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Paracetamol potentiates stress-induced gastric ulceration in rats

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Abstract—The effect of paracetamol on gastric ulcers produced by restraint at 4°C for 2 h (stress) was studied in rats. Paracetamol treatment s.c or p.o., with a dose as high as 250 mg kg^{-1}, did not produce any haemorrhagic lesions in the glandular mucosa. Oral administration with 250 mg kg^{-1}, however, significantly reduced the mast cell count in the gastric glandular mucosa and potentiated haemorrhagic ulceration but not mast cell degranulation caused by stress. The potentiating action was maximum when paracetamol was given between 15 and 30 min before stress. Ranitidine, astemizole, dimethylsulphoxide, sucralfate and verapamil did not protect against the adverse action of paracetamol on stress-evoked lesions. This study suggests that paracetamol worsens stress-induced stomach ulceration by an action which appears not to be due to histamine release, free radical production or intracellular calcium disturbance in the gastric mucosa.

Although it has been reported that paracetamol can prevent the formation of gastric mucosal lesions induced by ethanol or acidified aspirin in animals and man (Seegers et al 1978; Konturek et al 1982; Stern et al 1984; Poon et al 1987), Graham & Smith (1985) and Nakagawa & Okabe (1987) failed to show that paracetamol has cytoprotective activity against aspirin, ibuprofen and ethanol-evoked mucosal lesions. Those findings suggest that the gastric action of paracetamol may differ from that of other antipyretic analgesic drugs which damage the mucosa (Konturek et al 1981; Cho & Ogle 1987; Szabo & Cho 1988) and also worsen stress-evoked ulceration (Cho & Ogle 1987). Although the pharmacological actions of paracetamol on gastric mucosal damage by ethanol have been studied, its influence on the gastric effects of stress has not yet been examined. The latter aspect is important because paracetamol is taken for pain which could be accompanied by psychological stress. We have, therefore, investigated the effects of this drug on stomach ulceration produced by cold-restraint stress in rats.

Materials and methods

Male Sprague-Dawley rats, 180–200 g, housed at constant temperature (23 ± 1°C) and humidity (65–70%) were fed a pellet (Ralston Purina Company) diet and had free access to tap water. Food was withdrawn 48 h before experimentation, but free access to a solution of 8% w/v sucrose in 0·2% w/v NaCl was permitted; this drinking solution was removed 1 h before the rats were used. Paracetamol (Sigma) was suspended in 4% w/v Tween 80 (Sigma) solution, and 125 or 250 mg kg^{-1} was given either s.c. by injection or p.o. via an intragastric tube 30 min before restraining the animals individually in close-fitting cylindrical wire cages at 4°C for 2 h (stress) (Senay & Levine 1967). Non-stressed controls were returned to their starvation cages in the room where they were normally housed. All animals were killed by a sharp blow on the head 2 h after stress. In an experiment to explore the effects of varying the time of pretreatment with paracetamol, the drug was given orally at 15, 30, 60 or 90 min before stress. The stomach was then removed, opened along the greater curvature and the mucosal surface of the glandular segment examined under an illuminated magnifier (× 3). Each lesion was measured along its greatest length; in the case of petechiae, five of these were taken as the equivalent of a 1 mm ulcer. The sum of the lesion lengths in each group of animals was divided by its number and expressed as the mean ulcer index. The glandular segment of the stomach was then fixed in 4% w/v lead acetate (E. Merck) for 48 h before processing the tissue and staining the mast cells with toluidine blue (E. Gurr Ltd). The number of metachromatically stained mast cells in the upper one third of the glandular mucosa was counted in 42 oil immersion fields (magnification 1000 ×) (Cho & Ogle 1978a; Ogle & Lau 1979).

In separate experiments, astemizole (Janssen), ranitidine (Glaxo), dimethylsulphoxide (Sigma), verapamil (Knoll) or sucralfate (Chugai) was given s.c. or p.o. and this was followed by oral administration of paracetamol 250 mg kg^{-1}. Thirty minutes later, these rats were stressed for 2 h and killed; the severity of mucosal ulceration was then determined.

All values were expressed as means ± s.e.m. and the data analysed for statistical significance by Student's unpaired two-tailed *t*-test.

