Cytoprotective Action of Cetraxate against HCl-Ethanol-Induced Gastric Lesion in Rats

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Abstract—The protective effect of cetraxate, an antiulcer and antigastritis agent, on HCl-ethanol-induced gastric lesions was investigated in rats. Oral administration of 1 ml of HCl-ethanol (60% ethanol in 150 mM HCl) induced within 1 hr linear hemorrhagic necrosis in the gastric mucosa. Either oral or intraperitoneal treatment with cetraxate (30–300 mg/kg) significantly inhibited such macroscopic gastric lesions in a dose-related manner, and the inhibition at the oral highest dose (300 mg/kg) was practically complete. Histological analysis also confirmed that cetraxate effectively prevented deep mucosal necrosis, but showed that it was without protective effect on the surface epithelial disruption and submucosal edema in response to HCl-ethanol. The antilesion activity of cetraxate was of statistically significance for at least 3 hr after a single injection, and it was hardly affected by the removal of the gastric contents just prior to application of the necrotizing agent. However, subcutaneous treatment of rats with indomethacin (5 mg/kg) resulted in a partial but significant attenuation in the protection afforded by cetraxate, suggesting that dual mechanisms related and unrelated to endogenous prostaglandins may be involved in its protective activity. The results demonstrate that cetraxate is a potent cytoprotective agent effectively preventing the formation of gastric mucosal necrosis induced by HCl-ethanol.

Cetraxate (p-hydroxyphenyl-propionic ester of tranexamic acid) is an effective drug for the therapy of either gastritis or gastric ulcer diseases (1, 2). In addition to its healing property, it has been reported that cetraxate was capable of protecting the rat gastric mucosa against various types of acute experimental injury produced by water-immersion stress, serotonin, aspirin or taurocholate (3-6). Although cetraxate has been shown to exert an inhibitory effect on the gastric secretion in pylorus-ligated rats (3), many pharmacological and pathophysiological experiments indicate that the prevention by cetraxate of experimental gastric lesions may be attributable to its ability to reinforce the gastric defensive factors such as mucosal microcirculation (5), mucus glycoproteins (7, 8) and prostaglandins (9, 10). Little information, however, is available concerning the cytoprotective action of this antiulcer agent on the gastric mucosa.

In the present study, in an attempt to evaluate the cytoprotective activity of cetraxate, we examined its effect on the gastric lesion formation induced in rats by acidified ethanol, a severe gastric necrotizing agent. Additional studies were also conducted to explore the histological and ultrastructural aspects of its protective property against the necrotizing agent.

Materials and Methods

Animals: Male Sprague-Dawley rats (Shizuoka Agricultural Cooperative Association for Laboratory Animals), weighing 160 to 200 g, were used in all experiments. The animals were housed in raised mesh-bottom cages to prevent coprophagy, and they were maintained on standard laboratory chow (F-2, Funabashi Farm) and tap water ad libitum. Before the experiments, they were starved
overnight but allowed free access to water.

**HCl-ethanol-induced gastric lesions**: Gastric mucosal lesions were induced by oral administration of 1 ml of 60% ethanol (v/v) in 150 mM HCl (HCl-ethanol) (11, 12), and the animals were sacrificed 1 hr later. Subsequently, their stomachs were removed, inflated by injecting 10 ml of 2% buffered formalin, and immersed in 2% formalin for 20 min to fix the gastric wall. Each preparation was then incised along the greater curvature and examined for the presence of mucosal lesions. The length (mm) of each necrotic lesion was measured, summed per stomach, and used as a lesion index. Cetraxate (Daiichi) was suspended in 0.5% carboxymethylcellulose and administered either orally or intraperitoneally at doses ranging from 30 to 300 mg/kg at 30 min prior to the HCl-ethanol instillation, unless otherwise stated. The injection volume was kept constant at 5 ml/kg and an equal volume of the vehicle was administered to control groups in the same manner. Some groups of rats were treated subcutaneously with indomethacin (Sigma), suspended in saline with a trace of Tween 80 (Nakarai), at a dose of 5 mg/kg at 1 hr before the HCl-ethanol treatment.

**Removal of gastric contents**: To examine the influence of possible dilution of HCl-ethanol by gastric contents accumulated after the cetraxate treatment, the gastric contents were removed just before application of the necrotizing agent. In brief, after a midline laparotomy under light ether anesthesia, the stomach was exteriorized and an incision was made in the forestomach. Subsequently, the gastric contents were removed by gentle suctioning using a glass syringe with a 18G needle (Terumo), and the incision was closed. Immediately after the closure of the abdominal incision, HCl-ethanol was administered.

