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Inhibitors of gastric lesions in the rat

LINDA J. HAYDEN, G. THOMAS AND G. B. WEST*

Department of Paramedical Sciences, North East London Polytechnic, Romford Road, London, E15 4LZ, U.K.

The production by stress of gastric lesions in rats was inhibited by metiamide and by mepyramine. Lesions induced by indomethacin treatment were inhibited by mepyramine but not by metiamide. Those induced by aspirin treatment in pylorus-ligated rats were not affected by either antihistamine drug. Oral glutamine inhibited lesion production in all three systems whereas aspirin orally markedly potentiated it. Sodium salicylate inhibited both indomethacin-induced lesions and those produced by aspirin in pylorus-ligated rats. On the other hand, copper salicylate inhibited stress-induced lesions and it, like copper aspirinate, also markedly reduced the extent of lesions produced by aspirin. On the basis of these results, stress-induced lesion production offers a suitable animal model for testing anti-ulcer drugs as it is, like most human gastric ulcers, inhibited by H_2-receptor inhibitors like metiamide.

Oral aspirin produces gastric haemorrhagic lesions in rats which are exaggerated by previous ligation of the pyloric sphincter (Okabe, Takeuchi & others, 1974a). Pretreatment of rats with oral glutamine greatly reduces the production of these lesions (Takagi & Okabe, 1968; Okabe, Takeuchi & others, 1974b) without reducing the anti-inflammatory activity of aspirin (Tanaka, Kiyohara & others, 1974). Others (e.g. Djahanguira, Abtani & Hemmati, 1973) have reported that pyloric ligation inhibits aspirin-induced lesions but in our hands it always exaggerated it.

Ezer, Palosi & others (1976) recently reported that sodium salicylate antagonized the lesion production not only by aspirin but also by several other non-steroidal anti-inflammatory drugs. Sodium salicylate may exert this action by blocking the inhibitory effect of aspirin on prostaglandin synthetase as sodium salicylate has little effect on this enzyme.

Copper complexes of aspirin and many clinically used anti-arthritic drugs also exhibit anti-lesion activity (Sorenson, 1976) and some of these were reported to have greater anti-inflammatory activity than the corresponding sodium salts. Nevertheless, Rainsford & Whitehouse (1976) were unable to confirm this latter finding and Boyle, Freeman & others (1976) also failed to show increased activity of the copper complexes.

We have compared the effects of oral doses of glutamine, antihistamines, salicylates and copper complexes of salicylates on the production of gastric lesions in rats by aspirin after pyloric ligation, by indomethacin, and by exposure to stress. It was hoped to advance knowledge of the mode of action of anti-ulcer compounds as well as to determine which of the three methods of lesion production is a suitable animal model for testing new drugs.

* Correspondence.

METHODS

Groups of at least 8 Charles River Wistar rats (120–150 g) were used. Three methods were used for studying the production of gastric lesions.

(1) Gastric lesions induced by aspirin after pyloric ligation

After food had been withdrawn from rats for 24 h, pyloric ligation was performed under ether anaesthesia using the method of Shay, Komarov & others (1945). Immediately after recovery, the drug under test or its vehicle (0·5% w/v gum tragacanth, 10 ml kg^{-1}) was administered orally, followed 10 min later by oral aspirin (200 mg kg^{-1}). This procedure produced a consistent degree of gastric lesions 6 h later in the control experiments when the rats were killed. To determine the lesion index, the stomachs of the animals were removed and opened along the line of greatest curvature, then washed in warm water, and examined under a 3-fold magnifier. The lengths of the lesions were measured and summated to give a total lesion score (in mm) for each animal, the mean count for each group being calculated. In control rats, this was consistently about 20. Inhibition or potentiation of the production of the lesions by the drug was then expressed as a % value.

(2) *Gastric lesions induced by indomethacin*

After food had been withdrawn from rats for 16 h, the drug under test or its vehicle was administered orally 10 min before the subcutaneous injection of indomethacin (40 mg kg). The rats were killed 6 h

later and their stomachs removed and examined as before. The mean lesion count for control groups was consistently about 25 and the percentage inhibition or potentiation for each test group was calculated as before.

