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GASTROENTEROLOGY

Gastric antisecretory and antiulcer effects of simvastatin in rats

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Abstract

Background and Aim: Recently, statins have appeared to have additional benefits beyond their lipid lowering effects, which has led to the interest in the use of this class of drugs outside the realm of cardiovascular disease. Simvastatin (SIM) is a commonly prescribed statin with anti-inflammatory and antioxidant properties. Excessive generation of oxygen-derived free radicals (ODFR) and proinflammatory mediators has been implicated in the pathogenesis of gastric ulcers. This investigation aimed to study the effect of SIM on experimentally induced gastric acid secretion and ulcer formation.

Methods: Adult Wistar rats were divided into experimental groups containing six animals. Acid secretion studies were undertaken using pylorus-ligated rats pretreated with SIM (20, 40, and 60 mg/kg). The effect of orally administered SIM was also studied on indomethacin- and ethanol-induced gastric ulcers. The levels of myeloperoxidase (MPO), non-protein sulfhydryls (NP-SH), nitric oxide (NO), antioxidant enzymes, and gastric wall mucus were measured in the glandular stomach of rats following ethanol-induced gastric lesions.

Results: Administration of SIM significantly and dose-dependently inhibited the volume of gastric secretion and the acidity. Pretreatment with SIM significantly reduced the formation of indomethacin- and ethanol-induced gastric lesions. The antiulcer activity of SIM was associated with significant attenuation of adverse effects of ethanol on gastric wall mucus, NP-SH and MPO. SIM modified the gastric NO levels and reversed the ethanol-induced decrease in glutathione-S-transferase and increase in superoxide dismutase and catalase.

Conclusions: These findings clearly suggest the involvement of proinflammatory agents and ODFR in the pathogenesis of gastric lesions. The gastroprotective effects of SIM are mediated by inhibition of neutrophils activity, reduction of oxidative stress, and maintenance of vascular integrity. This study was conducted in rats; its relevance to human gastric ulcers is not known and warrants further study.

Introduction

The pathogenesis of gastric ulcers is complex and multifactorial. It is generally believed that adequate gastric mucosal blood flow is crucial for preventing the back-diffusion of gastric acid and maintaining gastric mucosal integrity.[1] Gastric circulation is regulated by local metabolic factors such as prostaglandins, leukotrienes, platelet aggregating factor, and other endogenous chemical mediators in mucosa.[2] During the past decade, the endothelial-derived relaxation factor nitric oxide (NO) has been recognized as one of the important mediators for the regulation of gastric mucosal microcirculation, repair, and integrity.[3–5] Inhibition of NO synthesis has been shown to produce acute gastric mucosal damage,[6,7] whereas enhancement of NO synthesis exerts gastroprotective effects.[8] Ischemic insult to intestinal mucosa is accompanied by enhanced generation of oxygen-derived free radicals (ODFR) that may initiate a chain of reactions in membrane-bound lipids causing lipid peroxidation and cellular injury.[9,10] An imbalance between gastrotoxic agents and protective mechanisms results in acute inflammation leading to gastric mucosal injury.[11]

Statins are a group of drugs defined as inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and have been recognized as the most effective therapeutic agents for reducing serum cholesterol levels. Interestingly, statins appear to have additional benefits beyond their lipid lowering effects, which has led to the interest in the use of this class of drugs outside the realm of cardiovascular disease.[12–14] Simvastatin (SIM) is a commonly prescribed statin with anti-inflammatory[15,16] and antioxidant[17–20] properties. SIM promotes endogenous NO production,[21–23] decreases platelet aggregation and inhibits thromboxane formation.[24] Recently, SIM has been shown to protect against ischemic reperfusion injury of lung,[25] kidney,[26] heart,[27] and brain[28] in experimental animals. Although the antiulcer activity of a lipid-lowering drug probucol has been demonstrated in rats,[29] the effect of SIM on gastric mucosal injury had remained unexplored. This investigation was therefore aimed to study the

effect of SIM on experimentally induced gastric acid secretion and ulcer formation in rats.

Materials and methods

Wistar rats of either sex, weighing 220 ± 20 g, fed on a standard chow diet were maintained in a temperature and humidity controlled room at 12 h light/dark cycles. The animals were divided into experimental groups of six animals each. The distribution of animals into groups and the treatment allotted to each group were randomized. The protocol of animal study was approved by Research and Ethical Committee of Armed Forces Hospital, Riyadh, Saudi Arabia, and the guidelines of animal care were strictly adhered during animal maintenance and experimentation.

