EFFECT OF POLYAMINES ON ACIDIFIED ETHANOL-INDUCED GASTRIC LESIONS IN RATS

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Abstract—The participation of polyamines and nonprotein sulfhydryls in the gastric cytoprotective mechanisms was studied using gastric mucosal lesions produced by acidified ethanol in rats as an experimental model. Treatment with prostaglandin E₂ (PGE₂), but not cimetidine, prevented the formation of gastric mucosal lesions. Oral administration of cadaverine, spermidine and spermine prevented the lesion formation by acidified ethanol in a dose-dependent manner. Indomethacin or acetazolamide had no influence on the cytoprotective effect of spermine, whereas sulfhydryl blockers such as iodoacetamide and N-ethylmaleimide partially blocked it. Sulfhydryl compounds such as cysteine, reduced glutathione (GSH), and cysteamine prevented the lesion formation induced by acidified ethanol. The concentration of nonprotein sulfhydryls in the gastric mucosa was significantly decreased at 1 hr after administration of acidified ethanol, and this decrease was partially prevented by spermine or PGE₂. These results suggest that the cytoprotective effect of spermine may not be mediated by endogenous prostaglandins or alkaline secretion in the gastric mucosa, but may be partially related to endogenous sulfhydryl compounds.

A number of recent observations suggest that prostaglandins play an important role in the integrity of the gastric mucosa. Recently, Wright et al. (1) reported that gastric mucosal PGE levels were reduced in patients with gastric ulcer disease. Various types of exogenous prostaglandins have a protective effect on the gastric mucosa against necrotizing agents such as absolute ethanol, strong acid or alkali, and boiling water (2, 3). The effect, so-called cytoprotection, is independent of the inhibition of acid secretion. The mechanisms of cytoprotection have not been fully elucidated, although stimulation of gastric mucosal alkaline secretion (4) and gastric chloride transport (5) have been suggested as contributing factors. The present study was done to examine whether the endogenous substances other than prostaglandins are involved in the gastric protective mechanisms in rats. Treatment with polyamines was found to prevent gastric mucosal lesions induced by acidified ethanol.

Materials and Methods

Procedures to produce gastric lesions: Male Sprague-Dawley rats, weighing 250–350 g, were fasted for 24 hr before the experiments, but allowed free access to water. Various concentrations (20–80%) of ethanol in 150 mM HCl were given orally in 1 ml, and the animals were sacrificed with an overdose of ether 1 hr later. The stomachs were removed and opened along the greater curvature. The length of each lesion was measured under a binocular microscope (×10), and the lesion index was expressed as the sum of the length of these lesions.

Measurement of gastric mucosal non-protein sulfhydryls: Animals were killed by
decapitation between 2 and 4 p.m. The glandular part of the stomach was removed, washed with ice-cold saline and then frozen on liquid nitrogen. The mucosal layer was separated from the muscle layer with a sharp blade. The concentration of non-protein sulfhydryls was determined according to the method of Ellman (6). The tissue sample was placed in 20 volumes of 1 mM EDTA and homogenized with a high speed homogenizer (Ultra-Turrax) for 15 sec. Proteins were removed by centrifugation at 5000 g for 15 min after addition of a quarter volume of 25% HPO₃, and 0.2 ml of the supernatant fluid was neutralized with 0.1 N NaOH and mixed with 1 ml of 0.5 M phosphate buffer at pH 8.0. Distilled water was added to bring the volume to 2.4 ml and then 0.1 ml of Ellman’s reagent (4 mM 5,5’-dithiobis [2-nitrobenzoic acid] in 0.1 M phosphate buffer at pH 7.0) was added. The absorbance was immediately measured at 412 nm.

Drug treatments: Drugs used were cimetidine (synthesized in our laboratories), PGE₂ (Wako), indomethacin (Sigma), acetazolamide (Sigma), putrescine dihydrochloride (Nakarai), cadaverine dihydrochloride (Nakarai), spermidine trihydrochloride (Nakarai), spermine tetrahydrochloride (Nakarai), cysteine (Nakarai), cysteamine hydrochloride (Nakarai), GSH (Kojin), N-ethylmaleimide (Nakarai) and iodoacetamide (Nakarai). Polyamines and cysteamine were orally administered as a water solution in doses converted into free base. N-ethylmaleimide (solution in saline), iodoacetamide (solution in saline) and indomethacin ( suspension in 0.5% methylcellulose) were administered subcutaneously. Cimetidine, cysteine and GSH were intraperitoneally administered as a saline solution. PGE₂ (solution in 1% ethanol) was administered orally.

Statistical analysis: The data were analyzed by means of Student’s t-test.

