Gastric Cytoprotection by Pirenzepine in Rats: Evaluating Method for Cytoprotective Activity by Antisecretory Agents

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Accepted April 15, 1985

Abstract—The effects of antisecretory agents, pirenzepine, atropine and cimetidine, on gastric mucosal lesions induced in rats by ethanol, HCl (0.6 N), HCl-acidified 50% ethanol and HCl-acidified 50 mM sodium taurocholate (TCA) were comparatively studied with PGE2. The involvement of gastric acid in the formation of ethanol-induced necrosis was also studied. PGE2 and pirenzepine inhibited necrosis induced by all necrotizing agents at the non-antisecretory doses, and the cytoprotective effect of pirenzepine was not abolished by indomethacin. Atropine and cimetidine did not inhibit HCl-induced necrosis even at the antisecretory dose. Atropine and cimetidine at the antisecretory dose inhibited necrosis induced by ethanol, but did not inhibit the red streaks. The ethanol-induced necrosis was also inhibited by neutralizing intragastric H+ with Tris buffer. In gastric fistula rats, alkalinization of the lumen was observed by exposure to ethanol, but necrosis was not produced. There is a close relationship between the necrosis and intragastric acid. Thus it is assumed that gastric acid is involved in the formation of ethanol-induced necrosis. It was suggested that pirenzepine possesses cytoprotective action which is not related to endogenous PGs. On the other hand, the antiulcer actions of atropine and cimetidine may be due, in a part, to antisecretory effects.

"Gastric cytoprotection" proposed by Robert et al. (1) is one of the properties of prostaglandins (PGs) which is considered to be entirely independent of their antisecretory effects (2, 3). Robert et al. (1, 4) and other investigators (5, 6) also reported that gastric antisecretory agents such as anticholinergic agents, H2-antagonists, and antacids did not exhibit cytoprotection. Recently, some investigators have reported that other gastric antisecretory agents such as cimetidine, atropine, ranitidine, probanthine and pirenzepine exhibited gastric cytoprotection (7–11). Thus, there are different results as regards to the cytoprotection by antisecretory agents.

In the present study, the cytoprotective effects of pirenzepine, atropine and cimetidine were studied in comparison with prostaglandin E2 (PGE2) using HCl-acidified necrotizing agents in order to exclude the influence of the antisecretory effects of these drugs. Further, since only the formation of necrosis induced by ethanol was inhibited by all antisecretory agents used in the present study, the influence of acid on the formation of ethanol-induced necrosis was also evaluated.

Materials and Methods

Animals: Male SD rats, weighing 150–180 g, were fasted and kept for 22 hr in individual wire bottom cages to prevent coprophagy. Water was withheld for 6 hr prior to the experiments.

Gastric secretion: Animals were anesthetized with ether, and pylori were ligated according to Shay's technique (12). Drugs were administered i.d. or s.c. immediately after pylorus ligation. The animals were kept in individual cages which had wire net bottoms to prevent coprophagy. Four hr
later, pylorus-ligated rats were sacrificed with an overdose of pentobarbital. The stomach was removed and gastric juice was collected. The volume was measured, and gastric acidity was measured by titration with 0.01 N NaOH to pH 7.0 using a glass electrode pH-meter. Acid output was expressed as μEq of HCl per 4 hr.

Effects of pirenzepine, atropine, cimetidine and PGE2 on necrotizing agents-induced gastric mucosal lesions: One ml of necrotizing agents such as 99.5% ethanol, HCl (0.6 N), 50% ethanol-0.4 N HCl, and 50 mM sodium taurocholate-0.4 N HCl were given orally. One hr later, the stomach was isolated and gastric mucosal damage was observed. The gastric mucosal damage was expressed as the total length of the major axis in the gastric mucosal lesions. Drugs were administered p.o. or s.c. 30 min before oral administration of necrotizing agents.

Effect of cimetidine on gastric lumen H+: Animals were anesthetized with urethane (1.2 g/kg, i.p.) and the stomach was removed carefully and rapidly. Ten ml of saline was infused into the stomach, and the gastric content was collected through the polyethylene tubing inserted into the stomach from the pylorus. The pH and acidity of the collected gastric content were measured in the same way as already described.

Effect of addition of HCl or neutralization by Tris buffer on the ethanol-induced gastric mucosal lesions: HCl was added to ethanol in concentrations of 15–240 μEq/ml and was orally administered. One hr later, the stomach was removed. In order to neutralize gastric H+, 0.2 ml of Tris buffer (1 M) at pH 7.4 was orally administered 5 min before oral administration of 99.5% ethanol (1 ml). Fifteen min later, rats were sacrificed with urethane. The stomach was removed and the gastric mucosal lesions were examined in the same way as already described.

