Effect of 16,16-Dimethyl Prostaglandin E₂ on Gastric Surface Epithelial Cell Damage Induced by 20% Ethanol in Rats

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Abstract—Prostaglandins protect against the gross damage of gastric mucosa induced by 50–100% ethanol, but do not protect surface epithelial cells (SEC) from necrosis. Since this induced damage to SEC is so severe, we attempted to determine the effects of a prostaglandin on slightly induced SEC damage and gastric potential difference (PD) in response to low concentrations of ethanol. The necrotizing effects of graded concentrations of ethanol (10–50%) to SEC on the rat gastric mucosa were studied by scanning electron and light microscopy. Intragastric instillation of 20% ethanol (v/v, 1 ml/100 g body wt.) to pylorus-ligated rats for 10 min induced slight and reproducible SEC damage consisting mainly of the apical cell membrane erosion of SEC. Pretreatment with 16,16-dimethyl prostaglandin E₂ (dmPGE₂, 3 or 30 μg/kg, p.o. or s.c.) afforded protection of the SEC from 20% ethanol-induced damage. However, the cytoprotective effects of dmPGE₂ were abolished when gastric contents were emptied prior to 20% ethanol instillation. Intragastric instillation of ethanol immediately reduced PD in a concentration-related manner. dmPGE₂ (3 or 30 μg/kg, s.c.) had no effect on the reduction of gastric PD after 20% ethanol treatment and the recovery of reduced PD to normal levels. We conclude that dmPGE₂ has no cytoprotective effect on 20% ethanol-induced SEC damage in rat gastric mucosa.

The ability of prostaglandins to protect the gastric mucosa against a number of damaging agents is well reviewed (1). Histologically, it was revealed that prostaglandins did not protect the surface epithelial cells (SEC) themselves, but rather protected the cells located beneath the SEC (2–4). In the case of ethanol, the concentration and volume of ethanol used were considerably high and large; i.e., 50% to 100% (v/v) of ethanol was given in a volume of 1 to 4 ml per animal (1, 5, 6). Therefore, it is possible that the SEC damage induced by ethanol under such conditions occurs too rapidly and is too severe to be given protection by the prostaglandins.

In the present work, we attempted to determine (A) the minimum concentration of ethanol which induces slight and reproducible damage to the SEC of the rat stomach and (B) the effect of 16,16-dimethyl prostaglandin E₂ (dmPGE₂) on the SEC damage and reduction of gastric potential difference (PD) induced by a lower concentration of ethanol.

Materials and Methods

Male Sprague-Dawley rats (200–240 g) were deprived of food for 24 hr before the experiments. Water was given freely for the initial 22 hr, but was withheld for 2 hr before the start of the experiments. Four to 8 animals were used for each study.

Ethanol-induced gastric mucosal damage:
To determine the minimum concentration of ethanol required to produce SEC damage, the following experiment was performed. With the rats under ether anesthesia, the abdomen was incised, the pylorus ligated, and the
abdomen closed. Three min later, 10%, 20%, 30% or 50% ethanol (v/v) was given p.o. by gastric intubation in a volume of 1 ml/100 g body wt. Control animals were given distilled water alone. These animals were killed 10 min later. The stomach was removed, opened along the greater curvature, and extended on a glass plate with a liquid adhesive (Aron Alfa, Toa Gosei Kagaku). The surface of each stomach was washed by spraying with saline at 4°C to remove adhering mucus and debris. Scanning electron microscopic studies were done as previously described (4). Briefly, the tissues were fixed in phosphate-buffered 1.2% paraformaldehyde – 2.5% glutaraldehyde – 0.03% trinitrophenol solution for 3 hr at 4°C. Tissue samples were then dehydrated using a graded series of ethanol solutions, and finally placed in isoamyl acetate for 18 hr. The tissues were then critical point dried with CO2 (Hitachi, HCP-2), mounted on aluminum stubs, and then vacuum coated with a palladium-platinum ion sputter (Eicho, IB-3). The samples were examined under a scanning electron microscope (SEM, Hitachi, S-510). The SEC damage after ethanol treatment was determined by scanning two portions of the corpus which were divided into two parts as described previously (4). The severity of SEC damage was arbitrarily divided into five degrees as follows: Damage index 0: no visible change in SEC, Damage index 1: several shallow apical erosions of SEC, Damage index 2: many deep apical erosions of SEC, Damage index 3: extensive damage of SEC without exposure of the lamina propria, Damage index 4: focal exfoliation of SEC, exposing lamina propria, Damage index 5: widespread exfoliation of the SEC, showing a honeycomb structure. The mean values of four parts of the corpus served as the damage index per one stomach. A person with no knowledge of which treatment an animal had received measured all the samples.

