

Comparison of prostaglandin E₂ and ranitidine in prevention of gastric bleeding by aspirin in man

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SUMMARY This study was designed to compare the effect of oral administration of PGE₂ (0.5 mg/kg) and ranitidine in larger (100 mg/dose) or smaller (10 mg/dose) doses on aspirin-induced gastric microbleeding and DNA loss determined chemically in gastric washings in eight healthy subjects. Aspirin (0.5 g) given four times daily greatly increased the rate of gastric bleeding and DNA loss and pretreatment with PGE₂ or ranitidine in larger doses almost completely prevented these changes. Smaller non-antisecretory doses of ranitidine also reduced the rate of bleeding and DNA loss but to a lesser degree than PGE₂. This study confirms that oral PGE₂ has a protective action on gastric mucosa exposed to aspirin and that this property is also shared by ranitidine, a potent histamine H₂-receptor antagonist.

Aspirin and other non-steroidal anti-inflammatory agents have been shown to cause varying degrees of gastric mucosal damage and bleeding in experimental animals^{1,2} and in humans.³⁻⁷ The mucosal damage caused by aspirin depends upon the presence of luminal acid, which facilitates the entry of this agent into the mucosal cells by non-ionic diffusion resulting in osmotic, buffering, and metabolic dysfunctions.⁸

The mechanism of aspirin-induced gastric mucosal damage is not fully explained but it has been related to the deficiency of mucosal prostaglandins because this agent is known to suppress the biosynthesis of prostaglandins⁹ and pretreatment with exogenous prostaglandins prevents the formation of gastric lesions and bleeding by aspirin.^{1,2,10-12} This property of prostaglandins of protecting the mucosa ('cytoprotection') against damage by aspirin and other noxious agents appears to be separate and unrelated to inhibition of gastric acid secretion, as it can be demonstrated at doses of prostaglandins many times smaller than those affecting gastric acid secretion.¹³

This study was undertaken to compare the cytoprotective effects of PGE₂ with those of ranitidine, a new potent histamine H₂-receptor antagonist, on aspirin-induced gastric bleeding and DNA loss in healthy subjects.

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Methods

SUBJECTS

This study was performed on eight male volunteers who were in good physical health and did not complain of any gastrointestinal problems. Their average age was 23 years (range 19-27 years) and weight 68 kg (range 55-74 kg). The study was approved by the Human Research Committee and informed consent was obtained from each subject.

Each subject was examined several times with five day intervals between examinations. The following experimental conditions were applied at random: (1) placebo tablets four times daily; (2) aspirin, 0.5 g, four times daily; (3) PGE₂, 0.5 mg, followed 15 minutes later by 0.5 g aspirin, four times daily; (4) ranitidine, 10 mg, followed 15 minutes later by 0.5 g aspirin, four times daily, and (5) ranitidine, 100 mg, followed 15 minutes later by 0.5 g aspirin, four times daily. Each treatment was given for two days; on the first day four tablets were given at three to four hour intervals and on the second day only one tablet early in the morning (about 5 am). Five hours later, blood and DNA lost into the stomach was measured by washing out the gastric contents and chemically measuring the recovered blood and DNA.

PROCEDURE

Examination was carried out after an overnight fast. Each subject intubated his stomach with a rubber nasogastric tube (16 French gauge). To measure the

rate of gastric bleeding, a solution of glucose (100 g/l) and trisodium citrate (9.8 g/l) with phenol red (40 mg/100 ml) as a non-absorbable marker was put into the stomach and recovered after fixed intervals in accordance with the method of Fisher and Hunt.¹⁴ For an initial washout, 100 ml of the test solution was instilled into the stomach and the subjects syphoned out their gastric contents, which were rejected. Then another 100 ml of test solution was instilled and nine minutes later syphoning of the gastric contents was started. When this was apparently complete, another 100 ml of solution was instilled and recovery started nine minutes later and so on. At the mid-point of the 10 minute interval between three successive washouts, 5 ml of a solution of phenol red was swallowed. The proportion of this marker was recovered five minutes later by washing out with a test solution of glucose (to slow gastric emptying) and trisodium citrate (used as a buffer). These were used to correct for the volume lost through the pylorus during the study period.^{15 16}

The volume of each recovery sample was determined and then after 10 minutes of homogenisation on a vortex stirrer the haemoglobin and DNA were measured. Haemoglobin was estimated by the method of Fisher and Hunt¹⁴ based on the peroxidase action of haemoglobin in the presence of hydrogen peroxide. Phenol red was measured spectrophotometrically.¹⁵ In addition, the content of DNA in each washout sample was determined by the modified diphenylamine method of Croft and Lubran.¹⁷ Results of gastric bleeding rate and DNA loss over a 30 minute period were expressed as ml/30 min and mg/30 min, respectively. Serum salicylate concentrations were measured by the method of Saltzman.¹⁸

In separate tests, gastric acid secretion was measured after placebo, ASA, PGE₂, ranitidine, or

their combination. For this purpose, a nasogastric tube was introduced and a basal 15 minute collection of gastric juice was performed and then placebo, aspirin (0.5 g), PGE₂ (0.5 mg), ranitidine (10 or 100 mg), PGE₂ + aspirin or ranitidine + aspirin was ingested, and gastric aspiration stopped for 45 minutes. After the period of interruption, gastric aspiration was restarted, the first five minute collection was rejected and the aspiration was then continued for the next five consecutive hours. Secretory outputs in the final hour of gastric collection were used to determine the effects of PGE₂ and ranitidine on basal gastric secretion. Each secretory test was performed on a different experimental day. Gastric acid contents were measured as previously described.¹⁹

Results are expressed as the means \pm SEM. Student's *t* test was used to determine the significance of the difference between means, with a difference of $p < 0.05$ being considered significant.

