Gastroenterologia Japonica
Vol. 24, No. 5

Printed in Japan

—Original Article—

Role of superoxide and hydroxyl radicals in rat gastric mucosal injury induced by ethanol

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Summary: It has been reported that oxygen-derived free radicals play an important role in the pathogenesis of mucosal injury in the small intestine as well as in the stomach. The aims of this study were to test whether ethanol-induced damage in the rat stomach was prevented by the administration of (1) superoxide dismutase (SOD; a scavenger of superoxide radicals), (2) allopurionol (ALP; an inhibitor of xanthine oxidase), (3) dimethyl sulfoxide (DMSO; a scavenger of hydroxyl radicals). SOD significantly decreased the ulcer index from 100±8.5% (control) to 39.6±8.2% ($P<0.001$). Ethanol-induced damage was reduced by the administration of ALP by 37.4% ($P<0.01$). DMSO also diminished the ulcer index from 100±8.5% (control) to 31.6±5.8% ($P<0.01$). Histochemical studies supported these results. A scanning EM study, however, revealed that surface epithelial cells were not protected by SOD against ethanol-induced damage. These results demonstrated that SOD, ALP and DMSO had the ability to protect gastric mucosa against ethanol-induced injury. Accordingly, oxygen-derived free radicals may be involved in the pathogenesis of ethanol-induced gastric mucosal damage. Surface epithelial cells, however, were not protected even by SOD against ethanol-induced injury. *Gastroenterol Jpn 1989;24:488–493*

Key words: *ethanol-induced damage; free radicals; gastric mucosa; protective effect; scanning electron micrograph*

Introduction

There is substantial evidence that oxygen-derived free radicals play an important role in the pathogenesis of the injury of various tissues including the digestive system[1-4].

It has been reported by Granger and his colleagues[5-8] that oxygen-derived free radicals play a leading role in ischemia-induced intestinal damage. Recently, it has been demonstrated that the hepatic injury produced by Corynebacterium parvum or diet-induced hemorrhagic pancreatitis are mediated by oxygen-derived free radicals[9,10].

Itoh and Guth[11] reported that superoxide dismutase (SOD; a scavenger of superoxide radicals) and allopurinol (ALP; an inhibitor of xanthine oxidase), but not dimethyl sulfoxide (DMSO; a scavenger of hydroxyl radicals) prevented hemorrhagic shock-induced gastric lesions in the rat. Furthermore, Perry and his colleagues[8] demonstrated that treatment with allopurinol and SOD as well as DMSO significantly diminished the ischemia-induced lesions in the cat stomach. Most of these reports suggest that ischemia or shock-induced lesions are mediated by oxygen-derived free radicals. Cochran et al[12] demonstrated protection by DMSO against stress-induced rat gastric mucosal injury the mechanism of which involves

Receive December 8, 1988. Accepted March 17, 1989.
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This paper was presented in 87th Annual Meeting of American Gastroenterological Association (1986).

reaction with ischemia-induced cytotoxic free radicals. Hiraishi et al[13] reported oxygen metabolite-induced cytotoxiry in cultured rat gastric mucosal cells.

It has been recently reported that free radicals may play a role in ethanol-induced gastric mucosal lesions in rats[14,15]. However, no morphological study as to whether surface epithelial cells of gastric mucosa are protected by free radical scavengers or xanthine oxidase inhibitor has been performed yet.

Materials and Methods

Animals

Male Sprague-Dawley rats (Doken, Ibaragi, Japan) weighing 200-250 g were used. The animals were fasted in individual wire-bottom cages for 24 hours prior to the experiments. Water was also withheld for 3 hours before starting the studies.

Studies

SOD (15000 U/kg; Sigma) was administered intravenously 15 min. prior to ethanol instillation. ALP (100 mg/kg; Sigma) was given orally once a day for two days in advance. DMSO (20 mg/kg; Sigma) was administered intravenously 30 min. prior to ethanol administration. Following these procedures, absolute ethanol (5 ml/kg) was instilled intagastrically. After 60 min. of ethanol instillation, animals were sacrificed with ether and the stomachs were removed and then opened along the greater curvature.

As parameters of gastric damage, ulcer index (length of red streaks; percent of control) and light microscopic studies (HE & PAS) as well as electron microscopic studies (EM) were employed.

