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Failure of prostaglandin E_2 and its 16,16-dimethyl analogue to prevent the gastric mucosal damage induced by Paf

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1 Intravenous and orally administered prostaglandin E_2 (PGE_2) and 16,16-dimethyl PGE_2 (dm PGE_2) protect the rat gastric mucosa from injury induced by oral administration of acidified 40% ethanol. The effects of pretreatment with these prostaglandins on platelet activating factor (Paf)-induced gastric damage has now been investigated in the rat.

2 A 10 min infusion of Paf (50 or 100 ng kg^{-1} min^{-1}, i.v.) resulted in dose-related vasocongestion of the gastric mucosa.

3 Intravenous pretreatment with $dmPGE_2$ (20 μg kg^{-1}) failed to prevent the gastric damage induced by the higher dose of Paf. Pretreatment with PGE_2 (10–100 μg kg^{-1}) or $dmPGE_2$ (1–20 μg kg^{-1}), either orally or intravenously, also failed to prevent the gastric vasocongestion induced by the lower dose of Paf. On the contrary, significant augmentation of Paf-induced damage was observed with several of the doses of PGE_2 and $dmPGE_2$.

4 These studies demonstrate that the protective properties of PGE_2 and $dmPGE_2$ in the gastric mucosa do not extend to damage induced by Paf.

Introduction

The endogenous phospholipid, platelet-activating factor (Paf), is the most potent gastric ulcerogenic agent yet described (Rosam *et al.*, 1986) and may play a role as a mediator of the gastrointestinal ulceration associated with septic shock (Wallace & Whittle, 1986a). The mechanism of Paf-induced gastric necrosis is not yet known, although the possibility of mediation through effects of Paf on platelets has been discounted in the rat (Rosam *et al.*, 1986). Histological evidence suggests that vascular stasis may be the precipitating event, since intravenous infusion of Paf causes extensive vasocongestion in the stomach. This vasocongestion does not appear to be due to constrictor actions of Paf on the submucosal microcirculation (Whittle *et al.*, 1986), but could be related to the haemoconcentration following Paf administration or to microvascular blockage by Paf-induced neutrophil aggregates (Wallace & Whittle, 1986b).

Prostaglandins, particularly those of the E_2 series, have potent protective (Robert *et al.*, 1979) and vasodilator (Main & Whittle, 1975) actions in the gastric mucosa. Pretreatment with PGE_2 or 16,16-dimethyl PGE_2 ($dmPGE_2$) has been shown to prevent the vascular stasis and the deep mucosal necrosis induced by topical application of ethanol in the rat stomach (Wallace *et al.*, 1982; Lacy & Ito, 1982). It was thus possible that prostaglandins would also prevent Paf-induced vascular stasis, and thereby protect the gastric mucosa from damage. In the present study, we have examined the effects of several doses of PGE_2 and $dmPGE_2$, administered both orally and intravenously, on the gastric mucosal damage induced by Paf.

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Methods

Male, Wistar rats (250 g) were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and a carotid artery and a femoral vein cannulated. Systemic arterial blood pressure (BP) was monitored with a pressure transducer via the carotid cannula. A steady baseline BP was recorded for at least 10 min before the injection or infusion of drug. PGE_2 (10–100 μg kg^{-1}) and $dmPGE_2$ (5–20 μg kg^{-1}) were administered orally (1.0 ml kg^{-1}) 20 min before beginning the infusion of Paf. Intravenous bolus injections (2.0 ml kg^{-1}) of PGE_2 (25–100 μg kg^{-1}) and $dmPGE_2$ (1–10 μg kg^{-1})

were given 15–20 min before Paf infusion. At this time the BP response to these prostaglandins had returned to basal levels. Paf was infused intravenously (50 or 100 ng kg^{-1} min^{-1}) for 10 min and the BP was monitored for 30 min after completion of the Paf infusion.

At the end of the experiment, the stomach was removed, opened along the greater curvature, rinsed and pinned out flat on a wax block which was immersed in fresh neutral-buffered formalin. Macroscopically-visible hyperaemia and haemorrhage were assessed independently by two observers who were unaware of the treatment. Each stomach was scored on a 0 to 5 scale according to the following criteria: 0 = normal; 1 = diffuse hyperaemia; 2 = moderate focal hyperaemia; 3 = severe focal hyperaemia; 4 = severe focal hyperaemia and haemorrhage; 5 = severe focal hyperaemia with extensive regions of haemorrhage. There was a highly significant correlation between the scores assigned by the two assessors ($r = 0.92$; $P < 0.001$; $n = 69$).

