Effects of Antimuscarinic Agents and Prostaglandin E2 on the Gastric Mucosal Lesions Induced by Necrotizing Agents and Water-Immersion Stress in Rats

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Abstract—The role of antimuscarinic action in gastric mucosal protection against necrotizing agents and the role of such mucosal protection in antiulcerogenic action were studied in rats with i.v. administered antimuscarinic agents. Pirenzepine, as well as PGE2, prevented the gastric mucosal lesions induced by all necrotizing agents (99.5% ethanol, 0.6 N HCl, 0.15 N NaOH, 0.4 N HCl-50 mM taurocholate), but atropine did not prevent the HCl-induced lesions. Cimetidine inhibited only the ethanol-induced lesions even at the antisecretory dose. Higher doses of pirenzepine (5-fold) and atropine (10-fold) were required to inhibit the gastric secretion in Shay rats than in vagally stimulated rats. There was no difference between the antisecretory doses of cimetidine in Shay rats and vagally stimulated rats. PGE2 (0.03–0.1 mg/kg) did not affect gastric secretion. The protective doses of pirenzepine and atropine against mucosal lesions induced by necrotizing agents were similar to the dose in inhibiting vagally stimulated acid secretion and water-immersion stress-induced lesions. PGE2 (100 μg/kg) did not prevent the water-immersion stress induced gastric lesions. These results suggested that antimuscarinic agents protect the gastric mucosa from necrotizing agents via a blocking action on the activation of the intrinsic cholinergic nerve. However, antiulcerogenic action is more deeply concerned with antisecretory action than cytoprotection.

Prostaglandins show gastric cytoprotection without inhibition of gastric acid secretion (1, 2). Gastric mucosal lesions induced by necrotizing agents such as ethanol, HCl and NaOH are used generally for estimation of cytoprotection. Recently, we found that antimuscarinic agents, pirenzepine and atropine, prevented the gastric mucosal lesions induced by ethanol, HCl-acidified taurocholate and HCl-acidified ethanol, although cimetidine prevented the ethanol-induced lesions alone (3). This finding indicates that the antimuscarinic action may be involved in the protective action on gastric mucosal lesions induced by necrotizing agents.

In this study, the effects of antimuscarinic agents on the gastric mucosal lesions induced by necrotizing agents and the acid secretion induced by vagal stimulation were examined in an attempt to define the role of antimuscarinic action in protection of gastric mucosa. In addition, the role of such mucosal protection of antimuscarinic agents in antiulcerogenic action was studied using the water-immersion stress model.

Materials and Methods

Animals
Male SD rats, weighing 140–180 g, were deprived of food and water and kept for 24 hr in individual wire bottom cages to prevent coprophagy.

Necrotizing agent-induced gastric mucosal lesions
One ml of necrotizing agents such as HCl...
(0.6 N), 99.5% ethanol, NaOH (0.15 N), or 0.4 N HCl. 50 mM sodium taurocholate were given orally. One hr later, animals were sacrificed by an overdose of pentobarbital. The stomach was removed and gastric mucosal lesions were examined. The lesions were quantified as the total length of the major axis in the gastric mucosal lesion, as described by Takagi and Okabe (4). Drugs were administered i.v. 30 min before oral administration of necrotizing agents.

Gastric secretion

Shay rats: Animals were anesthetized with ether, and pylori were ligated according to Shay’s technique (5). Drugs were administered i.v. immediately after pylorus ligation. The animals were kept in individual cages with wire net bottoms to prevent coprophagy. Four hours later the pylorus-ligated rats were sacrificed with an overdose of pentobarbital. The stomach was removed and gastric juice was collected. After centrifugation, 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.

Vagally stimulated rats: Animals were anesthetized with urethane (1.2 g/kg, i.p.), and a polyethylene tube was cannulated into the trachea. The duodenum was exposed through a midline incision, and a polyethylene cannula was inserted into the stomach through the duodenum. The esophagus was ligated at the cervical level. The stomach was first lavaged with saline then filled with 2 ml of warm saline (37±0.5°C). The saline was exchanged every 15 min for 45 min before and during the vagal stimulation; gastric acidity was measured as described above. For vagal stimulation, the bilateral vagus nerves in the neck were cut. The peripheral end of the left vagus nerve was electrically stimulated for 1 hr (10 V, 10 Hz, 2 msec).

Water-immersion stress-induced gastric mucosal lesions

Immediately after the drug administration, i.v., the animals were placed in a restraint cage and immersed in a water bath at 23±0.5°C to the depth of the xiphoid (4). After 6 hr, the stomach was isolated and the gastric mucosal lesions were examined as described above.

Drugs

Pirenzepine dihydrochloride (C. H. Boehringer Sohn) and atropine sulfate (Wako) were dissolved and diluted in physiological saline. Cimetidine (Sigma) was initially dissolved in 0.3 N HCl, adjusted to pH 7 with 0.3 N NaOH and diluted with physiological saline. Prostaglandin E2 (Funakoshi) was dissolved in a few drops of ethanol (99.5%) and diluted with physiological saline. All drugs were freshly prepared before the experiments and administered in a volume of 0.1 ml/100 g.