The aqueous solution of ulcerogens and SIM were freshly prepared before administration. SIM was administered orally in doses of 20, 40, and 60 mg/kg body weight daily for a period of 7 days, while the last dose was given 1 h before gastric secretion and ulcer studies. The animals were sacrificed, and their stomachs were removed and opened along the greater curvature. After washing with saline, the gastric lesions were quantified by a person blinded to the treatment protocol. The ulcers were scored according to the method devised by Valcavi *et al*.[30] The circular ulcers induced by indomethacin were assessed on the basis of their diameters: deep circular ulcers more than 8 mm diameter = 10; 7–8 mm = 8; 6–7 mm = 7; 5–6 mm = 6; 4–5 mm = 5; 3–4 mm = 4; 2–3 mm = 3; 1–2 mm = 2; and <1 mm = 1. Deep linear ulcers 10 mm or more in length were scored 3. The scores of each single lesion were then summed up for determination of the ulcer index. Patched lesions of the stomach induced by 100% ethanol were scored according to the method described by Schiantarelli *et al*.,[31] using the following scale; 0 = normal mucosa; 1 = hyperemic mucosa or up to three small patches; 2 = four–10 small patches; 3 = more than 10 small or up to three medium-sized patches; 4 = four–six medium-sized patches; 5 = more than six medium-sized or up to three large patches; 6 = four–six large patches; 7 = seven–10 large patches; 8 = more than 10 large patches or extensive necrotic zones. 'Small' was defined as up to 2 mm across (maximum diameter), 'medium-sized' as between 2 and 4 mm across, and 'large' as more than 4 mm across.

Gastric acid secretion studies: pylorus ligated (Shay) rats

The animals were fasted for 36 h with access to water ad libitum before the pylorus was ligated under ether anesthesia, care being taken not to cause bleeding or to occlude blood vessels.[32] The animals were sacrificed 6 h after pylorus ligation. The stomachs were removed and its contents were collected, with the volume measured, centrifuged, and subjected to analysis for titratable acidity against 0.01 N NaOH to pH 7 for total acid output calculation.

Indomethacin-induced gastric lesions

Indomethacin was suspended in 1% carboxy methylcellulose in water and administered by gavage at the dose of 30 mg/kg body weight.[33] The animals were sacrificed 6 h after indomethacin administration.

Gastric lesions induced by ethanol (cytoprotection studies)

The animals were administered 1 mL of 100% ethanol by gavage.[34] One hour after the administration of ethanol, the animals were sacrificed and examined for lesions in the stomachs. The assays of gastric wall mucus, myeloperoxidase (MPO), and glutathione (GSH) in the stomach were done as follows.

Determination of gastric wall mucus

Gastric wall mucus was determined according to the modified procedure of Corne *et al*.[35] The glandular segment of the stomach was separated from the lumen of the stomachs, weighed and transferred immediately to 10 mL of 0.1% w/v Alcian blue solution (in 0.16 mol sucrose solution buffered with 0.5 mL sodium acetate at pH 5). Tissue was stained for 2 h in Alcian blue and excess dye was removed by two successive rinses with 10 mL of 0.25 mol sucrose. Dye complexed with the gastric wall mucus was extracted with 10 mL of 0.5 mol magnesium chloride, which was intermittently shaken for 1 min at 30 min intervals for 2 h. Four milliliters of blue extract were then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3000 *g* for 10 min and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of wet glandular tissue was then calculated.

Determination of MPO

MPO activity in the gastric mucosa was measured according to the methods described earlier.[36] Preweighed tissue was homogenized (1:10 wt/vol) in 0.5% hexadecyltrimethyl ammonium bromide (ICN, Cleveland, OH, USA) in 50 mmol potassium phosphate buffer (pH 6.0) before sonication in an ice bath for 20 s. Three freeze/thaw cycles were performed followed by sonication (20 s in ice bath). The samples were centrifuged at 17 000 *g* (5 min, 4°C) and MPO in the supernatant was assayed by mixing 0.1 mL of supernatant with 2.9 mL of 50 mmol/1 potassium phosphate buffer (pH 6.0) containing 0.167 mg/mL O-dianasidine dihydrochloride (ICN, Cleveland, OH, USA) and 0.0005% hydrogen peroxide (Riedel, Seelze, Germany). The change in absorbance at 460 nm was measured for 4 min using UV-visible spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan).