Two blocks of mucosal tissue (8 mm × 3 mm) were excised from standardized regions of the dorsal and ventral corpus of each stomach. Paraffin sections (4–5 μm) of these tissue blocks were stained with haematoxylin and eosin by routine techniques for light microscopy. The sections were then coded to eliminate observer bias and scored on a 0 to 5 scale according to the following criteria: 0 = normal; 1 = focal regions of subepithelial vasocongestion; 2 = extensive vasocongestion limited to the subepithelial vessels; 3 = vascular congestion of isthmus region; 4 = vascular stasis of entire depth of mucosa; 5 = extensive vasocongestion of mucosa and/or submucosa; haemorrhage; necrosis.

In order to confirm that PGE_2 and $dmPGE_2$ would exert protective actions in another model of gastric damage, additional experiments were performed in which the effects of oral or intravenous pretreatment with these prostaglandins on the damage induced by acidified ethanol were examined. PGE_2 (25 μg kg^{-1}) or $dmPGE_2$ (5 μg kg^{-1}) were administered orally or intravenously to rats ($n = 3$ to 11 per group). Twenty minutes later, 1.0 ml of 40% ethanol (v/v) in 0.1 M HCl was administered orally. The gastric damage was scored 10 min later as described above.

Since the damaging effects of Paf may be attributable in part to its capacity to increase vascular permeability, the effects of Paf (50 ng kg^{-1} min^{-1}) and $dmPGE_2$ on plasma exudation were studied ($n = 5$ to 7 per group). ^{14}C-labelled bovine serum albumin (0.5 μCi; Amersham) was administered via the femoral cannula. Basal blood samples were taken from the carotid cannula 5 min before and 5 min after intravenous administration of $dmPGE_2$ (5 μg kg^{-1}) or vehicle. Additional blood samples were taken 2.5 and 10 min after starting the Paf infusion and 15 min after completion of the infusion. A 100 μl portion of each blood sample was mixed with 1.0 ml of saline and centrifuged for 2 min (9000 g). The supernatant was then added to 10 ml of scintillation mixture and the amount of radioactivity present determined by counting on a β-spectrometer.

Reagents

Platelet-activating factor (1-O-alkyl-2-O-acetyl-*sn*-glyceryl-3-phosphoryl-choline) was obtained from Sigma Chemical Company. An aliquot of the stock 2 mg ml^{-1} solution of Paf in chloroform was evaporated under a stream of nitrogen and reconstituted in 0.25% (w/v) bovine serum albumin in 0.9% (w/v) saline. Paf was infused at a rate of 0.05 ml min^{-1}. PGE_2 and $dmPGE_2$ (Upjohn) were stored in absolute ethanol (10 mg ml^{-1}) at −30°C. Aliquots were removed when required, dried under nitrogen, redissolved in Tris buffer (50 mM; pH 7.5 at 25°C) and diluted with saline.

Statistical analysis

Results are expressed as mean ± s.e.mean. Comparisons between groups of parametric data were made by Student's *t* test for unpaired data. Comparisons between groups of non-parametric data were made by the Wilcoxon Rank Sum test. With all analyses, an associated probability of 5% or less ($P < 0.05$) was considered to be significant.

Results

Effects of prostaglandins on gastric damage induced by acidified ethanol

The oral administration of acidified ethanol induced extensive haemorrhagic necrosis of the corpus region of the gastric mucosa (mean damage score of 5 ± 0; $n = 11$). Intravenous or oral pretreatment with either PGE_2 or $dmPGE_2$ significantly ($P < 0.01$) reduced the extent of gastric damage (Table 1).

