J. Pharm. Pharmacol. 1980, 32: 544–546 Received January 30, 1980

Decrease in aspirin-induced gastric mucosal damage in rats by oral administration of the cytotoxic drugs melphalan and methotrexate

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Gastric mucosal damage by aspirin and chemotherapeutic drugs was studied in Wistar rats. Aspirin 60 mg given by stomach tube caused substantial gastric mucosal damage as judged by visual examination of the stomachs removed four hours later. Melphalan and methotrexate given daily for four days had no significant macroscopic effect on the gastric mucosa, but reduced the damage caused by aspirin. This protective effect may involve a stimulation of prostaglandin synthesis by the stomach, increased mucus secretion, and/or inhibition of acid secretion.

Aspirin is a valuable drug commonly consumed for its analgesic, anti-inflammatory and antipyretic properties, and is therefore likely to be taken by patients with neoplastic disease. Furthermore, there is experimental evidence suggesting that aspirin or similar drugs may be of benefit in the management of cancer, either alone or in combination with cytotoxic drugs (see Bennett 1979; Bennett et al 1979). Since aspirin and other anti-inflammatory drugs can damage the gastric mucosa, and cytotoxic agents damage rapidly dividing tissues which include the gastric mucosa, the safety of these drug combinations should be examined. The results described below show that instead of greater gastric mucosal damage when these drugs were combined, the cytotoxic agents reduced the damage caused by aspirin.

METHODS

Wistar rats of either sex (from OLAC, Bicester, Oxford) weighing approximately 200 g and fed on 41B Diet (Oxoid, Hampshire) were given melphalan and methotrexate (low doses 0·7 mg kg^{-1} and 1 mg kg^{-1} respectively; high doses 2·8 mg kg^{-1} and 4 mg kg^{-1} respectively) in 2 ml raspberry syrup, the vehicle used in our previous experiments (Bennett et al 1979). Controls received 2 ml raspberry syrup alone. All drug administrations were made daily using a soft rubber stomach tube. On the fourth day, after allowing only water for 12 h, 60 mg aspirin suspended in the syrup was given orally to half of each group in addition to their other drugs.

Four hours later the rats were killed by inhalation of chloroform. The stomachs were removed, and in some experiments the volumes were determined to the nearest 0·5 ml by displacement of 0·15 M NaCl. After cutting open along the greater curvature, the gastric mucosa was inspected briefly and then rinsed gently in 0·15 M NaCl before a more careful examination. Mucosal damage was assessed visually without knowledge of the treatment given, using the points system described by Rees et al (1979).

Stomachs from other rats treated with the chemotherapeutic drugs alone were not examined as above but were extracted for prostaglandins. Each rat was stunned and bled, and the excised stomach was dissected and separated into corpus and antrum, and fundus. The tissue was rinsed in Krebs solution, cut finely with scissors, and rinsed again in Krebs solution. One half was homogenized in acid ethanol to indicate 'basal' amounts of prostaglandin, and the other half was homogenized in Krebs solution to allow formation of prostaglandins from endogenous precursors released by disruption of the tissue (Bennett et al 1973). Prostaglandin-like substances were then extracted by the method of Unger et al (1971) and bioassayed against PGE_2 on the rat fundus strip (Gilmore et al 1968, as modified by Bennett et al 1973). Since the homogenate in Krebs solution contains 'basal' plus newly synthesized material we refer to this extract as 'total' prostaglandin. The results are expressed as median values with semiquartile ranges in parentheses, and analysed statistically using the Mann–Whitney U-test.

RESULTS

The results summarized in Table 1 are from five separate experiments. Since male and female rats

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Table 1. Gastric mucosal damage scores.

Groups	n	Scores
Syrup controls	20	0 (0–0)
Chemotherapy alone, low dose	35	0 (0–0)
Chemotherapy alone, high dose	18	0 (0–0)
Aspirin alone	40	6·5 (3–10)
Aspirin + low dose chemotherapy	14	4·5 (2–6)
Aspirin + high dose chemotherapy	30	0 (0–1)

Chemotherapy (melphalan + methotrexate 0·7 + 1 mg kg^{-1} or 2·4 + 4 mg kg^{-1} daily) lessened aspirin-induced gastric mucosal damage in rats ($P = 0.052$ and $P < 0.0001$ respectively).

gave similar results with aspirin alone (median damage scores both 6·5, with semiquartile ranges of 4–8 and 3–12 respectively), the results for both sexes were combined. Chemotherapy alone produced few or no gastric lesions (Table 1) but surprisingly protected against the effect of aspirin. The high doses of melphalan and methotrexate markedly lessened the aspirin-induced damage, and a similar trend was suggestive with the lower doses.

