separation from unreacted [^{3}H] L-arginine by cation-exchange chromatography, the product [^{3}H] L-citrulline was eluted through the column by 1 mL of deionized water and collected into a scintillation vial. The samples were counted for the amount of radioactivity using a liquid scintillation counter (2 000 CA, Packard, USA). The data obtained were corrected for background counts obtained by a similar procedure but with heat-inactivated mucosal tissue. The final results were represented as cpm/min/mg protein. Constitutive NO synthase activity was obtained by subtracting the inducible NO synthase activity by the total NO synthase activity.

Determination of LTC_4 level in gastric mucosa

The pre-weighed mucosal samples were homogenized for 15 s under ice-cold condition in phosphate buffer (50 mmol/L, pH 7.4) with indomethacin (28 μmol/L), to prevent any neoformation of cyclooxygenase products during the extraction process. The homogenized samples were then centrifuged at 1 400 r/min for 15 min at 4 °C. An aliquot of 100 μL from the supernatant was withdrawn for protein assay[16]. The assay was carried out by using a LTC_4 [^{3}H] RIA kit (NEN Dupont, USA.). A standard curve was constructed with a range of 0.025-1.6 ng/100 μL. The final values of the samples obtained were represented as pg/mg protein.

Determination of SOD activity in gastric mucosa

The pre-weighed mucosal samples were homogenized for 20 s under ice-cold condition in phosphate buffer (50 mmol/L, pH 7.4). The homogenized samples were then centrifuged at 20 000 *g* for 15 min at 4 °C. An aliquot of 100 μL from the supernatant was withdrawn for protein assay[16].

The SOD activity in tissue homogenates was determined by the NBT reaction[22]. A 10 μL of the homogenates was added to a solution mixture containing 150 μL each of 2 mmol/L xanthine, 2 mmol/L EDTA and 0.5 mmol/L NBT, 500 μL of Na_2CO_3 and 75 μg/mL of BSA. After being made up to a 2.85 mL solution with phosphate buffer, the mixture was incubated for 3 min in a 25 °C bath. Following that, 150 μL of 0.12 μmol/L xanthine oxidase was added to the mixture and 3 mL solution was incubated further at 25 °C for 20 min. The reaction was terminated by adding 1 mL of 0.8 mmol/L $CuCl_2$ solution to each tube. The inhibition of NBT reduction in each sample was determined spectrophotometrically at 560 nm and the final value of SOD activity was represented as units/mg protein.

Drugs

[^{3}H] L-arginine (specific activity = 36.1 Ci/mmoL) and the [^{3}H] LTC_4 RIA kit were purchased from NEN DuPont (Boston, MA, USA.). L-NAME, DL-dithiothreitol, soybean trypsin inhibitor, leupeptin, aprotinin, phenylmethanesulfonyl fluoride, L-valine, L-arginine and DL-citrulline were all products of Sigma Chemicals (St. Louis, MO, USA.).

Statistical analysis

All the results were expressed as mean±SE of 6 animals per group. The means were compared by the analysis of variance followed by unpaired Student's *t* test. Differences were considered statistically significant if the *P* value was less than 0.05.

RESULTS

Adaptive cytoprotection of mild irritants against 50% ethanol-induced macroscopic lesion formation in gastritis rats (Table 1)

Under gastritis condition, there was a general aggravating effect on the macroscopic ethanol-evoked gastric mucosal damage in all experimental groups, when compared with the damaging effects of 500 mL/L ethanol in normal animals. The degree of protection by 200 mL/L ethanol and 50 g/L NaCl in gastritis rats was lessened when compared to that in normal animals, while the gastroprotection of 0.3 mol/L HCl was completely relieved in gastritis animals. As in normal animals, L-NAME pretreatment alleviated the protective action of 200 mL/L ethanol in gastritis animals. Nonetheless, the protective action of 50 g/L NaCl was preserved under gastritis condition.

