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Acceleration of recovery of gastric epithelial integrity by 16,16-dimethyl prostaglandin E_2

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1 The effects of pretreatment with 16,16-dimethyl prostaglandin E_2 ($DmPGE_2$) on the recovery of gastric mucosal 'barrier' parameters after ethanol-induced damage were studied using an *ex vivo* chamber preparation in the rat.

2 $DmPGE_2$ (4–40 $\mu g\,kg^{-1}$) significantly reduced the extent of haemorrhagic damage to the gastric mucosa induced by the topical application of 50% ethanol followed by 0.05 M hydrochloric acid.

3 The lowest dose of $DmPGE_2$ tested (4 $\mu g\,kg^{-1}$) had no effect on the ethanol-induced changes in transmucosal potential difference (PD) or K^+ efflux. However, $DmPGE_2$ at doses at 20 or 40 $\mu g\,kg^{-1}$ significantly reduced the changes in these indices of epithelial integrity.

4 The recovery of PD and K^+ efflux to control levels after ethanol-injury was accelerated by $DmPGE_2$.

5 With the two higher doses of $DmPGE_2$ (20 and 40 $\mu g\,kg^{-1}$) there was a significantly ($P < 0.001$) lower level of epithelial discontinuity, measured histologically, in samples taken at the end of the experiment.

6 These results suggest that at the higher doses, $DmPGE_2$ confers some protection to the gastric epithelium as well as accelerating the recovery of epithelial integrity after damage induced by ethanol.

Introduction

While the actions of prostaglandins in preventing gastric haemorrhage and necrosis induced by a variety of agents are well established (see Miller, 1983, for review), the effects on the gastric epithelium remain unclear. Lacy & Ito (1982) demonstrated that so-called 'cytoprotective' doses of a prostaglandin E_2 (PGE_2) analogue, 16,16-dimethyl PGE_2 ($DmPGE_2$) prevented ethanol-induced gastric necrosis, but had no protective effects on the epithelial cells that line the lumen of the stomach. Similar findings were described by Wallace *et al.* (1982) for PGE_2. These results have since been corroborated by several other studies (Morris *et al.*, 1984a; Schmidt *et al.*, 1985; Tarnawski *et al.*, 1985; Whittle & Steel, 1985).

In a recent study, it was found that $DmPGE_2$ could, at the dose of 20 $\mu g\,kg^{-1}$, significantly reduce the extent of ethanol-induced damage to the rat gastric epithelium (Whittle & Steel, 1985). Mucosae pretreated with these high doses of $DmPGE_2$ and challenged with ethanol *in vivo* had significantly less leakage of lysosomal and cytoplasmic enzymes when incubated *in vitro* and a significantly greater extent of intact epithelium when examined histologically. The conclusions of this study, however, were that it was unclear if damage to the epithelium had actually been prevented or if damage had occurred and the repair process had been accelerated by $DmPGE_2$ pretreatment.

The process of rapid re-epithelialization (or restitution) by emigration of cells from the gastric pits was first noted in an *in vivo* model after ethanol-induced mucosal damage (Morris & Wallace, 1981). Similar observations have since been reported in other *in vivo* (Lacy & Ito, 1984; Morris & Harding, 1984; Ito & Lacy, 1985) and *in vitro* models (Svanes *et al.*, 1982; Rutten & Ito, 1983; Critchlow *et al.*, 1985). It is possible that this process is accelerated by prostaglandin pretreatment. Thus, Morris *et al.* (1984a) noted an accelerated recovery of transmucosal potential difference (PD) and potassium efflux towards control levels in salicylate-damaged rat stomachs that had been pretreated with PGE_2. Tarnawski *et al.* (1985), using $DmPGE_2$, observed a similar increase in the recovery of PD and in the recovery of epithelial continuity during the 2–3 h following absolute ethanol-induced gastric damage. PD has been suggested

to be a good index of epithelial integrity (McGreevy, 1984) and its recovery after damage has been found to parallel closely the epithelial restitution process (Morris & Wallace, 1981; Rutten & Ito, 1983).

In the present study, the effects of $DmPGE_2$ on ethanol-induced damage were studied in an *ex vivo* chamber preparation of the rat stomach. This model allows the measurement of indices of epithelial integrity (PD and K^+ efflux) at the time when ethanol is applied to the mucosa and during the ensuing period of time when restitution occurs. Hence, this model was employed to separate any protective effect of $DmPGE_2$ from an acceleration of the repair process.

Methods

Male, Wistar rats of 225–250 g were used. The rats were deprived of food, but not water for 18 h before an experiment.

