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Developmental Changes in Gastric Mucus Gel Thickness: Responsiveness to 16,16-Dimethyl Prostaglandin E_2 and Mucosal Protection in the Rat

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ABSTRACT. The gastric mucosa of newborn rats is sensitive to the damaging effects of acid and ethanol. We measured gastric mucus gel thickness in newborn, suckling, and weaned rats by inversion microscopic observation. The thickness in newborn rats was 52.2 ± 6.7 μm and increased with age, reaching 96.8 ± 5.6 μm in 8-wk-old rats ($p < 0.001$). Oral administration of 16,16-dimethyl prostaglandin E_2 at concentrations of 10 and 100 μg/kg body weight increased mucus thickness in 8-wk-old rats but had no effect in 1-wk-old rats. We also assessed the effect of 16,16-dimethyl prostaglandin E_2 on the prevention of gastric mucosal damage induced by ethanol. Oral 16,16-dimethyl prostaglandin E_2 reduced damage in 8-wk-old rats, but there was no effect in 1-wk-old rats. These data suggest that the susceptibility of newborn rats to gastric mucosal injury may be related to the relative thinness of the gastric mucus gel layer and the failure of prostaglandins to increase the mucus gel layer thickness. (*Pediatr Res* 31: 193–195, 1992)

Abbreviations

PG, prostaglandin
$dPGE_2$, 16,16-dimethyl prostaglandin E_2

Gastroduodenal mucosal lesions are relatively frequent in neonates (1–3) and may cause serious bleeding. The gastric mucosa of newborn rats is more susceptible to injury after intragastric ingestion of HCl (4) and ethanol (5) than the mucosa of adult rats. Gastric mucosal lesions may result from any disruption in the balance between aggressive factors (acid and pepsin) and defensive factors such as mucus layer thickness, bicarbonate secretion, blood flow, and epithelial renewal (6). Although gastric acid and pepsin concentrations are relatively reduced in the neonatal human (7, 8) and rat (9, 10), luminal concentrations of PG that stimulate defensive functions (11–16) are also reduced in the neonatal human (17) and rat (5). Recently, Thorn and Tepperman (18) showed that PGE_2 administration to rat pups receiving PG-depleted milk partially corrected the increase in gastric permeability induced by 250 mN HCl. Thus, there may be some deficiency in defensive factors that predispose neonates to mucosal injury.

In this study, we investigated age-related changes in mucus layer thickness and its responsiveness to PG in developing rats. We also studied the effect of PG on ethanol-induced gastric mucosal damage, using PG doses identical to those used to test mucus thickness.

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MATERIALS AND METHODS

Measurement of mucus gel layer thickness. Newborn (3 d), suckling (1 and 2 wk), and weaned (4 and 8 wk) Wistar rats were used. They were fasted 30–36 h (3-d- and 1- and 2-wk-old rats) or 40–48 h (4- and 8-wk-old rats). Water was allowed *ad libitum* until 30–36 h before the rats were killed. Preliminary studies showed that those time intervals are the shortest times that insure stomachs empty of food. Preliminary studies also showed that the mucus gel thickness in newborn rats given water containing 0.3% NaCl and 5% glucose every 12 h before death was not significantly different from the thickness in the newborn rats that were not given the solution. Newborn and suckling rats were put in a box. Half of the bottom of the box was warmed to 37–40°C, and half was at room temperature (25°C). Rats were free to choose a position in the box. Twenty-four h before study, weanling rats were placed in cylindrical wire mesh cages to prevent coprophagy. Rats were killed by intrathecal or intraperitoneal injection of pentobarbital after a light ether anesthesia.

The method reported by Kerss *et al.* (19) was used with slight modifications to measure surface mucus thickness. The stomach was removed and opened along the lesser curvature. The tissue, luminal surface up, was mounted on a filter paper (Whatman 1), and a section was cut with a pair of parallel sharp razor blades separated by a distance of 1.0 mm. Tissues were immersed in 0.9% NaCl. The section was pinned on a rubber cube with care to insure that it was precisely vertical. Throughout the procedure, extreme care was taken not to distort the mucosa by stretching or manipulation.

The section pinned vertically to the rubber cube was placed in a thin well filled with 0.9% NaCl and observed by an inverse microscope (Olympus CK, Olympus, Tokyo, Japan; ×100 magnification). The distance between the solution-mucus interface and mucus-epithelial interface was measured with an eyepiece graticule. In some studies, we used dark-field illumination and phase contrast, as recommended by Kerss *et al.* (19). However, these techniques were not superior to the simple method, so we did not use them in most experiments.

In each tissue strip, measurements of mucus thickness were

completed at six or more sites each 1.0 mm apart, and mean thickness was calculated for each strip.

To study the effect of PG on mucus thickness, we gavage fed $dPGE_2$ (Sigma Chemical Co., St. Louis, MO) (0.01–10 μg/mL; 10 mL/kg body weight) or an equal volume of 0.9% NaCl intragastrically 30 min before the rats were killed. All strips were made and measured by a single investigator, who was not aware of the test solution given to the rat.