Table 1 Effect of mild irritants on 500 mL/L ethanol-induced macroscopic lesion formation in gastric mucosa of normal and gastritis rats, and modulation by pretreatment with L-NAME

Pretreatment	Macroscopic lesion areas (mm^2)	
	Normal	Gastritis
H_2O (Control)	41.17±7.85	134.17±19.23^{d}
200 mL/L EtOH	2.00±0.93^{b}	31.33±5.96^{b,f}
50 g/L NaCl	0±0^{b}	15.00±3.80^{b,f}
0.3 mol/L HCl	0±0^{b}	124.33±20.60^{f}
L-NAME+H_2O	42.50±10.65	125.40±17.75^{f}
L-NAME+200 mL/L EtOH	32.33±9.95^{a}	98.83±7.22^{h,f}
L-NAME+50 g/L NaCl	0±0^{b}	32.25±7.49^{b,f}
L-NAME+0.3 mol/L HCl	0±0^{b}	104.25±12.20^{h}

Values are mean±SE (n = 6), ^{b}P<0.001 *vs* corresponding H_2O group without mild irritant. ^{a}P<0.05, ^{f}P<0.001*vs* corresponding group without drug pretreatment. ^{d}P<0.01, ^{h}P<0.001 *vs* corresponding group without gastritis (Normal).

Adaptive cytoprotection of mild irritants against 50% ethanol-induced histological damage in gastritis rats (Table 2)

The type II and type III histological damages induced by 500 mL/L ethanol were generally aggravated in the gastric mucosa of gastritis animals. In addition, the histological cytoprotection of 200 mL/L ethanol that could be observed in normal animals (the total, type I and type II histological damages, which was alleviated by the pretreatment with L-NAME) was completely

Table 2 Effect of mild irritants on 500 mL/L ethanol-induced histological damage in gastric mucosa of normal and gastritis rats, and modulation of protective action of 200 mL/L ethanol by pretreatment with L-NAME

Treatment	Histological damage (% total mucosal thickness)							
	Normal				Gastritis			
	Total	Type I	Type II	Type III	Total	Type I	Type II	Type III
H_2O (Control)	70.80±1.26	16.23±1.07	21.92±1.01	31.36±1.67	91.13±4.31^{f}	14.99±1.14	27.37±1.83^{e}	48.77±3.69^{f}
200 mL/L EtOH	59.61±2.04^{b}	11.12±0.97^{h}	15.27±1.10^{h}	33.20±1.80	92.23±3.89^{j}	14.17±1.26	25.06±1.77^{j}	53.00±3.16^{j}
50 g/L NaCl	73.16±1.82	15.37±1.26	22.64±1.54	35.15±2.07	90.73±3.15^{f}	12.11±1.02	28.33±1.60^{e}	50.29±3.40^{f}
0.3 mmol/L HCl	66.37±2.21	18.20±1.55	20.01±1.98	28.16±3.04	89.83±3.71^{f}	13.72±1.33	25.69±1.52^{e}	50.40±2.56^{j}
L-NAME+H_2O	74.2±1.96	16.41±1.72	21.19±1.70	36.62±3.21	94.27±3.06^{f}	15.21±1.31	29.61±1.97^{f}	49.42±3.90^{e}
L-NAME+200 mL/L EtOH	69.75±2.11^{d}	15.55±1.41^{a}	20.48±1.88^{c}	33.71±2.86	95.10±3.62^{f}	13.78±1.56	27.49±1.81^{e}	53.82±4.11^{f}

Values are mean±SE (n = 6), ^{h}P<0.01, ^{b}P<0.001 *vs* corresponding H_2O group without mild irritant. ^{a}P<0.05, ^{d}P<0.01 *vs* corresponding group without drug pretreatment. ^{e}P<0.05, ^{f}P<0.01, ^{j}P<0.001 *vs* corresponding group without gastritis (Normal).