Effects of intravenous infusion of Paf

Intravenous infuson of Paf (50 or 100 ng kg^{-1} min^{-1}) for 10 min resulted in hyperaemia of the glandular region of the stomach. The hyperaemia was generally diffuse, but in some mucosae there were focal patches of more severe hyperaemia and haemorrhage, particularly with the higher dose of Paf. The mean macroscopic scores for the Paf groups (50 and 100 ng kg^{-1} min^{-1}) were 1.4 ± 0.3 ($n = 6$) and 3.8 ± 0.3 ($n = 4$), respectively, both significantly ($P < 0.01$) greater than observed in the rats which

Table 1 Effects of prostaglandin E_2 (PGE_2) and 16,16-dimethyl PGE_2 ($dmPGE_2$) on the gastric damage induced by acidified 40% ethanol

Pretreatment	*Route*	n	*Gastric damage score*
None	—	11	5.0 ± 0
PGE_2 (25 μg kg^{-1})	p.o.	4	3.5 ± 0.5†
	i.v.	4	1.5 ± 0.3†
$dmPGE_2$ (5 μg kg^{-1})	p.o.	6	1.3 ± 0.1†
	i.v.	3	1.1 ± 0.1†

PGE_2 or $dmPGE_2$ were administered intravenously (i.v.) or orally (p.o.) 20 min before oral administration of 1.0 ml of 40% ethanol (v/v) in 100 mM HCl. Macroscopic gastric damage scores are shown as the mean ± s.e.mean of (n) experiments. †$P < 0.01$ compared to the control group (Wilcoxon Rank Sum Test).

received only the vehicle (macroscopic score of 0 ± 0; $n = 3$).

Histologically, Paf produced regions of vasocongestion which was for the most part limited to the superficial, subepithelial blood vessels (histological scores of 2.0 ± 0.3 ($n = 6$) and 3.8 ± 0.5 ($n = 5$) for the 50 and 100 ng kg^{-1} min^{-1} doses, respectively). In some regions where the vasocongestion extended deeper than the superficial vessels, extravasation of erythrocytes and necrosis was evident. Both intravascular and extravascular accumulations of neutrophils were observed, particularly in the submucosa.

Effects of oral pretreatment with prostaglandins

Oral pretreatment with $dmPGE_2$ (20 μg kg^{-1}) had no significant effect on the macroscopically-visible damage induced by intravenous infusion of Paf (100 ng kg^{-1} min^{-1}), with a mean score of 3.8 ± 0.6 ($n = 4$). Since a much lower dose of $dmPGE_2$ was effective in reducing gastric damage induced by acidified ethanol (Table 1), it seemed unlikely that PGE_2 or lower doses of $dmPGE_2$ would have significant effects on the damage induced by this dose of Paf. Accordingly, all subsequent studies were performed using the lower dose of Paf (50 ng kg^{-1} min^{-1}).

Oral administration of PGE_2 or $dmPGE_2$ failed to reduce the gastric damage induced by Paf. For instance, at doses of 25 and 50 μg kg^{-1}, PGE_2 caused a significant ($P < 0.01$) increase in the histological score (Figure 1), while $dmPGE_2$ significantly ($P < 0.01$) augmented Paf-induced damage when administered at doses of 10 or 20 μg kg^{-1} (Figure 2).