**Histological study**: Groups of 3 rats each were sacrificed by an overdose of ether at 1 hr after HCl-ethanol treatment. The specimens of gastric wall were cut obliquely through the entire extent of glandular mucosa from the forestomach to the pylorus. They were fixed in 10% buffered formalin and processed for histological sections stained with periodic acid Schiff (PAS)-aurantia using routine techniques.

For scanning electron microscopic study, gastric mucosal specimens were fixed in 2.5% glutaraldehyde buffered in 0.1 M phosphate buffer (13), washed in phosphate buffer, and osmicated in 1.0% osmium tetroxide for 2 hr. After dehydration in a graded series of ethanol solutions, the specimens were dried at critical point by liquid carbon dioxide substitution, placed on spinner stubs, and coated with gold to a depth of 20 nm in a polaron SEM coating unit. They were viewed in a S-800 scanning electron microscope (Hitachi) operated at 20 kV.

**Statistical analysis**: The data are represented as means±S.E. of 6 rats per groups. The statistical analysis was carried out using Dunnett's multiple comparison test (14) for unpaired variates, and P-values less than 0.05 were regarded as significant.

**Results**

**Effect of cetraxate on HCl-ethanol-induced gastric lesions**: Oral administration of HCl-ethanol to rats resulted in the development of streak, hemorrhagic mucosal necrosis in the glandular stomach, especially in the corpus mucosa, at least within 1 hr (Fig. 1a). Pretreatment of rats with cetraxate at doses ranging from 30 to 300 mg/kg, either orally or intraperitoneally, effectively prevented the development of the gastric mucosal damage in a dose-related manner, as evidenced by significant and marked reductions in lesion indices (Figs. 2a and 2b). The degree of protection by oral cetraxate appeared to be greater than that by the intraperitoneal one. The effect of orally administered cetraxate was significant at the dose of 30 mg/kg or more, while a significant effect was achieved at doses over 100 mg/kg when given intraperitoneally. In addition, the inhibition at 30, 100 and 300 mg/kg of oral cetraxate was 32 (P<0.05), 74 (P<0.01) and 93% (P<0.01), respectively, and that of intraperitoneal treatment was 30, 57 (P<0.05) and 72% (P<0.01), respectively.

So far as macroscopically examined, the prevention of gastric lesion formation by cetraxate at the oral highest dose (300 mg/kg) appeared practically to be complete (Fig. 1b).

**Light microscopic findings**: The histo-
pathological alterations in the stomachs of control animals given HCl-ethanol alone can be summarized as follows: (a) severe disruption and desquamation of the surface epithelial cells, (b) deep necrotic lesions and (c) edema associated with a marked leucocyte infiltration in the submucosal layer (Figs. 3a and 3b). Pretreatment with 300 mg/kg, p.o., of cetraxate at 0.5 hr before HCl-ethanol treatment failed to prevent the disruption of the surface epithelial cells and the submucosal edema with leucocyte accumulation. However, the formation of deep necrotic lesions in the mucosa was absent or drastically reduced in cetraxate-treated animals (Fig. 3c).

**Scanning electron microscopic findings:** Instillation of HCl-ethanol produced a prominent disruption of the surface epithelial cells with extrusion of the contents of the gastric glands as casts of interconnected necrotic cells (Figs. 4a and 4b), resulting in the formation of large craters in areas of denuded lamina propria. Pretreatment of rats with 300 mg/kg, p.o., of cetraxate was without protective effect on the disruption of the surface epithelial cells after the HCl-ethanol instillation (Fig. 4c), but effectively reduced the exfoliation of the injured epithelial cells from the mucosal surface.

**Duration of the gastric cytoprotection:** As shown in Fig. 5, oral treatment with cetrax-
Fig. 3. Histological findings of rat gastric mucosa at 1 hr after the treatment with HCl-ethanol. In the normal gastric mucosa (a), no pathological alteration is present. On the other hand, deep mucosal necrosis and severe submucosal edema associated with leucocyte infiltration are observed in the stomach of a rat given the vehicle alone before the HCl-ethanol treatment (b). In the cetraxate-treated animal (c), deep necrotic lesion is absent, but submucosal edema is still observed. Cetraxate was administered at 300 mg/kg, p.o., at 30 min before the HCl-ethanol treatment. (PAS-aurantia. x20).