Results

Gastric mucosal lesions induced by acidified ethanol: The relationships between ethanol concentration and the intensity of the gastric lesions were studied (Fig. 1). Gastric lesions were hardly produced by 20% ethanol in 150 mM HCl, but appeared to a similar degree when the ethanol concentration exceeded 40%. This suggests that a threshold concentration exists in the resistance of gastric mucosa to ethanol.

Figure 2A shows gastric lesions produced by oral administration of 1 ml of 60% ethanol in 150 mM HCl. The lesions were located predominantly in the glandular portion, which showed black or reddish brown lines accompanied by hemorrhages. Acidification of ethanol accelerated the development and aggravated the severity of the gastric lesions compared with equivalent concentrations without HCl (data not shown). Histological examination of the tissues showed necrotic lesions in the mucosal layer, which usually reached the muscularis mucosae, and edema

![Fig. 1](Image)

Fig. 1. Effect of ethanol in various concentrations on the formation of gastric lesions. One milliliter of ethanol plus 150 mM HCl was given orally, and animals were killed 1 hr later. Each column is the mean ± S.E. of six animals. No significant difference was found between the 40, 60, 80% ethanol groups.
in the submucosal layer (Fig. 2B). The intensity of necrosis in the lesion area was more severe than that induced by absolute ethanol alone (data not shown).

Effects of cimetidine, PGE$_2$ and polyamines on the gastric lesions induced by 60% ethanol in 150 mM HCl: As shown in Fig. 3, cimetidine, at 20 mg/kg given intraperitoneally 1 hr before administration of 60% ethanol in 150 mM HCl, showed no effect on the intensity of the lesions, while 100 $\mu$g/kg PGE$_2$ given orally 30 min before acidified ethanol markedly prevented their formation. Polyamines such as cadaverine, spermidine and spermine, given orally at 50 mg/kg 30 min before acidified ethanol, significantly prevented lesion formation, but putrescine did not.

The cytoprotective effects of cadaverine, spermidine and spermine were dose-dependent in the dose range of 30 to 100 mg/kg (Fig. 4).

Effects of indomethacin, acetazolamide and sulphydryl blocking agents on the cytoprotection by spermine: Indomethacin given subcutaneously at 5 mg/kg 90 min before spermine administration had no effect on the cytoprotective action of spermine. Indomethacin alone slightly, though significantly, aggravated the lesions induced by 60% ethanol in 150 mM HCl. Pretreatment with acetazolamide at 120 mg/kg given intraperitoneally 1 hr before spermine did not affect the cytoprotection, and acetazolamide alone also potently prevented lesion formation (Fig. 5).

Iodoacetamide, given subcutaneously at 20 mg/kg 30 min before spermine, or N-
ethylmaleimide at 10 mg/kg subcutaneously administered together with spermine, partially blocked the cytoprotection by spermine. N-ethylmaleimide alone significantly aggravated the lesions, but iodoacetamide did not appreciably affect them (Fig. 6).

Effects of sulfhydryl compounds on acidified ethanol-induced gastric lesions: Figure 7 shows the effect of sulfhydryl compounds such as cysteine, GSH and cysteamine on the gastric lesions induced by 60% ethanol in 150 mM HCl. All significantly prevented the lesion formation when given at 100 mg/kg 30 min before acidified ethanol. The protective effect of cysteamine was dose-dependent in the range of 10 to 100 mg/kg.

Changes in gastric mucosal nonprotein sulfhydryl levels induced by treatments: The concentration of nonprotein sulfhydryls in the gastric mucosa was significantly reduced.
Fig. 7. Effect of sulfhydryl drugs on acidified ethanol-induced gastric lesions. Each column is the mean±one S.E., and the number of animals is given in parentheses. Statistical significance of difference from the control: *P<0.02, **P<0.01, ***P<0.001.

Table 1. Relationship between the concentration of nonprotein sulfhydryls in the gastric mucosa and the severity of the acidified ethanol-induced gastric lesions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nonprotein sulfhydryls (μmoles/g wet weight)</th>
<th>Gastric lesion index (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (Starvation for 24 hr)</td>
<td>5.30±0.18 (7)</td>
<td>—</td>
</tr>
<tr>
<td>60% EtOH in 150 mM HCl</td>
<td>3.92±0.30** (7)</td>
<td>88.3±7.7 (10)</td>
</tr>
<tr>
<td>Spermine 100 mg/kg p.o. + EtOH-HCl</td>
<td>4.84±0.23† (7)</td>
<td>2.0±1.4†† (9)</td>
</tr>
<tr>
<td>Control (Starvation for 24 hr)</td>
<td>6.40±0.30 (7)</td>
<td>—</td>
</tr>
<tr>
<td>60% EtOH in 150 mM HCl</td>
<td>5.02±0.42* (7)</td>
<td>78.3±5.6 (11)</td>
</tr>
<tr>
<td>PGE₂ 100 μg/kg p.o. + EtOH-HCl</td>
<td>6.05±0.22† (7)</td>
<td>1.2±0.6†† (8)</td>
</tr>
</tbody>
</table>

Each value represents the mean±S.E., and the number of animals is given in parentheses. Statistical significance of difference from the control: *P<0.02, **P<0.01. Statistical significance of difference from the group given acidified ethanol alone: †P<0.05, ††P<0.001.

at 1 hr after administration of 60% ethanol in HCl. This reduction could be partially counteracted by 100 mg/kg spermine or 100 μg/kg PGE₂ given orally 30 min before acidified ethanol (Table 1).

