Effect of Cetraxate, a Mucosal Protective Agent, on Gastric Mucosal Blood Flow and Gastric Clarithromycin Concentration in Nicotine-treated Rats

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ABSTRACT. Our previous study demonstrated that combination treatment with cetraxate plus omeprazole, amoxicillin, and clarithromycin is effective for the eradication of *Helicobacter pylori* in smokers. To evaluate the effect of cetraxate on gastric mucosal blood flow (GMBF) and the gastric concentration of clarithromycin in nicotine-treated rats, 10 rats were divided into two groups given nicotine with or without cetraxate, and GMBF was measured by laser Doppler blood flowmetry. Another 36 rats were divided into three groups (control, nicotine, and nicotine+cetraxate). Clarithromycin was administered intraduodenally and nicotine was administered after 30 minutes, with cetraxate being given 30 minutes later. The gastric mucosal clarithromycin concentration was measured. After cetraxate administration, GMBF increased significantly in the nicotine+cetraxate group compared with the nicotine group ($p<0.05$). The mucosal clarithromycin concentration increased in the nicotine+cetraxate group compared with the nicotine group, but the difference was not significant. Our results indicate that cetraxate increased GMBF in nicotine-treated rats.

Key words : gastric mucosal blood flow — nicotine — cetraxate — clarithromycin

The association between cigarette smoking and the development of gastric mucosal lesions has long been discussed,[1-3] and clinical studies have indicated that peptic ulcer disease and ulcer recurrence is common in smokers.[4-7] Smoking is generally considered to stimulate acid secretion, an aggressive factor, leading to increased gastric acidity.[8,9] Several studies have shown that nicotine or smoking also causes a decrease in gastric mucosal blood flow (GMBF), which is an important defensive factor.[10-14] It is thus considered that smoking is an important factor in the formation of peptic ulcers.[3-6]

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There is now strong evidence that *Helicobacter pylori* (*H. pylori*) infection also plays an important role in the pathogenesis of peptic ulcer disease,[15,16] and many studies have shown that eradicating *H. pylori* can markedly decrease the ulcer relapse rate.[16,17] A number of authors have reported that the *H. pylori* eradication rate achieved using a proton-pump inhibitor in combination with amoxicillin and clarithromycin is more than 90%,[18,19] but this means that such treatment still fails in 5-10% of patients. Our previous study demonstrated that eradication of *H. pylori* by treatment with omeprazole, amoxicillin, and clarithromycin (OAC therapy) was less effective in smokers than in non-smokers.[20] Thus, it is also considered that smoking is one of the causes of *H. pylori* eradication failure. Our recent study demonstrated that treatment for one week with cetraxate plus OAC therapy is effective for the eradication of *H. pylori* in smokers.[21] Cetraxate was introduced in 1976 as an antiulcer drug with a mucosal protective effect[22] and it is thought to increase GMBF.[10,23-25]

The aim of the present study was to evaluate the pharmacological effect of cetraxate on GMBF and the gastric clarithromycin concentration in nicotine-treated rats.

MATERIALS AND METHODS

Animals : Experiments were carried out on male Spraque-Dawley rats weighing 200-300 g. The rats were fasted for 18 hours before the experiments, but were allowed free access to water. Cetraxate, 4-(2-carboxyethyl)phenyltrans-4-amino-methylcyclohexanecarboxylate hydrochloride was purchased from Daiichi Pharmaceutical Co. (Tokyo, Japan).

Experiment 1 : Measurement of GMBF after nicotine administration

A preliminary experiment was carried out to determine the optimum nicotine dose for the rats. Twelve 10-week old rats were divided into four groups of three each (Group 1 : control ; Group 2 : nicotine at 10 μg/kg/min ; Group 3 : nicotine at 30 μg/kg/min ; Group 4 : nicotine at 100 μg/kg/min). After each rat was anesthetized with urethane (1.2 g/kg), a lucite chamber was fixed to the body of the stomach at laparotomy. The probe (Type N, ADVANCE CO., LTD., Tokyo, Japan) was placed on the lucite chamber, and GMBF was recorded using a laser Doppler blood flowmeter (AFL21, ADVANCE CO., LTD., Tokyo Japan). Data were monitored by a recorder (VP6621A, Matsushita Communication Industrial CO., LTD., Yokohama, Japan) for 75 minutes at 5-min intervals from 30 min after the start of intravenous infusion of nicotine. Saline alone was infused instead of nicotine in the control group (1.5 ml/hr).