Results

The effects of PGE₂ and ranitidine alone or in combination with aspirin on basal acid secretion are shown in the Table. PGE₂ in a dose of 0.5 mg given orally did not affect acid secretion. Ranitidine in a smaller dose (10 mg) also did not significantly influence gastric secretion, whereas a larger dose (100 mg) produced significant inhibition of acid outputs.

Figure 1 shows the rates of gastric bleeding for placebo, aspirin alone, and aspirin in combination with PGE₂ or ranitidine. Each value is an estimate of the volume of blood lost in a 30 minute period based on the sum of recoveries during three consecutive 10 minute periods. In tests with placebo, the mean recovery of phenol red varied between 62% and 78% of the liquid consumed five

Table Basal acid outputs (mmol/h) in fifth hour after oral administration of placebo, aspirin, PGE₂, ranitidine and combination of aspirin + PGE₂ or ranitidine

Initials	Placebo	Aspirin (0.5 g)	PGE ₂ (0.5 g)	Ranitidine		Aspirin + PGE ₂	Aspirin + ranitidine
				(10 mg)	(100 mg)		
LA	1.17	1.27	1.12	1.35	0.15	1.37	1.62
OW	2.38	2.95	1.78	2.12	0.36	3.54	1.92
MW	1.62	2.25	2.10	1.47	0.04	1.85	1.63
FT	3.10	3.30	3.54	2.83	0.47	3.52	2.95
ST	1.45	1.85	2.35	1.62	0.38	1.42	1.63
WK	1.25	1.56	2.10	1.05	0.24	1.12	1.48
KW	2.76	3.15	2.47	2.47	0.12	2.95	3.56
ZB	2.33	1.32	1.85	1.93	0.08	1.60	1.49
X	2.01	2.21	2.16	1.85	0.23	2.17	2.03
SEM	0.27	0.31	0.26	0.22	0.60	0.37	0.29

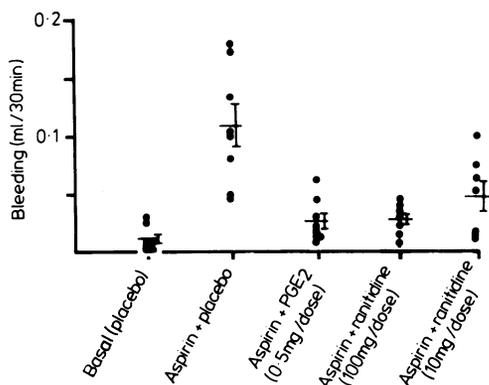


Fig. 1 Mean bleeding rate after placebo and aspirin alone or in combination with PGE₂ or ranitidine in a smaller or larger dose. Mean \pm SEM of eight tests; eight subjects.

minutes previously. Similar recoveries of volumes instilled were observed in tests with aspirin alone or aspirin combined with PGE₂ or ranitidine. The mean placebo bleeding rate was about 0.014 ± 0.004 ml/30 min and this increased several times after ingestion of aspirin. Pretreatment with PGE₂ or ranitidine in a larger dose caused a reduction in the blood loss to a level not significantly different from placebo value. Ranitidine in a smaller, non-antisecretory dose (10 mg) also reduced significantly the aspirin-induced bleeding rate but to a smaller degree than a larger, antisecretory dose (100 mg).

Figure 2 presents the results of mean DNA loss under basal conditions and after aspirin alone or combined with PGE₂ as in Fig. 1. DNA loss in the three successive washing periods had a tendency to decline and the data are expressed as 30 minute outputs, being the sum of DNA outputs in three 10 minute periods. Aspirin significantly increased DNA loss and this was reduced by PGE₂ or ranitidine in a larger dose to a level not significantly different from basal value. Ranitidine in a smaller dose was less effective.

The serum salicylate level that was obtained the morning of gastric washing was about 142 ± 37 μ g/ml in tests with aspirin alone, and it was not significantly different from that after pretreatment with PGE₂ or ranitidine.

