The Effect of Cimetidine on Adaptive Cytoprotection by Mild Irritant Dose of HCl in the Rat Gastric Mucosa

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ABSTRACT — While cimetidine (CIM) is strikingly effective in inhibiting gastric acid secretion, its effect on the defensive mechanisms of the gastric mucosa has been controversial. The aims of the present study were to test if administration of CIM at an antisecretory dose is protective against acid-induced injury and to assess its effect on adaptive cytoprotection induced by non-necrotizing concentrations of HCl in rats. A dose of 100 mg/kg of CIM was administered once, or twice a day for 5 days intraperitoneally. To study the effect of CIM on HCl-induced damage, 0.6 N HCl was given orally one hour after the last administration of CIM. To study the effect of CIM on adaptive cytoprotection, 0.35 N HCl was given orally one hour after the last administration of CIM. Fifteen minutes later, 0.6 N HCl was given orally. Thirty minutes after the administration of 0.6 N HCl, the stomach was removed and ulcer indices were calculated. Pretreatment with CIM did not prevent 0.6 N HCl induced gastric damage. Prior administration of 0.35 N HCl significantly reduced ulcer indices caused by 0.6 N HCl. Short or long term treatment with CIM did not have significant effects on the reduction of ulcer indices. These results suggest that CIM at an antisecretory dose neither acts as a protective agent nor modulates the protective process of the gastric mucosa.

Recently histamine H_2 receptor blocking agents (H_2 blockers) have been widely used in the therapy of peptic ulcers. While H_2 blockers are effective as ulcer-healing agents, the frequency of relapse has been reported to be relatively high (1). On the other hand, prostaglandins (PG) are important defensive agents of the gastric mucosa. Exogenously administered PG prevents gastric damage induced by necrotizing agents (cytoprotection, 2). Low concentrations of necrotizing agents (mild irritants) also show similar effects against strong irritants by stimulation of endogenous formation of PG (adaptive cytoprotection, 3). The effect of H_2 blockers on PG production has been controversial (4–7). H_2 blockers might decrease endogenous PG formation or change the response of the gastric mucosa to mild irritants. The purpose of our study was to assess the effect of short and long term administration of cimetidine at an anti-secretory dose on HCl-induced gastric damage and adaptive cytoprotection.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Doken, Ibaragi, Japan) weighing 150–180 g were used. The animals were fed with liquid form food (Clinimeal, Eizai, Tokyo, Japan) for 24 hours and then fasted for 24 hours prior to the experiments. Water was also discontinued 3 hours before starting the studies.

Studies

Saline (control), 100 mg/kg cimetidine (CIM), once (short term) or 100 mg/kg CIM, twice a day for 5 days (long term) was given orally.

The effect of CIM on gastric acid secretion: One hour after treatment with CIM, rats were killed and their stomachs removed. Intra-gastric pH was measured by pH indicator strips (Merck, Dokkstadt, F.R. Germany).

The effect of CIM on HCl-induced gastric damage: One hour after treatment with CIM, 1 ml of 0.6 N HCl was given orally through metal tubing attached to a 6-ml syringe.

The effect of CIM or indomethacin on adaptive cytoprotection: One hour after treatment with CIM or 5 mg/kg of indomethacin, rats received 1 ml of 0.35 N HCl. Fifteen minutes later, 1 ml of 0.6 N HCl was given. These agents were administered orally.

Macroscopic mucosal lesions

The stomachs were rinsed with tap water to remove mucus. The length of hemorrhagic lesions was measured by an observer who was unaware of the treatment. Results were expressed as the total length of hemorrhagic lesions in mm.

Light microscopic studies

The opened stomachs were cut transversely 5 mm distal to the ridge formed by the fundic portion, at widths of 2 mm. These samples were fixed in 10% buffered formalin. Hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) staining were performed.

Scanning electron microscopic studies

The samples were fixed for 3 hours in 0.1 M phosphate buffer (pH 7.2), containing 2% glutaraldehyde. After they were washed three times with 0.1 M phosphate-buffered saline, they were refixed in 0.1 M phosphate-buffered 1.0% osmic acid for 1 hour. Samples were dehydrated in a graded series of ethanol solutions and then placed in isoamyl acetate solution for 18 hours. Tissues were critical-point dried with CO_2 and coated with platinum palladium. They were observed under a scanning electron microscope (Hitachi S450 LB).