(3) Gastric lesions induced by stress

Female rats were used for this test as these were found to be more sensitive than males. Food was withdrawn for 16 h, after which the drug under test or its vehicle was given orally and the animals were placed vertically in individual restraint cages in water at 22° for 1 h. They were then removed and injected intravenously with azovan blue dye (30 mg kg^{-1}). Ten min later, they were killed and their stomachs removed. An injection of formol-saline (2% v/v) was then made into the totally-ligated stomachs for storage overnight. Then they were opened and examined as before, the dye assisting in the evaluation of the lesion score. The mean lesion count for control groups was consistently about 25.

Drugs used. Glutamine, mepyramine (May and Baker) and metiamide (Smith, Kline and French) were all suspended in gum tragacanth. Aspirin, sodium salicylate, copper salicylate and copper aspirin (gifts of Dr J. R. J. Sorenson) were suspended in either gum tragacanth, Tween 80, or distilled water. The three vehicles were used for the salicylates as the copper complexes have been stated to be stable only at pH values close to neutrality. In our hands, however, the choice of suspending agent did not change the activity of the compound (cf. Sorenson, 1977).

RESULTS

Inhibition by glutamine and antihistamines

Large oral doses of glutamine significantly reduced the degree of lesions produced by all 3 methods (Table 1). Mepyramine, a selective H_1-histamine receptor blocking agent, inhibited indomethacin-induced and stress-induced lesions but had no effect on aspirin lesions in pyloric-ligated rats. Lesions produced by indomethacin were particularly susceptible. On the other hand, metiamide, a selective H_2-histamine receptor blocking agent, was effective only against stress-induced lesions and then doses as low as 4 mg kg^{-1} orally were inhibitory (Table 1). It was surprising that metiamide did not modify indomethacin-induced lesions as others (e.g. Whittle, 1976) have reported on the effectiveness of metiamide, an anti-secretory drug.

Table 1. *Mean percentage inhibition (± s.e.m.) by glutamine and antihistamine drugs of gastric lesions produced in rats by three methods.*

Drug dose (mg kg^{-1})	Lesion production: Aspirin	Indomethacin	Stress
Glutamine			
250	28·6 ± 9·2	46·0 ± 4·2*	29·1 ± 7·4
500	80·8 ± 11·6*	58·1 ± 4·7*	74·1 ± 8·4*
Mepyramine			
10	10·7 ± 5·3	42·7 ± 2·8*	28·0 ± 6·7
25	18·1 ± 5·1	60·9 ± 5·8*	77·0 ± 5·0*
100	21·6 ± 7·8	68·3 ± 4·9*	79·8 ± 10·2*
Metiamide			
4	8·3 ± 1·7	6·5 ± 2·0	44·6 ± 2·9*
10	12·8 ± 2·0	12·0 ± 2·4	84·6 ± 5·2*
25	9·2 ± 2·5	9·7 ± 1·9	87·2 ± 8·8*
100	19·9 ± 5·9	10·5 ± 2·9	98·4 ± 7·7*

* = Significant inhibition at $P = <0·05$.

Effect of salicylates

Sodium salicylate orally significantly inhibited the production of lesions by aspirin and indomethacin, confirming some of the observations of Ezer & others (1976), but it increased lesion production by stress (Table 2). Copper salicylate orally at doses one-tenth those of sodium salicylate also inhibited aspirin-induced lesions but it potentiated the action of indomethacin and inhibited stress-lesion production (Table 2). Whereas aspirin (sodium acetylsalicylate) increased the degree of lesions produced by all 3 methods, the copper complex of aspirin, like copper salicylate, inhibited aspirin-induced lesions and potentiated indomethacin lesions at doses one-tenth those of aspirin. However, the production of lesions by stress was significantly potentiated by copper aspirin at doses as low as 5 mg kg^{-1} orally.

Table 2. *Effect of salicylates on gastric lesion production in rats by three methods.* Values are percentage changes ± s.e.m. (controls = 100%).