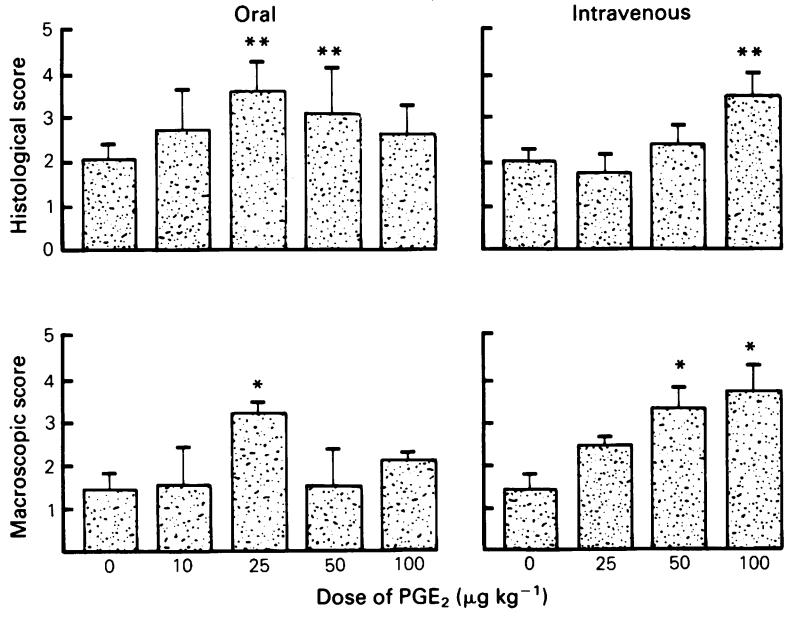

Figure 1 Effects of oral or intravenous pretreatment with prostaglandin E_2 (PGE_2) on Paf-induced gastric mucosal damage, scored both histologically and macroscopically. Paf (50 ng kg^{-1} min^{-1}) was infused for 10 min. Each column represents the mean of 4 to 11 experiments; vertical lines show s.e.mean. Asterisks denote groups which differ significantly from the control group (*$P < 0.05$; **$P < 0.01$; Wilcoxon Rank Sum Test).

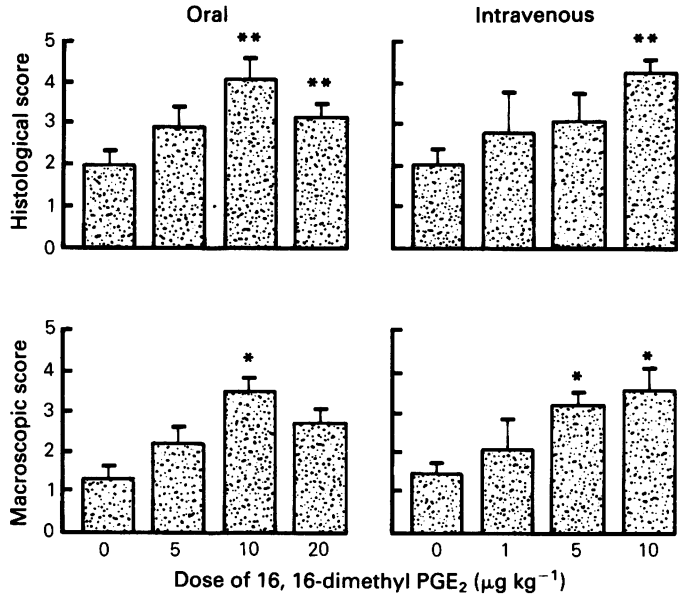

Figure 2 Effects of oral or intravenous pretreatment with 16,16-dimethyl prostaglandin E_2 on Paf-induced gastric mucosal damage, scored both histologically and macroscopically. Paf (50 ng kg^{-1} min^{-1}) was infused for 10 min. Each column represents mean of 4 to 11 experiments; vertical lines show s.e.mean. Asterisks denote groups which differ significantly from the control group (*$P<0.05$; **$P<0.01$; Wilcoxon Rank Sum Test).

Effects of intravenous pretreatment with prostaglandins

Intravenous pretreatment with $dmPGE_2$ (1–10 μg kg^{-1}) or PGE_2 (25–100 μg kg^{-1}) resulted in dose-related increases in the macroscopic and histological scores over that observed with Paf alone (Figures 1 and 2). In these groups, the hyperaemia was of a more focal nature than seen with Paf alone and was often associated with regions of overt bleeding.

Effects of Paf and prostaglandins on systemic arterial blood pressure

Resting BP prior to any injections of prostaglandins or Paf was 119 ± 4 mmHg ($n = 75$). Intravenous infusion of Paf (50 ng kg^{-1} min^{-1}) caused a rapid decrease in BP of 78 ± 5 mmHg which remained depressed throughout the 10 min period of the infusion (Figure 3). During the 30 min after completion of the infusion, the BP steadily increased, and by the end of the experiment, had recovered to 85 ± 2% of resting levels.

Intravenous administration of PGE_2 or $dmPGE_2$ caused a decrease in BP (Δ 40–60 mmHg) which rapidly recovered to resting levels within 10 min. The initial Paf-induced decrease in BP was significantly reduced ($P<0.05$) by pretreatment with PGE_2 (25 or 50 μg kg^{-1}) or $dmPGE_2$ (5 μg kg^{-1}).