Determination of non-protein sulfhydryls (NP-SH)

Gastric mucosal NP-SH levels were measured according to the method reported by Owen.[37] The glandular part of stomach was homogenized in ice-cold perchloric acid (0.2 mol) containing 0.01% of EDTA. The homogenate was centrifuged at 10 000 *g* for 10 min. The enzymatic reaction was initiated by adding 100 µL of clear supernatant in a spectrophotometric cuvette containing 800 µL of 0.3 mmol reduced nicotinamide adenine dinucleotide phosphate (NADPH), 100 µL of 6 mmol 5,5-dithiobis-2-nitrobenzoic acid (DTNB), and 10 µL of 50 units/mL glutathione reductase (all the above three reagents were freshly prepared in phosphate buffer at pH 7.5). The absorbance was measured over a period of 4 min at 412 nm at 30°C. The NP-SH level was deter-

mined by comparing the change of absorbance (ΔA) of the test solution with the ΔA of the standard solution.

Determination of antioxidant enzymes

Stomach tissues were homogenized (10% w/v) in ice-cold phosphate buffer (0.1 mol, pH 7.4). The supernatants collected after centrifugation (10 000 *g*, 20 min, 4°C) were used for the assay of enzymes' activities.

The method of Habig *et al*.[38] was used with some modifications to estimate the activity of glutathione S-transferase (GST). In a final volume of 2 mL, the reaction mixture consisted of 0.1 mol phosphate buffer, 1 mmol reduced glutathione, 1 mmol 1-chloro-2,4-dinitrobenzene (CDNB), and tissue homogenate. The GST activity determined as nmol CDNB conjugate formed min/mg protein using a molar extinction coefficient of 9.6×10^3 M^{-1} cm^{-1}.

Superoxide dismutase (SOD) activity was determined according to the method described by Marklund and Marklund.[39] The reaction mixture consisted of 0.5 mL of tris-buffer (50 mM; pH-8.2), 0.5 mL pyragallol (0.5 mmol), 0.5 mL EDTA (1 mmol), and in different volumes, 0.025 mL, 0.05 mL, 0.075 mL, and 0.1 mL of tissue homogenate. The change in absorbance was recorded at 420 nm. Activity was reported by its ability to inhibit 50% reduction of pyragallol and the result is expressed as mU/min/mg protein.

Catalase (CAT) activity was assayed by the method devised by Claiborne.[40] The reaction mixture consisted of 0.1 mol phosphate buffer (pH 7.4), 0.019 M H_2O_2 and tissue homogenate in a total volume of 3 mL. The enzyme activity was expressed as μmol H_2O_2 consumed/min/mg protein.

Determination of NO

NO was estimated according to Navarro-Gonzálvez's method.[41] Tissue homogante (300 μL) was deproteinized by adding 250 μL of 75 mmol/L $ZnSO_4$ solution, stirring and centrifuging at 10 000 *g* for 1 min at room temperature, after which 350 μL of 55 mmol/L NaOH were added. Again, the solution was stirred and centrifuged at 10 000 *g* for 3 min and the supernatant was recovered, followed by the dilution of an aliquot (500 μL) with 1.5 mL of glycine buffer (pH 9.7). Cadmium granules were rinsed three times with distilled water and swirled in a 5 mmol/L $CuSO_4$ solution in glycine buffer for 5 min. The copper-coated granules were to be used within 10 min. Cadmium granules (1.5 mg) were added to reduce the nitrate into nitrite. After continuous stirring for 10 min at room temperature, 500 μL of the reduced sample was taken in a separate tube and 500 μL of Gries reagent (1% sulfanilamide, 0.1% N-(1-Naphthyl) ethylene-diamine, in 5% H_3PO_4) was added to it; this was kept at room temperature for 20 min for development of pink color. The absorbance was measured at 540 nm. Calibrators at various concentrations were prepared by diluting 20 mmol/L stock solution of $NaNO_2$. The results were expressed as nmol NO/g of tissue.

Histology of ethanol-induced gastric lesions

The stomach was opened along the greater curvature, washed with saline and fixed in 10% neutral buffered formalin for 24 h. The specimens were then processed overnight for dehydration and clearing steps, using an automatic tissue processor (Shandon Southern 2 L Processor MKII; Runcorn, Cheshire, UK). The specimens were embedded in paraffin blocks and sections of 5 μm thickness were stained with hematoxylin–eosin for light microscopy observations.