Drug dose (mg kg^{-1})	Lesion production: Aspirin	Indomethacin	Stress
Sodium salicylate			
50	22·2 ± 6·0	23·2 ± 5·2	11·6 ± 4·1†
100	78·2 ± 9·4*	63·9 ± 5·2*	30·4 ± 3·9*†
200	95·4 ± 7·9*	84·6 ± 8·2*	41·8 ± 6·2*†
Copper salicylate			
10	25·8 ± 8·3	173·3 ± 12·6*†	71·1 ± 6·7*
20	52·4 ± 8·1*	185·6 ± 14·9*†	84·6 ± 5·9*
Copper aspirin			
5	41·7 ± 5·9*	28·0 ± 5·3†*	60·2 ± 8·0*†
10	69·5 ± 9·0*	184·6 ± 18·5*†	151·9 ± 13·7*†
Aspirin			
50	23·0 ± 8·0†	207·6 ± 20·0*†	60·8 ± 8·9*†
100	49·7 ± 8·7*†	305·9 ± 20·2*†	277·3 ± 21·2*†

* = Significant differences from controls ($P = <0·05$).
† = Potentiation of lesion production.

DISCUSSION

The production of gastric lesions by the three methods used in the present work probably results from different mechanisms. Oral aspirin in pyloric-ligated rats possesses a local action during 6 h, as subcutaneous or intraperitoneal aspirin fails to produce lesions. The gastric mucosal barrier normally exerts a protective function and this is broken by aspirin. Its hexosamine content (Narumi & Kanno, 1972) and the rate of incorporation of *N*-acetyl-glucosamine into the mucosa (Dekanski, Macdonald & Sacra, 1975) may be reduced. Aspirin is also a good inhibitor of prostaglandin synthetase and this action may also be involved in its mucosal effect. However, recent work by Cashin, Dawson & Kitchen (1977) shows that the potential of a compound to produce lesions is not always related to its ability to inhibit prostaglandin synthetase activity.

Subcutaneous indomethacin exerts its action on the gastric mucosa during 6 h and it is a systemic effect. Bhargava, Gupta & Tangri (1973) identified a release of catecholamines from the adrenal medulla after indomethacin and these are known to exert a regulatory effect on the actions of prostaglandins (Vane, 1971). Mepyramine was effective against indomethacin in our experiments and the dose required was low (10 mg kg^{-1}) so an involvement of H_1-histamine receptors cannot be eliminated. H_2-Histamine receptors are probably not involved as metiamide was ineffective, surprisingly, even at doses of 100 mg kg^{-1}. Poor absorption from the suspending agent may be the cause of its ineffectiveness.

Stress lesions produced over 1 h are complex and involve, in part, both H_1 and H_2-histamine receptors. Catecholamines may also be involved in the production of these lesions as they are released during stress. Davenport (1964) suggested that the aspirin lesions may result from increased back diffusion of H^+ ions and efflux of Na^+ ions but short-term stress does not change the back diffusion of acid (Gereby & Guth, 1972).

As the evidence as to the cause of lesion production is unclear, the different profiles of activity of the three salicylates under test are also difficult to explain. Copper aspirin differs from aspirin only in antagonizing lesion production by aspirin and the presence of the copper ion in the molecule probably stabilizes the mucosal membrane. Copper salicylate differs from copper acetylsalicylate only in reducing lesion production by stress and so is probably well absorbed during the 1 h of stress. Finally, sodium salicylate differs from copper salicylate in potentiating stress-induced lesions but inhibiting indomethacin lesions, as does mepyramine. None of these salicylates produce lesions *per se* (at the doses used) and all three antagonize aspirin-produced lesions.

Although, on the basis of these results, it has not been possible to advance knowledge on the mode of action of anti-lesion compounds, stress-induced lesion production appears to offer a suitable animal model for testing the activity of these compounds. It alone, of the three methods tried, was influenced by low doses of metiamide, which, like cimetidine, is an H_2-histamine antagonist effective in most human gastric ulcers.

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