Macroscopic study

After rinsing the stomachs with tap water, the presence of necrotic lesins (length of red streaks) was counted by an observer who was unaware of the treatment. The results were expressed as a percentage of controls.

Light microscopic study

The opened stomachs obtained from six rats were cut transversely 5 mm distal from the ridge of the fundic glandular portion at a width of 5 mm. These specimens were fixed in 10% buffered formalin and stained with hematoxylin-eosin.

Scanning electron microscopy

The specimens were obtained from the rat fundic area and fixed for 3 hours in 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.2). After they were immersed in 0.1 M cacodylate buffer with 3.4% sucrose (pH 7.2) for more than 24 hours, they were refixed in 0.1 M cacodylate buffered 1.0% osmic acid for 1.5 hours. The samples were dehydrated using a graded series of ethanol (50%, 70%, 80%, 90%, 95%, and 100%) and then immersed in iso-amyl acetate solution. They were dried by the critical point method and coated with platinum palladium. They were observed by a scanning electron microscope (Hitachi S450 LB).

Statistical analysis

Statistical significance of ulcer indices was evaluated by Student's t-test. The results were expressed as means±SEM.

Results

Macroscopic study

Intragastric administration of 1 ml of 100% ethanol (ET) to control produced extensive red streaks (hemorrhagic necrosis) in the gastric fundic area (**Fig. 1a**). In contrast, SOD and ALP as well as DMSO pretreatment remarkably reduced the length of red streaks in comparison to controls (**Fig. 1b-d**). The quantitative results of macroscoic studies in respective groups are presented in **Figure 2**. (1) SOD significantly decreased the ulcer index from 100±8.5% (control) to 39.6±8.2% ($P<0.01$). (2) Ethanol-induced mucosal damage was reduced by the administration of ALP by 37.4% ($P<0.01$). (3) DMSO also diminished ulcer index from 100±8.5% (control) to 31.6±5.8% ($P<0.01$).

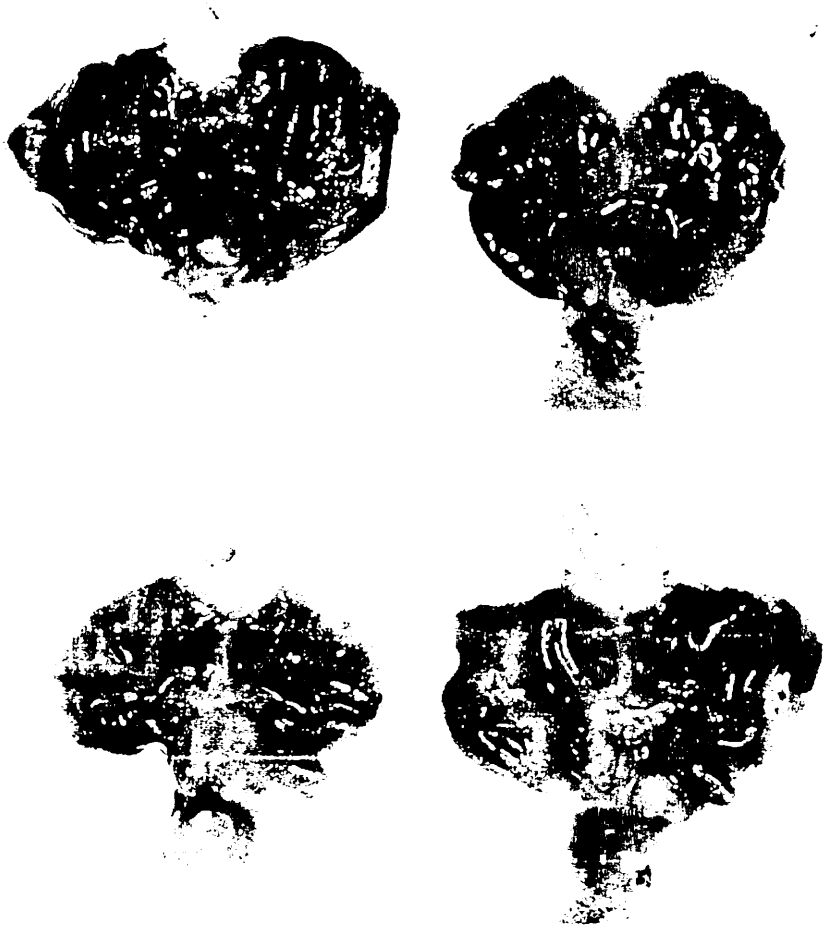

Fig. 1 The gross appearance of rat gastric mucosa.
a: Ethanol-induced gastric damage.
b: Superoxide dismutase (SOD)-pretreated stomach prior to ethanol injestion.
c: Allopurinol (ALP)-pretreated stomach.
d: Dimethyl sulfoxide (DMSO)-pretreated stomach.