Experiment 2 : Measurement of GMBF after cetraxate administration

Ten 10-week old rats were divided into two groups of five each (nicotine group and nicotine+cetraxate group). Nicotine (30 μg/kg/min) was infused intravenously and measurement of GMBF was started after 30 min. Cetraxate (10 mg/ml) or distilled water was given intravenously at 30 min after starting nicotine administration. GMBF was measured by the same method as in Experiment 1.

Experiment 3 : Influence of cetraxate on gastric concentration of clarithromycin

Thirty-six seven-week old rats were divided into three groups of 12 each (control group, nicotine group, and nicotine + cetraxate group). Clarithromycin (20 mg/kg) was administered intraduodenally, and nicotine (30 μg/kg/min) or saline was given intravenously after 30 minutes. Cetraxate (300 mg/kg) or 0.5% mythylcellulose (5 ml/kg) was administered at 30 min after starting the nicotine infusion. The rats were killed by cervical dislocation after another 30 minutes, and the stomach was extracted. After the stomach was opened along the greater curvature, two mucosal biopsy specimens were taken from the body of the stomach using a punch with an 8-mm internal diameter. The concentration of clarithromycin (μg/g) in the mucosal tissue was measured by liquid chromatography-mass spectrometry (LC-MS)[26] after extraction with an organic solvent.

Statistical analysis

Results are expressed as the mean ± standard error (S.E.). Intergroup differences were evaluated by the Student's *t*-test or Scheffe's test, and $p<0.05$ was considered to indicate significance.

RESULTS

Experiment 1 : Measurement of GMBF after nicotine administration

The results are summarized in Fig 1. In Group 1 (the control group), GMBF showed little change throughout the observation period. After the start of nicotine administration (10 μg/kg/min), it was slightly decreased in Group 2, but no significant change was recognized compared with the

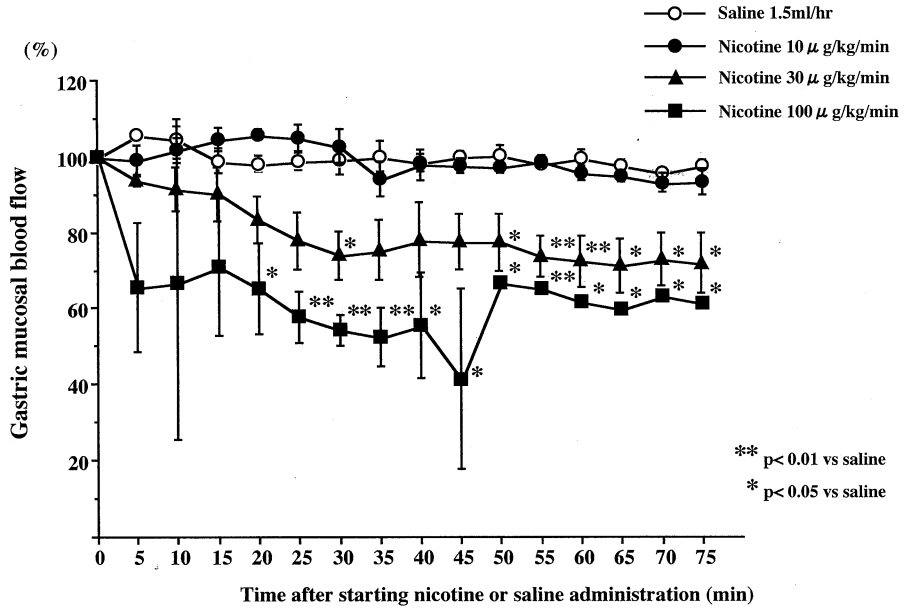

Fig 1. Effect of nicotine on gastric mucosal blood flow (GMBF) in anesthetized rats. After the start of nicotine administration, GMBF was dose-dependently reduced by nicotine.

control group. However, Group 3 (nicotine at 30 μg/kg/min) showed a slow decrease of GMBF over time, and this decline was significant after 30 min of nicotine infusion (GMBF decreased from 99.2±3.8% to 73.9±6.5%, $p<0.05$). GMBF became a condition which was almost fixed from after the nicotine administration for 35 minutes. After nicotine infusion at 100 μg/kg/min in Group 4, there was a dose-dependent decrease in GMBF compared with that in Group 3. The GMBF of Group 4 was significantly lower than that of the control group after 20 minutes of nicotine administration ($p<0.05$, 0.01). However, in Group 4, one rat died after 15 minutes and another after 50 minutes of nicotine infusion. From the above results, the optimum nicotine dose was determined to be 30 μg/kg/min. In addition, the optimum time for cetraxate administration was determined to be 30 min after starting nicotine infusion.