Table 3 Effect of mild irritants and/or 500 mL/L ethanol on constitutive and inducible nitric oxide synthase activity in gastric mucosa of normal and gastritis rats

	Nitric oxide synthase activity (cpm/min/mg protein)							
	H_2O		200 mL/L EtOH		50 g/L NaCl		0.3 mol/L HCl	
	Constitutive	Inducible	Constitutive	Inducible	Constitutive	Inducible	Constitutive	Inducible
Basal normal	266.01±22.10	96.97±12.72	768.98±93.17^{b}	107.66±17.17	734.68±74.67^{b}	84.13±19.26	328.77±48.23	97.52±16.12
500 mL/L EtOH normal	132.18±16.49^{d}	106.55±13.19	326.54±33.43^{a,h}	143.51±15.71	282.67±23.86^{a,d}	109.67±12.32^{a}	160.86±25.07	137.24±16.13
Basal gastritis	31.56±7.20^{f}	856.58±93.82^{f}	31.13±10.47^{f}	746.05±94.07^{f}	41.26±11.64^{f}	649.89±81.83^{f}	22.27±8.01^{f}	949.87±106.22^{f}
500 mL/L EtOH gastritis	56.21±8.64^{j}	972.29±123.73^{f}	39.12±3.09^{f}	888.88±129.36^{f}	42.72±10.29^{f}	853.36±103.64^{f}	49.82±10.58^{j}	875.24±122.44^{f}

Values are mean±SE (n = 6), ^{b}P<0.001 *vs* corresponding H_2O group without mild irritant treatment. ^{a}P<0.05, ^{h}P<0.01, ^{d}P<0.001 *vs* corresponding group without 500 mL/L EtOH treatment (Basal). ^{j}P<0.01, ^{f}P<0.001 *vs* corresponding group without gastritis (Normal).

Table 4 Effect of mild irritants and / or 50% ethanol on superoxide dismutase (SOD) activity in gastric mucosa of normal and gastritis rats

	SOD activity (units / mg protein)			
	H_2O	200 mL / L EtOH	50 g / L NaCl	0.3 mol / L HCl
Basal normal	32.08±2.20	36.07±3.18	38.01±1.55	37.53±3.06
500 mL / L EtOH normal	37.20±2.51	41.14±1.82	40.48±0.99	41.33±3.78
Basal gastritis	41.92±2.42[a]	47.51±3.27[a]	48.03±2.06[b]	50.56±3.88[a]
500 mL / L EtOH gastritis	50.85±4.46[a]	49.76±3.36[a]	57.68±6.04[a]	54.92±4.74[a]

Values are mean±SE (n = 6), [a]P<0.05, [b]P<0.01 *vs* corresponding group without gastritis (Normal).

Table 5 Effect of mild irritants and / or 50% ethanol on leukotriene C_4 (LTC_4) level in gastric mucosa of normal and gastritis rats

	LTC_4 level (pg / mg protein)			
	H_2O	200 mL / L EtOH	50 g / L NaCl	0.3 mol / L HCl
Basal normal	13.33±3.08	5.77±0.86[a]	4.19±0.67[a]	4.28±0.81[a]
500 mL / L EtOH normal	29.28±5.46[c]	10.01±1.87[b]	9.15±3.05[b]	8.17±2.42[b]
Basal gastritis	35.08±6.28[e]	17.05±4.22[a,e]	12.88±3.68[a,e]	14.86±3.60[a,e]
500 mL / L EtOH gastritis	43.84±3.79	18.86±4.66[b]	17.70±3.06[d]	21.28±6.33[a]

Values are mean±SE (n = 6), [a]P<0.05, [b]P<0.01, [d]P<0.001 *vs* corresponding H_2O group without mild irritant. [c]P<0.05 *vs* corresponding group without 500 mL / L EtOH treatment (Basal). [e]P<0.05 *vs* corresponding group without gastritis (Normal).

relieved. Five percent NaCl and 0.3 mol/L HCl did not exert any cytoprotective action in the histological level whatsoever.

Effects of mild irritants on NO synthase activity in gastric mucosa of gastritis rats, during basal condition or followed by 500 mL/L ethanol challenge (Table 3)

The condition of gastritis caused a profound elevation of inducible NO synthase activity as well as a reduction of constitutive NO synthase activity in all experimental groups. The elevation of constitutive NO synthase activity induced by 200 mL/L ethanol or 50 g/L NaCl was also alleviated in gastritis rats, with the inducible NO synthase activity remained unaltered by mild irritants. On the other hand, the overall inhibitory effect of 500 mL/L ethanol on mucosal constitutive NO synthase activity was relieved in gastritis animals, of which the activation of the constitutive isozyme by 200 mL/L ethanol and 50 g/L NaCl was also completely prevented.