Study of ethanol-induced gastric lesion. To investigate ethanol-induced gastric mucosal injury, we used 1- and 8-wk-old rats. Ethanol was diluted with water to specified concentrations and was given intragastrically in a volume of 15 mL/kg body weight. After 60 min, we killed the animal and removed the stomach. The stomach was opened along the greater curvature and washed gently with tap water. A glass slide was placed over the mucosal surface to improve the measurements of mucosal damage. The gastric mucosal lesions were photographed. The damaged area and total glandular area were measured on the photograph by an image analysis division (Cardio 200, Kontron, Osaka, Japan). The damaged area was quantified as a lesion index, the percentage of total glandular area that was damaged.

To assess the effect of PG on the lesion index, $dPGE_2$ (0.1–10 μg/mL; 10 mL/kg body weight) or an equal volume of 0.9% NaCl was given intragastrically 30 min before the ethanol ingestion.

Values are expressed as the mean ± SEM. The data were analyzed statistically by the analysis of variance, Dunnett's multiple comparison, or *t* test.

RESULTS

Figure 1 demonstrates the view of the mucus layer through the inverse microscope. The interface between the solution and mucus and that between the mucus and epithelium was easily identified. Table 1 shows the effect of age on the thickness of the mucus layer. The thickness increased with age ($p < 0.001$). The value for 8-wk-old rats (96.8 ± 5.6 μm) was nearly twice that in 3-d-old rats (52.2 ± 6.7 μm).

Table 2 shows the effect of $dPGE_2$ on the mucus thickness in 1- and 8-wk-old rats. Intragastric administration of $dPGE_2$ at concentrations of 1 and 10 μg/mL (10 and 100 μg/kg body weight) increased the thickness ($p < 0.01$ and $p < 0.05$, respectively) in 8-wk-old rats, but not in 1-wk-old rats.

Figure 2 demonstrates the 75% ethanol-induced mucosal injuries in an 8-wk-old rat (Fig. 2, *left*). In preliminary studies, we found that damage is concentration-dependent in both 1- and 8-wk-old rats. However, the concentrations that induce the same amount of damage are different. Therefore, in studies to assess the protective effect of PG, we found the concentrations of ethanol resulting in a consistent injury. We used different concentrations of ethanol: 50% for 1-wk-old rats and 75% for 8-wk-old rats.

Oral administration of $dPGE_2$ 30 min before ethanol, 1 μg/mL, decreased the damage in 8-wk-old rats ($p < 0.005$) (Table 3; Figure 2, *right*). In contrast, there was no protective effect of $dPGE_2$, 0.1–10 μg/mL, in 1-wk-old rats.

Table 1. *Developmental changes in mucus gel thickness in rat stomach*

Age	Number of animals	Thickness (μm)*
3 d	6	52.2 ± 6.7†
1 wk	6	45.0 ± 3.2
2 wk	6	49.7 ± 4.9
4 wk	6	94.0 ± 9.5
8 wk	6	96.8 ± 5.6

* Each value is the mean ± SEM from a minimum of six determinations/tissue strip.

† The difference between groups is statistically significant ($p < 0.001$, analysis of variance).

Table 2. *Effect of $dPGE_2$ on gastric mucus gel thickness in 1- and 8-wk-old rats*

Concentration of $dPGE_2$ (μg/kg)	1 wk		8 wk	
	n	Thickness (μm)*	*n*	Thickness (μm)*
0 (Control)	5	54.8 ± 5.5	13	91.7 ± 5.1
0.1	5	51.8 ± 6.7	6	80.5 ± 8.2
1	6	55.7 ± 4.6	6	115.1 ± 8.2
10	5	64.1 ± 10.2	8	135.8 ± 12.6†
100	5	60.9 ± 10.0	8	127.3 ± 12.6‡

* Each value is the mean (± SEM) from a minimum of six determinations/tissue strip.

† Statistical difference from control, $p < 0.01$ (Dunnett's multiple comparison).

‡ Statistical difference from control, $p < 0.05$ (Dunnett's multiple comparison).

Fig. 2. Gastric mucosal injury induced by 75% ethanol with (*right*) or without (*left*) PG pretreatment. The rat was given $dPGE_2$ or an equivalent volume of 0.9% NaCl orally 30 min before intragastric ethanol ingestion. The stomach was removed and opened along the greater curvature 60 min after the ethanol injection. A glass slide was put on the surface so that hemorrhagic lesions were clearly visible.