There was slight and reproducible SEC damage with the administration of 20% ethanol. Therefore, we attempted to determine whether or not dmPGE2 given p.o. or s.c. protects the SEC against 20% ethanol-induced damage. Two types of experiments were performed. First, the animals were given dmPGE2 (3 or 30 μg/kg, Ono) or the vehicle alone either p.o. or s.c. Thirty min later, these animals were anesthetized with ether, the pylorus ligated and 20% ethanol instilled. Second, we found in a preliminary study that the administration of dmPGE2 (3 or 30 μg/kg) either p.o. or s.c. resulted in an accumulation of gastric contents 30 min later: 1.0±0.2 or 1.5±0.4 ml/rat after p.o. administration (0.2±0.1 ml/rat in saline control group) and 0.3±0.1 or 1.2±0.2 ml/rat after s.c. administration (0.1±0.1 ml/rat in saline control group). Therefore, gastric contents were emptied through a small hole made in the forestomach 30 min after vehicle or dmPGE2 treatment to avoid any dilution of ethanol. This emptying procedure itself did not damage the SEC in the stomach. The small hole was then closed, the stomach was replaced, and the abdomen closed, and 20% ethanol given p.o. The animals were killed 10 min after ethanol instillation and the stomachs were examined for SEC damage. In additional experiments, light microscopic studies were done to determine the extent of damage in the gastric mucosa after 20% ethanol administration and the effect of dmPGE2 on the damage. The stomachs of animals given distilled water alone, 20% ethanol alone, or dmPGE2 (3 or 30 μg/kg, p.o.) plus 20% ethanol for 10 min after pylorus ligation were fixed in 10% formalin solution for light microscopic studies. After fixation, samples measuring 3×10 mm were obtained from the corpus at a region located 3 mm below the limiting ridge. The tissue sections were stained with eosin and hematoxylin.

Measurements of gastric PD: Measurement of gastric PD was performed with the rats under urethane anesthesia (1.25 g/kg, i.p., Nakarai) as described (7, 8). Briefly, the stomach was perfused at a flow rate of 1 ml/min with saline (154 mM NaCl) that was gassed with 100% O2, heated at 37°C, and kept in a reservoir. The PD was determined using two agar bridges, one positioned in the stomach and the other in the abdominal cavity. Changes in PD were continuously monitored on a Hitachi recorder (Model 056). Approximately 1 hr after PD had stabilized,
the perfusion system was interrupted, and the solution in the stomach was withdrawn. The stomach was then exposed for 10 min to 2 ml of a graded concentration of ethanol (10, 20, 30 or 50%, v/v). After application of ethanol, the stomach was rinsed with saline, another 2 ml of saline was instilled, and the perfusion was resumed. The PD was continuously measured throughout a 2 hr experimental period. The effect of dmPGE2 (3 or 30 μg/kg) on the alterations in PD in response to 20% ethanol was determined by giving this agent s.c. 30 min before the ethanol treatment. Control animals received the vehicle alone.

Analysis of data: Data were presented as the mean±S.E.M. of 4–8 animals per group. Damage indices from scanning electron microscopic study were analyzed by the χ²-test and the other parameters (PD and gastric contents) were compared by a two tail Dunnett’s multiple comparison test (analysis of variance) (9). A 5% level of significance was used throughout.

Results

Ethanol-induced SEC damage: The p.o. administration of ethanol produced SEC damage in the stomach in a concentration dependent manner (Fig. 1A, B). Ten % ethanol produced apical erosions of several or many SEC. The damage index was 1.9±0.1, and it was not significantly different from the control values. The administration of 20% ethanol induced extensive apical erosions of many SEC without exposure of the lamina propria. The damage index was 2.5±0.1, the value is significantly higher than those observed in the control group. The typical damage observed 10 min after administration of 30% or 50% ethanol was an extensive deep apical erosion of SEC associated with a focal to widespread exfoliation of SEC and exposure of the lamina propria. The damage index was 3.6±0.1 or 4.6±0.2, respectively. Thus, the damage induced by 20% ethanol appears to be minimal and reproducible. The administration of 10% and 20% ethanol induced no macroscopic damage to the SEC, but concentrations of ethanol over 30% produced visible hyperemic damage in the stomach. Light microscopic studies also demonstrated that 20% ethanol induced a slight damage to the SEC, but exerted little or no influence on the cells under the SEC as compared to the control (Fig. 2A, B).

Effects of dmPGE2 on 20% ethanol-induced SEC damage: Pretreatment with 3 or 30 μg/kg of dmPGE2 either p.o. or s.c. protected the gastric SEC against 20% ethanol-induced damage. However, the protective effects of dmPGE2 were abolished when gastric contents were emptied prior to 20% ethanol instillation (Figs. 3 and 4). Similar to the SEM study, the light microscopic study also demonstrated that dmPGE2 did not protect the SEC from damage induced by 20% ethanol (Fig. 2C).