Effects of mild irritants on SOD activity in gastric mucosa of gastritis rats, during basal condition or followed by 500 mL/L ethanol challenge (Table 4)

SOD activity was significantly increased in all gastritis animals, when compared to normal rats. On the other hand, neither the mild irritants nor 500 mL/L ethanol significantly altered SOD activity in the gastric mucosa.

Effects of mild irritants on LTC_4 level in gastric mucosa of gastritis rats, at basal condition or followed by 500 mL/L ethanol challenge (Table 5)

In general, there was a higher LTC_4 level in the gastric mucosa of all gastritis animals. Nevertheless, all three mild irritants significantly reduced the level of LTC_4 in the gastric mucosa of both normal and gastritis rats. Fifty percent ethanol significantly increased the amount of mucosal LTC_4 in normal animals, but not in gastritis animals. The ability of mild irritants to reduce mucosal LTC_4 level remained persistent in all 500 mL/L-ethanol-treated groups.

DISCUSSION

Previous studies investigating on the phenomenon of gastric adaptive cytoprotection were conducted in normal animals. The present investigation demonstrated the adaptive cytoprotection of mild irritants in gastritis rats. It is known that after deep mucosal injury involving extensive hemorrhage and tissue destruction, as in the case of gastritis induced by 80% ethanol, epithelial restitution still occurred[23]. The term "hemorrhagic gastritis" is a term used to describe the appearance of subepithelial hemorrhage in the stomach. Some patients with gastric subepithelial hemorrhage could be expected to have associated histologic gastritis. Hence, histological protection and the preservation of vascular integrity were the two main criteria of gastroprotection[24]. Our findings indicated that the gross macroscopic protective actions of 200 mL/L ethanol and 50 g/L NaCl remained persistent in the gastritis stomach. The loss of protective effect of 0.3 mol/L HCl under gastritis condition implied that a normal functional mucosa could be essential for the protective mechanism of the mild acid. We previously reported that adaptive cytoprotection by 0.3 mol/L HCl was completely blocked by vagotomy, implicating that innervation of an intact vagus nerve was one of the prerequisites of this mild irritant to induce physiological responses in the mucosal oxyntic cells[25]. Similarly, normal integrity of the mucosa that was interfered under gastritis condition may also be important for the protective action of HCl. Apart from that,50 g/L NaCl and 0.3 mol/L HCl failed to preserve the mucosal cells histologically, even in non-gastritis normal animals, leaving 200 mL/L ethanol to be the only agent that could exert "true cytoprotection" at the surface glandular level. Nevertheless, the loss of histological protective ability of 200 mL/L ethanol during gastritis condition also suggests that the initiation of its histologic adaptive cytoprotection may also require an intact glandular mucosa to operate. Clinically, the histologic gastritis that attributed to ethanol was somehow related to the underlying presence of *H pylori*, although ethanol did not seem to initiate *H pylori*-associated histologic gastritis directly[26]. Treatment of *H pylori* was associated with almost complete normalization of histologic findings[27]. In other words, 200 mL/L ethanol may only be responsible for the improvement of macroscopic gastric mucosal lesion formation by surface epithelial restitution, while *H pylori* eradication is capable of restoring the deep subepithelial mucosa in histologic gastritis.

