Cetraxate Improves Ethanol-Induced Gastric Mucosal Congestion in Anesthetized Dogs

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ABSTRACT — Gastric mucosal microcirculatory disturbance in experimental animals is stressed as an important factor in the development of gastric ulceration induced by ethanol. In this study, we used a reflectance spectrophotometry system to investigate the effect of cetraxate on ethanol-induced gastric mucosal hemodynamics in anesthetized dogs. Forty percent ethanol caused a significant increase in the mucosal blood volume and a significant decrease in mucosal hemoglobin oxygenation. The changes in these parameters indicated mucosal congestion and tissue hypoxia. Topical administration of cetraxate (200 mg in 10 ml saline) prevented the increase in mucosal blood volume and decrease in mucosal hemoglobin oxygenation which had been induced by ethanol loading in the stomach. In conclusion, cetraxate showed a protective effect on mucosal microcirculation that resulted in the maintenance of the gastric mucosal integrity against ethanol loading in the gastric mucosa.

Keywords: Reflectance spectrophotometry system, Gastric mucosal hemodynamics, Gastric mucosal oxygenation, Cetraxate

Several authors investigated the mechanism of ethanol-induced gastric mucosal injury in experimental animals and humans and reported that a high concentration of ethanol causes mucosal congestion, resulting in mucosal injury (1–4). We have reported that a level of more than 20% ethanol causes mucosal congestion and tissue hypoxia, resulting in mucosal lesions in a dose-dependent manner (5). Furthermore, the degree of tissue hypoxia in anesthetized rats correlates well with the extent of mucosal injury (5, 6). In rat gastric mucosa, cetraxate (4-(2-carboxyethyl)phenyl trans-4-amino-methylcyclohexanecarboxylate HCl) has a protective effect against various types of acute experimental injury induced by water-immersion stress, serotonin, aspirin, taurocholate and HCl-ethanol (7–10). These results suggest that the effect of cetraxate may be attributed to its ability to reinforce gastric defensive factors such as mucosal microcirculation, mucus glycoproteins and prostaglandins. However, the mechanism of gastric mucosal protective action in the presence of cetraxate remains obscure. In this study, we used a reflectance spectrophotometry system to investigate the effect of cetraxate on gastric mucosal hemodynamic changes induced by topical administration of 40% ethanol solution in the gastric mucosa in anesthetized dogs.

MATERIALS AND METHODS

Four male adult mongrel dogs weighing approximately 10 kg each were fasted for 24 hr, then anesthetized with ketamine HCl (18 mg/kg, i.v.). Tracheal intubation was performed to reduce the risk of aspiration, and the dogs were allowed to ventilate spontaneously. An electronic endoscope system was used (Olympus V10Z, Olympus Co., Tokyo). After endoscopic observation of the stomach to ensure the absence of gastric lesions, a sensor probe (diameter = 2.8 mm) from the reflectance spectrophotometry system (TS-200, Sumitomo Electric Co., Osaka, Japan) was inserted through a biopsy channel and allowed to gently touch the gastric mucosa where the drug, saline or ethanol was to be sprayed. The indices of mucosal blood volume (IHb) and hemoglobin oxygen saturation (ISO2) were estimated from the spectrum, as reported previously (11–13). In brief, the index of mucosal blood volume was calculated from the absorption difference between 569 and 650 nm of the spectrum, because this difference correlates well with the hemoglobin content in the gastric mucosa. The index of hemoglobin oxygen saturation (ISO2) was calculated from the spectrum using three wavelengths (569, 577 and 586 nm) and the following
The TS-200 reflectance spectrophotometry system is equipped with a computer and can calculate IHb and ISO2 within 1 sec. The figures for IHb and ISO2 in our study were therefore automatically and simultaneously printed out every second. The largest value was obtained when the sensor probe was touching the most suitable area on the gastric mucosal surface, with individual figures taken at 5–10-sec intervals to obtain this value.

After a 10-min stabilizing period, cetraxate (200 mg in 10 ml saline) or saline was administered topically on the gastric mucosa at the lesser curvature of the lower corpus of the stomach via a polyethylene tube inserted through the biopsy channel. Twenty minutes later, 10 ml of 40% ethanol was sprayed on the same area of the gastric mucosa via a polyethylene tube, and the hemodynamic changes were followed for 10 min.