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Results

Paracetamol or its vehicle, Tween 80, given either s.c. or p.o. did not produce any observable haemorrhagic lesions in the gastric glandular mucosa of the non-stressed controls (Table 1A). The lesions, if any, were only in the form of petechiae. However, oral administration of 250 mg kg^{-1} significantly reduced the mast cell count (by about 30%) in the upper third of the glandular mucosa. Cold-restraint stress itself produced severe haemorrhagic ulcers and reduced the mast cell count by about 34% (controls pretreated with vehicle s.c.) and 45% (controls pretreated with vehicle p.o.) (Table 1B). Oral administration of paracetamol dose-dependently and significantly (with 250 mg kg^{-1}) potentiated the severity of ulceration but not the mast cell degranulation produced by stress. The potentiation of lesion formation was found to be maximum when the drug was given between 15 to 30 min before stress, and the effect started to decline thereafter (Table 2).

Table 3 shows the results of drug pretreatment on the aggravation of stress-induced ulceration by p.o. administration of paracetamol 250 mg kg^{-1}. Astemizole, ranitidine, dimethylsulphoxide, verapamil or sucralfate administration did not significantly affect the ulcer index.

Discussion

This study demonstrates that oral administration of paracetamol 125 or 250 mg kg^{-1}, in doses which have been shown to protect against ethanol-induced lesion formation (Poon et al 1987), worsens stress-induced ulceration. Although paracetamol p.o. has been found to increase endogenous prostaglandin synthesis, which is one of the protective mechanisms against ethanol-induced mucosal lesions (Robert et al 1979; Konturek et al 1982), this eicosanoid has been reported not to influence stress-induced gastric ulceration when given in doses which completely antagonize the relatively more severe mucosal damage by ethanol (Ogle et al 1985a). The present findings support past observations which indicate that the aetiology of mucosal lesions due to ethanol and stress is different. Tween-80

Table 1. The effects of paracetamol (given s.c. or p.o. 30 min before stress) on stress (restrained at 4°C for 2 h)-induced ulceration and mast cell degranulation and in the glandular mucosa of rat stomachs.

Pretreatment	No. of rats	Ulcer index (mm)	Mast cell count
A. Non-stressed (unrestrained at 22°C)			
4% Tween 80 (5 mL kg^{-1} s.c.)	6	0·12 ± 0·03	57·1 ± 2·1
Paracetamol (125 mg kg^{-1} s.c.)	6	0·11 ± 0·04	54·9 ± 3·1
Paracetamol (250 mg kg^{-1} s.c.)	6	0·15 ± 0·09	59·1 ± 4·3
4% Tween 80 (5 mL kg^{-1} p.o.)	6	0·23 ± 0·06	56·3 ± 5·1
Paracetamol (125 mg kg^{-1} p.o.)	6	0·10 ± 0·07	51·5 ± 8·8
Paracetamol (250 mg kg^{-1} p.o.)	6	0·55 ± 0·22	36·3 ± 3·9*
B. Stressed (restrained at 4°C)			
4% Tween 80 (5 mL kg^{-1} s.c.)	9	3·53 ± 0·83†	37·7 ± 4·4†
Paracetamol (125 mg kg^{-1} s.c.)	9	3·77 ± 1·20†	28·8 ± 3·4†
Paracetamol (250 mg kg^{-1} s.c.)	9	5·68 ± 1·30†	36·9 ± 2·9†
4% Tween 80 (5 mL kg^{-1} p.o.)	9	4·09 ± 0·81†	30·8 ± 4·6†
Paracetamol (125 mg kg^{-1} p.o.)	9	7·61 ± 1·77†	31·3 ± 2·2†
Paracetamol (250 mg kg^{-1} p.o.)	9	10·50 ± 2·03*†	25·8 ± 4·2

Values shown are the means ± s.e.m. o.i.f. = oil immersion fields (1000 ×).
* $P < 0.05$ when compared with the corresponding Tween 80-pretreated control (p.o.).
† $P < 0.05$ when compared with the corresponding nonstressed group in A.

was used as a vehicle for the preparation of paracetamol suspension because the drug was taken up better in this substance, which is not different from a less irritant vehicle, e.g. methylcellulose, in its ability to produce mucosal injury (Cho & Ogle 1987). Both chemicals produce only petechiae in the glandular mucosa.