Statistical analysis

Student's *t*-test was used to determine the statistical significance of the data; values of $P < 0.05$ were regarded as significant. Results

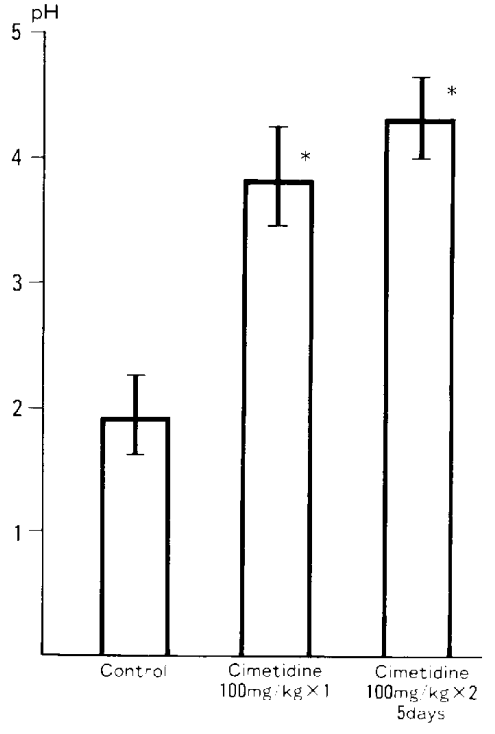

Fig. 1. Effect of short or long term treatment with cimetidine on gastric pH. Cimetidine was administered intraperitoneally. *$P < 0.01$ vs. control.

were expressed as the mean ± S.E.M.

RESULTS

The effect of CIM on gastric acid secretion

Intraperitoneal administration of 100 mg/kg CIM significantly increased gastric pH from 1.9 ± 1.3 (control) to 3.8 ± 0.3. At 1 hour after long term treatment with CIM, gastric pH was also increased significantly to 4.4 ± 0.3 (Fig. 1).

Macroscopic study

The effect of CIM on HCl-induced gastric damage: Intragastric administration of 1 ml of 0.6 N HCl produced black streaks of hemorrhagic necrosis in the gastric fundic area as shown in Fig. 2A. While there was a suggestion that short or long term treatment with CIM decreased gastric damage induced by 0.6 N HCl, the difference was not statistically significant (control, 55.5 ± 5; short term CIM, 47 ± 5; and long term CIM, 49 ± 4, Fig. 3). Thus CIM had neither a preventive nor an

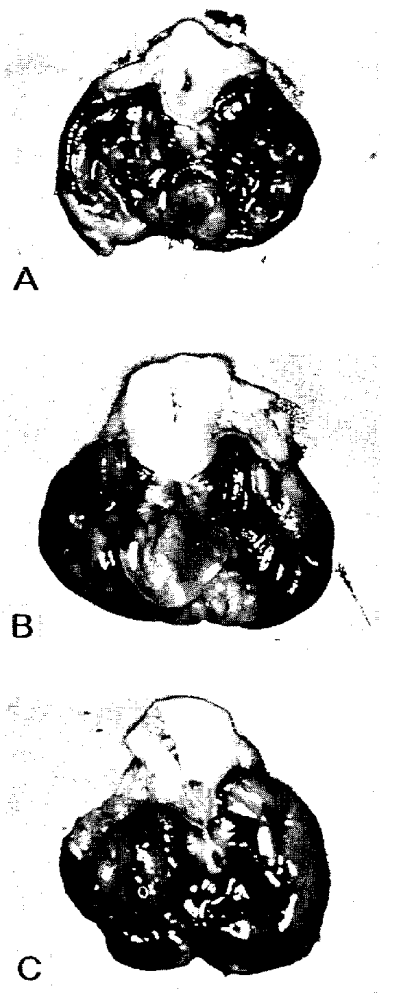

Fig. 2. Macroscopic appearance of short or long term treatment with cimetidine (CIM) against 0.6 N HCl-induced damage. A: 0.6 N HCl, B: 100 mg/kg CIM, i.p. + 0.6 N HCl, C: 100 mg/kg CIM, twice/day × 5 days.