Experiment 2 : Measurement of GMBF after cetraxate administration

The results of Experiment 2 are shown in Fig 2. GMBF fell to about 70% of the baseline value when nicotine was given at 30 μg/kg/min for 30 min in both the nicotine group and the nicotine + cetraxate group. The subsequent response of GMBF in the nicotine group was similar to that in Experiment 1. After cetraxate administration, however, GMBF increased over time in the nicotine + cetraxate group. At 30 min after cetraxate administration, there was a significant increase of GMBF in the nicotine + cetraxate group (from 60.9±4.0% to 79.9±7.0%, $p<0.05$) compared with the nicotine group.

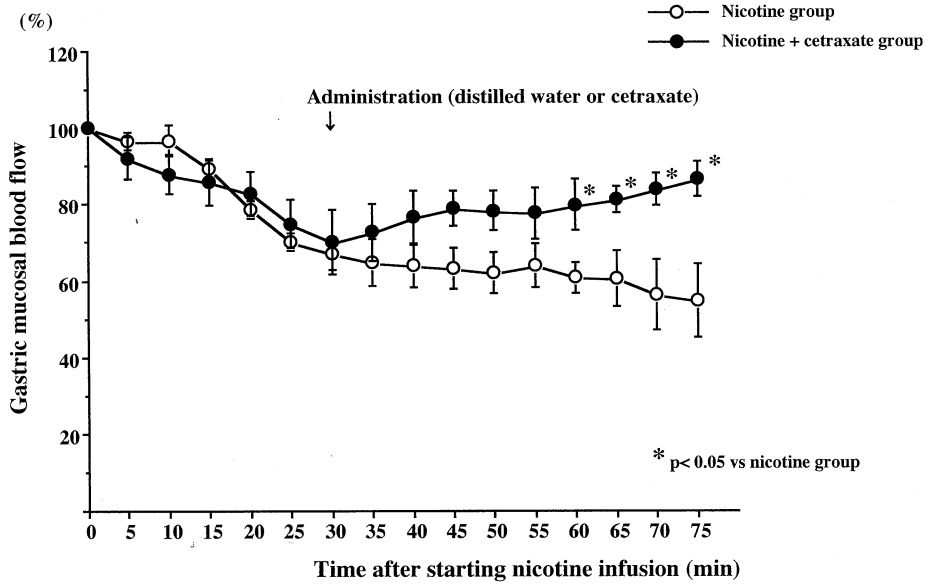

Fig 2. Effect of cetraxate on the nicotine-induced decrease of gastric mucosal blood flow (GMBF) in anesthetized rats. After cetraxate administration, GMBF increased over time in the nicotine+cetraxate group.

Experiment 3 : Influence of cetraxate on the gastric concentration of clarithromycin

The results of Experiment 3 are shown in Fig 3. The gastric clarithromycin concentration was lower in the nicotine group than in the control group (12.4 ± 1.2 μg/g vs 14.9 ± 2.3 μg/g), but the difference was not significant. It was higher in the nicotine + cetraxate group than in the nicotine group (15.9 ± 3.5 μg/g vs 12.4 ± 1.2 μg/g), but again the difference was not significant.

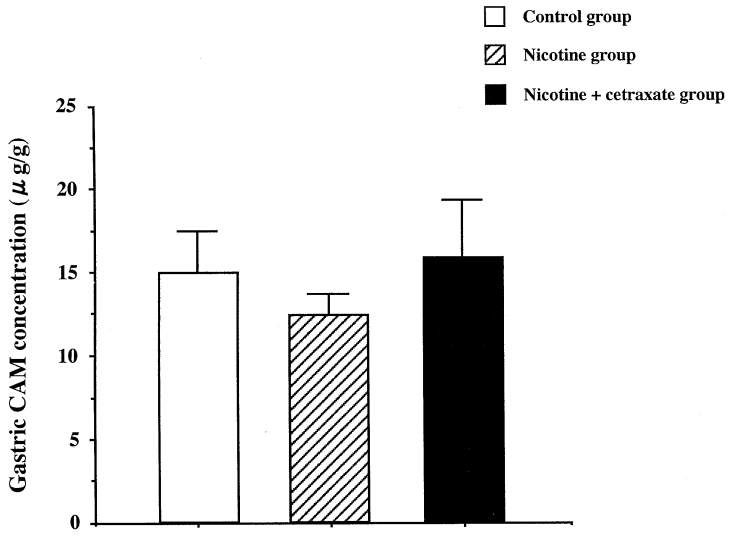

Fig 3. Effect of cetraxate on the gastric mucosal clarithromycin concentration in nicotine-treated rats. The gastric clarithromycin concentration was higher in the nicotine + cetraxate group than in the nicotine group, but the difference was not significant. CAM : clarithromycin, MC : methylcellulose

DISCUSSION

Our study demonstrated that GMBF was dose-dependently reduced by nicotine, while cetraxate, which increases microcirculation as its main action, significantly increased GMBF in anesthetized rats receiving nicotine.

