Gut, 1976, **17**, 33-36

Effect of prostaglandin 15(R)15 methyl-E_2 methyl ester on aspirin and taurocholic acid-induced gastric mucosal haemorrhage in rats

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SUMMARY The effect of orally administered prostaglandin 15(R)15 methyl-E_2 methyl ester on aspirin and taurocholic acid-induced gastric mucosal haemorrhage has been studied in rats. The incidence of haemorrhage induced by aspirin (26·7 mM), 64 mg/kg, together with taurocholic acid (2·5 mM), was significantly reduced from 53·6% to 19·5% by the addition of the prostaglandin (9·9 μM), $P < 0.01$. The incidence of haemorrhage induced by aspirin alone (53·3 mM), 128 mg/kg, was significantly reduced from 80% to 20% by the addition of prostaglandin (9·9 μM), $P < 0.002$. These results indicate the possible use of synthetic prostaglandins in the prevention of aspirin-induced gastric pathology.

It has long been known that aspirin ingestion in man produces gastric irritation which, on occasion, may progress to severe gastric erosions with haemorrhage. Semple and Russell (1975) have shown that the incidence of gastric mucosal bleeding induced by aspirin in rats is significantly increased by the addition of conjugated bile acids, especially taurocholic acid.

Aspirin, in addition to other anti-inflammatory agents, has been shown to inhibit the synthesis of prostaglandins *in vitro* (Ferreira *et al.*, 1971; Smith and Willis, 1971; Vane, 1971). Naturally occurring prostaglandins, especially E_1, E_2, and A_1, when given systemically, markedly inhibit gastric acid secretion in animals (Robert *et al.*, 1967; Wilson and Levine, 1969) and in man (Classen *et al.*, 1970; Wilson *et al.*, 1971). Prostaglandin E_1 has also been shown to inhibit the production of gastric ulceration in rats induced by stress after pyloric ligation (Robert *et al.*, 1968). However, naturally occurring prostaglandins are rapidly degraded in the stomach when given orally and are largely ineffective by this route. In recent years, resistant synthetic analogues have been produced and can therefore be given orally with effect. Various workers have shown that certain of these analogues have an even greater inhibitory effect on gastric secretion than the naturally occurring prostaglandins; this has been demonstrated in animals (Robert and Magerlein, 1973) and in man (Carter *et al.*, 1973; Lippmann, 1974b). A more marked inhibitory effect has also been shown on gastric ulcer formation in the rat induced by indomethacin (Lippmann, 1974a) and by stress due to pyloric ligation (Carter *et al.*, 1974).

We have studied the effects of the synthetic analogue prostaglandin 15(R)15 methyl-E_2 methyl ester on aspirin and taurocholic acid-induced gastric mucosal bleeding in rats.

Methods

SOLUTIONS

Finely ground aspirin (Evans' Medical Company, Liverpool, England) was made up in a suspension of 1% carboxymethylcellulose (Evans' Medical Company). Solutions of taurocholic acid (Sigma Chemicals Ltd., London) were prepared in glucose saline (56 mM glucose and 33 mM sodium chloride). The test solutions consisted of equal parts of carboxymethylcellulose with or without aspirin, and glucose-saline with or without taurocholic acid. The combined solutions contained aspirin (26·7 mM) and taurocholic acid (2·5 mM). Two aspirin solutions (26·7 mM and 53·3 mM) and four taurocholic acid solutions (2·5 mM, 5 mM, 10 mM and 20 mM) were used in the study. The control solution consisted of

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Received for publication 20 October 1975

glucose saline and carboxymethylcellulose as above without aspirin or taurocholic acid.

The prostaglandin used in the study was the synthetic analogue prostaglandin 15(R)15 methyl-E_2 methyl ester (Upjohn Company). This was made up with absolute alcohol to a concentration of 10 mg prostaglandin in 1 ml alcohol and stored at a temperature of −20°C. The prostaglandin was added to the solutions immediately before use as described below.

ANIMALS

Male Sprague-Dawley rats were used in the study. They were housed in wire-bottomed cages in an attempt to prevent coprophagia and kept in a windowless room with a 12 hour light cycle. They were fasted for 24 hours before the start of the study, although they were allowed free access to tap water.