Statistics

Data were analyzed by ANOVA, followed by Dunnett's multiple comparison tests. Differences with a *P*-value less than 0.05 were considered statistically significant.

Results

Effect of SIM on the gastric secretion in 6 h pylorus-ligated (Shay) rats

In control rats, pylorus ligation for 6 h resulted in accumulation of 10.3 ± 0.64 mL of gastric secretion (Table 1). The volume of gastric secretion in the rats treated with 40 mg/kg and 60 mg/kg of SIM significantly reduced to 7.2 ± 0.77 mL and 5.5 ± 1.04 mL, respectively (ANOVA $F = 4.76$, $P < 0.01$). A significant decrease in total acid output was observed in the rats treated with 20 mg/kg (604 ± 75.0 mEq), 40 mg/kg (476 ± 69.4 mEq), and 60 mg/kg (267 ± 46.1 mEq) of SIM, as compared to 868 ± 63.2 mEq in control group (ANOVA $F = 15.28$, $P < 0.001$) (Table 1).

Effect of SIM on indomethacin-induced gastric mucosal damage

The administration of indomethacin resulted in production of gastric lesions mainly in the glandular stomach in all the animals. The lesion area in the control group was found to be 27.0 ± 1.5 mm^2. Pretreatment of rats with SIM in the doses of 20 mg/kg (lesion area, 10.8 ± 2.1 mm^2), 40 mg/kg (lesion area, 8.8 ± 1.8 mm^2), and 60 mg/kg (lesion area, 8.1 ± 0.8 mm^2) significantly decreased the intensity of indomethacin-induced ulcers (ANOVA $F = 28.12$, $P < 0.001$, Fig. 1).

Effect of SIM on ethanol-induced gastric lesions

The treatment of rats with ethanol produced extensive gastric lesions in the glandular mucosa of stomach in 100% of the control animals. These lesions were characterized by multiple hemorrhagic red bands (patches) of different size along the axis of the glandular

Table 1 Effect of simvastatin (SIM) on gastric secretion and acidity in 6 h pylorus ligated (Shay) rats

Treatment	Dose of SIM (mg/kg)	Volume of gastric secretion (ml)	Total acid output (mEq)
Control†	0	10.3 ± 0.6	868 ± 63
SIM	20	8.0 ± 1.0	604 ± 75*
SIM	40	7.2 ± 0.7*	476 ± 69**
SIM	60	5.5 ± 1.0**	267 ± 46**

*$P < 0.05$ and **$P < 0.01$ *vs* control group using Dunnett's test.
†Ligation only. Values are means ± standard error of means.

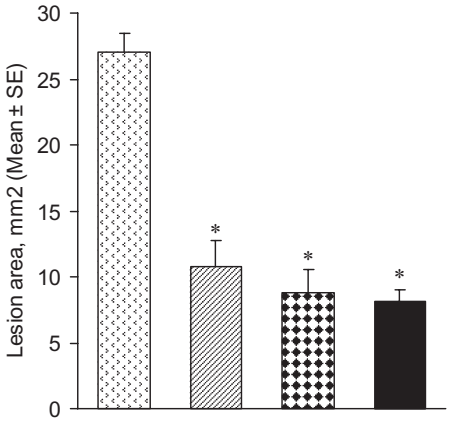

Figure 1 Effect of simvastatin (SIM) on gastric mucosal damage induced by indomethacin in rats (six animals in each group). *$P < 0.001$ *vs* SIM 0 mg/kg (indomethacin only) group using Dunnett's test. (▫), SIM 0 mg/kg; (▨), SIM 20 mg/kg; (▩), SIM 40 mg/kg; (■), SIM 60 mg/kg.

stomach (Fig. 2). The ulcer index was found to be 7.1 ± 0.3 in control animals (Fig. 3). Pretreatment of rats with SIM in the doses of 20 mg/kg (ulcer index, 4.8 ± 0.6), 40 mg/kg (3.6 ± 0.6), and 60 mg/kg (2.5 ± 0.4) significantly inhibited the formation of gastric lesions (ANOVA $F = 15.62$, $P < 0.001$). Histological examination of gastric mucosa showed the appearance of ethanol-induced lesions in the form of gastric pits with detachment of the surface epithelium (Fig. 4). Epithelial cells appeared to be vacuolated and microvessels elongated. Pretreatment with SIM dose-dependently prevented ethanol-induced mucosal damage (Fig. 4).