Gross gastric dilatation was observed in all the rats receiving chemotherapy (only high-dose group measured), and some dilatation occurred with aspirin alone. The gastric volumes, as measured by fluid displacement, were: controls 2 (2–2) ml; aspirin 5 (4–5) ml; chemotherapy 9·5 (8–11) ml. These differences were statistically significant ($P < 0.001$), but the tendency for even greater dilatation with chemotherapy + aspirin (11·5 (8–13) ml) was not significantly greater than with chemotherapy alone ($P > 0.2$). In addition to dilatation, visual inspection indicated that mucus secretion was increased in rats given chemotherapy, but this was not measured. Increased 'total' and 'synthesized' amounts of prostaglandin-like material were extracted from fundic tissue of rats treated with cytotoxic drugs, and a similar tendency occurred with corpus and antrum (Table 2).

DISCUSSION

The concurrent use of aspirin and related compounds with cytotoxic drugs in patients with malignant disease may be desirable to treat painful or inflammatory conditions unrelated to cancer. In addition, aspirin is given to reduce the side effects of cancer therapy (such as diarrhoea following irradiation for uterine cancer, Mennie et al 1975; or mucositis from irradiation of tumours in the head or neck), or to enhance the antitumour effect of cytotoxic drugs (Powles et al 1978). Studies in animals may lead to other uses of drugs which inhibit prostaglandin synthesis. In mice, administration of the anti-inflammatory drug flurbiprofen together with radiotherapy ± chemotherapy increased the anti-tumour effect (Bennett et al 1979), and following tumour resection flurbiprofen increased the survival of mice given chemotherapy (Berstock et al 1979). The effect of anti-inflammatory drugs and chemotherapeutic agents on the gastric mucosa is therefore of particular interest, and the protection against aspirin-induced gastric mucosal damage is both surprising and somewhat reassuring. It remains to be seen whether or not gastric mucosal protection occurs in man, but our findings suggest that such a study is ethical if both types of drugs are thought to be therapeutically desirable.

Gastric mucosal protection may involve various factors, including dilution of the aspirin, mucus secretion, and other effects which may be due to stimulation of prostaglandin synthesis. The increased volume of gastric contents following treatment with chemotherapeutic drugs probably reduced the intragastric concentration of aspirin. However, there was presumably a reduction in gastric emptying which increased the contact time of aspirin and its hydrolysis products with the gastric mucosa. A protective factor may be the increased secretion of mucus, which was apparent on visual inspection. Increased synthesis of prostaglandin-

Table 2. Prostaglandin-like material extracted from rat stomach.

	Fundus			Corpus + antrum		
Treatment groups	Basal	Synthesized	Total	Basal	Synthesized	Total
Controls: median	18·5	116	140	215	625	850
semiquartiles	(65–56)	(38–135)	(61–180)	(190–240)	(620–1350)	(800–1550)
Chemotherapy: median	25	265	270	140	1550	1715
semiquartiles	(1–32)	(160–300)	(190–300)	(108–195)	(1400–1800)	(1600–1900)
P	0·928	0·0062	0·016	0·055	0·055	0·078

Following treatment for 4 days with melphalan 2·8 mg kg^{-1} and methotrexate 4·0 mg kg^{-1} daily, the amounts of extracted prostaglandin-like material (expressed as ng PGE_2 equivalents/g wet tissue) tended to be higher than in controls.

like material occurred in fundic tissue, and a similar trend occurred in the corpus + antrum. This prostaglandin-like material may have stimulated mucus secretion (Fung et al 1974). However, we do not know how much prostaglandin was formed by the mucosa and muscle respectively, since we homogenized the whole stomach wall. This could have partly masked a greater effect in the mucosa, particularly in the antrum where the muscle forms a large proportion of the wall. The relative amounts of prostaglandins formed by rat gastric mucosa and muscle are not known, although in human stomach most of the prostaglandin-like material measured by bioassay is formed by the mucosa and submucosa (Bennett et al 1968, 1973). Our techniques do not permit measurement of prostacyclin, which would not survive our extraction procedure, or its degradation product 6-keto-$PGF_{1\alpha}$ which only weakly stimulates the rat stomach assay tissue (Omini et al 1977). However, prostacyclin may be the most appropriate prostanoid to measure since it is thought to be the major product formed by rat fundus (Moncada et al 1978).

Prostaglandins are synthesized by other tissues in response to chemotherapeutic drugs (Levine 1977). In the gastric mucosa these prostaglandins may be 'cytoprotective' (Robert 1977), and may be important for the inhibition of aspirin-induced damage. Exogenous prostaglandins or their analogues protect rat stomach from the effect of aspirin (Carmichael et al 1976). Increased formation of prostaglandins may reduce gastric acid secretion (Robert 1977) and this could alter the ionization and local absorption of aspirin. The reduction by cytotoxic drugs of aspirin-induced gastric mucosal damage may therefore involve several factors, particularly those affected by prostaglandins.

Acknowledgements

We thank the CRC and MRC for support.

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