Effect of ethanol on gastric secretion in gastric fistula rats: Animals were anesthetized with ether and the trachea was cannulated. The esophagus was tied without disturbing the vagus nerves. An acute gastric fistula was placed into the stomach from the duodenum through the pylorus. The acute gastric fistula rats were kept in Bollman cages. The stomach was gently rinsed with warmed saline, and then 2 ml of saline was instilled into the stomach. Instilled saline was exchanged every 15 min. One ml of necrotizing agent (99.5%, 50% ethanol or 50% ethanol-0.4 N HCl) was infused into the stomach through the fistula, and it was collected 15 min later. Subsequently, the stomach was rapidly rinsed with 1 ml of saline in order to add to the necrotizing solution that had been instilled into the stomach for 15 min. Again 2 ml of saline was filled into the stomach and exchanged for fresh saline every 15 min. The animals were sacrificed 45 min later. The stomach was removed and the gastric mucosal lesions were examined. The volume and pH of the collected gastric solution were measured.

Drugs: Pirenzepine dihydrochloride (C.H. Boehringer Sohn), atropine sulfate (Sigma) and cimetidine (SK and Fujisawa) were dissolved and diluted in physiological saline. Prostaglandin E2 (Sigma) was dissolved in a few drops of ethanol (99.5%) and diluted with physiological saline. Indomethacin (Sigma) was dissolved in 0.5% NaHCO3 and immediately injected s.c. Drugs were freshly prepared before the experiments and administered in a volume of 0.1 ml/100 g, b.w.

Statistics: All data were expressed as the mean±S.E. Statistical significance was evaluated by Student’s t-test.

Results

Gastric secretion

Intraduodenal administration of pirenzepine and atropine showed dose-dependent inhibition of gastric acid secretion in Shay’s rats (Fig. 1). The high doses of pirenzepine (12.5 mg/kg), atropine (2.5 mg/kg) and cimetidine (100 mg/kg) significantly inhibited gastric secretion, but the low doses of pirenzepine (1 mg/kg) and atropine (0.25 mg/kg) had no effect (Fig. 1). Pirenzepine at a dose of 6 mg/kg tended to inhibit gastric acid secretion, but not significantly. Subcutaneous administration of pirenzepine, atropine and cimetidine also inhibited gastric acid secretion in Shay’s rats (Table 1). The high doses of pirenzepine (12.5 mg/kg),
atropine (6 mg/kg) and cimetidine (200 mg/kg) markedly reduced the volume of gastric juice and acid output (Table 1). On the other hand, intraduodenally (0.1 mg/kg) or subcutaneously (0.2 mg/kg) administered prostaglandin E2 had no effect on the gastric acid secretion at the doses that we used in this study.

Effects of pirenzepine, atropine, cimetidine and PGE2 on necrotizing agents-induced gastric mucosal lesions

i. Ethanol-induced gastric mucosal lesions: Oral administration of ethanol (1 ml, for 1 hr) induced necrosis and red streaks in the gastric mucosa (Fig. 2A and B). Though ethanol-induced necrosis was inhibited by antisecretory agents such as pirenzepine, atropine and cimetidine, the doses required to inhibit ethanol-induced necrosis were different from each other. In the case of oral administration, pirenzepine (6 mg/kg) as well as PGE2 (0.1 mg/kg) inhibited both ethanol-induced necrosis and red streaks without the inhibition of gastric acid secretion. Atropine (2.5 mg/kg) and cimetidine (100 mg/kg) showed the inhibition of necrosis in the antisecretory doses, but the red streaks were not inhibited by cimetidine. On the other hand, subcutaneous administration of these agents at the antisecretory doses inhibited ethanol-induced necrosis but not the red streaks (Table 1). PGE2 inhibited necrosis as well as red streaks without the inhibition of acid secretion.

ii. 0.6 N HCl-induced necrosis: Gastric mucosal necrosis induced by HCl (0.6 N) was inhibited 82% by oral administration of pirenzepine (6 mg/kg) without inhibition of gastric acid secretion (Fig. 3). Subcutaneous administration of pirenzepine also inhibited necrosis (inhibitory activity: 61%) at the antisecretory dose (Table 1). However, neither atropine nor cimetidine showed inhibition of necrosis even at the antisecretory doses by both oral and subcutaneous administration (Fig. 3, Table 1). PGE2 prevented necrosis by both oral and subcutaneous administration, and the inhibitory activities were 79.3% and 75.0%, respectively.