Effect of SIM on ethanol-induced changes in gastric wall mucus

The treatment of rats with ethanol significantly decreased the Alcian blue binding capacity of gastric wall mucus (676 ± 23.0 μg Alcian blue/g of tissue) as compared to control rats (969 ± 28.9 μg/g). Pretreatment of animals with SIM in the doses of 20 mg/kg (833 ± 36.7 μg/g), 40 mg/kg (872 ± 25.3 μg/g), and 60 mg/kg (924 ± 31.9 μg/g) significantly enhanced the Alcian blue binding capacity of gastric mucosa in a dose-dependent manner (ANOVA $F = 15.04$, $P < 0.001$, Fig. 5a).

Effect of SIM on ethanol-induced depletion of gastric mucosal NP-SH

The level of NP-SH in the gastric mucosa of control animals was 658.3 ± 69.1 nmol/g of tissue. The NP-SH were significantly decreased to 132.5 ± 43.8 nmol/g of tissue following the administration of 100% ethanol. Pretreatment of rats with SIM in the dose of 60 mg/kg (NP-SH, 314 ± 63.4 nmol/g) significantly inhibited ethanol-induced depletion of NP-SH (ANOVA $F = 16.57$, $P < 0.001$, Fig. 5b).

Effect of SIM on ethanol-induced changes in gastric MPO activity

Changes in gastric accumulation of leukocytes following ethanol-induced lesions were evaluated by measuring gastric MPO activity, which was found to be significantly increased as compared to control mucosa (Fig. 5c). Pretreatment with SIM significantly attenuated ethanol-induced increase in gastric MPO activity in rats (ANOVA $F = 11.29$, $P < 0.001$).

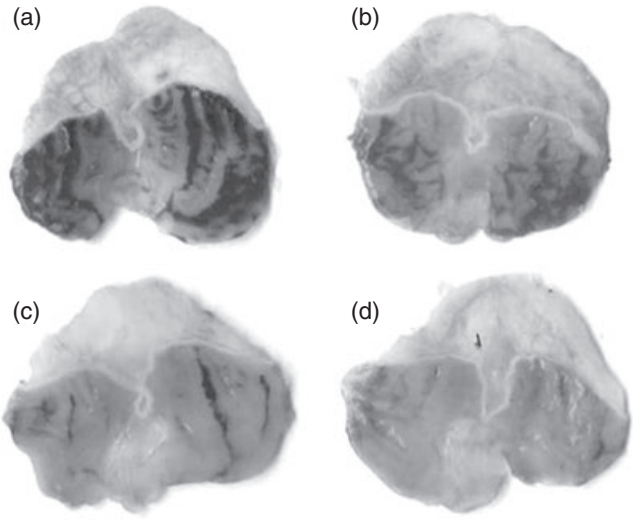

Figure 2 Morphological appearance of ethanol-induced band like hemorrhagic lesions in the stomach of rats treated with (a) vehicle, (b) SIM 20 mg/kg, (c) SIM 40 mg/kg and (d) SIM 60 mg/kg.

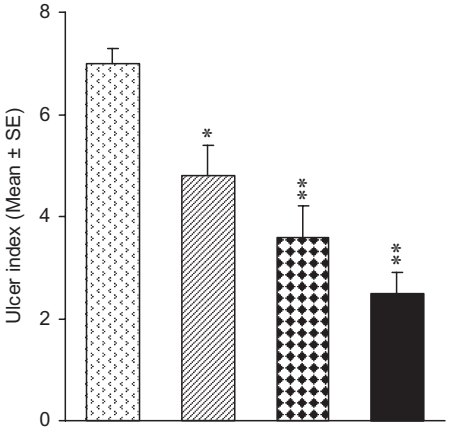

Figure 3 Effect of simvastatin (SIM) on gastric mucosal damage induced by ethanol in rats (six animals in each group). *$P < 0.01$ and **$P < 0.001$ *versus* SIM 0 mg/kg (ethanol only) group using Dunnett's test. (▫), SIM 0 mg/kg; (▨), SIM 20 mg/kg; (▩), SIM 40 mg/kg; (■), SIM 60 mg/kg.

