Gastric mucosal protection with selective inhibition of thromboxane synthesis

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SUMMARY Because thromboxane synthesis enhances gastric mucosal damage we have investigated whether the thromboxane synthesis inhibitor dazmegrel might be protective to the mucosa. Dazmegrel at a dose of 1 and 5 mg per rat (4.8 and 23.8 mg/kg) significantly reduced the damage caused by acidified taurocholate. In parallel experiments dazmegrel exerted a selective and dose dependent inhibition of *ex vivo* thromboxane synthesis by gastric fragments over the dose range in which protection was observed. As dazmegrel can be given to man, these experiments suggest that investigation of mucosal protection would be justified.

At present there is considerable interest in the use of prostaglandins to treat peptic ulceration and in the role of 'cytoprotection' of the gastroduodenal mucosa in this process.¹ Exogenous prostaglandins can prevent or limit gastric mucosal damage provoked by ethanol, bile acids and non-steroidal anti inflammatory drugs in the rat^{1,2} and there are more limited comparable data in man.¹ There is growing evidence that similar protection can be achieved by stimulating endogenous prostaglandin synthesis.³⁻⁵

In contrast with the E and I prostaglandins, thromboxane A₂ has properties which can be detrimental to gastric mucosal integrity. Thromboxane A₂ is vasoconstrictor and enhanced synthesis produced by continuous infusion of arachidonic acid causes the rapid development of extensive gastric erosions in chambered rat stomachs exposed to acidified taurocholate.⁶ This damage could be prevented by the use of benzyl imidazole, a thromboxane synthetase inhibitor. We have therefore investigated the protective potential of dazmegrel (Fig. 1), another thromboxane synthetase inhibitor available for human use,⁷⁻⁹ in a different model of gastric mucosal damage, and have defined its effects on synthesis of TXB₂ and PGE₂ in gastric mucosa.

Methods

MATERIALS

Dazmegrel (3-(-1H-imidazol-1-yl-methyl)-2-methyl-1H-indole-1-propanoic acid, MW283) was supplied by Pfizer UK Ltd and used at three concentrations (2 mg/ml, 10 mg/ml, and 50 mg/ml) in phosphate buffered saline (PBS), 0.05 M pH 7.4.

Prostaglandin E₁ was supplied by Upjohn, Kalamazoo and made up to 150 mg/ml in PBS from ethanolic stock solutions (1 mg/ml). Sodium taurocholate (Sigma UK) was dissolved in 0.2 N hydrochloric acid to a final concentration of 100 mM. All solutions were freshly prepared immediately before the start of each experiment and coded to ensure that the investigator was unaware of their identities.

Fig. 1 Chemical structure of dazmegrel (molecular weight 283).

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Male Wistar rats weighing between 200 and 220 g were used in all experiments. The rats were housed in restraining cages to prevent coprophagy, were fasted for 20 hours and were deprived of water for four hours before each group of experiments.

GASTRIC MUCOSAL DAMAGE

Groups of rats were treated as shown in Figure 2. Dazmegrel in PBS or PBS alone were administered by gavage in volumes of 0.5 ml through a 5 FG orogastric tube. Three doses of dazmegrel were used, 1 mg/rat (4.8 mg/kg, $n=8$), 5 mg/rat (23.8 mg/kg, $n=8$) and 25 mg/rat (119 mg/kg, $n=8$). Two hours later 1 ml acidified taurocholate or distilled water was administered by gavage and after a further hour the animals were killed by CO₂ narcosis. Seven rats were pretreated with PGE₁, 75 µg in 0.5 ml PBS 15 minutes before acidified taurocholate and killed one hour later. In each experiment positive control (PBS followed by taurocholate) and negative controls (PBS followed by water) were included.

ASSESSMENT OF DAMAGE

After death the stomachs were carefully removed, and opened along the greater curve, and flattened on glass. Damage was assessed by two observers independently, using a numerical grading score of 0–4. Grade 0 was no macroscopic abnormality, grade 1 a few spots of mucosal haemorrhage, grade 2 spots of mucosal haemorrhage with short confluent streaks, grade 3 long confluent streaks of mucosal haemorrhage, grade 4 confluent streaks of mucosal haemorrhage with surface blood. The assessors were

Fig. 2 Diagram illustrating the protocols followed with time (in hours) from start of starvation period shown at the top of the figure. Protocol (a) was used for all studies of morphological damage and (b) for assessments of prostanoid synthesis.

neither aware of the treatments received nor the assessments of the other investigator. Complete agreement by the two observers was 76% and there were never differences of greater than one score between them. Where there was disagreement, average scores were used. Statistical analysis was by the Mann Whitney U test.

