16,16-Dimethyl Prostaglandin E₂ Protects Gastric Mucosal Surface Epithelial Cells from Indomethacin-Induced Damage in Rats

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Abstract—The protective effect of 16,16-dimethyl prostaglandin E₂ (dmPGE₂) against early damage induced by indomethacin in the rat gastric mucosal surface epithelial cells was studied using a scanning electron microscope. Indomethacin (10 or 25 mg/kg, p.o.) induced a widespread exfoliation of the surface epithelial cells and an exposure of the lamina propria both in the corpus and antrum within 1 hr after the administration. Pretreatment with dmPGE₂ (0.3, 3 or 30 µg/kg, p.o.) 30 min before indomethacin (25 mg/kg) dose-dependently inhibited these damages. The effects of dmPGE₂, at least on the surface epithelial cells in the corpus, appear to be related to the prevention of damage formation itself and is unrelated to the enhancement of reconstitution of once damaged mucosa. Enhanced gastric motility by indomethacin was potently inhibited by pretreatment with 3 and 30 µg/kg of dmPGE₂, but not with 0.3 µg/kg. dmPGE₂ pretreatment (30 µg/kg) significantly decreased the absorption of indomethacin (25 mg/kg) when determined 10 min after giving indomethacin, but did not affect it when determined 30 and 60 min later. We conclude that dmPGE₂ protects gastric mucosal surface epithelial cells from indomethacin injury at an early stage, partly by inhibiting gastric motility.

We (1) have reported that 16,16-dimethyl prostaglandin E₂ (dmPGE₂) significantly protected surface epithelial cells (SEC) of the rat stomach against damage induced by indomethacin. At that time, we determined the protective effects of dmPGE₂ 4 hr after the administration of indomethacin. It was recently demonstrated that damage to the SEC induced by ethanol led to a rapid reconstitution (i.e., within 1 to 2 hr) (2) and that dmPGE₂ enhanced the repair process (3, 4). Therefore, there was a question as to whether or not the protective effect of dmPGE₂ is reproducible even when examined at a much earlier time after indomethacin treatment (1). We studied the dose-response and time-course of gastric mucosal cell damage in the rat in response to indomethacin and also the effect of dmPGE₂ on the initial damage using a scanning electron microscope. To elucidate the mechanism of action of dmPGE₂, the influence of this agent on gastric motility and absorption of indomethacin was determined.

Materials and Methods

Male Sprague-Dawley rats (180–220 g) were deprived of food but allowed free access to water for 24 hr before the experiments. They were kept in raised mesh-bottom cages to prevent coprophagy. Each group studied included 5–10 rats.

Damage induction: Dose-response and time-course studies were carried out as follows. Indomethacin (Sigma), suspended in saline with a trace of Tween 80, was given p.o. by gastric intubation at 1, 5, 10 and 25 mg/kg in a volume of 0.5 ml/100 g body weight, and then the rats were killed 1 hr later. Since the dose-response study demon-
strated that 25 mg/kg of indomethacin induced the maximum damage 60 min later, this dose was used in the time-course study. The rats given 25 mg/kg of the agent were killed immediately, and 10, 30, 60 and 120 min after the administration. The stomachs were removed and opened along the greater curvature. Scanning electron microscopic study of the stomach was done according to our methods (5). Briefly, the stomach was put into phosphate-buffered 1.2% paraformaldehyde-2.5% glutaraldehyde-0.03% trinitrophenol for 3 hr at 4°C. The tissues were then dehydrated using a graded series of ethanol, placed in isomyl acetate for 18 hr, critically point dried with CO₂ (Hitachi, HCP-2), and vacuum coated with a palladium-platinum ion sputter (Eicho, IB-3). The samples were then examined under a scanning electron microscope (Hitachi, S-510). Four parts in the corpus and two parts in the antrum were individually scanned, and only the most severe damage in each part was recorded. The mean values of the four parts of the corpus and two parts of the antrum served as the damage index per stomach.