Calculation of ED50

ED50 values to halve the gastric acid output (in Shay rats or vagally stimulated rats) or the severity of mucosal lesions were calculated by the Litchfield-Wilcoxon method.

Results

Necrotizing agents-induced gastric mucosal lesions

Oral administration of 99.5% ethanol (1 ml, for 1 hr) or 0.15 N NaOH produced the visually-confirmed red streaks and necrosis in rat gastric mucosa. Either 0.6 N HCl or TCA produced gastric necrosis alone.

The i.v. administration of pirenzepine (0.1–1.6 mg/kg) and PGE2 (0.01–0.1 mg/kg) prevented dose-dependently the gastric mucosal lesions induced by all necrotizing agents, including ethanol- or NaOH-induced red streaks (Table 1). Atropine (0.01–0.3 mg/kg) also prevented dose-dependently the gastric mucosal lesions induced by ethanol, NaOH, and TCA, but it prevented only 28% of HCl-induced necrosis even at the highest dose of 3 mg/kg (Table 1). On the contrary, cimetidine (12.5–50 mg/kg) prevented dose-dependently the ethanol-induced gastric mucosal necrosis, though it did not prevent ethanol-induced red streaks and gastric mucosal necrosis induced by the other necrotizing agents (Table 1).

Gastric secretion

Shay rats: The i.v. administration of pirenzepine, atropine and cimetidine inhibited dose-dependently the gastric acid secretion in Shay rats (Figs. 1, 2 and 3).
ED50 values for pirenzepine, atropine, cimetidine and prostaglandin E₂ (i.v.) on the gastric mucosal lesions induced by necrotizing agents in rats

<table>
<thead>
<tr>
<th></th>
<th>Pirenzepine (n=6–11)</th>
<th>Atropine (n=4–8)</th>
<th>Cimetidine (n=5–11)</th>
<th>PGE₂ (n=5–9)</th>
</tr>
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<tbody>
<tr>
<td>Ethanol</td>
<td></td>
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<tr>
<td>Necrosis</td>
<td>0.100 (0.044–0.229)</td>
<td>0.019 (0.006–0.060)</td>
<td>36.0 (20.5–63.1)</td>
<td>0.022 (0.016–0.031)</td>
</tr>
<tr>
<td>Red streaks</td>
<td>0.245 (0.126–0.478)</td>
<td>0.045 (0.016–0.128)</td>
<td>n.e.</td>
<td>0.019 (0.012–0.029)</td>
</tr>
<tr>
<td>0.6N-HCl</td>
<td>0.300 (0.127–0.707)</td>
<td>n.e.</td>
<td>n.e.</td>
<td>0.031 (0.015–0.065)</td>
</tr>
<tr>
<td>0.15N-NaOH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>0.390 (0.264–0.576)</td>
<td>0.096 (0.039–0.235)</td>
<td>n.e.</td>
<td>0.025 (0.012–0.052)</td>
</tr>
<tr>
<td>Red streaks</td>
<td>0.640 (0.380–1.080)</td>
<td>0.101 (0.036–0.283)</td>
<td>n.e.</td>
<td>0.022 (0.011–0.044)</td>
</tr>
<tr>
<td>50 mM-taurocholate</td>
<td>0.310 (0.125–0.766)</td>
<td>0.037 (0.013–0.107)</td>
<td>n.e.</td>
<td>0.011 (0.003–0.041)</td>
</tr>
</tbody>
</table>

n.e.: No effect

Fig. 1. Effects of intravenous administration of pirenzepine on gastric secretions and gastric mucosal lesions in rats. ●, △: Gastric secretion in Shay rats and vagally stimulated rats. ◊, ■: Gastric mucosal necrosis induced by ethanol and 0.6N HCl. ○: Gastric mucosal lesions induced by water-immersion stress. Points are means for six to nine animals.

ED50 values for pirenzepine, atropine and cimetidine (the doses produced 50% inhibition of acid output for 4 hr) were 1.23 (95% confidence limit: 0.35–4.31), 0.12 (0.03–0.45) and 50.0 (18.8–133.0) mg/kg, respectively. Atropine was about 10 times potent as pirenzepine in inhibiting acid output. On the other hand, i.v. administered PGE₂ (0.01, 0.03 and 0.1 mg/kg) inhibited only 18–26% of the gastric acid output.

Vagally stimulated rats: Intravenously administered pirenzepine, atropine, and cimetidine inhibited dose-dependently the increase in gastric acid secretion induced by vagal stimulation (Figs. 1, 2 and 3). The estimated ED50 values for pirenzepine, atropine and cimetidine were 0.240 (95% confidence limit: 0.068–0.850), 0.017 (0.010
Atropine was about 10 times as potent as pirenzepine in inhibiting acid output. The i.v. administration of PGE2 at 0.03 mg/kg had no effect; and at the dose of 0.1 mg/kg, it inhibited only 30% of the gastric acid secretion.