Oral administration of PGE_2 or $dmPGE_2$ had no effect on resting BP. Only the highest dose of $dmPGE_2$ (20 μg kg^{-1}) tested significantly reduced ($P<0.01$) the initial Paf-induced fall in BP (Figure 3).

Effects of Paf and prostaglandins on plasma exudation

Intravenous infusion of Paf resulted in a significant ($P<0.01$) loss of ^{14}C-labelled albumin from the blood (29.3 ± 2.2% in samples taken 15 min after Paf) (Figure 4). Pretreatment with $dmPGE_2$ (5 μg kg^{-1} i.v.) did not significantly affect the Paf-induced leakage of radiolabelled albumin.

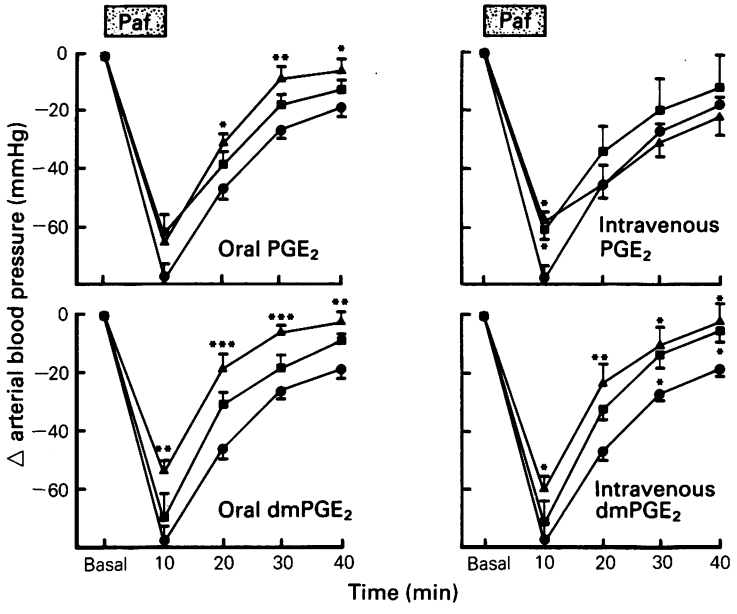

Figure 3 Effects of oral or intravenous pretreatment with prostaglandin E_2 (PGE_2) or 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$) on systemic arterial blood pressure response during and after a 10 min intravenous infusion of Paf (50 ng kg^{-1} min^{-1}), as indicated by the stippled bars. Each point represents the mean of 4 to 11 experiments; vertical lines show s.e.mean. The control group, receiving Paf alone, is shown in each panel (●). PGE_2 was administered orally and intravenously at doses of 50 μg kg^{-1} (■) and 100 μg kg^{-1} (▲). 16,16-dimethyl PGE_2 was administered orally at doses of 10 μg kg^{-1} (■) and 20 μg kg^{-1} (▲) and intravenously at doses of 1 μg kg^{-1} (■) and 5 μg kg^{-1} (▲). Asterisks denote groups which differ significantly from the control group ($^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; Student's t test).

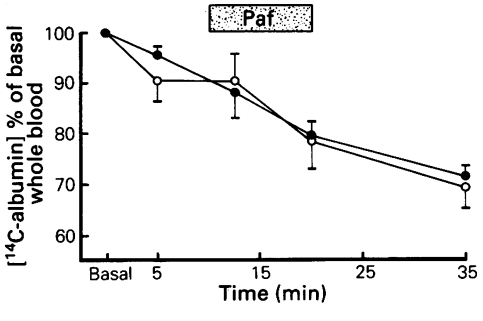

Figure 4 Effects of intravenous pretreatment with 16,16-dimethyl prostaglandin E_2 (5 μg kg^{-1}; ○) on Paf-induced plasma leakage. Plasma leakage from the blood was measured using ^{14}C-labelled bovine serum albumin as a marker. Each point represents the mean of 4–6 experiments; vertical lines show s.e.mean. There were no significant differences between the group pretreated with the prostaglandin analogue and the control (●) group.