Effect of SIM on ethanol-induced changes in gastric NO

Administration of ethanol significantly reduced gastric NO levels (ANOVA $F = 26.50$, $P < 0.001$). The lower and medium doses of SIM significantly reversed the effect of ethanol on gastric NO, whereas the high dose of SIM insignificantly increased gastric NO levels (Table 2).

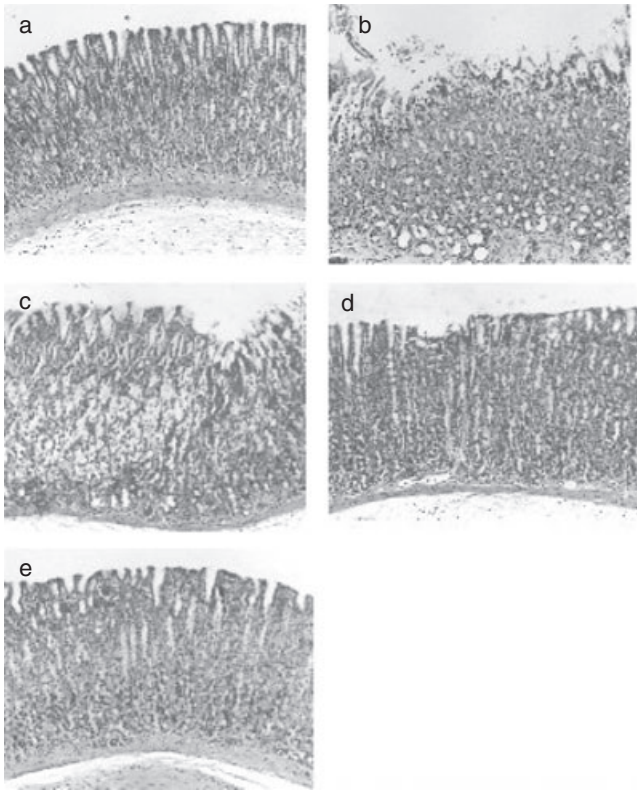

Figure 4 Light micrographs showing the effect of SIM on ethanol-induced gastric lesions. Administration of ethanol produced lesions in the form of gastric pits with detachment of the surface epithelium; epithelial cells appeared to be vacuolated and microvessels elongated (b) as compared to normal mucosa (a). Pretreatment of rats with SIM 20 mg/kg (c), 40 mg/kg (d), and 60 mg/kg (e) dose-depenently protected against ethanol-induced lesions.

Effect of SIM on ethanol-induced changes in gastric antioxidant enzymes

Administration of ethanol significantly decreased gastric mucosal GST (ANOVA $F = 7.39$, $P < 0.001$) and increased SOD (ANOVA $F = 98.17$, $P < 0.001$) and CAT (ANOVA $F = 3.99$, $P < 0.05$) activities (Table 2). All the three doses of SIM significantly reversed the effect of ethanol on GST, whereas only medium and high doses of SIM significantly reduced ethanol-induced increase in SOD activity. All the three doses of SIM failed to produce any significant effect on ethanol-induced changes in CAT (Table 2).

Discussion

In the present study, the effect of SIM was studied on gastric acid secretion using the Shay model,[32] which is a simple and reliable method for predicting the antisecretory activities of various agents.[42–44] Pretreatment with SIM reduced the acidity and volume of gastric secretions in Shay rats (Table 1). The increase in gastric acidity is considered an important contributing factor in the pathogenesis of gastric ulcers.[45] NO plays an important role in regulating acid secretion and maintaining the integrity of gastric mucosa against hyperacidity or exposure to ulcerogens.[43,46,47] The beneficial effects of NO on wound repair may be attributed to its functional influences on angiogenesis and inflammation.[48] The ulcerogenic effects of indomethacin and ethanol are accompanied by impaired gastric blood flow and vascular injury.[49–51] Drugs with the ability to reduce acid secretion[52,53] and/or improve microcirculation[54–56] have been shown to attenuate gastric lesions. NO has been recognized as a basic mediator in the regulation of gastric mucosal microcirculation.[3–5] However, NO is a double-edged weapon exerting either protective or destructive effects depending on the extent of NO synthesis. It has been demonstrated that NO generated from endothelial NO synthase (eNOS) plays an important role in gastric ulcer formation and healing,[57] whereas NO generated from inducible NO synthase (iNOS) participates in ulcer formation through the production of ODFR and their cytotoxic action.[58] We observed that the high dose of SIM (60 mg/kg) was not as potent as the low and medium doses of SIM in improving gastric NO levels (Table 2). This may be explained by considering the effects of SIM in increasing eNOS and inhibiting iNOS for optimal regulation of NO production.[59] Thus, the gastroprotective effects of SIM may be attributed to a proper regulation of NO[21,23,59] and a tandem elimination of ODFR.[17,19]