iii. Gastric mucosal lesions induced by acidified 50% ethanol or 50 mM sodium taurocholate (TCA): Oral administration of HCl (1 ml of 0.4 N HCl, for 1 hr) alone did not produce gastric mucosal lesions, but that in combination with 50% ethanol or 50 mM sodium taurocholate (TCA) produced marked necrosis (Figs. 4 and 5). Acidified 50% ethanol-induced mucosal lesions were prevented by oral administration of pirenzepine.
Table 1. Effects of subcutaneous administration of pirenzepine, atropine, cimetidine and prostaglandin E₂ on the gastric secretion and gastric mucosal lesions induced by necrotizing agents in rats

<table>
<thead>
<tr>
<th>Drugs (mg/kg)</th>
<th>Acid output (μEq/4 hr)</th>
<th>Ethanol necrosis</th>
<th>Ethanol red streaks</th>
<th>0.6N HCl</th>
<th>0.4N HCl-50% ethanol</th>
<th>0.4N HCl-50 mM taurocholate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>259.9±43.4 (7)</td>
<td>31.6± 6.7 (5)</td>
<td>35.9±12.9 (5)</td>
<td>47.0±7.2 (11)</td>
<td>88.5± 6.0 (17)</td>
<td>49.5± 7.1 (7)</td>
</tr>
<tr>
<td>PZ 12.5</td>
<td>32.7± 5.3** (6)</td>
<td>0** (5)</td>
<td>11.8± 2.8 (5)</td>
<td>18.1±3.3** (8)</td>
<td>35.6± 4.7** (13)</td>
<td>22.0± 5.3** (7)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>80.7±10.6 (10)</td>
<td></td>
<td>52.8±11.4 (6)</td>
</tr>
<tr>
<td>At 6.0</td>
<td>8.5± 2.9** (6)</td>
<td>1.0± 1.0** (5)</td>
<td>11.0± 5.6 (5)</td>
<td>32.5±8.7 (6)</td>
<td>43.9± 4.3** (8)</td>
<td>13.0± 3.4** (6)</td>
</tr>
<tr>
<td>Control</td>
<td>31.8±10.0 (6)</td>
<td>79.3± 7.5 (6)</td>
<td>58.7±9.4 (6)</td>
<td>68.1±11.6 (6)</td>
<td></td>
<td>46.8± 9.0 (5)</td>
</tr>
<tr>
<td>Cim 200</td>
<td>24.9±10.2** (6)</td>
<td>7.8± 3.1* (6)</td>
<td>55.2±12.0 (6)</td>
<td>38.7±8.0 (6)</td>
<td>68.8± 5.7 (6)</td>
<td>32.5± 5.5 (6)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>42.8± 7.7 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PG 0.2</td>
<td>210.3±28.8 (7)</td>
<td>3.8± 2.5* (6)</td>
<td>2.2± 0.7** (6)</td>
<td>14.7±4.0** (6)</td>
<td>4.3± 1.2** (6)</td>
<td>2.2± 0.7** (6)</td>
</tr>
</tbody>
</table>

PZ: pirenzepine, At: atropine, Cim: cimetidine, PG: prostaglandin E₂. Values are expressed as the mean±S.E. The numbers in parentheses show the number of rats. *, **: Significant different from the control (*: P<0.05, **: P<0.01).
and PGE2 (0.1 mg/kg) in non-antisecretory doses (inhibitory activity: 42.4% and 73.7%, respectively) (Fig. 4). However, oral administration of atropine and cimetidine had no effect on acidified 50% ethanol-necrosis. Subcutaneously administered atropine (6.0 mg/kg), which exerted antisecretory action, inhibited to 45.9% necrosis induced by HCI-acidified 50% ethanol. Pirenzepine (12.5 mg/kg, s.c.) significantly reduced necrosis to about 40% of the control. Cimetidine did not prevent necrosis (Table 1). On the other hand, a 60 min pretreatment with indomethacin (10 mg/kg, s.c.) before HCI-acidified 50% ethanol produced more severe damage (148.4±10.9 mm, n=5) in gastric mucosa than that induced by acidified 50% ethanol alone (99.1±5.8 mm, n=7). However, pirenzepine (12.5 mg/kg, s.c.) with indomethacin pretreatment reduced the necrosis (92.2±11.8 mm, n=5) to the control level.

The gastric mucosal damage induced by acidified 50 mM sodium taurocholate (TCA) was not so severe as that induced by acidified 50% ethanol (Fig. 6). Oral administration of pirenzepine (1 mg/kg) and atropine (0.25 mg/kg) even at non-antisecretory doses inhibited necrosis induced by TCA, and the inhibitory activities were 51.8% and 61.8%, respectively. Subcutaneous administration of
both drugs, which showed the inhibition of gastric acid secretion, also prevented TCA-induced necrosis (Table 1). However, cimetidine in antisecretory doses had no effect on necrosis induced by TCA (Fig. 5, Table 1). PGE2 (0.1 or 0.2 mg/kg) inhibited TCA-induced necrosis by both oral and subcutaneous administration.