Cigarette smoking is an important cause of gastric mucosal injury, and it delays ulcer healing, promotes intractability, and promotes recurrence.[4-7] Nicotine stimulates acid secretion, an aggressive factor, leading to an increase in gastric acid output and pepsin secretion.[8,9] It is important to effect stomach and duodenal mucosa of the smoking on the protection, and nicotine in cigarettes is main factor of the action expression. Following recent technical advances, interest has also developed in the effect of smoking on GMBF, which is an important mucosal defensive factor. There have been conflicting reports about the influence of smoking or nicotine on GMBF.[10-14,27-29] Animal studies performed using laser Doppler flowmetry have failed to detect any reduction in GMBF after either acute[27] or repeated[28] administration of nicotine. Additionally, Robert *et al*[29] reported that hypotension reduced GMBF, but intravenous nicotine or cigarette

smoke in the doses tested did not. However, another animal study[10] and human studies[11-14] have shown that nicotine or smoking can decrease GMBF. Guslandi *et al*[14] reported that GMBF in heavy smokers was significantly reduced compared with that in light smokers or non-smokers. The present study also demonstrated that GMBF was dose-dependently reduced by nicotine in rats.

In 1976, cetraxate was introduced as an antiulcer drug with a mucosal protective effect.[22] Kurebayashi *et al*[30] demonstrated that cetraxate is a potent cytoprotective agent that can effectively prevent gastric mucosal necrosis induced by HCl. ethanol in rats. Its efficacy is ascribed to promotion of an increase in GMBF.[10,23-25] Animal experiments have shown that cetraxate can significantly reverse the reduction of GMBF induced by smoking.[10] McCready *et al*[31] reported that smoking reduces the prostaglandin E_2 level in the gastric lumen and this may explain why smoking is a risk factor for peptic ulcer. After studying rats under stress, Tachi *et al*[24] suggested that the effect of cetraxate might depend on enhancement of nitric oxide synthase activity and prevention of a decrease in the mucosal prostaglandin content, resulting in an increase of GMBF. Additionally, Kuroiwa *et al*[25] showed that cetraxate reversed decrease in mucosal prostaglandin, resulting in an increase of GMBF in rats subjected to water immersion stress. Thus, enhancement of nitric oxide synthase activity and an increase in gastric mucosal prostaglandin may prevent a reduction in GMBF and thus avoid gastric mucosal damage. The present study also demonstrated that cetraxate significantly increased GMBF in nicotine-treated rats, suggesting that this drug might lead to increased GMBF and improved microcirculation through the above-mentioned mechanisms.

Our previous study demonstrated that *H. pylori* eradication by OAC therapy was less effective in smokers than in non-smokers.[20] A number of studies have shown that smoking is associated with failure to achieve *H. pylori* eradication.[32-34] Our hypothesis is that smoking decreases GMBF and thus may reduce the efficacy of treatment by decreasing the delivery of antibiotics to the gastric mucosa. Because clarithromycin is frequently used for *H. pylori* eradication therapy, we examined the effect of nicotine on the gastric mucosal concentration of this antibiotic. We found that GMBF was significantly lowered by nicotine administration and it was significantly highered by cetraxate administration in rats. However, the mucosal clarithromycin concentration was not significantly lowered by nicotine administration and also it was not significantly highered by cetraxate administration in our study. Our previous study demonstrated that treatment for one week with cetraxate plus OAC therapy is effective for the eradication of *H. pylori* in smokers.[21] Because the present study showed that cetraxate could not reverse the effect of nicotine on the concentration of mucosal clarithromycin, cetraxate may achieve higher levels of amoxicillin in the gastric mucosa of smokers and thereby improve the eradication rate. We were unable to investigate the measurement of amoxycillin in this study. In the future, we will perform a similar investigation using amoxycillin, because amoxycillin is another antibiotic frequently used for the eradication of *H. pylori*. Additionally, we will perform a similar investigation using

high dose-nicotine which was significantly lowered the concentration of mucosal clarithromycin in rats.

In conclusion, our study demonstrated that cetraxate, a mucosal protective agent, significantly increased GMBF in nicotine-treated rats.

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