Discussion

The rate of gastric bleeding can be assessed either indirectly by labelling the blood with radioactive chromium and measuring the radioactivity of stools as an index of blood loss, or directly by determining the haemoglobin contents in gastric washouts as

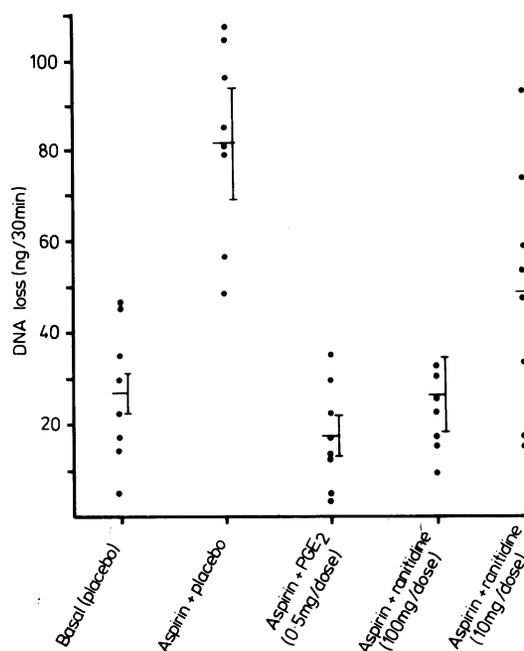


Fig. 2 Mean DNA loss after placebo and aspirin alone or in combination with PGE₂ or ranitidine in a smaller or larger dose. Mean \pm SEM of eight tests; eight subjects.

recently proposed by Fisher and Hunt.¹⁴ Using the radiochromium method in healthy subjects it was found that oral aspirin markedly increased faecal blood loss, and that this could be prevented dose-dependently by concurrent treatment with oral PGE₂.^{10, 20} Similarly, PGE₂ and its methylated analogue were reported to prevent occult bleeding during indomethacin treatment of patients with rheumatic diseases.²¹ In studies with the radiochromium method it is, however, difficult to ascribe the blood recovered in the stool to any particular site of the gastrointestinal tract or to any particular day of treatment. The alternative method for estimating bleeding—that is, measurement of the blood recovered from the stomach by washing it out at three successive 10 minute intervals¹⁴ allows the determination of blood loss directly into the stomach: this is probably the major source of gastrointestinal bleeding after administration of aspirin. In addition, it is a simple, reliable, and sensitive method which does not involve the radio-labelled material. Using this technique, Hunt and Franz¹¹ recently reported that oral PGE₂ significantly reduced gastric bleeding caused by aspirin and our results agree with that report. As PGE₂ was previously shown and confirmed in this study to be

without any influence on gastric acid or mucus secretion, when given orally to humans even at higher doses,²² all these results with the exception of aspirin-induced bleeding by oral PGE₂ represent clinical evidence of gastric cytoprotection.¹³

The major question remains as to whether gastric cytoprotection is a unique feature of prostaglandins, as suggested by Robert,¹³ or whether other compounds used in the treatment of ulcer disease share this property. Recent studies on animals show that conventional antisecretory agents such as cimetidine¹ or ranitidine¹² seem to exhibit gastric cytoprotection but this is a controversial problem^{13 23} and no attempts have so far been made to determine the possible cytoprotection of H₂-receptor antagonists.

The dose of aspirin used in the present report was found in our previous study⁷ to cause marked endoscopic mucosal lesions and haemorrhages accompanied by a dramatic reduction in the ability of gastric mucosa to biosynthesise PGE₂. In addition, aspirin and other anti-inflammatory agents were found to enhance the exfoliation of gastric epithelial mucus cells and to increase gastric DNA loss, which can be taken as a measure of the degree of shedding of these cells into the gastric lumen.^{24 25} Our present finding that exogenous PGE₂ can prevent gastric bleeding and DNA loss caused by aspirin could be simply attributable to the replacement therapy with prostaglandins which appears to be necessary for the gastrointestinal epithelial cells to maintain their integrity. Obviously, this explanation cannot be applied to the cytoprotective effect of ranitidine. As shown in this study, excessive gastric bleeding and DNA loss produced by aspirin can be greatly reduced by ranitidine not only in a larger inhibitory dose but also in a smaller dose, which does not affect acid secretion. As H₂-receptor antagonists do not influence the mucosal generation of prostaglandins¹² it is unlikely that endogenous prostaglandins could contribute to this effect. It is possible that H₂-blockers share the cytoprotective property without involving mucosal generation of prostaglandins, as has already been demonstrated in animals.^{1 12} The hypothesis that H₂-blockers may have a cytoprotective effect is too novel to be accepted without seeking for some other explanation. It is possible that the repeated application of small doses of ranitidine, which was shown to have a protracted antisecretory action,²⁰ could cause sufficient reduction in gastric acid secretion to decrease the damaging effect of oral aspirin. Whether or not the protective property of a small dose of ranitidine is due to its undetectable antisecretory effect or represents true cytoprotection, our finding indicates that ranitidine may

have therapeutic potential in the prevention of aspirin-induced gastric mucosal injury and bleeding. This agrees with a recent report that cimetidine is effective in reducing dyspepsia and peptic ulcerations in arthritic patients taking anti-inflammatory drugs.²⁶ Further studies are needed to demonstrate whether H₂-receptor blockers used at low dosage could have a prophylactic role in patients undergoing long-term treatment with aspirin and other anti-inflammatory agents.

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