Effect of a Combination of Ecabet Sodium and Cimetidine on Experimentally Induced Gastric Lesions and Gastric Mucosal Resistance to Ulcerogenic Agents in Rats

Mine Kinoshita,* Tsunehisa Noto, and Hajime Tamaki

Pharmacological Research Laboratory, Tanabe Seiyaku Co., Ltd., 2–2–30, Kawagishi, Toda, Saitama 335, Japan. Received June 13, 1994; accepted October 22, 1994

We studied the effect of single oral administration of ecabet sodium (ecabet), a gastroprotective agent, in combination with the histamine H₂-receptor antagonist cimetidine on gastric acid secretion, mucosal prostaglandin E₂ (PGE₂) production and experimentally induced acute hemorrhagic gastric lesions in rats. The effect of repeated administration of ecabet in combination with cimetidine on the vulnerability of gastric mucosa to the ulcerogenic agents 0.6 N HCl and aspirin was also studied. In pylorus-ligated rats, oral administration of cimetidine reduced gastric acid secretion, whereas ecabet did not affect the cimetidine-induced reduction in acid secretion. On the other hand, ecabet increased the capacity of gastric mucosa to synthesize PGE₂, while cimetidine showed no effect on this parameter either in the presence or absence of ecabet. Both ecabet and cimetidine inhibited the formation of aspirin-induced gastric mucosal lesions, and the combination of ecabet and cimetidine showed a more potent inhibition than either drug alone. After cessation of repeated administration of cimetidine, but not ecabet, the 0.6 N HCl- and aspirin-induced gastric lesions were significantly aggravated. The co-administration of ecabet improved the cimetidine-induced aggravation of these gastric lesions. These results suggest that ecabet in combination with cimetidine augments the antilucre effect of cimetidine and improves the cimetidine-induced increase in gastric mucosal vulnerability to the ulcerogenic agents.

Key words ecabet sodium; cimetidine; gastric acid secretion; PGE₂; gastric mucosal resistance

Peptic ulcer is considered to be a result of the impairment of the balance of aggressive factors (acid and pepsin) and defensive factors (gastric mucosal blood flow, alkaline secretion and gastric mucus secretion, etc.) in the stomach.¹ Histamine H₂-receptor antagonists, which are represented by cimetidine and ranitidine, brought a remarkable improvement in the treatment of peptic ulcer by their potent antisecretory effect.² On the other hand, there are patients who are resistant to H₂-receptor antagonists and show high relapse rates after the healing with this antagonist.³ ⁴ The antilucre agent ecabet sodium (ecabet) has been reported to prevent the formation of acute gastroduodenal ulcers and promote the healing of chronic ulcers in animals and man.⁵ ⁶ ⁷ ⁸ Enhancement of the defensive factors through an increase in gastroduodenal prostaglandin E₂ (PGE₂) and prostacyclin has been proposed to be one of the mechanisms underlying the therapeutic action of ecabet.⁵ ⁶ ⁷ ⁸ ⁹ ¹⁰ The combination of ecabet and cimetidine has been reported to be clinically beneficial in the treatment of patients resistant to H₂-antagonists.¹¹ However, the efficacy of the combination of these two drugs has not been exactly defined.

In the present study, we examined effects of the combination of ecabet and cimetidine on the gastric acid secretion, gastric mucosal PGE₂ generation, and experimentally induced gastric mucosal lesions. In addition, the influence of repeated administration of ecabet and cimetidine on the vulnerability of gastric mucosa to ulcerogenic factors was studied. Aspirin-induced acid-dependent and 0.6 N HCl-induced acid-independent gastric lesions were used as the acute hemorrhagic lesion models in the present study.