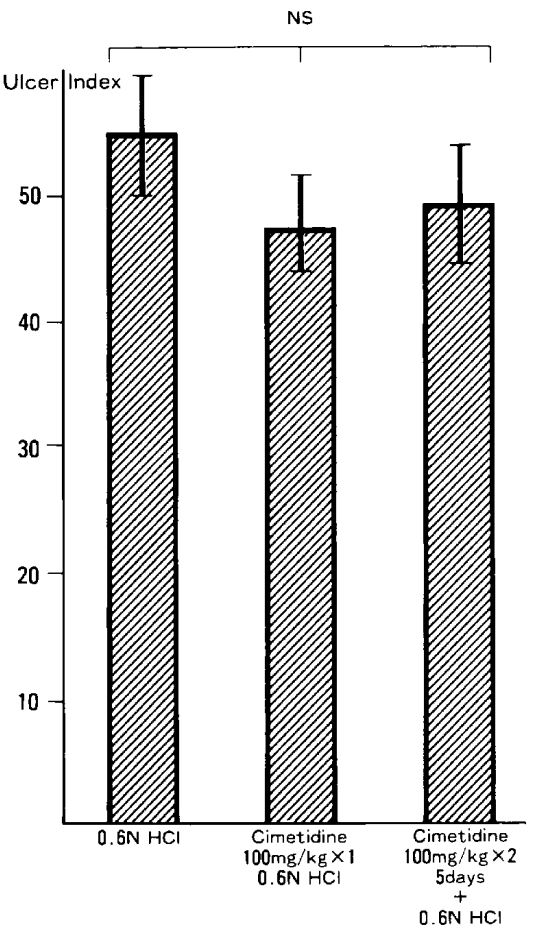

Fig. 3. Effect of short or long term treatment with cimetidine against 0.6 N HCl-induced damage. Each bar represents the mean ± S.E.M. (n = 4). NS: Not significant.

adverse effect on HCl-induced gastric damage (Fig. 2, B and C).

The effect of CIM or indomethacin on adaptive cytoprotection: Pretreatment with 0.35 N HCl remarkably reduced the length of black streaks (Fig. 4A). Quantitatively, pre-administration of 0.35 N HCl reduced ulcer indices caused by 0.6 N HCl from 55 ± 5 to 8 ± 3. Short or long term treatment with CIM did not have a significant effect on the reduction of ulcer indices by 0.35 N HCl (short time CIM, 9 ± 3 and long term CIM, 7 ± 2, Fig. 5). However, oral administration of indomethacin decreased the preventive effect of 0.35 N HCl quantitatively (Fig. 5) and macroscopically (Fig. 4D).

Light microscopic study

Hemorrhagic necrosis of rat gastric mucosa was induced by 0.6 N HCl. Deep mucosal lesions caused by 0.6 N HCl was prevented by 0.35 N HCl, but extensive desquamation of surface epithelium was observed, compared to normal controls (Fig. 6). Thus, 0.35 N HCl did not protect surface epithelial cells of the gastric mucosa. Short and long term treatment with CIM did not have any effect on the disruption of surface epithelial or deep mucosal lesions induced by 0.6 N HCl.

Scanning electron microscopic study

Normal gastric mucosa consisted of regularly arranged intact surface epithelial cells on scanning electron microscopy (SEM, Fig. 6B). While 0.35 N HCl almost completely prevented the gastric damage induced by 0.6 N HCl macroscopically, microscopically extensive exfoliation of surface epithelial cells (Fig. 6C) and denuded lamina propria were observed on SEM (Fig. 6D). Pretreatment with CIM did not protect surface mucous cells.