Effects of ethanol on gastric PD: Exposure of the stomach for 10 min to 2 ml of various concentrations of ethanol led to a reduction in the PD in a concentration-related manner. The magnitude of reduction was 7.3, 15.3, 22.2 and 26.0 mV immediately after exposure to 10%, 20%, 30% and 50% ethanol, respectively (Fig. 5A). The PD reduced by ethanol was restored with time after removal of ethanol from the stomach, and this process was also evident in a concentration-related manner. The reduced PD caused by 10% and 20% ethanol was followed by a rapid reversion to practically normal levels (>80% of control) within 20 min after exposure, whereas those caused by 30% and 50% ethanol were gradually restored to 67.6% and 57.1% of the control levels within 1 hr, respectively.

Effects of dmPGE2 on gastric PD: Pretreatment of the animals with dmPGE2 (s.c.) did not significantly affect the PD response caused by 20% ethanol, at either 3 or 30 μg/kg (Fig. 5B). In the animals given 30 μg/kg of dmPGE2, the PD was reduced from -37.5±1.5 mV to -21.5±1.4 mV immediately after exposure to 20% ethanol, the magnitude of PD reduction (16 mV) being not significantly different from that (15.3 mV) seen after ethanol exposure in control animals. Neither doses of this agent affected the process of PD recovery; i.e., the restoration in PD at 30 min and 60 min after exposure to 20% ethanol was not significantly different between the control and dmPGE2-treated groups.
Discussion

The primary barrier and protective layer of the stomach is the layer of SEC and its associated gastric mucus. SEC damage that breaks the "gastric mucosal barrier" but is not accompanied by macroscopic hemorrhage or extensive hyperemia probably occurs relatively frequently during the normal course of food and drug ingestion (6). However, SEC damage is considered to be prerequisite for further development to visible lesions when other factors are added such as deficiency of endogenous prostaglandins, gastric acid secretion, decrease of mucosal blood flow and so on (10, 11). In foregoing
studies, we showed that dmPGE2 protects SEC against aspirin-, indomethacin- and vagal nerve stimulation induced damage but not against ethanol-induced damage (4, 11, 12). In the case of ethanol, however, there was a possibility that the damage produced by 50% ethanol occurred instantaneously and severely so that dmPGE2 was not protective. The present studies in rats confirm findings of other workers regarding the damaging effect of graded concentrations of ethanol on the SEC of the gastric mucosa in mice and dogs (13, 14). Concentration response experiments revealed that 20% ethanol is the threshold which will induce slight and reproducible damage to the SEC.
of the rat gastric mucosa with a 100% incidence. At that concentration, the damage is confined to the SEC itself and an exposure of the lamina propria seldom, if ever, occurs. This seems to be the early step of ethanol induced damage.

Twenty % ethanol is known to inhibit the necrotic lesions of the rat stomach, although too innocuous to cause visible morphologic injury (15–17). The phenomenon is induced by stimulation of endogenous prostaglandin generation and called “adaptive cytoprotection”. However, 20% ethanol moderately but consistently injured SEC as shown in the present study. These mean that mild irritants including 20% ethanol produce SEC injury, and by so doing, generation of endogenous prostaglandins may be triggered and induce the adaptive cytoprotection (18). Furthermore, adaptive cytoprotection seems to be the phenomenon which inhibits the development to macroscopically visible mucosal lesions.

Pretreatment of animals with intact stomachs with dmPGE₂ (both p.o. and s.c.) significantly protected the SEC against 20% ethanol-induced damage. However, the agent (both p.o. and s.c.) had no protective effect on the SEC induced by 20% ethanol when gastric contents were removed before ethanol instillation. These findings suggest that the cytoprotective effect of dmPGE₂ observed in intact stomach is simply due to the dilution of 20% ethanol and delaying contact with SEC by increased gastric juice volume.

Other investigators (2, 3, 19) found that while dmPGE₂ or PGF₂α failed to protect the SEC against 50% or 100% ethanol-induced damage, these prostaglandins significantly protected the cells (including proliferating zone cells) located deep in the gastric mucosa. Our data and those of others indicate that ethanol seems to exert a necrotizing effect in different modes to the SEC and cells located beneath the SEC. Robert et al. (20) reported
that 100% ethanol labelled with $[^{14}C]$ given p.o. rapidly penetrated all the gastric mucosal layers, thereby suggesting that both the SEC and cells located in the deep portion might be in contact with the same concentration of ethanol. Elucidation of the mechanism by which prostaglandins protect the cells in the deep portion but do not protect the SEC against ethanol-induced damage is vital for a better understanding of the term "cytoprotection".

Morphological changes after a graded concentration of ethanol were reflected in the changes in PD; i.e., the PD was reduced in a concentration-related manner. Prostaglandin pretreatment (s.c.) failed to prevent the reduction in PD response to 20% ethanol. These functional findings would also support the proposal that prostaglandins do not protect the gastric mucosa from ethanol-induced damage.

We conclude that dmPGE$_2$ has no cytoprotective effect against ethanol on the SEC of the rat stomach, even in cases of slight damage induced by 20% ethanol.

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