Discussion

PGE₂, in a dose which was shown not to affect the acid secretion (3), prevented the formation of gastric lesions in rats induced by the administration of acidified ethanol. Cimetidine, administered similarly, did not affect the severity of lesion formation. This dose of cimetidine was verified to almost completely inhibit the gastric acid secretion in preliminary experiments. These results agree with those of Robert et al. (2, 3) that the cytoprotection by prostaglandins is independent of the inhibition of gastric acid secretion.

Polyamines such as cadaverine, spermidine and spermine as well as PGE₂ prevented the formation of gastric lesions by acidified ethanol. Robert et al. (7, 8) reported that
pretreatments with mild irritants given orally prevented the gastric lesions produced by necrotizing agents through the adaptive cytoprotection via endogenous prostanoids, and this cytoprotection was diminished by indomethacin. We examined the possibility that polyamines might act as mild irritants in the gastric mucosa and found that indomethacin does not influence the action of spermine. On the other hand, gastric HCO3- transport has been suggested to have a role in gastric mucosal protection (9-11). 16,16-Dimethyl PGE2 and PGF2a stimulate alkaline secretion in amphibian gastric mucosa (12), which is thought to be affected by carbonic anhydrase (9). In fact, acetazolamide, an inhibitor of carbonic anhydrase, has been found to interfere with the PGE2 action of preventing indomethacin-induced gastric erosions (4). However, the present results show that the cytoprotective effect of spermine against acidified ethanol-induced gastric lesion is not influenced by acetazolamide. These results suggest that the cytoprotective action of spermine is not mediated by endogenous prostanoids or the stimulation of alkaline secretion. Unexpectedly, treatment with acetazolamide alone prevented the lesions induced by acidified ethanol: the mechanism is unknown at present.

Recently, Boyd et al. (13) reported that the gastric mucosa contains a high concentration of GSH, comparable to that in the liver. Cellular GSH is generally associated with the prevention of tissue damage by various reactants including peroxides and free radicals (14, 15). Diethyl maleate (16) or cold-restraint stress (13), which decreases the gastric mucosal GSH, produces acute gastric lesions in rats. A recent report (17) demonstrated that sulfhydryl blockers reverse the cytoprotection by PGF2a against absolute ethanol-induced gastric lesions. These reports suggest that the gastric sulfhydryl compounds, primarily GSH, play an essential role in the gastric cytoprotective mechanisms. We are interested in whether or not the gastric sulfhydryls affect the cytoprotective effect of spermine. The effect of spermine was partially blocked by sulfhydryl blockers such as iodoacetamide and N-ethylmaleimide in doses smaller than those reported to reverse the cytoprotection by PGF2a (17). In contrast, sulfhydryl compounds prevented the acidified ethanol-induced gastric lesions. The results agree with the report by Szabo et al. (17) that sulfhydryl compounds offer protection against gastric damage by absolute ethanol. The acidified ethanol-induced lesions were accompanied by the concomitant decrease in gastric mucosal nonprotein sulfhydryls, and pretreatment with either spermine or PGE2 prevented both the decrease of nonprotein sulfhydryls and the intensity of the lesions in the gastric mucosa. The decrease of nonprotein sulfhydryl levels in the gastric mucosa has been reported to develop prior to the initiation of major gastric lesions (17), and hence the maintenance of nonprotein sulfhydryl levels by spermine or PGE2 seems to be the cause of the cytoprotective effect of these compounds, rather than its result.

Intracellular polyamines such as putrescine, spermidine and spermine have been suggested as important factors in cell proliferation including DNA, RNA and protein synthesis (18). One of their functions is concerned with the stabilization of DNA (19, 20) and RNA (21). Trophic agents such as pentagastrin (22) and epidermal growth factor (23) prevent the formation of gastric lesions produced by stress or aspirin plus HCl. This protective action seems to be closely related to prevention of the reduction of DNA synthesis in the gastric mucosa by ulcerogens. The cytoprotective effect of polyamines may also be related to the gastric mucosal DNA or protein synthesis.
At present, the cytoprotective mechanism of spermine is not clear, but the present results suggest that it may partly be due to the maintenance of gastric mucosal non-protein sulfhydryl levels.

References