Effect of cimetidine on gastric lumen H+

The existence of gastric lumen H+ in intact rats was confirmed, and the amount was 12.7±1.10 nEq/stomach (n=6). Cimetidine (200 mg/kg, s.c., for 30 min) significantly reduced the amount of gastric lumen H+ to about 30% (3.94±0.99 nEq/stomach, n=5). Effects of addition of HCl or neutralization of gastric lumen H+ by Tris buffer on the ethanol-induced necrosis

When 0.2 ml of Tris buffer (1 M at pH 7.4), which is sufficient to neutralize more than 13 nEq of HCl (total amount of gastric lumen H+ in intact rats considered by the above result), was orally administered 5 min before ethanol administration, the ethanol-induced necrosis was significantly reduced compared with the control (Table 2). The red streaks induced by ethanol were also inhibited by Tris buffer. On the other hand, there was a significant correlation between addition of HCl in the dose range from 15 to 240 nEq/ml and gastric necrosis (r=0.71, n=23, P<0.01). However, no correlation was obtained between HCl and gastric red streaks or total gastric lesions.

Effect of ethanol on gastric secretion in gastric fistula rats

Intragastric administration of 99.5% ethanol or 50% ethanol (1 ml, for 15 min) markedly produced red streaks in the gastric mucosa of gastric fistula rats, but neither bleeding nor necrosis was observed (Fig. 6). The basal pH of the gastric solution, which
was washed out by saline, was about 4.0, and the gastric lumen pH was increased over 7.0 immediately after exposure to either 99.5% or 50% ethanol. However, intragastric administration of 50% ethanol-0.4 N HCl (1 ml, for 15 min) produced no red streaks, but produced necrosis with bleeding (Fig. 6). In this case, lumen alkalinization was also observed but at a later time, when compared with that after exposure to 50% ethanol alone.

Discussion

PGE₂ and pirenzepine at the non-antisecretory doses prevented the formation of necrotic lesions induced by all the following necrotizing agents: ethanol, 0.6 N HCl, acidified 50% ethanol and acidified 50 mM taurocholate (TCA). Atropine and cimetidine at the antisecretory dose inhibited the gastric lesions induced by ethanol, but such drugs at the non-antisecretory doses did not inhibit the gastric lesions induced by ethanol. Furthermore, atropine did not inhibit HCl-induced necrosis, and cimetidine inhibited neither HCl nor HCl-acidified necrotizing agents-induced necrosis even at the antisecretory doses. These results suggest that there is a possibility that the inhibition of ethanol-induced necrosis by atropine and cimetidine may be related to their anti-secretory effects.

In the second experiment, therefore, the role of acid in the formation of ethanol-induced necrosis and red streaks was studied. Lacy and Ito (13) also reported that visually-confirmed red streaks and necrotic lesions were induced by ethanol. Though the stomach of intact rats contained about 13 μEq of H⁺, it was significantly decreased by cimetidine. Furthermore, a close correlation between the addition of exogenous H⁺ and ethanol-induced necrosis was shown. The

Table 2. Effect of neutralization with Tris buffer on the ethanol-induced gastric mucosal lesions in rats

<table>
<thead>
<tr>
<th></th>
<th>Gastric mucosal lesion (mm)</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>necrosis</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>6</td>
<td>20.5±5.4</td>
</tr>
<tr>
<td>Tris buffer (1 M), 0.2 ml</td>
<td></td>
<td>6</td>
<td>4.5±1.3*</td>
</tr>
</tbody>
</table>

Values are expressed as the mean±S.E. *: Significantly different from the control (P<0.05).
ethanol-induced necrosis was inhibited by neutralization of the residual intragastric H⁺ with Tris buffer. In the present study with gastric fistula rats that were administered 50% ethanol–0.4 N HCl, bleeding and apparent necrosis were observed at an early stage. On the other hand, only red streaks were developed after intragastric administration of 99.5% ethanol or 50% ethanol in the gastric fistula rats, whose stomachs seemed to contain a very low concentration of acid due to washing of the intragastric contents. At the same time, gastric alkalinization was observed. It is known that gastric lumen alkalinization is induced by mucosal damaging agents like ethanol, taurocholate and aspirin. This phenomenon is considered to play an important role, not in preventing the formation of necrosis, but in aiding the recovery from gastric mucosal damage (14).