DESIGN OF STUDY

Between 30 and 40 animals were studied at any one time. Between 11 am and noon on the morning of the study the appropriate volume of solution was introduced into each animal's stomach by means of a non-flexible metal oral needle. The volume administered was proportional to the weight of the animal, a 150 g animal receiving 2 ml of solution. The doses of aspirin administered thus were 64 mg/kg or 128 mg/kg. Gastric lavage was not performed before each study. Of the test solutions studied on each particular day, equal numbers of animals received that test solution with or without prostaglandin (9·9 μM), 50 μg/kg. A suitable number of control animals were also intubated each day, and given the control solution. Rats were killed with ether four hours after the administration of the solutions and the stomachs were removed, opened along the greater curvature, and examined for the presence of gastric haemorrhagic mucosal lesions. Any haemorrhagic area larger than 1 mm in its greatest dimension was considered a positive result, no account being taken of the number or extent of erosions or the degree of gastric haemorrhage. All stomachs were examined by at least two observers who were unaware which test solution was given to each animal.

The results of the study were analysed statistically by the Chi-square test, using Yates's correction.

Results

The administration of taurocholic acid (2·5 mM) in addition to aspirin (64 mg/kg), significantly increased the incidence of bleeding from 17·8% to 53·6% as shown in Fig. 1 ($P < 0·01$).

The effect of prostaglandin on bleeding induced by aspirin (64 mg/kg) together with taurocholic acid (2·5 mM) is shown in Fig. 2. There were 41 animals in each group and the incidence of bleeding was significantly reduced from 53·7% to 19·5% by the addition of prostaglandin ($P < 0·01$).

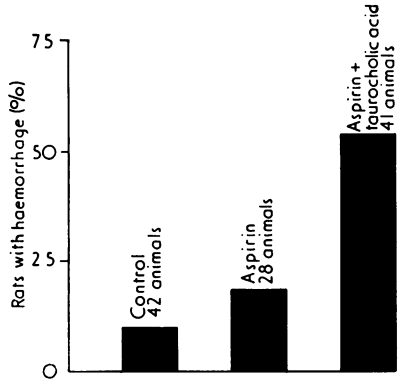

Fig. 1 *Incidence of haemorrhage in rats induced by aspirin (64 mg/kg), taurocholic acid (2·5 mM), and aspirin (64 mg/kg) and control solutions.*

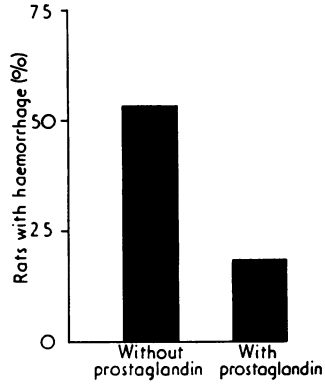

Fig. 2 *Effect of prostaglandin on haemorrhage induced by aspirin (64 mg/kg) and taurocholic acid (2·5 mM) (41 animals used in each group).*

The incidence of bleeding induced by aspirin at a dose of 64 mg/kg was considered to be too low to attempt to demonstrate any significant reduction by the addition of prostaglandin. The effect of prostaglandin on an increased dose of aspirin (128 mg/kg) was thus investigated and the results are shown in Fig. 3. There were 25 animals in each group and the incidence of bleeding was significantly reduced from 80·0% to 20·0% by the addition of prostaglandin ($P < 0·002$).

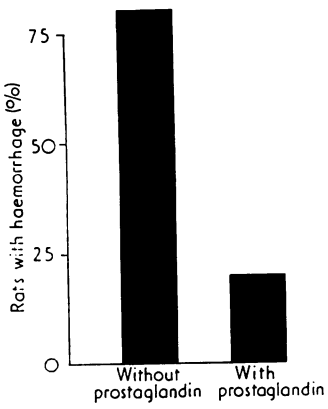

Fig. 3 *Effect of prostaglandin on haemorrhage induced by aspirin* (128*mg/kg*) (25 *animals in each group*).

The effect of taurocholic acid alone in the production of gastric bleeding was also investigated at concentrations of 2·5 mM, 5 mM, 10 mM and 20 mM results also being shown in the Table. Although the numbers in some of the groups were small, the incidence of haemorrhage, even with high concentrations, was not large enough to attempt to demonstrate any significant reduction with the addition of prostaglandin.