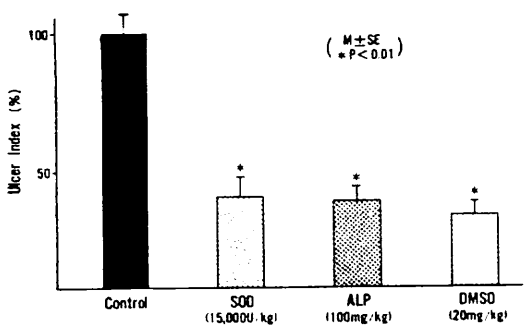

Fig. 2 Effect of superoxide dismutase (SOD), allopurinol (ALP) and dimethyl sulfoxide (DMSO) on ethanol-induced gastric demage in rat. Each bar represents the mean±SEM (control; n=10: others; n=6). Asterisks indicate statistically significant change *P<0.01 compared to control).

Histological study

Figure 3 shows the representative features of the results of a light microscoic study of gastric fundic mucosa (hematoxylin-eosin staining), using six animals respectively. In the control group, the surface epithelium was completely desquamated and intramucosal hemorrhage was observed (**Fig. 3b**). In contrast, SOD, ALP, and DMSO pretreatment prior to ET instillation markedly diminised the mucosal damage caused by 100% ET, although the surface of the mucosa was slightly exfoliated when compared with the control group (**Fig. 3c**). Hemorrhage was not observed in this group.

Fig. 3 Photomicrograph of hematoxylin and eosin-stained section of rat gastric fundic mucosa (×70). a b c
a: Normal fundic mucosa.
b: Fundic mucosa exposed to absolute ethanol for 60 min.
c: Fundic mucosa pretreated with SOD prior to absolute ethanol administration.