Our results showed that SIM had a significant and dose-dependent protective effect against indomethacin-induced gastric lesions (Fig. 1). Gastropathy associated with non-steroidal anti-inflammatory drugs (NSAIDs) is a major public health problem.[60,61] NSAIDs-induced gastric lesions are accompanied by increased gastric acidity, imbalance of arachidonic acid metabolites,[62,63] elevated oxidative stress,[10] and enhanced neutrophil activity.[64] SIM-induced gastroprotective effects may be attributed to its ability to reduce acidity (Table 1), decrease thromboxane formation,[24] scavenge free radicals,[17,19] and inhibit neutrophil activity.[65]

Pretreatment with SIM significantly protected gastric mucosa against ethanol-induced injury (Figs 2–4). The cytoprotective effect of SIM was accompanied by attenuation of ethanol-induced increase in MPO (a marker of neutrophil activity), depletion of gastric wall mucus, and NP-SH (Fig. 5). Neutrophils are the major inflammatory cell type infiltrating the injured mucosa following exposure to ethanol.[66] Strategies to counteract the infiltration and/or activation of neutrophils have been shown to protect animals against gastric ulcers.[67,68] Activated neutrophils injure the microvasculature via the release of ODFR and/or proteases including elastase, collagenase, and cathepsin G.[69,70] A significant decrease in gastric NP-SH following ethanol administration indicated massive generation of ODFR (Fig. 5). Our findings are in agreement with earlier reports showing depletion of sulfhydryls in ethanol-induced gastric lesions.[71,72] The treatment of rats with glutathione depletors has been shown to significantly potentiate ulcerogen-induced gastric mucosal injury,[73] whereas increase in mucosal NP-SH exerts a gastroprotective effect.[74,75] Administration of ethanol significantly reduced gastric GST (Table 2). The GSH-GST system is ubiquitous for the metabolic detoxification of xenobiotics. The enzyme GST destroys the toxins by covalently linking GSH (NP-SH) to the toxic species, forming a less reactive GSH-S-conjugate which is catabolilzed and excreted.[76,77] In addition to catalyzing GSH conjugation, GST also exhibits glutathione peroxidase activity, which suggests a role in protection against oxidant injury. Both GSH and GST act in concert and their replenishment by SIM could have a direct implication in protection against ethanol-induced oxidative stress and ulceration.