Fig. 4. Scanning electron microscopic findings of rat gastric mucosa after the administration of HCl-ethanol. In the normal mucosa (a), individual surface epithelial cells and the lumina of the gastric glands are clearly visible. The instillation of HCl-ethanol resulted 1 hr later in the severe disruption and complete desquamation of surface epithelial cells (b). In the cetraxate-pretreated rat (c), the surface epithelial cells are disrupted, whereas the exfoliation of the damaged epithelial cells is effectively prevented. Cetraxate was administered at 300 mg/kg, p.o., at 0.5 hr before the instillation of the necrotizing agent. (x 500)

Cetraxate at 3 hr before the HCl-ethanol instillation also produced a significant and dose-related prevention of the gastric lesion formation, the inhibition being 24 and 69% (P<0.01) at 100 and 300 mg/kg, respectively. On the other hand, at 6 hr post-cetraxate in the same
dosage, there was no significant inhibition of gastric lesion formation.

Influence of dilution by gastric contents: In a preliminary experiment, we found that the gastric contents in the rats treated with the vehicle alone were less than 0.1 ml, but those in the cetraxate-treated animals were elevated to about 0.4 ml at 30 min after the injection. Therefore, the gastric contents were removed just prior to the instillation of HCl-ethanol (at 30 min after cetraxate treatment) in order to exclude the possible influence of physical dilution with the increased contents of the necrotizing agent and to substantiate the pharmacological effect of cetraxate. The results are illustrated in Fig. 6. The removal of gastric contents did not affect the protective activity of cetraxate against the necrotizing action of HCl-ethanol on the gastric mucosa. Indeed, the inhibition was 66% (P<0.05) and 82% (P<0.01) at doses of 100 and 300 mg/kg, p.o., respectively.

Effect of indomethacin on the efficacy of cetraxate: To investigate the possible involvement of endogenous prostaglandins in the gastric mucosal protection afforded by cetraxate, its inhibitory effect on the development of HCl-ethanol-induced injury was examined in the rats pretreated with indomethacin, which is known to be a potent inhibitor of prostaglandin synthesis. Subcutaneous treatment of rats with 5 mg/kg of indomethacin at 1 hr before the HCl-ethanol

Fig. 5. Duration of the cytoprotection by cetraxate against the gastric lesion formation induced by HCl-ethanol in rats. Cetraxate was given orally at 3 or 6 hr before the HCl-ethanol-administration. Each column and vertical bar represent the mean±S.E. (N=6). **P<0.01 vs. control.

Fig. 6. Effect of cetraxate on HCl-ethanol-induced gastric lesions in rats. The gastric contents of rats were removed at 30 min after the oral treatment with cetraxate. It was evident that the protective effect of cetraxate was not caused by a physical dilution of the necrotizing agent with gastric contents. Each column and vertical bar represent the mean±S.E. (N=6). **P<0.01 vs. control.
treatment hardly affected the severity of gastric mucosal lesions in response to the necrotizing agent. Figure 7 illustrates the preventive effect of orally administered cetraxate on HCI-ethanol-induced gastric necrosis in rats pretreated with or without indomethacin. The protective effect of cetraxate was partially, but significantly attenuated by indomethacin pretreatment. However, even in the presence of indomethacin, the inhibition at the higher dose (300 mg/kg, p.o.) remained significant (69%, P<0.01).

**Fig. 7.** Effect of indomethacin pretreatment on the protective effect of cetraxate against HCI-ethanol-induced gastric lesions in rats. Indomethacin was given at 5 mg/kg, s.c., at 1 hr before the oral administration of cetraxate. Each column and vertical bar represent the mean±S.E. (N=6). *P<0.05, **P<0.01 vs. control. □P<0.05 vs. cetraxate at 100 mg/kg, p.o., without indomethacin.

**Discussion**

Prostaglandins and their analogues, administered either orally or parenterally, inhibit the formation of macroscopic and deep microscopic mucosal necrosis induced by various necrotizing agents, including acidified or concentrated ethanol (11, 15-18). This protective action of prostaglandins, which is apparently unrelated to their antisecretory property, has been called “cytoprotection” (16-18). The results of the present study clearly demonstrated that cetraxate, like prostaglandins, effectively protected the rat gastric mucosa against the insult of HCI-ethanol. The inhibition by this agent of macroscopic gastric lesions appeared to be nearly complete at the highest dose (300 mg/kg, p.o.). It seems likely that cetraxate exerted such protection mainly through a systemic action since it was effective whether given orally or intraperitoneally. However, the antisecretory effect of oral cetraxate appeared to be more potent than that of the parenteral one at all doses tested (30-300 mg/kg), suggesting that a local direct effect upon the gastric mucosa may additionally contribute to the effect of orally administered cetraxate.