Discussion

Prostaglandins of the E series and their methylated analogues have been shown to inhibit the formation of gastric erosions induced in rats by pylorus ligation, physical restraint, steroids, reserpine, 5-hydroxytryptamine, bile salts, ethanol and non-steroid anti-inflammatory drugs (see Hawkey & Rampton, 1985, for review). In the present study, PGE_2 and $dmPGE_2$, given either orally or intravenously, significantly reduced gastric damage induced by orally-administered acidified ethanol, but failed to prevent Paf-induced gastric damage. Furthermore, several doses of PGE_2 and $dmPGE_2$ caused an augmentation of Paf-induced gastric damage.

The vasodilator actions of prostaglandins have been implicated in their mechanism of augmenting the Paf response in various tissues. McGivern & Basran (1984) observed a synergistic increase in the wheal volume response following intradermal injections of Paf and PGE_2 into the human forearm. A similar potentiation

of vascular permeability due to a combination of prostaglandins and Paf has also been demonstrated in the guinea-pig skin and lungs (Basran *et al.*, 1982). Previously, Williams & Peck (1977) had demonstrated the ability of prostaglandins, ADP, adenosine and isoprenaline to potentiate plasma exudation induced by bradykinin in rabbit skin, an effect attributed to the local vasodilator properties of these agents. In the present study, $dmPGE_2$ ($5\,\mu g\,kg^{-1}$) did not significantly augment Paf-induced vascular permeability, as measured by ^{14}C-albumin leakage. However, it is possible that there was a local, additive interaction between the prostaglandins and Paf in the gastric mucosa, since the vascular congestion induced by Paf was accentuated by both PGE_2 and $dmPGE_2$. Although both PGE_2 and $dmPGE_2$ have vasodilator actions on the gastric mucosal microcirculation (Main & Whittle, 1975), the mechanism of this interaction does not appear to be attributable to such actions of these prostaglandins. The infusion of Paf was started at a time when the systemic hypotensive actions of the prostaglandins, presumably a reflection of their peripheral vasodilator action, had dissipated. Furthermore, if the vasodilator action of the prostaglandins was responsible for the augmentation of Paf-induced gastric damage, a more characteristic dose-response relationship could have been expected. As can be seen from Figures 1 and 2, no such relationship existed when the prostaglandins were given by the oral route. The nature of the interactions of these prostanoids with different tissues or receptors, which could underlie the apparent bell-shaped dose-response curves, are not understood.

The reason for the failure of the prostaglandins to protect against Paf-induced damage is not clear, due in part to the lack of understanding of the mechanism underlying the gastric damage. We have previously suggested that Paf-induced haemoconcentration and neutrophil aggregation may contribute to the gastric mucosal damage (Rosam *et al.*, 1986; Wallace & Whittle, 1986b). It is clear from the ^{14}C-albumin experiments that $dmPGE_2$ did not significantly reduce Paf-induced haemoconcentration. Conversely, pretreatment with glucocorticoids or the dual lipoxygenase/cyclo-oxygenase inhibitor, BW755C, significantly reduced both Paf-induced gastric damage and haemoconcentration (Wallace & Whittle, 1986c). These latter findings suggested that lipoxygenase products of arachidonic acid may be important mediators of some of the pathological effects of Paf. Without quantitative data, it is not possible to state whether or not the prostaglandin pretreatments had any effect on Paf-induced neutrophil aggregation. It is interesting, however, that Camussi *et al.* (1983) were able to prevent Paf-induced neutrophil aggregation *in vivo* in the rabbit by an infusion of prostacyclin. Attempts to test prostacyclin in the present study were unsuccessful, since in preliminary studies the concomitant intravenous administration of the two hypotensive agents was lethal. Although the necrotic damage to the small intestine following intravenous injection of a high dose of Paf was reported to be attenuated by intravenous infusion of PGE_1 ($2\,mg\,kg^{-1}\,min^{-1}$), the large doses used warrants cautious interpretation of these findings (Hsueh *et al.*, 1986).

Previous studies in this laboratory demonstrated that the gastric damaging actions of Paf were unlikely to be due solely to the hypotension produced by this agent (Rosam *et al.*, 1986; Wallace & Whittle, 1986b). The present findings that both of the prostaglandins significantly reduced Paf-induced hypotension without providing any protection in the gastric mucosa adds further support to this conclusion. These studies also demonstrate that the protective actions of PGE_2 and $dmPGE_2$ do not extend to all experimental models of gastric mucosal damage.

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