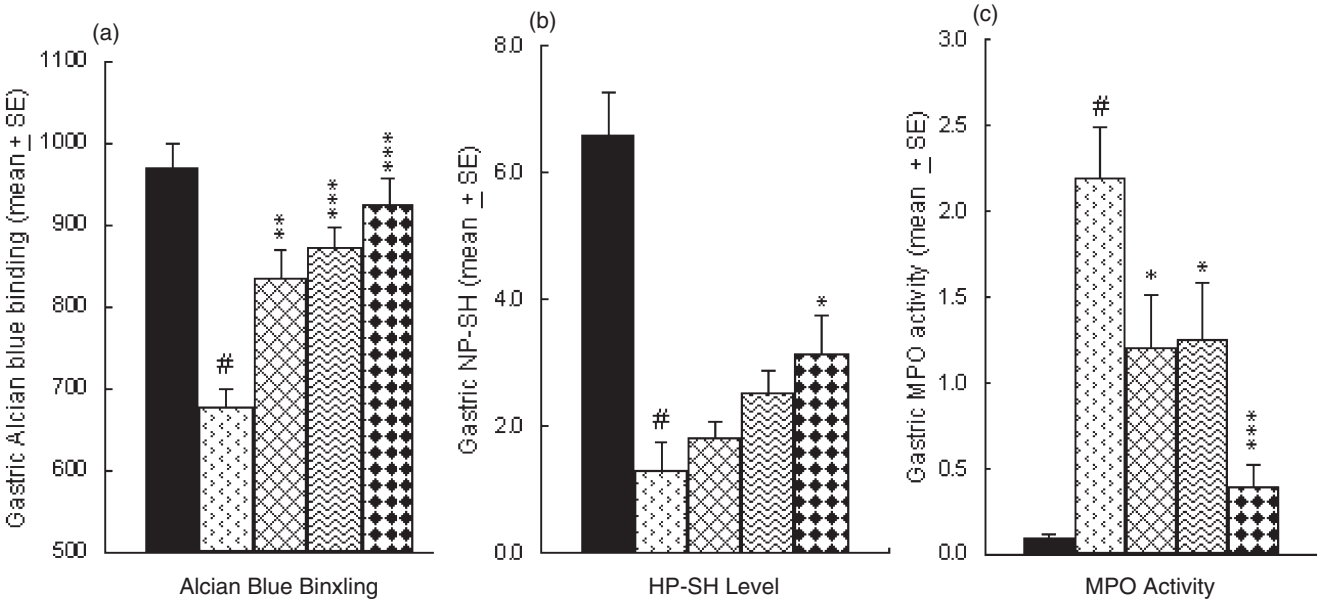

Figure 5 Effect of simvastatin (SIM) on ethanol induced changes in (a) Alcian blue binding capacity, (b) NP-SH levels, and (c) MPO activity and in gastric mucosa of rats. #$P < 0.01$ and #$P < 0.001$ *vs* control group, and *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ *vs* SIM-only group (Dunnett's test). (■), control; (▤), EtOH+SIM 0 mg/kg; (▨), EtOH+SIM 20 mg/kg; (▩), EtOH+SIM 40 mg/kg; (◧), EtOH+SIM 60 mg/kg.

Table 2 Effect of simvastatin (SIM) on ethanol-induced changes in gastric mucosal nitric oxide and antioxidant enzymes

Treatment	Dose of SIM (mg/kg)	NO	GST	SOD	CAT
Control	0	61.7 ± 5.9	238 ± 18	179 ± 10	61.0 ± 4.5
Ethanol	0	19.6 ± 2.3##	121 ± 11##	593 ± 29##	97.3 ± 7.1#
Ethanol + SIM	20	34.1 ± 1.9*	196 ± 22*	549 ± 16	91.0 ± 7.2
Ethanol + SIM	40	35.9 ± 2.1*	215 ± 10**	291 ± 19***	90.9 ± 8.1
Ethanol + SIM	60	25.2 ± 0.6	202 ± 15**	208 ± 18***	85.9 ± 6.4

Values are means ± standard error of means. The units of biochemical observations are as follows: nitric oxide (NO), nmol NO formed/g tissue; glutathione-S-transferase (GST), nmol CDNB formed/g tissue; superoxide dismutase (SOD), mU SOD/mg protein; catalase (CAT), μmol H_2O_2 consumed/g tissue/min.

#$P < 0.01$ and ##$P < 0.001$ *vs* control group; *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ *vs* Ethanol (ulcer only) group using Dunnett's multiple comparison test.

The metabolism of ethanol generates superoxide radicals (O_2^-) which may in turn promote lipid peroxidation.[68,78] The enzyme SOD dismutes O_2^- into H_2O_2, which is scavenged by catalase and glutathione peroxidase. Administration of ethanol significantly increased SOD and CAT activities (Table 2). A significantly high SOD activity in ethanol-treated rats indicates increased production of O_2^-, as an elevated O_2^- level is thought to increase the concentration of cellular SOD.[79] Increased levels of SOD and CAT in response to noxious stimuli play an important role in the protection of oxidative stress.[80–82] Pretreatment with medium and high doses of SIM protected rats against ethanol-induced generation of O_2^-, as evidenced by the significantly lower activity of SOD in these animals (Table 2). SIM is a potent scanvenger of ODFR[17,19] and improves enzymatic antioxidant parameters like SOD, CAT, and glutathione peroxidase.[18,20] Thus, the cytoprotective effect of SIM against ethanol-induced gastric injury may be linked with its antioxidant[17,19] and/or anti-inflammatory properties.[15,16,65]

In conclusion, the findings of this study clearly demonstrate the protective effects of SIM against chemically induced gastric lesions. Maintenance of vascular integrity, inhibition of neutrophils activity and reduced oxidative stress may define the gastroprotective effects of SIM in experimental ulcers.

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