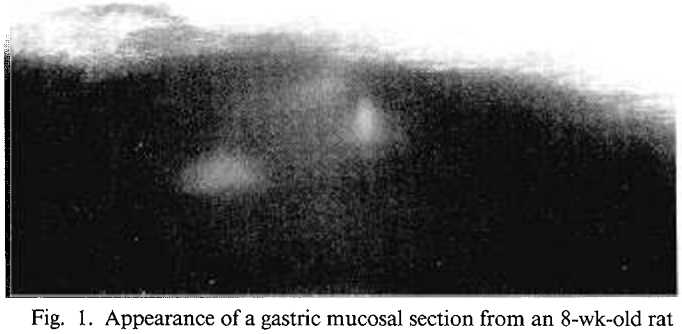

Fig. 1. Appearance of a gastric mucosal section from an 8-wk-old rat under an inverse microscopic observation (×100 magnification). Three distinct phases composing solution (*top*), mucus gel layer, and mucosa (*bottom*) can be clearly seen.

DISCUSSION

The relative importance of the mucus gel in gastric mucosal protection has been a subject of debate (6, 11, 12, 20–29). The mucus gel generates an unstirred layer that prevents lumenal hydrogen ions from reaching the epithelial surface. Mucus mixes with bicarbonate secreted from epithelial cells to create a five-unit pH gradient from the lumen to the epithelial surface (6, 11, 22–25). However, there are some contradictory findings. There may be no direct relationship between the thickness of the mucus gel and cytoprotection by PG in rats (26). Cystamine stimulates

Table 3. *Effect of $dPGE_2$ on ethanol-induced gastric mucosal injury in 1- and 8-wk-old rats*

Concentration of $dPGE_2$ (μg/kg)	1 wk		8 wk	
	n	Lesion index (%)*	*n*	Lesion index (%)*
Control	10	23.2 ± 5.9	6	41.7 ± 9.9
1	6	20.4 ± 8.6		
10	7	26.0 ± 7.0	5	0.6 ± 0.3†
100	7	29.7 ± 11.6		

* Each value is the mean ± SEM.

† Significantly different from control, $p < 0.005$ (*t* test).

mucin secretion and is cytoprotective. In the study by Lamont *et al.* (27), N-ethylmaleimide, a known inhibitor of cytoprotection by cystamine, had no effect on mucin secretion, whereas indomethacin inhibited mucin secretion by cystamine but did not influence cytoprotection. $dPGE_2$ protected gastric mucosa from 100% ethanol without preventing entry of the ethanol into the gastric mucosa (28).

The present study showed that the baseline thickness of mucus is decreased in newborn rats, who are more susceptible to ethanol-induced injury (5). Further, the increase in mucus thickness after $dPGE_2$ did not occur in the newborn rats, who are not protected by $dPGE_2$. These data are consistent with the possibility that mucus may be involved in the mucosal protection. These data, however, do not demonstrate a causal relationship between mucus thickness and cytoprotection. There are likely to be other relevant age-related changes, such as increased mucosal circulation or bicarbonate secretion.

Dial and Lichtenberger (4) showed that acid (0.6 N HCl) induced more severe gastric mucosal damage in newborn rats than in adult rats. In preliminary studies, we showed that both acid and ethanol caused more severe damage in neonatal rats (5), suggesting that the mechanisms involved are not specific to acid but more generalized. Dial and Lichtenberger (4) showed that PG prevent mucosal injuries by acid in suckling rats only after they reach 21 d of age. Our present data, which showed that PG did not protect against ethanol-induced damage in 7-d-old neonatal rats, confirm that PG do not protect the gastric mucosa in younger rats. These data suggest that less effective cytoprotection by PG might be responsible for the fragility of gastric mucosa in the newborn rat.

The mechanisms proposed for PG-mediated mucosal protection include increases in mucus secretion, bicarbonate secretion, mucosal circulation, or epithelial cell renewal (6, 11, 12, 24). Our data showed that the $dPGE_2$ does not increase the mucus thickness and has no protective effect in the suckling rat, whereas it increases the thickness and prevents damage in older rats. Our data, therefore, are consistent with the supposition that mucus secretion might mediate the protective effect of PG. However, as mentioned above, the effect of mucus is still controversial, and it is possible that other factors are also involved. Inasmuch as we examined only mucus thickness in the present study, the involvement of other factors remains to be investigated.

Gastrointestinal bleeding is common in the critically ill human newborn (1–3). PGE_2 concentrations in gastric aspirates were lower in human neonates than in adults (17). Moreover, PGE_2 concentrations in the secretions from premature and critically ill infants, who are at high risk of developing ulcers, are lower than those in healthy babies (17, 30).

Adults with peptic ulcer disease, especially during treatment with nonsteroidal antiinflammatory drugs, can be treated with $dPGE_2$ (Arbocet; Upjohn, Kalamazoo, MI) (13, 29). However, our present data suggest that there may be an age-related delay in the protective effects of $dPGE_2$. Moreover, pharmacologic amounts of PG interfere with the closure of the ductus arteriosus in the newborn. Although these animal experiments may not be directly applicable to man, they suggest that there will be no indication for the use of PG in the prevention and treatment of gastric mucosal lesions in the newborn.

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