The reflectance spectra were obtained every 5 min throughout the experiments.

Data were shown as means ± S.D. After analysis of the variance, Fisher’s latest “significant difference” test was performed. An associate probability smaller than 5% was considered to indicate a significant difference.

**RESULTS**

The IHb and ISO2 values in the 10-min stabilizing periods were 105.2 ± 4.8 and 39.4 ± 1.6% in the control group and 104.0 ± 5.6 and 43.2 ± 2.0% in the cetraxate group, respectively. There were no significant differences in IHb and ISO2 values between the groups.

As shown in Figs. 1 and 2, cetraxate caused a transient increase in the IHb level, with a peak at 5 min and a return to the stabilizing period level at 20 min after cetraxate administration. There was no significant difference in the IHb level at each interval when compared with the values in the control group. In contrast, ISO2 did not show any significant changes for the 20-min period after either saline or cetraxate administration.

Topical administration of 40% ethanol on the gastric mucosa caused a significant increase in IHb and a significant decrease in ISO2 in the control group when compared with values in the stabilizing period. No significant changes were found in IHb and ISO2 in the cetraxate group even after ethanol loading on the gastric mucosa. When the two groups were compared at 5 min and 10 min after ethanol administration, the changes in IHb and ISO2 in the control group were significantly greater than those in the cetraxate group. The area

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**Fig. 1.** Effect of cetraxate pretreatment on changes in the index of gastric mucosal blood volume (IHb) before and after 40% ethanol loading on the gastric mucosa in anesthetized dogs. Open circles are the control group and closed circles are the cetraxate group. The values are shown as percentages compared with the value obtained during the stabilizing period. N = 4 in each group. +: P < 0.05 vs. the value in stabilizing period. *: P < 0.05 vs. the values in control group.

**Fig. 2.** Effect of cetraxate pretreatment on the changes in the index of oxygen saturation of hemoglobin in gastric mucosa before and after ethanol loading on the gastric mucosa in anesthetized dogs. Open circles are the control group and closed circles are the cetraxate group. The values are shown as percentages compared with the value obtained during the stabilizing period. N = 4 in each group. +: P < 0.05 vs. the value in stabilizing period. *: P < 0.05 vs. the values in control group.
under the curve (AUC) for IHb and ISO2 for 10 min after ethanol loading were also significantly greater in the control group than in the cetraxate group (Table 1). These data indicate that 40% ethanol-induced mucosal congestion was clearly higher in the control group than in the cetraxate group.

### Table 1. Mean decreases of IHb and ISO2 after ethanol administration in the control and cetraxate-treated groups

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<th>Percent changes in</th>
<th>AUC* in</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>IHb</td>
<td>ISO2</td>
<td>IHb</td>
</tr>
<tr>
<td>Control group</td>
<td>16.7 ± 5.0</td>
<td>-19.2 ± 4.8</td>
<td>119.8 ± 14.0</td>
</tr>
<tr>
<td>Cetraxate group</td>
<td>7.1 ± 2.1*</td>
<td>4.0 ± 2.4*</td>
<td>53.8 ± 5.5*</td>
</tr>
</tbody>
</table>

n = 4 in each group. Data are shown as mean ± S.D. The changes in IHb and ISO2 were calculated and expressed as a percentage when compared with the values obtained during the stabilizing period. *: P < 0.05 vs. control group. *: Area under the curve (AUC) was estimated from Figs. 1 and 2 and expressed in arbitrary units.