MATERIALS AND METHODS

Animals and Drugs  Male Sprague-Dawley rats (Charles River Co., Japan) weighing 170–225 g were used. Animals were fasted for 24 h before use, but allowed access to water ad libitum. In the 0.6 N HCl-induced gastric mucosal lesion model, no water was given during the last 16 h of the starvation period. Ecabet was synthesized in the Organic Chemistry Research Laboratory of Tanabe Seiyaku Co., Ltd. Cimetidine was purchased from Sigma Chemical Co., aspirin from Nacalai Tesque Inc., and ¹²⁵I-radioimmunoassay kit for PGE₂ from New England Nuclear Co. Drugs were either dissolved or suspended in distilled water in a volume of 5 ml/kg and administered orally.

Gastric Acid Secretion (Shay Rat) Gastric acid secretion was measured in pylorus-ligated rats.¹¹ The drugs were orally administered 90 min prior to ligation. Gastric contents were collected 3 h after pyloric ligation under ether anesthesia. After centrifugation (900 x g, 10 min), the volume of the supernatant was measured as that of gastric juice and the acid concentration was determined by titration against 0.1 N NaOH to an endpoint of pH 7.0 with an autotitrator (TTT 85; Radiometer Copenhagen).

PGE₂ Generation by Rat Gastric Mucosa (Ex Vivo) The capacity of gastric mucosa to synthesize PGE₂ was measured as described by Boughton-Smith and Whittle.¹² Thirty minutes after oral administration of the test drug, the stomach was excised under ether anesthesia, and immediately placed in ice-cold saline. Then, the gastric mucosa was separated from the underlying muscle layer with fine scissors. The mucosa was transferred to an Eppendorf microtube, chopped with scissors for 1 min in

© 1995 Pharmaceutical Society of Japan
500 μl of 0.1 M phosphate buffer (pH 7.4) at 4°C, and centrifuged at 12000 × g for 1 min (Eppendorf centrifuge 5412). The pellet was suspended in 500 μl of the same buffer, and shaken vigorously using a Vortex mixer (3000 rpm) for 1 min at room temperature. After addition of 40 μl of 1% NaHCO₃ containing 10 μg indomethacin, the suspension was centrifuged at 12000 × g for 1 min. The concentration of PGE₂ in the supernatant was determined using the specific ¹²⁵I-PGE₂ radioimmunoassay kit.

0.6 N HCl- and Aspirin-Induced Gastric Mucosal Lesions Either 30 min after oral administration of 1 ml/rat of 0.6 N HCl, or 4 h after oral administration of aspirin (200 mg/kg), the stomach was excised under ether anesthesia. The stomach was filled with 10 ml of 1% formalin to fix the gastric wall. The area (mm²) of 0.6 N HCl-induced hemorrhagic lesions and the length (mm) of aspirin-induced lesions were measured, summed up, and used as the lesion index, respectively. For single administration, the drugs were orally given 30 min before administration of 0.6 N HCl or aspirin. For repeated administration, the drugs were orally given twice (9:00 a.m. and 14:00 p.m.) daily for 14 consecutive days. Eighteen h after the last dosing, 0.6 N HCl or aspirin was administered.

Statistical analysis Statistical analysis was performed by analysis of variance followed by Bonferroni's method. A p value less than 0.05 was considered to be statistically significant. All data were presented as means ± S.E.M.

RESULTS

Effects on Gastric Secretion in Pylorus-Ligated Rats Oral administration of cimetidine (25 and 100 mg/kg) dose-dependently reduced the gastric juice volume and acid concentration in pylorus-ligated rats as shown in Fig. 1. Ecabet (100 mg/kg, p.o.) affected neither the basal gastric secretion nor the cimetidine-induced reduction of gastric secretion.

Effects on Gastric Mucosal PGE₂ Generation (Ex Vivo) Oral administration of ecabet (25 and 100 mg/kg) dose-dependently increased the capacity of the gastric mucosa to synthesize PGE₂, whereas cimetidine (100 mg/kg, p.o.) did not give any effect on either the control or the ecabet-induced increase in gastric mucosal PGE₂ generation (Table I).

Effects on Gastric Mucosal Lesions In the 0.6 N HCl-induced gastric lesion model, cimetidine (100 mg/kg, p.o.) showed no effect on the lesion index (Fig. 2). On the other hand, ecabet (25 and 100 mg/kg, p.o.) exhibited a dose-dependent prevention against 0.6 N HCl-induced gastric mucosal lesions. This prevention was not influenced by simultaneous administration of cimetidine (Fig. 2).