Gastric chamber preparation

The rat was anaesthetized with sodium pentobarbitone (60 $mgkg^{-1}$, i.p.) and an *ex vivo* gastric chamber prepared as described in detail previously (Wallace *et al.*, 1982). This preparation allowed direct observation of the gastric mucosa with the vascular and neural connections intact. The pylorus and oesophagus were ligated. The stomach was opened by an incision along the greater curvature and clamped to a Perspex chamber. This chamber had a capacity of 6.0 ml. The mucosa was gently rinsed with warm (37°C) 0.3 M mannitol to remove any debris present in the stomach. Each experiment lasted 70 min, consisting of five 10 min periods and one 20 min period. A fresh solution (5.0 ml) was added to the chamber at the beginning of each period. The solution added to the chamber at the beginning of period 2 was allowed to bathe the mucosa for 20 min. The composition of the 'luminal' bathing solutions in each group were as follows: (1) 0.3 M mannitol, (2) 0.3 M mannitol ± $DmPGE_2$ (4, 20 or 40 $\mu g\,kg^{-1}$), (3) 50% ethanol (v/v), 4–6) 0.05 M hydrochloric acid in 0.2 M mannitol. This protocol was selected because in previous studies with the gastric chamber model (Morris & Wallace, 1981; Wallace *et al.*, 1982) it was found to elicit a reproducible level of damage. While in the chamber, the solutions were stirred by a Perspex paddle (1 cm above the mucosal surface) turning at approximately 200 r.p.m. At the end of each period the luminal solution was aspirated with a syringe and weighed to determine changes in volume. These solutions were immediately frozen for subsequent analysis of potassium ion concentration. Transmucosal potential difference (PD) was measured continuously throughout the experiment (see below).

At the end of each experiment the mucosa was photographed with colour transparency film (Ektachrome 160). The extent of haemorrhagic damage was quantified from these transparencies in a randomized, blind manner. The images on the transparencies were projected onto 29 × 21 cm paper and traced. The total area of glandular mucosa and the area of haemorrhagic damage were measured with a planimeter. The gastric damage score was calculated as the % of glandular mucosa showing haemorrhagic damage.

After the mucosa was photographed, the gastric pedacle was cut and the mucosa was immersed in neutral buffered formalin. Samples (2 × 10 mm) of the fundus (corpus) were excised with a scalpel and transferred to fresh fixative. These samples were taken from the same regions on all mucosae; i.e. the mid-fundus of both the ventral and dorsal sides of the stomach. The samples were processed by routine techniques before being embedded in paraffin. Sections (5–8 μm) were stained with haematoxylin and eosin and mounted on glass slides. The slides were coded and randomized to prevent observer bias. Using a graticule mounted in the eyepiece of a light microscope, the entire length of each section was measured, as were the regions in which the layer of surface epithelial cells was intact. Regions exhibiting haemorrhagic erosions were not included in the calculations. For each slide, the % of total length (approximately 10 mm) that had an intact epithelium was calculated.

Transmucosal potential difference

Electrode bridges were constructed of polyethylene tubing (o.d. 1 mm) filled with 4% agar/saturated KCl. One bridge was placed in the gastric chamber, approximately 1 cm above the mucosal surface. A second bridge was placed in the peritoneal cavity below the chamber preparation. The peritoneum was flooded with 0.9% w/v NaCl solution (saline). The free ends of the two bridges were inserted into beakers containing identical solutions of 3M KCl. Identical Ag/AgCl electrodes were placed in the two beakers. The leads of the electrodes were connected to a Comark d.c. Millivoltmeter and to a Rikadenki chart recorder. The polarity of the electrodes was set such that, under control conditions, the mucosal to serosal potential difference was negative.

Potassium ion determinations

The concentraton of potassium ions in the samples of luminal solutions were determined by flame photometry (Corning model 405). The concentration in the sample was multiplied by the final volume of the luminal solution to give the net flux of K^+.

Drugs

16,16-Dimethyl prostaglandin E_2 ($DmPGE_2$), obtained from Upjohn Co., Kalamazoo, U.S.A., was

stored at −20°C as a stock solution in absolute ethanol (1 mg ml^{-1}). On the day of an experiment, fresh solutions were prepared by drying an aliquot of the stock solution under nitrogen and redissolving in saline. Ethanol (BDH; Analar grade) was diluted to 50% (v/v) with distilled water.