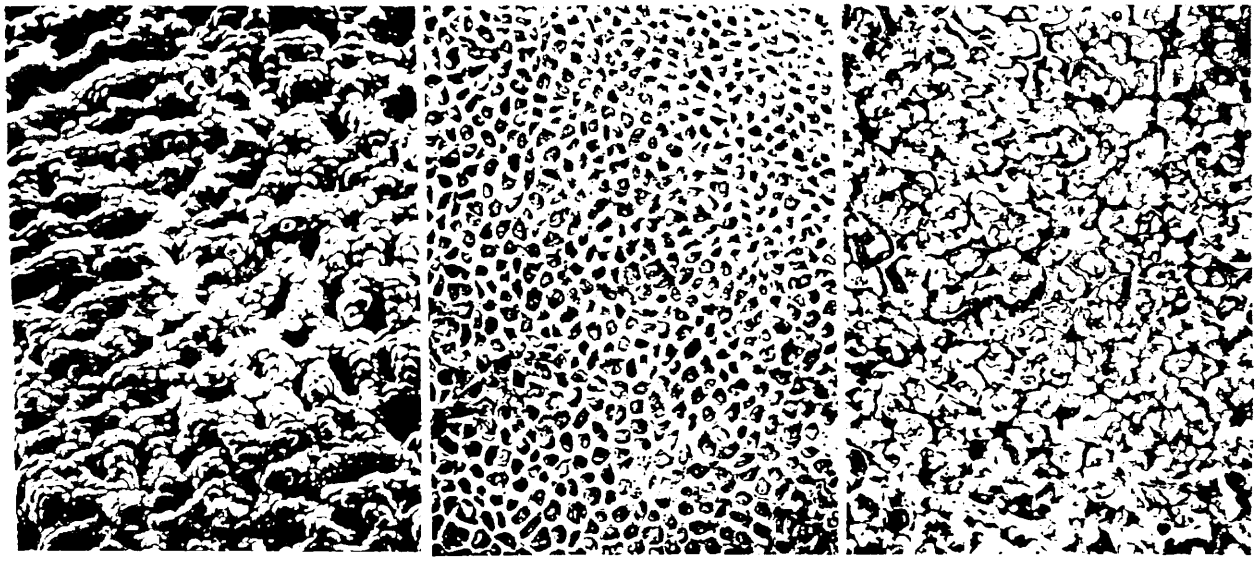

Fig. 4 Scanning electron micrograph of rat gastric fundic mucosa (×560). a | b | c
a: Normal rat gastric mucosa.
b: Fundic mucosa exposed to absolute ethanol for 60 min.
c: Fundic mucosa pretreated with superoxide dismutase (SOD) before ethanol ingestion.

Scanning electron microscopy:

Scanning electron microscopy demonstrated the extensive exfoliation of the surface epithelial cells and the denuded lamina propria observed after ethanol treatment (Fig. 4b).

The surface epithelial cells of the rat gastric

mucosa, however, were partially, but not completely, protected by pretreatment with SOD against ethanol-induced damage, whereas they seemed to be protected by such agents macroscopically as well as light-microscopically (**Fig. 4c**). These findings were similar in every group, respectively.

Discussion

The data obtained in this study demonstrated that SOD and ALP as well as DMSO significantly and remarkably reduced the gastric damage produced by administration of absolute ethanol. The source of free radicals in ethanol-induced gastric damage has not yet been elucidated. It is well known that production of superoxide occurs in vivo after activation and stimulation of macrophages and neutrophils[3]. Superoxide, however, may also be produced by tissues endogenously. Granger and his colleagues[5] hypothesized that the primary source of free radicals in ischemic lesions may be the enzyme xanthine oxidase. They also emphasized that reperfusion after ischemia may be a very important factor for generation of free radicals[5-8]. In the case of ethanol-induced gastric damage, it can be speculated that such phenomena, i.e., ischemia and subsequent reperfusion, may occur at the level of microcirculation[16]. In addition, xanthine oxidase may be the major source of free radicals in ethanol-induced damage, because ALP significantly diminished the lesion in our study.

Although the source of free radicals in ethanol-induced gastric damage remains elusive, it is suggested that oxygen-derived free radicals may play an important role in this kind of damage[14,15]. It is controversial which free radical, superoxide or hydroxyl radical, plays a leading role in the gastric damage. Itoh and Guth[11] indicated that superoxide plays an important role in the formaiton of gastric lesions produced by ischemia plus HCl, since both ALP and SOD significantly protected against hemorrhagic shock-induced gastric lesions, whereas DMSO did not. In contrast, Perry and his colleagues[8] have reported that the hydroxyl radical appears to be the major oxygen radical contributing to ischemic damage. In our study, SOD and ALP as well as DMSO significantly and equally reduced the gastric damage produced by absolute ethanol. Thus we speculated that both superoxide and hydoxyl radical have important roles in the pathogenesis of ethanol-induced gastric damage.

Although the gastric mucosa seemed to be protected by SOD against ethanol-induced damage macroscopically and light-microscopically, it is not clear whether gastric surface epithelial cells per se are truly protected by these agents. In the case of the cytoprotective effect of prostaglandins against ethanol-induced injury, it was revealed that gastric surface epithelial cells were damaged even by pretreatment with prostaglandins, while the gastric deeper layer was protected[17,18]. We, therefore, attempted to perform an ultrastructural study using a scanning electron microscope (SEM). The results revealed that gastric surface epithelial cells were not protected by the administraion of SOD against the injury produced by ethanol. Accordingly, it is suggested that the deeper layer of gastric mucosa such as mucous neck cells or lamina propria including gastric microcirculation may play a key role in the mechanisms of gastric mucosal damage.

In summary, we demonstrated that SOD, ALP and DMSO had the ability to protect gastric mucosa against ethanol-induced injury. Consequently, it is suggested that oxygen-derived free radicals, both superoxide and hydroxyl radical, may be involved in the pathogenesis of ethanol-induced gastric mucosal damage. Further investigation of the mechanisms of gastric mucosal injury induced by ethanol, e.g., the role of mucous neck cells and the gastric microcirculation as well as the gastric mucin is required.

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