In general, inflammatory reactions were initiated and amplified by proinflammatory mediators from injured tissues as well as those being synthesized during the process[28]. These substances could result in further local tissue injury by release and activation of destructive enzymes as well as production of oxygen-derived free radicals. Thus, removal of oxygen-derived free radicals could stimulate the healing of ethanol-induced acute gastric mucosal injury in rats[29]. Increased superoxide generation was resulted from activation of polymorphonuclear leukocytes and macrophages, which were the stimuli that induced the production of NO by inducible NO synthase[30]. NO produced in a relatively high concentration by this inducible enzyme might react with oxygen or superoxide to yield more reactive oxidants, such as the peroxynitrite[31]. These secondary oxidants are believed to be responsible for most biological oxidative damages, and are often the targets of antioxidant defense. In the present study, inducible NO synthase activity was significantly increased for many folds in gastritis animals, which could be correlated with the aggravation in 500 mL/L ethanol-induced mucosal damage and the alleviation of the histological cytoprotection induced by 200 mL/L ethanol. Although the administration of 500 mL/L ethanol to normal rats did not cause any significant activation of inducible NO synthase activity, the activity of constitutive NO isozyme was inhibited. Production of NO from the calcium-dependent constitutive enzyme has been known to play a role in the modulation of gastric mucosal integrity[32]. The general suppression of this constitutive isozyme in gastritis mucosa could be due to the loss of endothelial integrity. Alternatively, the gastroprotection caused by 200 mL/L ethanol and 50 g/L NaCl, which concurrently maintained the constitutive NO synthase activity following 500 mL/L ethanol challenge in normal animals, must be due to the preservation of endothelium and hence to maintain vascular integrity. However, L-NAME pretreatment only reversed the protective action of 200 mL/L ethanol but not that of 50 g/L NaCl, suggesting that the involvement of NO from the constitutive form in the anti-lesion action of NaCl is still uncertain. Nevertheless, the suppression of constitutive NO synthase activity and the loss of its activation by 200 mL/L ethanol in the gastritis mucosa may explain why gastritis provoked ethanol ulceration and attenuated the protective action of 200 mL/L ethanol, respectively[33]. In addition, the damaged mucosa could activate the inducible NO isozyme and trigger the release of more free radicals, thus producing extensive tissue necrosis under gastritis condition.

Local SOD has been shown to abolish the gastric mucosal injury induced by the cytotoxic level of NO, possibly due to the prevention of peroxynitrite formation from interaction between superoxide and cytotoxic NO[34]. Our results demonstrated that under gastritis condition, SOD activity was significantly elevated. This could be a defensive mechanism of the gastric mucosa that would be activated when a tremendous amount of superoxide and other free radicals was produced following severe tissue injury. Indeed, acute administration of either mild irritants or 500 mL/L ethanol did not induce a similar increase in SOD activity in the gastric mucosa. Hence, the acute protective action of mild irritants in both normal and gastritis rats did not seem to involve the modulation of mucosal SOD activity.

It was reported in a human study that the basal release of PGE_2 and LTC_4 in alcoholics was higher than that in healthy volunteers, and that alcohol administration could cause an increase in PGE_2 and LTC_4 in healthy volunteers[35]. In addition, by using a rat model, the same group of investigators further proposed that the anti-lesion effect of some gastroprotective agents was related to the inhibition of LTC_4 formation and an increase in PGE_2 biosynthesis in the gastric mucosa[36]. Our findings in fact indicated that LTC_4 level in the mucosa was significantly increased in gastritis rats, which was similar to the state in alcoholics. Moreover, the challenge with 500 mL/L ethanol also stimulated an increase in LTC_4 level, as in the case of healthy volunteers. In fact, increased synthesis of mucosal PGE_2 in alcoholics could also indicate a defensive mechanism of the mucosa, mainly to counteract with the proinflammatory action of LTC_4 generated from the tissue. In the present study, mild irritants were able to induce a significant inhibition on the release of LTC_4 in both normal and gastritis mucosa. Reduction of this autacoid would attenuate vascular disturbances and decrease hemorrhagic lesions in the gastric mucosa[37].

In summary, aggravation in 500 mL/L ethanol-induced gastric mucosal damages under gastritis condition can be due to increased mucosal biosynthesis of inducible NO and LTC_4. The protective action of 200 mL/L ethanol could be restricted to the surface mucosal cells, thus having no effect on deeper histologic lesions. In general, deep histologic protection by mild irritants could not be found in gastritis animals. On the other hand, mild irritants could also act by reducing gastric mucosal LTC_4 level, which can lessen gross vascular injury and subsequently reduce hemorrhagic lesion even in gastritis mucosa.

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