These results suggested that intragastric acid was associated with the formation of ethanol-induced necrosis. It would be inadequate to use ethanol-induced necrosis as the experimental model for evaluation of cytoprotection by substances possessing inhibitory effects on gastric acid secretion or neutralizing effects on intragastric acidity. It is considered that such studies should be carried out under conditions where the influence of gastric acid is excluded with the models induced by HCl or HCl-acidified necrotizing agents.

As already mentioned, PGE₂ and pirenzepine at the non-antisecretory doses inhibited all experimental necrosis induced by ethanol, HCl and HCl-acidified necrotizing agents. It is thus obvious that pirenzepine possesses cytoprotective action, not only an antisecretory effect. Moreover, oral administration of pirenzepine showed such an inhibitory effect at a low dose compared with subcutaneous administration. This finding indicated that the cytoprotection by pirenzepine might be due to direct action on the gastric mucosa.

There are substances reported to have cytoprotective action, such as acetazolamide (15) and carbenoxolone (16). The cytoprotective action of these substances is considered to be related to endogenous prostaglandins. Carbenoxolone inhibits prostaglandins decomposition, and acetazolamide activates the biosynthesis of prostaglandins. Their cytoprotective effects are abolished by indomethacin, an inhibitor of prostaglandin biosynthesis. However, the cytoprotective action of pirenzepine was not abolished by indomethacin, and Konturek et al. (9) also reported that pirenzepine had no effect on the gastric mucosal PGE₂ and PG₁₂ level. Therefore, it was assumed that the cytoprotective mechanism of pirenzepine is not due to endogenous prostaglandins.

On the other hand, it was reported that imidazole, an inhibitor of thromboxane A₂ (TXA₂) biosynthesis (17, 18), inhibited aspirin-induced erosion (19) and that OKY-1581, a selective inhibitor of thromboxane biosynthesis, inhibited taurocholate plus HCl-induced necrosis (20). Furthermore, it was suggested that water-immersion stress ulcer was inhibited by indomethacin at a very low dose, which exerted an inhibitory action on TXA₂ biosynthesis (21), and by imidazole (22). These results indicate that TXA₂-induced gastric vasoconstriction and the decrease of gastric mucosal blood flow may be involved in the pathogenesis of certain ulcerative disorders of the stomach and that TXA₂ biosynthesis inhibitors may have some protective action against certain kinds of gastric mucosal lesions. We have suggested that pirenzepine possesses an inhibitory action on TXA₂ biosynthesis and this action of pirenzepine might be involved in the prevention of water-immersion stress ulcer (23). Therefore, this inhibitory effect of TXA₂ biosynthesis by pirenzepine may be partially related to its cytoprotective mechanism. Besides, it was reported that pirenzepine showed other pharmacological effects such as an increasing action on gastric mucosal blood flow (24, 25) and activation of mucus secretion (26). Although there is yet insufficient information to understand the mechanism of cytoprotection, Miller (3) has suggested the following effects as possible mechanisms for cytoprotection by prostaglandins: (1) prevention of gastric mucosal barrier disruption, (2) stimulation of mucus secretion, (3) enhancement of gastric mucosal blood flow, (4) stimulation of
nonparietal cell alkaline secretion, (5) stimulation of macromolecular synthesis, (6) stimulation of cellular transport processes (Na⁺, Cl⁻), (7) stimulation of c-AMP, (8) stabilization of tissue lysosomes, (9) dissolution of gastric mucosal folds, (10) maintenance of gastric mucosal sulfhydryl compounds, and (11) stimulation of surface active phospholipids. It is considered that factors (1) to (4) are the most important of all the factors. Therefore, the increasing action of gastric mucosal blood flow and activation of mucus secretion may also be involved in the cytoprotective mechanisms of pirenzepine. The antiulcer effect of pirenzepine might be exerted by its cytoprotective action in addition to its anti-secretory effect. Cytoprotection by pirenzepine may be involved in its antiulcer effect in addition to its anti-secretory effect.

Atropine inhibited necrosis induced by 0.4 N HCl-taurocholate at the non-antisecretory dose, but did not prevent HCl-induced necrosis even at the antisecretory dose. It is, therefore, assumed that atropine did not show gastric cytoprotection at least in the doses lower than the antisecretory dose. Even if atropine possesses weak cytoprotection, the action will only appear at antisecretory doses. Hence, atropine is presumed to derive its antiulcer action mainly from its anti-secretory effect. Since cimetidine inhibited neither necrosis induced by HCl nor HCl-acidified necrotizing agents at the antisecretory dose, it seems to have no cytoprotective action.

References