Solutions	*Without prostaglandin*		*With prostaglandin*	
Taurocholic acid (mM)	*No. of rats studied*	*% Bleeding*	*No. of rats studied*	*% Bleeding*
2·5	4	25	—	—
5	8	12·5	—	—
10	22	13·6	13	7·7
20	9	22·2	9	11·1
Controls	42	9·5	—	—

Table *Incidence of gastric mucosal haemorrhage induced by varying concentrations of taurocholic acid*

Discussion

The study shows that oral administration of the synthetic analogue prostaglandin 15(R)15 methyl-E_2 methyl ester significantly inhibits gastric mucosal bleeding in rats induced by aspirin alone and when taurocholic acid is administered with aspirin.

The damaging effect of aspirin on the gastric mucosa has been previously shown to involve several factors, including inhibition of the production of gastric mucus (Menguy and Masters, 1965), increasing exfoliation of cells from the gastric mucosa (Croft and Wood, 1967), and decreased platelet stickiness (Morris, 1967). Significant bleeding may also be due to subclinical ascorbic acid deficiency (Russell and Goldberg, 1968; Russell *et al.*, 1968). Davenport (1967) showed that aspirin caused back-diffusion of hydrogen ions and this has been considered to be an important precipitating factor in aspirin-induced gastric mucosal damage.

The concept of damage to the gastric mucosa by bile acids has been suggested by Grant *et al.* (1951) and reflux of bile into the stomach has been incriminated in the aetiology of gastric ulcer (Du Plessis, 1965; Rhodes, 1972). Taurocholic acid in the presence of H^+ and haemorrhagic shock produces extensive gastric mucosal injury (Safaie-Shirazi *et al.*, 1972). Bile acids in the stomach also appear to increase the likelihood of gastric mucosal haemorrhage after the ingestion of aspirin (Semple and Russell, 1975). Bile acids have also been shown to cause back-diffusion of hydrogen ions across the mucosal barrier (Davenport, 1968; Ivey *et al.*, 1971; Cochran *et al.*, 1975).

The finding that aspirin-like drugs have a marked inhibitory effect on prostaglandin synthesis (Ferreira *et al.*, 1971; Smith and Willis, 1971; Vane, 1971) led to the proposal that the action of these drugs might, at least in part, be due to this. Indeed, most, if not all, of the actions of aspirin and related drugs can be explained by the inhibitory effect on prostaglandin synthesis (Collier, 1971; Vane, 1971).

Gastric ulceration induced by pyloric ligation in the rat has been shown to be inhibited by the subcutaneous infusion of prostaglandin E_1 (Robert *et al.*, 1968), prostaglandin E_2 and prostaglandin F_2 (Usardi *et al.*, 1974). Gastric ulceration in the rat induced by indomethacin (Lippmann, 1974a) and by stress due to pyloric ligation (Carter *et al.*, 1974; Lippmann, 1974c) was inhibited by oral administration of synthetic prostaglandin analogues. Prostaglandins have also been shown to inhibit gastric erosions induced by whole bile in the rat (Mann, 1975).

The protective role of prostaglandins with regard to the integrity of the gastric mucosa might, at least in part, be due to a fairly marked inhibitory effect on basal and stimulated gastric secretion, thus allowing less hydrogen ions to be available for back-diffusion across the gastric mucosal barrier, which is damaged by aspirin-like drugs and bile acids. It also might be proposed that prostaglandins may help to re-establish the gastric mucosal barrier. However, recent work has shown that the synthetic prostaglandin 16,16 dimethyl-E_2 methyl ester also casues back diffusion of hydrogen ions when given orally but not when given intravenously in Heidenhain pouch preparations in dogs (O'Brien and Carter, 1975) and this may, in part at least, account for the antisecretory effect. Although controversy still exists, the action of prostaglandins on gastric

secretion appears to involve the cyclic AMP system (Wilson, 1974). The role of prostaglandins in protecting the gastric mucosal cells might be that of helping to mop up any H^+ that leaks back through the cell membrane in such a way as to prevent it damaging the parent cell. This action may be dependent on the cyclic AMP system.

Further work is still required to evaluate the relationship between aspirin-like drugs and prostaglandins, in particular synthetic analogues, and to establish if prostaglandins or their analogues have a place in the prevention and treatment of aspirin-induced gastric pathology in man.

The authors would like to thank Professor E. M. McGirr for the use of animal house facilities, and Upjohn Company, Kalamazoo, Michigan, for kindly donating the prostaglandin analogue.

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