The severity of damage of the gastric mucosa was arbitrarily divided into five degrees as follows: Damage index 0, almost no visible changes in the SEC; Damage index 1, several shallow apical erosions in the SEC; Damage index 2, many deep acical erosions of the SEC; Damage index 3, extensive damage of the SEC, no exposure of the lamina propria; Damage index 4, focal exfoliation of the SEC, exposing lamina propria; Damage index 5, widespread exfoliation of the SEC. A person with no knowledge of which treatment the animals had been given determined the damage in all tissues.

Absorption of indomethacin: To determine whether or not dmPGE₂ affects the absorption of indomethacin, plasma levels of indomethacin were determined according to Hucker et al. (9) and Bayne et al. (10). Briefly, blood samples were obtained from the abdominal aorta under ether anesthesia and were then centrifuged at 4°C for 20 min at 3,000 rpm. One ml of plasma was pipetted into a 50 ml glass-stopped centrifuge tube containing 4 ml of 0.5 M citrate buffer (pH 5.0). Twenty-five ml of n-heptane containing 3% isomyl alcohol was added, and the tube was shaken for 15 min, and centrifuged. Fifteen ml of the upper organic layer was transfered to another tube, 5 ml of 0.2 M Na₂CO₃ was added, shaken for 15 min, and centrifuged.
The concentration of indomethacin in the aqueous phase was determined using a spectrophotofluorometer system (Shimadzu, RF-530). Indomethacin was given p.o. at 25 mg/kg, and the rats were then anesthetized with ether 10, 30 and 60 min later for blood sampling. dmPGE2 was given p.o. at 30 μg/kg 30 min before indomethacin treatment. Control animals were given the vehicle alone. Eight animals were used in each group.

Analysis of data: Student's t-test was used to determine the statistical significance of the data, and P<0.05 was regarded as significant.

Results

Dose-response of indomethacin-induced SEC damage: Administration of 1 or 5 mg/kg of indomethacin induced no appreciable damage either in the corpus or antrum 60 min later (Fig. 1). In the case of 10 mg/kg, the damage in the SEC of the corpus was significantly more severe than in the control group, but not in the SEC of the antrum. The damage in the corpus was the most severe when 25 mg/kg of the agent was administered and consisted of a widespread exfoliation of the SEC and exposure of the lamina propria. The SEC in the antrum was also significantly damaged when 25 mg/kg of indomethacin was given.

Time-course of indomethacin-induced SEC damage: The SEC of both the corpus and antrum examined immediately and 10 min after administration of 25 mg/kg of indomethacin appeared intact (Fig. 2). However, there was an extensive damage of the SEC both in the corpus and antrum 30 min after administration (Figs. 2 and 3). Sixty min later, the damage in the corpus progressed to a widespread exfoliation of the SEC. The severity of damage observed 120 min later was much the same as observed 60 min later, i.e., the development of damage was maximal at 60 min. In contrast to the corpus, the damage in the antrum observed at 60 and 120 min later was much the same as that observed at 30 min after administration.

Effects of dmPGE2 on indomethacin-induced SEC damage: Pretreatment with 0.3, 3, 30 μg/kg of dmPGE2 significantly inhibited indomethacin-induced SEC damage (both in the corpus and antrum) in a dose-dependent manner (Fig. 4). At 3 and 30 μg/kg, the protection of the SEC in the corpus against damage induced by indomethacin was almost complete (Fig. 5).

Effects of dmPGE2 on gastric motility: Administration of 25 mg/kg of indomethacin gradually increased the amplitude of gastric contraction, and the response reached the

![Fig. 1. Dose-response study of damage in the surface epithelial cells of the rat stomach induced by indomethacin. Animals were killed 1 hr after p.o. administration of indomethacin. The development of damage was most evident at 25 mg/kg both in the corpus and antrum. Data represent the mean ± S.E.M.](image-url)
Fig. 2. Scanning electron micrographs showing damage in the surface epithelial cells of rat gastric corpus 10, 30, 60 and 120 min after p.o. administration of indomethacin (25 mg/kg) (10 and 30 min, x1000; 60 and 120 min, x100).