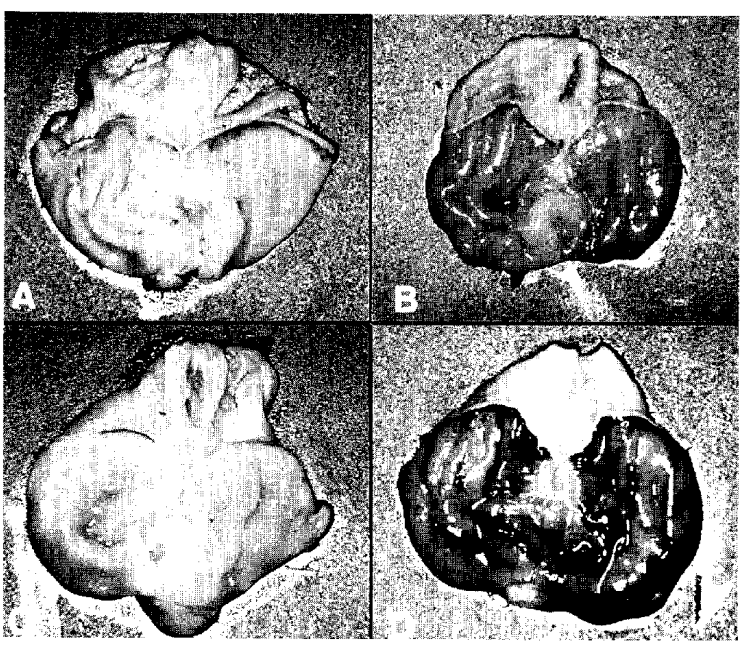

Fig. 4. Macroscopic appearance of the gastric mucosa in adaptive cytoprotection with or without short or long term treatment with cimetidine (CIM) and indomethacin (IND). A: 0.35 N HCl + 0.6 N HCl, B: 100 mg/kg CIM, i.p. + 0.35 N HCl + 0.6 N HCl, C: 100 mg/kg CIM, twice/day × 5 days + 0.35 N HCl + 0.6 N HCl, D: 5 mg/kg IND + 0.35 N HCl + 0.6 N HCl.

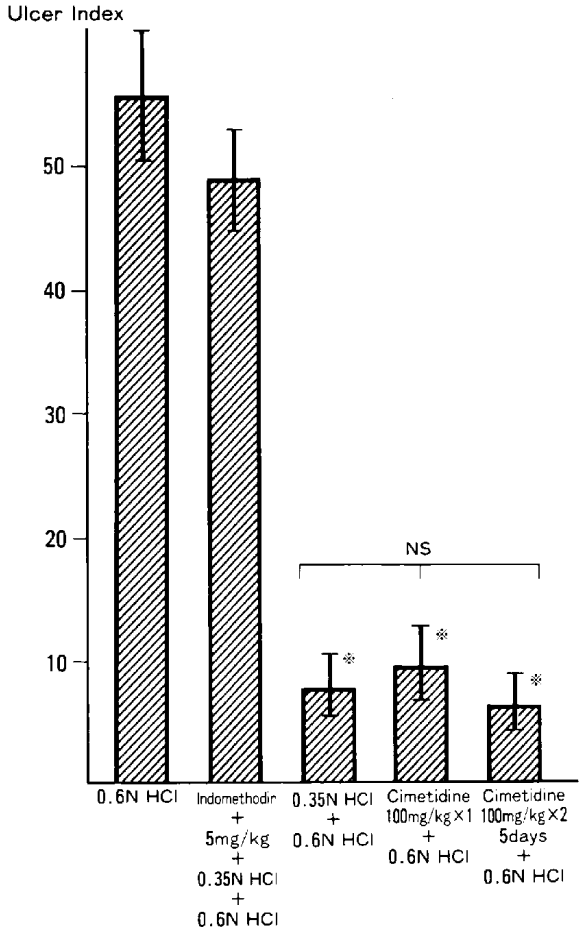

Fig. 5. Effect of short or long term treatment with cimetidine and indomethacin on adaptive cytoprotection. Each bar represents the mean ± S.E.M. (n = 7–9). *P < 0.01 vs. 0.6 N HCl. NS: Not significant.