Water-immersion stress-induced gastric mucosal lesions

The control rats had gastric mucosal lesions of 11.7±1.7 mm/rat (n=24) after water-immersion stress for 6 hr. The i.v. administration of pirenzepine, atropine and cimetidine prevented dose-dependently the gastric mucosal lesions (Figs. 1, 2 and 3). The estimated ED50 values for pirenzepine, atropine and cimetidine were 0.47 (95% confidence limit: 0.22–1.00), 0.015 (0.004–0.050) and 27.5 (17.0–44.5) mg/kg, respectively. Atropine was 30 times as potent as pirenzepine in inhibiting water-immersion stress-induced gastric lesions. On the other hand, intravenously administered PGE2 (0.01, 0.03 and 0.1 mg/kg) had no effect on the stress-induced gastric lesions.

Discussion

PGE2 and pirenzepine clearly prevented the gastric mucosal lesions induced by necrotizing agents such as ethanol, HCl, NaOH and TCA. Atropine prevented the gastric mucosal lesions induced by ethanol, NaOH and TCA, except for HCl. Cytoprotective doses of PGE2 did not inhibit acid secretion in Shay rats or vagally stimulated rats. Cimetidine even at dose levels which inhibited acid secretion prevented only the formation of mucosal necrosis induced by ethanol, but did not prevent those induced by HCl, NaOH and TCA. This result indicates that cimetidine does not possess cytoprotection in agreement with the other reports (1, 3, 6, 7).

The i.v. administration of pirenzepine...
showed the protective action against necrotizing agents from about one-tenth to one-half the dose compared to the antisecretory dose needed in Shay rats. For atropine i.v. administration, the protective action against necrotizing agents was produced using from about one-sixth to four-fifth the doses compared to the antisecretory doses in Shay rats. The distinction between a protective dose against necrotizing agents and an antisecretory dose of pirenzepine and atropine may suggest that the antisecretory action of antimuscarinic agents does not contribute to the protective action against necrotizing agents and that such antimuscarinic agents possess the cytoprotective action. However, pirenzepine and atropine at doses protecting gastric mucosa from ethanol, NaOH and TCA inhibited the increase in acid secretion induced by vagal stimulation. The doses of such antimuscarinic agents for antisecretory action in vagally stimulated rats were about one-fifth of that in Shay rats. Therefore, such protective action of antimuscarinic agents is not cytoprotection as defined by Robert (1).

Though pirenzepine prevented the gastric lesions induced by exogenous HCl, atropine had no effect. Therefore, pirenzepine possesses cytoprotection which is independent of its antimuscarinic action. The possible mechanisms of such cytoprotection by pirenzepine were discussed in our previous report (3). In experiments of cytoprotection, Shay rats are generally used for the estimation of the antisecretory action of drugs (1, 8, 9). Our results showed that the estimation of antisecretory action in such experiments should be performed in vagally stimulated rats, although the cause of the distinction between antisecretory doses in Shay rats and vagally stimulated rats was unclear.

It has been reported that the gastric mucosal lesions induced by necrotizing agents may be due to the activation of intrinsic cholinergic nerve terminals of the gastric wall (10, 11). Therefore, the protective action of pirenzepine and atropine against ethanol, NaOH and TCA may be due to antimuscarinic action in the intrinsic cholinergic neuron. Although cimetidine inhibited the gastric acid secretion in both Shay rats and vagally stimulated rats, it did not inhibit the gastric mucosal lesions induced by the necrotizing agents (except for ethanol). Therefore, the protective action of antimuscarinic agents against necrotizing agents may not be caused by the antisecretory action alone.

Water-immersion stress-induced gastric lesions is caused by the increase in acid secretion due to the activation of vagus nerve and the ischemia of gastric mucosa (12, 13). Pirenzepine and atropine prevented the water-immersion stress-induced gastric lesions; the inhibitory doses were the same as those needed to prevent the gastric acid output induced by vagal stimulation, but they did not agree with those in Shay rats. Cimetidine also prevented the water-immersion stress-induced gastric lesions at the antisecretory dose. However, PGE2 even at 100 μg/kg which is about five times higher than cytoprotective dose did not prevent the water-immersion stress-induced gastric lesions. Therefore, cytoprotection of PGE2 may not involve antulcerogenic action.

In conclusion, the antimuscarinic agents protect the gastric mucosa against the necrotizing agents via a blocking action on the activation of the intrinsic cholinergic nerve induced by necrotizing agents. Antiulcerogenic action is more deeply concerned with antisecretory action than cytoprotection.

References
4 Takagi, K. and Okabe, S.: The effects of drugs on the production and recovery processes of the