Fig. 3. Time course study of damage in the surface epithelial cells of the rat stomach induced by p.o. administration of indomethacin (25 mg/kg). The damage in the corpus and antrum was observed under a scanning electron microscope (SEM). Note that the damage was maximum 1 and 0.5 hr after indomethacin treatment in the corpus and antrum, respectively. Data represent the mean ± SEM.

maximum 50 min later (Fig. 6). While dmPGE₂ given at 0.3 µg/kg transiently reduced the normal amplitude of gastric contraction, it did not affect the enhanced gastric contraction by indomethacin. dmPGE₂ given at 3 and 30 µg/kg dose-dependently reduced the normal gastric contraction for 30 min. In addition, the agent significantly reduced the enhanced gastric contraction by indomethacin. At 30 µg/kg, the reduced gastric contraction was not reverted to the normal level even at 1 hr after indomethacin treatment.

Effects of dmPGE₂ on absorption of indomethacin: Plasma levels of indomethacin 10, 30 and 60 min after p.o. administration of 25 mg/kg of indomethacin were 44.0±4.9, 90.0±6.9 and 95.8±9.1 µg/ml, respectively. Pretreatment of 30 µg/kg of dmPGE₂ significantly decreased the plasma level of indomethacin 10 min after administration of the agent (26.8±6.2 µg/ml). However there
Fig. 4. Effects of 16,16-dimethyl prostaglandin E₂ (dmPGE₂) on damage in the surface epithelial cell of the rat stomach induced by indomethacin (25 mg/kg). Animals were killed 1 hr after p.o. administration of indomethacin. The damage was dose-dependently inhibited by pretreatment with dmPGE₂. Data represent the mean±S.E.M.

Fig. 5. Scanning electron micrographs showing the effects of dmPGE₂ on damage in the surface epithelial cells of the corpus in the rat stomach induced by indomethacin (25 mg/kg) (A: control, x100; B: 0.3 μg/kg, x400; C: 3 μg/kg, x1000; D: 30 μg/kg, x1000). dmPGE₂ was given p.o. 30 min before p.o. administration of indomethacin (25 mg/kg). Animals were killed 1 hr after indomethacin treatment. The surface epithelial cells in Figs. C and D appear to be intact.
Fig. 6. Effects of dmPGE$_2$ on rat gastric motility enhanced by indomethacin. Both indomethacin (25 mg/kg) and dmPGE$_2$ were given p.o. after stabilization of spontaneous gastric contraction. The amplitude of gastric contraction before and after indomethacin treatment was dose-dependently reduced by 3 and 30 µg/kg of dmPGE$_2$. Data represent the mean±S.E.M.

Discussion

We obtained evidence that indomethacin induces microscopic damage in the SEC of both the corpus and antrum of rats within 1 hr after treatment in a dose-dependent manner. The damage in the SEC of the corpus became severe with time as compared to findings in the antrum. In general, the gastric mucosal damage observed macroscopically 4 to 6 hr after treatment with indomethacin (25 mg/kg) occurs primarily in the corpus, but seldom, if ever, in the antrum (11, 12). Our microscopic observations suggest that the damage observed in the antrum either rapidly heals or remains in the same condition without developing to visible damage.

The appearance of damage induced by 10 or 25 mg/kg of indomethacin resembles that observed after treatment with ethanol (5). A honey-comb structure was observed in several areas of the corpus and antrum. We reported that dmPGE$_2$, even at 100 µg/kg, has no protective effect on ethanol-induced gastric damage under the same experimental conditions (5). In contrast, we found that dmPGE$_2$, even at 0.3 µg/kg, significantly inhibited the development of such damage in response to indomethacin. These data suggest that the pathogenesis of damage induced by ethanol and indomethacin apparently differs.