Statistical analysis

All data are expressed as mean ± s.e.mean. Comparisons between groups were performed using Student's two-tailed *t* test for unpaired data, where $P<0.05$ was taken as significant.

Results

Gross observations

Topical application of 50% ethanol followed by 0.05 M HCl produced haemorrhagic erosions covering 21.0 ± 4.3% ($n = 5$) of the glandular mucosa. This damage was confined largely to the fundic (corpus) region. All three doses of $DmPGE_2$ (4, 20 or 40 μg kg^{-1}) significantly ($P < 0.01$) reduced the extent of macroscopically-visible damage (Table 1). With all three doses of $DmPGE_2$ this reduction of damage appeared to be maximal, as the only haemorrhagic regions were those around the periphery of the chamber. During the period in which $DmPGE_2$ was in the chamber the mucosa invariably became flushed, changing from its typical pink colour to a deep red. After $DmPGE_2$ treatment mucosal folds were no longer visible.

Table 1 Macroscopic and histological assessment of gastric damage 40 min after application of 50% ethanol: effects of pretreatment with 16,16-dimethyl prostaglandin E_2

$DmPGE_2$ (μg kg^{-1})	*Haemorrhagic damage (%)*	*% of surface with intact epithelium*
0 (control)	21.0 ± 4.3	57.8 ± 3.4
4	2.2 ± 1.0*	57.6 ± 4.7
20	1.0 ± 1.0*	83.0 ± 3.0**
40	1.2 ± 0.7*	84.0 ± 3.3**

Haemorrhagic damage, expressed as the % of glandular mucosa, was scored macroscopically in a randomized, blind manner. The extent of intact epithelium (% of total length of epithelium) was quantified histologically (see Methods). Each group consisted of 5 to 9 rats. *$P < 0.01$; **$P < 0.001$ compared to the control group.

Histological observations

Topical application of 50% ethanol followed by 0.05 M HCl produced extensive damage to the surface epithelium and induced regions of deeper necrosis. The necrotic regions were characterized by vascular congestion and haemorrhage. The necrosis involved the entire depth of the mucosa, but did not penetrate the muscularis mucosae. Other histological features of the ethanol-induced damage were identical to those previously described in detail (Morris & Wallace, 1981). All three doses of $DmPGE_2$ almost completely prevented the mucosal necrosis and haemorrhage. Damage in these groups was limited to the surface epithelium. With the lowest dose of $DmPGE_2$ tested (4 μg kg^{-1}) the extent of damage to the surface epithelium was not significantly different from that in the control group (Table 1). Conversely, with $DmPGE_2$ at doses of 20 or 40 μg kg^{-1} the extent of damage to the surface epithelium was significantly ($P < 0.001$) reduced. The increase in the dose of $DmPGE_2$ from 20 to 40 μg kg^{-1} did not result in any change in the extent of epithelial continuity. Despite the large regions of intact epithelium in these groups, there was usually cellular debris and mucus present on the luminal surface.

Potential difference

During the first (resting) period, the PD in all four groups remained between −40 and −45 mV (Figure 1). Addition of $DmPGE_2$ to the chamber caused a gradual fall in PD of 5–10 mV that usually

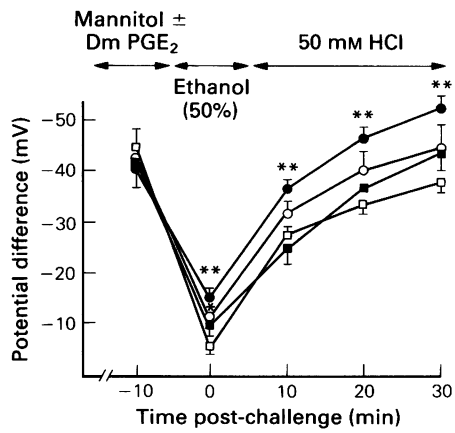

Figure 1 Effects of 16,16-dimethyl prostaglandin E_2 ($DmPGE_2$) pretreatment on the recovery of potential difference following ethanol challenge. (□) Control; (■) $DmPGE_2$, 4 μg kg^{-1}; (○) $DmPGE_2$, 20 μg kg^{-1}; (●) $DmPGE_2$, 40 μg kg^{-1}. Each point represents the mean, and vertical lines show s.e.mean potential difference over a 10 min period for 4–6 experiments. The s.e.mean has been omitted from some points for the sake of clarity. Asterisks indicate points which differ significantly from the corresponding control group (*$P < 0.01$; **$P < 0.001$). Solutions present in the chamber are indicated at the top of the figure.

recovered to basal levels by the end of the second period. Upon addition of 50% ethanol to the chamber, the PD fell abruptly. In the control group, the PD fell to -5 ± 1 mV and remained at this low level throughout the 10 min period. In the final 30 min of the experiments, when acid was present in the chamber, the PD gradually returned towards resting levels. In the control group, the PD recovered to only 85% of resting levels by the end of the experiment.