DISCUSSION

The effect of CIM on gastric acid secretion has been well-established, whereas the effect of CIM on the defensive mechanism of the gastric mucosa has been rather controversial. Prostaglandins that show "cytoprotective" effects against gastric damage induced by noxious agents (2) have been recognized as important defensive factors. It has been suggested that CIM decreases PG production in the rat (8). On the other hand, Branski et al. demonstrated that PG in the gastric mucosa of patients, suffering from duodenal ulcers, was significantly increased after CIM treatment (5). Whether CIM is cytoprotective against agents causing necrotizing gastric damage is controversial. Guth et al. reported that CIM was protective against lesser degrees of damage induced by aspirin (4). Other studies showed CIM to be not as protective against damage induced by absolute ethanol (9) or even to increase such damage (7). Others reported that gastric damage induced by water immersion stress was increased after long term treatment with CIM (10). Previously we demonstrated that CIM at an anti-secretory dose in vitro (11) did not have a significant effect

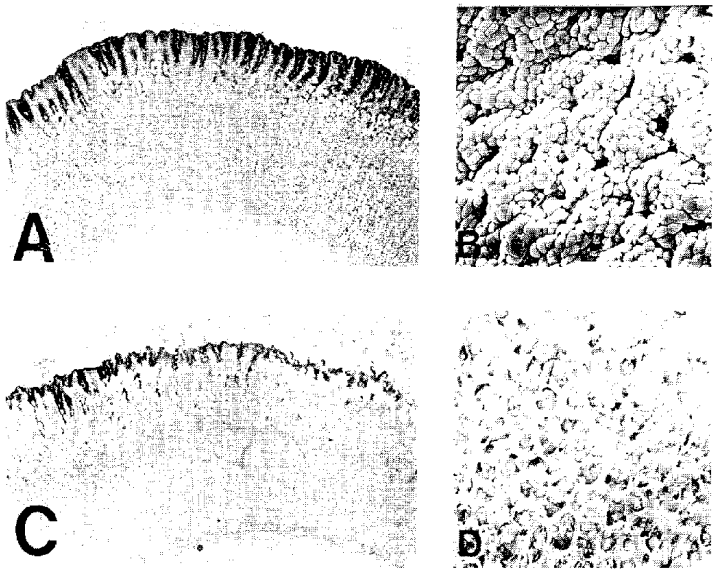

Fig. 6. Histological study of adaptive cytoprotection. A: Photomicroscopy of normal gastric mucosa (PAS staining, × 200), B: Scanning electron microscopy (SEM) of normal rat gastric mucosa (× 250), C: Photomicroscopy of rat gastric mucosa after 0.35 N HCl + 0.6 N HCl (periodic acid-Schiff staining, × 200), D: SEM of rat gastric mucosa after 0.35 N HCl + 0.6 N HCl.

on PG production nor protect against indomethacin-induced damage using cultured gastric mucous epithelial cells (12, 13). In the present study, short and long term treatment with CIM at an anti-secretory dose did not decrease HCl-induced hemorrhagic lesions. These results coincide with those of Lacy (9), although the damaging agents are different. Thus CIM even at an anti-secretory dose neither shows a "cytoprotective" effect nor modulates endogenous formation of PG in gastric mucosa.

Unlike with absolute ethanol (7) or water-immersion stress (10), CIM did not increase HCl-induced gastric damage. Since HCl is a mild irritant, suppression of HCl secretion by CIM might change the response to mild irritants after long term treatment as suggested by Goto et al. (10). The effects of short or long term treatments were tested in the present study. Indomethacin, a cyclooxygenase inhibitor, reversed the effects of adaptive cytoprotection, whereas neither short nor long term administration of CIM did so. These data suggest that CIM does not change the response of the gastric mucosa to a mild irritant even after long term treatment.

Other agents such as prostaglandins and sucralfate, while protecting against deep mucosal lesions induced by absolute ethanol, do not protect surface mucous cells of rat gastric mucosa against ethanol-induced injury (14–16). In the present study, surface mucous cells were not protected by adaptive cytoprotection using HCl as a mild irritant and also as a damaging agent. Short or long term treatment with CIM did not have a significant effect on the damage to surface mucous cells in adaptive cytoprotection.

In summary, we have demonstrated that short or long term administrations of CIM are not "cytoprotective" against HCl-induced damage in vivo and do not have adverse effects on "adaptive cytoprotection".

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