Both ecabet (6.5—100 mg/kg, p.o.) and cimetidine (3.3—50 mg/kg, p.o.) dose-dependently inhibited the formation of aspirin-induced gastric mucosal lesions and their ED₅₀ values were 29.6 and 9.1 mg/kg, respectively. The combination of ecabet and cimetidine at doses (ecabet 25 mg/kg; cimetidine 3.3 and 12.5 mg/kg), which by themselves showed only moderate inhibitions against the aspirin-induced lesions, showed more potent pro-
Effects of Oral Administration of Ecabt (25 mg/kg) and Cimetidine (Cim; 3.3 and 12.5 mg/kg) on Aspirin-Induced Gastric Lesions in Rats

Test drugs were given orally 30 min before oral administration of aspirin (200 mg/kg). Rats were killed 4 h after the administration of aspirin. Each column represents the mean ± S.E.M. for 12 rats. * and **: significant difference from the control (Cont.) at p<0.05 and p<0.01, respectively. #: significant difference from ecabt alone at p<0.05.

DISCUSSION

Cimetidine has been known to decrease gastric acid secretion by H₂-receptor antagonism in animals and man. However, the effect of cimetidine on gastric mucosal prostaglandins such as PGE₂ and prostacyclin, which play an important role in regulating the gastric defensive factors, has not been clearly defined; Kubota et al. reported that cimetidine increased PGE₂ production in a gastric specimen, and others reported that it was devoid of this effect. In the present study, cimetidine dose-dependently suppressed gastric acid secretion in pylorus-ligated rats, but did not affect the ex vivo synthesis of PGE₂ by gastric mucosa. Ecabt dose-dependently increased the ex vivo synthesis of PGE₂ by gastric mucosa, but did not affect the gastric acid secretion in pylorus-ligated rats. These findings indicate that ecabt and cimetidine modify different functions of the stomach; the former stimulates the synthesis of PGE₂ and the latter inhibits acid secretion. Furthermore, these actions were independent and their simultaneous administration did not affect each other's action.

The balance of defensive and aggressive factors is thought to be important in maintaining the gastric mucosal integrity. The formation of the gastric mucosal lesions by necrotizing agents such as 0.6 N HCl has been reported to be unrelated to the aggressive factors and to involve depression of gastric defense mechanisms. The pathogenesis of aspirin-induced gastric mucosal lesions has been demonstrated to involve gastric acid as well as the depression of gastric defensive factors due to deficiency in endogenous prostaglandins.

In the present study, when cimetidine was repeatedly administered to rats, the vulnerability of gastric mucosa to ulcerogenic agents, 0.6 N HCl or aspirin increased, as well as the observations reported by Goto et al. and Arakawa et al. Some reports have demonstrated that repeated administration of cimetidine causes the depression of gastric mucosal defensive factors like gastric mucosal prostaglandin content and mucus secretion. It has been suggested that both the cimetidine-induced increase...
in the vulnerability of the mucosa to the ulcerogenic agent and decrease in gastric mucosal defensive factors are related to the high ratio of ulcer recurrence after the cessation of long-term therapy of this drug. The gastroprotective agent ecabet prevents the cimetidine-induced aggravation in gastric lesions, suggesting the clinical advantage of the combination of ecabet with cimetidine to prevent a high ratio of ulcer relapse.

In conclusion, the present study demonstrated that the combination of ecabet and cimetidine is more effective than either drug alone in preventing the formation of gastric lesions involving both acid-related processes and depression of the gastric defense. In addition, repeated administration of cimetidine reduced the gastric mucosal resistance to the ulcerogenic agents, and co-administration of ecabet prevented the aggravating effect of cimetidine.

Acknowledgments The authors would like to thank Dr. S. Takeyama for reading the manuscript, and Ms. N. Saito and Mr. M. Jinbo for their skillful technical assistance.

REFERENCES