It has been reported that histamine H_1- and H_2-receptor antagonism, as well as calcium channel blockade, can prevent stress-induced gastric damage (Cho & Ogle 1978b; Ogle et al 1985b), indicating that both histamine release and calcium influx into the cells could play important roles in stress-evoked gastric ulceration. Paracetamol itself has been shown to increase histamine release from the mast cells, and this action is thought to be mediated by the action of free radicals (Mannaioni et al 1988). Although the higher p.o.-administered dose of paracetamol (250 mg kg^{-1}) reduced the mast cell count (but without significant lesion production in these non-stressed animals) in the present study, it did not lower further the mast cell count which was decreased by stress itself. Thus, it is unlikely that increased histamine release from these mast cells, and its subsequent action of H_1- and H_2-receptors, is responsible for the worsening effect of paracetamol p.o. on stress-provoked ulcers. This idea is substantiated by the finding that histamine H_1- and H_2-receptor blockade by astemizole and ranitidine, respectively, did not prevent the ulcerogenic effect of paracetamol. The similar degree of mast cell degranulation by paracetamol 250 mg kg^{-1} p.o. in non-stressed rats and by stress alone, but with only the latter group showing a significant increase in glandular mucosal ulceration, can be explained by the multifactorial aetiology of stress-induced ulcers (Cho & Ogle 1978a, b; Ogle & Lau 1979). In stress, there appears to be an intense surge of vagal overactivity with rapid degranulation of the mast cell resulting in a strong action by released histamine which summates with the other ulcerogenic effects of vagal stimulation. In the case of paracetamol, there is likely to be gradual mast cell degranulation and a slow leak of histamine to yield relatively low tissue amine concentrations which, in the absence of the other stress-evoked gastric effects, would not be ulcerogenic. However, a detailed discussion on the other inter-related mechanisms of stress is not relevant to the presently proposed action of paracetamol.

Dimethylsulphoxide, a free radical scavenger, has been reported to prevent ischaemia/reperfusion-induced gastric mucosal injury (Perry et al 1986; Kvietys et al 1988). As this chemical did not influence the ulcerogenic action of paracetamol combined with stress, it is unlikely that the paracetamol-stress-evoked mucosal lesions are caused by oxygen-derived free radicals.

It has been shown that stress increases calcium levels in the gastric muscle; verapamil, which reduces these ions to normal values, prevents stress-induced ulceration (Koo et al 1989). In the present study, verapamil also failed to antagonize the potentiating action of paracetamol on stress-evoked ulceration.

Table 2. The effects of paracetamol (given at varying periods before stress) on stress (restrained at 4°C for 2 h)-induced ulceration in the glandular mucosa of rat stomachs.

Pretreatment	Time (min) of administration before stress	No. of rats	Ulcer index (mm)
Combined vehicle control† (4% Tween 80, 5 mL kg^{-1})		16	4·9 ± 0·79
Paracetamol (250 mg kg^{-1})	15	8	17·3 ± 1·69*
Paracetamol (250 mg kg^{-1})	30	6	15·5 ± 2·54*
Paracetamol (250 mg kg^{-1})	60	8	11·1 ± 1·25*
Paracetamol (250 mg kg^{-1})	90	7	5·7 ± 1·09

Values shown are the means ± s.e.m.
* $P < 0.001$ when compared with the combined-vehicle control.
† The ulcer indices of Tween 80 pretreated at 15, 30, 60 or 90 min before stress were similar and therefore they were pooled and regarded as combined vehicle control.

Table 3. The effects of various pharmacological agents (given 32 min before stress) on the aggravation of stress (restrained at 4°C for 2 h)-induced ulceration by paracetamol (given p.o. 250 mg kg^{-1} 30 min before stress) in the glandular mucosa of rat stomachs.

Pretreatment	No. of rats	Ulcer index (mm)
Saline (2 mL kg^{-1} s.c.)	25	10·52 ± 1·14
Astemizole (1·5 mg kg^{-1} s.c.)	9	8·00 ± 2·10
Ranitidine (15 mg kg^{-1} s.c.)	7	10·60 ± 1·54
DMSO (100 mg kg^{-1} s.c.)	7	10·50 ± 2·32
Verapamil (2 mg kg^{-1} s.c.)	12	10·85 ± 1·40
Sucralfate (25 mg kg^{-1} p.o.)	6	8·23 ± 1·49

Values shown are the means ± s.e.m. DMSO = dimethylsulphoxide.

The finding that only oral administration of paracetamol potentiates stress-induced ulceration, indicates that the action may be a local one. However, this topical action could not be antagonized by sucralfate, which is known to enhance the integrity of the mucosal barrier and to prevent gastric damage by noxious agents (Szabo & Brown 1987). As paracetamol does not affect the mucus which adheres to the gastric mucosa (Poon et al 1987), it is possible that this drug exerts its action beyond the mucosal barrier. The drug may act through active metabolites formed in the liver (Flower et al 1985). Further studies are needed to clarify these observations.

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