The damaged tissue in the corpus, as induced 1 hr after indomethacin treatment, revealed no evidence of reconstitution. Accordingly, one can conclude that the effect of dmPGE$_2$ on the damage in the corpus was not due to the enhancement of reconstitution of once damaged cells, but rather due to the prevention of development of damage. In contrast to the corpus, the damage observed in the antrum reached its peak 30 min after indomethacin treatment. Therefore, it is difficult to state at this moment whether or not the effect of dmPGE$_2$ on the antral damage observed 1 hr later is caused by the prevention of damage formation itself or enhancement of reconstitution. The effect of dmPGE$_2$ on the damage developed 30 min...
after indomethacin treatment will be determined in our next study.

Several investigators including our own groups reported that indomethacin in a dose of 1 mg/kg or greater significantly reduced endogenous prostaglandin levels in the rat stomach (7, 13–17). It was postulated that the inhibition of prostaglandin synthesis in the gastric mucosa by indomethacin is an essential factor in lesion formation (13, 14). We showed in this study that indomethacin at 1 and 5 mg/kg did not induce any damage in the SEC of the rat gastric mucosa 1 hr later. These results suggest that a deficiency in endogenous prostaglandin itself is not a causal factor in the development of mucosal damage induced by indomethacin. Accordingly, the protective effect of dmPGE2 will not be simply due to a replacement of deficient endogenous prostaglandin levels in response to indomethacin.

It is known that various antisecretory agents and antacids prevent the formation of gastric lesions in response to indomethacin (18, 19). However, in a previous study, we found that dmPGE2 (3 and 30 µg/kg, p.o.) had no effect on gastric acid secretion in pylorus ligated rats (5). Therefore, dmPGE2 might protect SEC from indomethacin-induced damage by a mechanism other than inhibiting acid secretion.

Indomethacin is known to enhance gastric motility in rats (6, 7). Mersereau and Hinchey (6) suggested that this enhanced motility is related to the causal factor of gastric mucosal damage in response to indomethacin. In fact, they demonstrated that 4 µg/kg of dmPGE2, which apparently inhibited gastric motility, significantly protected the gastric mucosa from indomethacin-induced damage. Our present data also support their hypothesis because dmPGE2, at 3 and 30 µg/kg which markedly inhibited the enhanced gastric motility by indomethacin, significantly protected SEC from the damage. It is uncertain whether this enhanced gastric motility results in SEC damage through a rubbing of the gastric mucosa and/or disturbance of the gastric mucosal circulation by mucosal compression. However, our study showed that even 0.3 µg/kg of dmPGE2, which has no effect on the enhanced gastric motility by indomethacin, significantly inhibited the damage in the SEC of gastric mucosa. Therefore, in addition to the enhanced gastric motility, other mechanisms must be also involved in the earliest changes in the gastric mucosal cells after indomethacin treatment. Indeed, Rainsford and Willis (20) and Rainsford (21) reported that indomethacin given p.o. at 5 mg/kg to pigs induced damage of gastric mucosal capillaries within 10 to 15 min and suggested that this capillary damage develops to severe damage to surrounding tissues. In addition, indomethacin is reportedly known to reduce gastric mucosal blood flow (22, 23), mucus secretion (24) and bicarbonate secretion (25).

In contrast to the local effects of ethanol, indomethacin might induce such damage systemically because this agent induces visible damage even after i.p. or s.c. treatment (11, 26). Thus, dmPGE2 may interfere with the absorption of indomethacin, thereby resulting in inhibition of tissue alteration. Indeed, the plasma level of indomethacin 10 min later was reduced by about 40% by dmPGE2 treatment, which might be caused by an inhibition of gastric emptying rate. However, there was no difference in the plasma levels of the agent between the control and dmPGE2-treated groups 30 and 60 min later. Therefore, it is unlikely that the protective effect of dmPGE2 is due to the interference with absorption of indomethacin.

We conclude that dmPGE2 has a protective effect against early damage in the SEC of the rat gastric mucosa induced by indomethacin.

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