In the group pretreated with the lowest dose of $DmPGE_2$ ($4\,\mu g\,kg^{-1}$) the ethanol-induced fall in PD ($\Delta 35 \pm 2$ mV) was not significantly different from that in the control group ($\Delta 40 \pm 2$ mV). However, in the groups pretreated with higher doses of $DmPGE_2$ (20 and $40\,\mu g\,kg^{-1}$) the initial fall in PD induced by ethanol was significantly reduced ($\Delta 32 \pm 3$ mV, $P<0.05$ and $\Delta 25 \pm 3$ mV, $P<0.01$, respectively). During the 30 min following the period of ethanol application the PD gradually recovered, but never to the level of the resting period. With the lowest dose of $DmPGE_2$ ($4\,\mu g\,kg^{-1}$) the PD recovery was not significantly different from controls (Figure 1). Although $DmPGE_2$ at a dose of $20\,\mu g\,kg^{-1}$ did not significantly increase the PD compared with the control group, the PD had recovered to resting levels by 25 ± 3 min after removal of ethanol from the chamber. With the highest dose of $DmPGE_2$ tested ($40\,\mu g\,kg^{-1}$) the PD recovered much more rapidly than that in the controls, as reflected by the significantly higher ($P<0.01$) PD values in the final 30 min. The PD had recovered to resting levels by 11 ± 2 min after ethanol was removed from the chamber.

Potassium efflux

Net efflux of K^+ during the first (resting) period in all four groups ranged between 2 and 4 μEq (per 10 min). Each of the doses of $DmPGE_2$ tested produced a slight, but not significant, reduction in K^+ efflux during the second period. In the control group, application of 50% ethanol to the mucosa during the third period resulted in a highly significant increase (to 232 ± 24% of basal; $P<0.001$) in the net release of K^+ into the lumen (Figure 2). This release of K^+ decreased gradually towards resting levels during the final 30 min of the experiment. In period 7, the K^+ release in the control group was not significantly different from resting levels.

Pretreatment with $DmPGE_2$ caused a reduction in the ethanol-induced increase in net K^+ efflux which was significant with the higher doses used (20 and $40\,\mu g\,kg^{-1}$, $P<0.01$). In the final three periods, the K^+ efflux decreased towards resting levels in a dose-dependent manner (Figure 2). With the highest dose of $DmPGE_2$ tested ($40\,\mu g\,kg^{-1}$) the K^+ efflux never differed significantly from the resting values.

Discussion

These results demonstrate that the changes in gastric mucosal 'barrier' parameters (PD and net K^+ efflux) induced by topical application of 50% ethanol can be significantly reduced by $DmPGE_2$ at doses of 20 or $40\,\mu g\,kg^{-1}$. Furthermore, $DmPGE_2$ dose-dependently accelerated the recovery of these parameters to control levels. The results presented also confirm previous studies showing that a lower dose of $DmPGE_2$ ($4\,\mu g\,kg^{-1}$) could prevent ethanol-induced mucosal necrosis and haemorrhage without significantly reducing damage to the surface epithelium (Lacy & Ito, 1982; Whittle & Steel, 1985).

The term 'cytoprotection' has been used as an all-embracing term to describe the anti-ulcer properties of

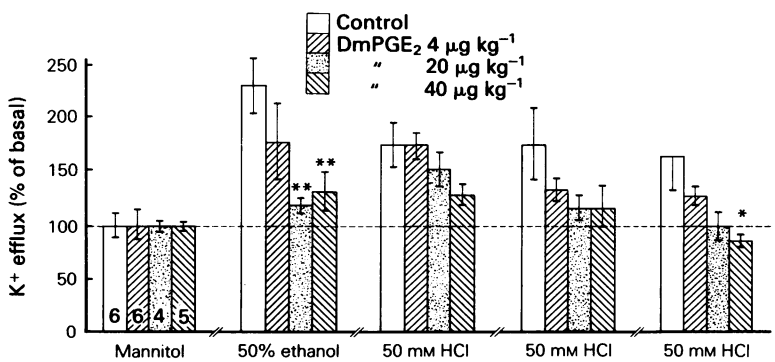

Figure 2 Effects of 16,16-dimethyl prostaglandin E_2 ($DmPGE_2$) pretreatment on the net efflux of K^+ before, during and after challenge of the chambered gastric mucosa with 50% ethanol. Columns represent the mean and vertical lines s.e.mean % basal efflux (i.e. that during the first period) for a 10 min period. Asterisks denote groups that differ significantly from the corresponding control group (*$P<0.05$; **$P<0.01$).