**DISCUSSION**

It has been reported that by endoscopic examination, ethanol-induced mucosal injury in humans can be found in approximately 5–10% of acute gastric mucosal lesions. Many authors have also reported on the mechanism of ethanol-induced mucosal injury in experimental animals (1–4) and stressed the important role of mucosal blood flow in the etiology of ethanol-induced mucosal lesions. However, because of technical difficulties, there are few reports on the use of endoscopy to examine gastric mucosal blood flow in dogs and humans. A laser Doppler velocimetry (PeriFlux 2, Periflux Inc., Sweden) was recently used under endoscopy. A reflectance spectrophotometry system has also been used to measure mucosal hemodynamics under endoscopy in anesthetized dogs. This system (TS-200 tissue spectrum analyzer, Sumitomo Electronic Co., Osaka, Japan) was equipped with a microcomputer and spectrum analyzing system. It has a touch sensor that automatically analyzes the spectrum when the tip of the sensor probe touches the gastric mucosa. With this system, the largest values of IHb and ISO2 can be obtained because the pressure on the gastric mucosal capillaries by the sensor tip decreases IHb and ISO2; and when the tip of the sensor does not touch the gastric mucosa, the reading of IHb and ISO2 decreases. Using the reflectance spectrophotometry system, Leung et al. reported that IHb increased and ISO2 decreased when the portal vein was ligated, and IHb and ISO2 decreased when the celiac artery was occluded in anesthetized rats (14). The former therefore indicated mucosal congestion and the latter indicated ischemia. ISO2 decreased under both conditions, which suggests that this parameter can be used as an index of tissue hypoxia. Using these parameters, it is possible to differentiate the mucosal ischemia and congestion of gastric mucosal capillaries. Under both conditions, the ISO2 index of tissue hypoxia, decreased. We have reported that when ethanol was administered topically to anesthetized rats, a relatively high concentration of ethanol (more than 30%) caused an increase in IHb and a decrease in ISO2 in a concentration-dependent manner. Thus, the topical administration of a high concentration of ethanol causes mucosal congestion and acute gastric mucosal lesions in a dose-dependent manner in anesthetized rats. Furthermore, the extent of mucosal lesions correlates well with the degree of decrease in ISO2 after ethanol loading on the gastric mucosa (5, 6). These results indicate that the severity of mucosal congestion affects the extent of mucosal lesions.

The topical administration of cetraxate, with a dose of 20 mg/kg body weight, caused a transient increase in IHb that returned to the stabilizing level in 20 min, but it did not reach a significant difference between the control and cetraxate groups because of the relatively large standard error. We reported a transient but significant increase in IHb in the human gastric mucosa in 10 min after topical administration of cetraxate (400 mg in 15 ml-saline) in (15). Thus, the effect of cetraxate on the gastric mucosal hemodynamics in anesthetized dogs showed the same tendency as that of the human study. Although the reason for the lack of significant IHb increase compared with the control group was unclear, the different species and experimental conditions used in the studies may have affected the results.

Hoshina et al. reported that cetraxate with a dose of 30 mg/kg, i.v. caused a significant increase in mucosal blood flow as measured by the hydrogen gas clearance method (16). Thus, cetraxate may increase the gastric mucosal blood flow in animals and humans. Kurebayashi et al. (10) reported that either oral or intraperitoneal treatment with cetraxate (30–300 mg/kg) significantly inhib-
mented gastric lesions induced by 60% ethanol in 150 mM HCl in a dose-related manner; and subcutaneous treatment of rats with indomethacin (5 mg/kg) resulted in a partial but significant attenuation in the protection afforded by cetraxate, suggesting that prostaglandins may be involved in this protective activity. Miyata et al. (17) also reported that cetraxate inhibited the ulcer formation induced by ethanol and the cytoprotective action of this drug.

In this study, we administered 40% ethanol 20 min after cetraxate administration. When the IHb had returned to the stabilizing period level, 40% ethanol administered topically to the gastric mucosa caused mucosal congestion in the control group. The cetraxate pretreatment almost completely attenuated the ethanol-induced mucosal congestion. Although the results suggest that cetraxate acts on the gastric mucosal capillaries and improves mucosal microcirculation, further investigations are needed to determine the precise action site of cetraxate.

We recently reported that endothelin-1 was released after topical ethanol loading on the gastric mucosa and may play an important role in the etiology of ethanol-induced gastric mucosal injury (18, 19). Therefore, next we will investigate the effect of cetraxate on endothelin-1 release after ethanol loading and determine the effect of cetraxate on chemical mediators induced by ethanol from the endothelium in gastric vessels.

In conclusion, cetraxate attenuated gastric mucosal congestion induced by topical administration of 40% ethanol on the gastric mucosa. Cetraxate thus acts to protect the mucosal microcirculation against ethanol, resulting in the maintenance of gastric mucosal integrity.

REFERENCES