THROMBOXANE AND PROSTAGLANDIN SYNTHESIS

Eight groups of rats were treated as shown in Figure 2. They were killed (by cord transection) two hours after dosing with dazmegrel. The wall of the body of the stomach was cut into small fragments of 3–4 mg (30–60 mg total) which were incubated at 37°C for 15 minutes in gassed (95% O₂ 5% CO₂) Krebs' solution on a rotating mixer. At the end of the experiment the incubated gastric fragments were removed and weighed. Indomethacin 10 µg/ml (final concentration) was added to the incubated supernatants which were stored at –50°C until analysed (within one month). TxB₂ and PGE₂ were measured by radioimmunoassay of unextracted supernatant, using methods described elsewhere^{10,11} and antiserum kindly donated by Dr L Levine (TxB₂) or bought from the Institut Pasteur (PGE₂). The effects of dazmegrel on TxB₂ and PGE₂ synthesis were evaluated by t test after two way analysis of variance for dose and experimental day.

Results

The rats were in good general condition before being killed and no rat died during any procedure.

GASTRIC MUCOSAL DAMAGE

The degree of mucosal damage was decreased by treatment with dazmegrel (Fig. 3). The median (range) grade of damage was 2.5 (2–4) with placebo ($n=8$) and fell to 1.5 (0–3) with dazmegrel 1 mg ($n=8$, $p<0.05$) and to 1 (0–3) with dazmegrel 5 mg ($n=8$, $p<0.01$) and to 2 (0.5–3.5) with dazmegrel 25 mg ($n=8$, NS). With PGE₁ the values were 1 (0–3), significantly lower than the comparable control values which were 2.5 (2–4) ($p<0.01$).

THROMBOXANE AND PROSTAGLANDIN SYNTHESIS

Synthesis of TXB₂ (mean and SEM) by gastric fragments from untreated rats was 84+6 pg/mg (mean and SEM). The values for PGE₂ were 330+36 pg/mg. There was dose dependant inhibition of TXB₂ synthesis by dazmegrel, by 23+8% (mean+SEM) of control values ($p<0.05$) with 1 mg/rat, by 34+5% ($p<0.01$) with 5 mg/rat and by 46+7% ($p<0.001$) with 25 mg/rat. The lower doses of dazmegrel had no

Fig. 3 Effect of three doses of dazmegrel on: Macroscopic damage after acidified taurocholate (top). Compared with placebo, damage decreased with all doses of dazmegrel (1 mg: $p < 0.05$, 5 mg: $p < 0.01$, 25 mg: NS). Synthesis of TxB_2 (middle). Compared with placebo TxB_2 synthesis was inhibited by 1 mg dazmegrel: $p < 0.05$, 5 mg dazmegrel: $p < 0.01$, 25 mg dazmegrel: $p < 0.001$. Synthesis of PGE_2 (bottom). Significant inhibition was seen with 25 mg dazmegrel only ($p < 0.05$). Individual data are shown. Line joining results from the treated rat to its untreated control.

significant effect on synthesis of PGE_2 (122+24% control at 1 mg/rat and 102+16% control at 5 mg/rat) but at 25 mg/rat there was 27+9% inhibition of PGE_2 synthesis ($p < 0.05$).

Discussion

In this study the thromboxane synthetase inhibitor dazmegrel has been shown to afford 'cytoprotection' in an animal model of gastric mucosal damage. The second series of experiments was designed to measure the effect of dazmegrel on prostanoid synthesis at precisely the moment when acidified taurocholate would have been applied in the first series. These experiments show a dose dependent

inhibition of thromboxane synthesis (measured as TxB_2) in gastric mucosa.

These combined observations thus make it very likely that inhibition of thromboxane synthesis was the mechanism by which mucosal protection occurred. Under some circumstances dazmegrel may cause enhanced prostaglandin synthesis.^{7,9,12,13} This appears to be a relatively limited effect but could be important for mucosal protection. In our experiments, significant enhancement of PGE_2 was not seen and, particularly when the middle dose was used, and this could not have accounted for the protection observed. We cannot say whether there was enhanced synthesis of any other prostanoid which we did not measure.

Gastric mucosal protection with two other TXSIs

and a thromboxane receptor antagonist has now been reported in studies with rats.^{4,6,14} In addition we have investigated the biochemistry of the 'cytoprotective' drug WHR2348A and shown it to be a powerful and specific inhibitor of thromboxane synthesis in the gastric mucosa.¹⁵ Taken as a whole, this evidence emphasises the importance of thromboxane synthesis in the gastric mucosa, and the therapeutic potential of TXSIs. The possibility that similar mechanisms are involved in the apparent 'cytoprotective' properties of such compounds as bismuth¹⁶ sucralfate^{17,18} or cimetidine¹⁹ would be worth investigating.

The precise clinical significance of our observations remains to be determined, as the contribution of 'cytoprotection' to ulcer healing by prostaglandins is currently undecided.¹ Inhibition of thromboxane synthesis, however, represents a novel approach to the development of possible ulcer healing agents which warrants further investigation. Likewise the enhancement of mucosal integrity achieved by TXSIs could be of value for maintenance treatment after healing of peptic ulcers. The particular interest of dazmegrel is that it has been given to man for prolonged periods without adverse effects. Our observations therefore justify further assessment of dazmegrel's mucosal protective properties in man using established techniques to measure microscopic bleeding²⁰ or cellular exfoliation.²¹

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