prostanoids. Recently, the use of this term has become controversial. 'Cytoprotective' doses of PGE_2 and $DmPGE_2$ have been shown to prevent macroscopic damage (i.e. haemorrhagic necrosis), but not to prevent damage to the surface epithelium or to the mucosal barrier (Wallace *et al.*, 1982; Lacy & Ito, 1982; Morris *et al.*, 1984a). A recent study from this laboratory showed that higher doses of $DmPGE_2$ appeared to reduce the areas of epithelial discontinuity induced by ethanol (Whittle & Steel, 1985). While it is likely that $DmPGE_2$ was, indeed, preventing damage to a significant portion of the epithelium, it was not clear if this prostanoid was also accelerating repair of damaged portions of the epithelium. In the present study, the PD and K^+ efflux data suggest that both a protection of the gastric epithelium and an acceleration of the restitution process were occurring with the higher doses of $DmPGE_2$ (20 and 40 $\mu g\,kg^{-1}$).

It is likely that the accelerated recovery of the barrier parameters reflected accelerated recovery of epithelial integrity. When a section of gastric epithelium is destroyed, such as by the topical application of ethanol or salicylate, cells in the gastric pits rapidly migrate along the exposed basal lamina to cover the denuded area (Morris & Wallace, 1981; Morris & Harding, 1984; Lacy & Ito, 1984). The recovery of barrier parameters closely parallels this restitution process (Morris & Wallace, 1981; Rutten & Ito, 1983). Potential difference is generated primarily by the surface epithelial cells (McGreevy, 1984) and it has been postulated that leakage of K^+ into the lumen primarily reflects surface epithelial cell damage (Morris *et al.*, 1984b; Carter *et al.*, 1985). The electrophysiological evidence for recovery of epithelial integrity is supported by the histological data, which showed that at the end of the experiments there was a significantly greater level of epithelial continuity in the groups pretreated with the higher doses of $DmPGE_2$.

It is possible that $DmPGE_2$ directly affects the rate of migration of cells from the gastric glands. However, a more plausible explanation is that $DmPGE_2$ accelerates the recovery of the gastric epithelium by reducing the extent of damage to cells that line the gastric glands. The 'protected' cells would thus have shorter distances to migrate to re-establish epithelial continuity. Tarnawski *et al.* (1985) have suggested that $DmPGE_2$ may promote epithelial recovery by protecting the cells in the proliferative zone of the mucosa.

The primary site of the protective actions of $DmPGE_2$ may be the mucosal vasculature. $DmPGE_2$ prevents the vascular congestion induced by ethanol (Lacy & Ito, 1982; Guth *et al.*, 1984) and in doing so, may aid in the maintenance of mucosal perfusion. In addition to supplying oxygen and nutrients to the cells involved in the restitution process, the intact blood supply would also enable clearance of the ethanol and acid diffusing into the mucosa. An effect of $DmPGE_2$ on the mucosal vasculature is supported by the observations of Svanes *et al.* (1984) that $DmPGE_2$ had no effect on the rate of restitution in an *in vitro* amphibian mucosal preparation. Effects of $DmPGE_2$ on mucus and bicarbonate secretion are unlikely to have contributed to the reduction of ethanol-induced damage, but may have contributed to the acceleration of the epithelial repair process.

In light of the present findings, it is clear that conclusions about the effects of $DmPGE_2$ on the gastric epithelium must be carefully qualified by reference to dosages. At a dose of 4 $\mu g\,kg^{-1}$, $DmPGE_2$ significantly reduced haemorrhagic necrosis induced by 50% ethanol, but conferred no protection to the epithelium (or the 'barrier'). Conversely, higher doses of $DmPGE_2$ (20 or 40 $\mu g\,kg^{-1}$) appeared to confer some protection to the surface epithelium, as reflected by the reduced PD drop and K^+ efflux, while enhancing the recovery of epithelial integrity. It is thus apparent from the present findings that a careful distinction must be made between prevention of damage and rapid repair of the gastric epithelium. Clearly prostaglandin 'cytoprotection' may involve not only protection of some portion of the mucosa, but also acceleration of the short-term repair processes.

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