Histological and ultrastructural analysis showed that HCI-ethanol instillation induced severe epithelial desquamation, deep mucosal necrosis and submucosal edema associated with leucocyte accumulation. These pathological changes in the gastric wall were essentially comparable to the earlier findings described in studies of concentrated ethanol (13, 17-19). Treatment with cetraxate failed to prevent the epithelial cell disruption and submucosal edema. However, it was confirmed that HCI-ethanol-induced mucosal necrosis in the deeper layer was absent or drastically reduced in cetraxate-treated rats. These histological characteristics of cetraxate’s protection are consistent with those of
prostaglandin cytoprotection against ethanol-induced gastric injury, whose main feature is an inhibition of macroscopic and deep microscopic mucosal necrosis (17-19).

Cetraxate has been reported to inhibit gastric secretion in pylorus-ligated rats at doses of 150 mg/kg, i.p. or more (3). In the present study, however, ethanol was administered into the stomach together with sufficient amounts of exogenous acid (150 mM HCl), excluding the possibility that the antisecretory activity may be responsible for the protective effect of cetraxate. Also, we found that the preliminary removal of the gastric contents prior to the lesion induction hardly affected its inhibitory effect on lesion formation. It seems therefore unlikely that the mucosal protection by cetraxate was mediated by an acid neutralization or a simple physical dilution of the necrotizing agent with the gastric contents, as had been suspected for certain compounds (13, 20, 21). Based on these pharmacological considerations, together with the present histological evidence, the mucosal protection afforded by cetraxate appears to be quite similar to prostaglandin cytoprotection. Thus, it may be justified to consider that cetraxate has a cytoprotective property.

Our data also show that the antileision activity of cetraxate was significantly attenuated by prior administration of indomethacin (5 mg/kg, s.c.), indicating that endogenous prostaglandins are, at least partly, involved in the mechanisms by which cetraxate protected the gastric mucosa against the injurious action of HCl-ethanol. This view may be further supported by the morphological similarities between the protection by cetraxate and that by prostaglandin. However, we also found that even in the presence of indomethacin the highest dose (300 mg/kg p.o.) of cetraxate was capable of producing a significant, pronounced inhibition (69%, P<0.01) of the lesion formation, suggesting that an additional mechanism unrelated to endogenous prostaglandins may also contribute to the cytoprotective property of this agent.

It has been well established that various mild irritants of the gastric mucosa, such as low concentrations of ethanol or HCl, were cytoprotective against the insults of strong necrotizing agents (22, 23), so called "adaptive cytoprotection". The protective effects of these mild irritants are considered to result from a stimulation of endogenous prostaglandin synthesis in the stomach, inasmuch as their effects were totally abolished in the presence of indomethacin. Likewise, as mentioned above, indomethacin treatment attenuated the protective activity of cetraxate against HCl-ethanol injury. However, unlike mild irritants, cetraxate was effective even when given parenterally. In addition, the protective effect of oral cetraxate was of significance for over 3 hr, whereas the protection by mild irritants totally disappeared at 3 hr after single application (22). It appears therefore appropriate to consider that the protective effect of cetraxate against HCl-ethanol damage is not due to a mild irritation upon the gastric mucosa, but rather to its pharmacological effects.

There has been evidence showing the ability of cetraxate to reinforce the gastric defensive factors in normal and pathological conditions (5, 7-10), including injury by a relatively low concentration (37.8%) of ethanol (24). Ethanol has been shown to produce gastric lesions with concomitant impairments in such factors as mucus (24, 25) and mucosal circulation (26). It seems thus conceivable that the cytoprotective action of cetraxate may be related to its effects on the mucosal protection, as has been proposed for prostaglandins (27-29). Recently, it has been proposed that gastric hypercontraction may play an important role in the pathogenesis of ethanol-induced gastric necrosis, and that an inhibition of such response may be a main cause of the cytoprotection by prostaglandins and other compounds (30-32). Inasmuch as cetraxate has been found to improve gastric hypermotility in a certain condition (5), the possibility can not be ruled out that the effect on gastric motility may contribute to its protective activity. Conclusive proof for the above possibilities requires further experiments.

In summary, the results of the present study indicate that cetraxate has a gastric cytoprotective activity similar to that of prostaglandins, and that this activity may be
mediated at least partly by endogenous prostaglandins. Although the detailed mechanism by which cetraxate exerts such protection remains to be elucidated, the mucosal protective property may in part account for its antigastric lesion effect in various experimental ulcer models.

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References


