Gastric mucosal ulceration induced in pigs by tablets but not suspensions or solutions of aspirin

K. D. RAINSFORD, *Department of Biochemistry, University of Tasmania Medical School, G.P.O. Box 252 C, Hobart, Tasmania, Australia* 7001

Gastric ulceration and haemorrhage are among the most serious side-effects associated with the use of aspirin (Jennings, 1965; Cooke, 1976). The exact basis of the relation between ingestion of the aspirin and the development of acute or chronic gastrointestinal ulcers remains unclear (Langman, 1970; Rainsford 1975a). While superficial mucosal damage (i.e. lesions) can be demonstrated in animals given high therapeutic doses of aspirin by mouth, ulceration *per se* is not observed except occasionally when massive (toxic) doses have been given for a long time (St. John, Yoemans & De Boer, 1973; Rainsford, 1975a). The results from these and other studies have given impetus to a search for other factors (e.g. ethanol or stress) which, in aspirin, may be involved in the development of gastrointestinal ulceration (Jennings, 1965; Rainsford, 1975b).

The effects of tablet formulations of aspirin or related drugs in the development of gastric ulceration in animals do not appear to have been reported. Tablets of aspirin could be potentially more ulcerogenic than suspensions because of the high concentration of an acidic drug (in a compressed dosage form) being in direct contact with the gastric mucosa. Some comparisons have been made of the effects of various aspirin preparations on the loss of blood from the gastrointestinal tract and of the gastroscopic appearance of the gastric mucosa in man (Wood, Harvey-Smith & Dixon, 1962; Leonards, 1976; Corrigan, Champion & others, 1977). However, the doses of drug and the duration of treatment used in some of these studies were much less than usually employed in the treatment of arthritic conditions. Also, some of the techniques employed in assessing gastrointestinal damage (e,g, ^{51}Cr blood loss technique) may not be a sufficiently accurate means of assessing the site or the degree of damage (including ulceration) in the gastrointestinal tract (Rainsford, 1975a). The feasibility of performing such studies to ensure the gastric safety of these preparations may have been limited because of the difficulty of dosing tablets to animals. The domestic pig is, however, a species which can be easily dosed with tablets. Moreover, it appears to have advantages in closely resembling that of man in gastrointestinal structure and function (Bustad & McClelland, 1966), and it is subject to gastric ulceration following exposure to various stressful stimuli of the kind encountered by man (Muggenburg, McNutt & Kowalczyk, 1964). Hence the effects of various tablet formulations have been compared with those of suspensions of aspirin on the gastrointestinal mucosa of pigs.

Female pigs (Large white × Landacre cross, 12–16 kg) were dosed orally for 10 days with: (1) various commercial formulations of aspirin (Table 1) at a dose equivalent to aspirin 100 mg kg^{-1}, (2) aspirin suspension (100 mg kg^{-1} day^{-1}), or (3) an equimolar mixture of aspirin (100 mg kg^{-1}) and sodium bicarbonate. The animals had free access to water but no food for 24 h before the final dose of the drug. They were killed by captive bolt 2 h later. The stomach and upper intestinal tract were washed free of food, the mucosal damage assessed (Rainsford, 1975b) and the stomachs were subsequently photographed. Sections of mucosa were selected for histological examination and following fixation in formal-saline, the tissue sections were stained with either haematoxylin and eosin, periodic acid Schiff's reagent (McManus, 1961) or alcian blue (Lev & Spicer, 1964).

Table 1. *Gastric mucosa damage by aspirin preparations in pigs.* Aspirin preparations were given orally to pigs at aspirin doses of 100 mg kg^{-1} day^{-1} for 10 days, either as (†) suspension or (**) solutions in 50 ml H_2O or as whole tablets alone. The animals were starved of food for 24 h and given final doses of the drugs 2 h before they were killed by a captive bolt and the number of lesions or ulcers and the severity of lesions (assessed on an arbitrary scale of 0–4+) in the gastric mucosa were determined (Rainsford, 1975b). Without exception, aspirin-dosed animals showed evidence of gastric mucosal damage. No damage was observed in the intestinal tract with the exception of one animal dosed with sustained-release aspirin (‡) which has 4 ulcers in the upper duodenum. *No statistically significant differences (Mann Whitney U-test, $P<0.05$) were observed in the number of lesions produced by the aspirin preparations.

Preparation (manufacturer)	No. of pigs	Number of haemorrhagic lesions (mean ±s.e.)	Average severity of lesions	No. of ulcers (mean ±s.e.)
None (control)	17	0	0	0
Aspirin suspension (Monsanto)†	5	32·0 ±17·3*	2·0	0
Aspirin (Monsanto) + equimolar sodium bicarbonate**	4	23·8 ±12·4*	2·5	0
Aspirin tablets B.P.	11	30·7 ±5·5*	3·4	2·7 ±1·5
Sustained-release aspirin tablets: Boots SR-A (Boots)‡	8	28·0 ±6·0*	3·0	3·8 +1·5
Enteric-ocated tablets: Ecotrin (Smith, Kline & French)	4	21·8* ±1·3	3·0	1·8 ±1·1
Aspirin + sodium bicarbonate + citrate; Aspro-Clear (Nicholas)**	4	27·3 ±6·4*	2·6	0·7 ±0·3

The results (Table 1) show that oral administration of tablets of aspirin to pigs for 10 days resulted in the development of chronic ulcers and extensive haemorrhagic lesions in the gastric mucosa. The damage was confined principally to the fundic (i.e. acid-pepsin secreting region of the greater curvature of the stomach. Occasionally mucosal lesions and ulcers were evident in the junction between the fundus and antrum and the antrum itself in animals given aspirin tablets. Haemorrhagic lesions and ulcers were occasionally observed in the pyloric region of pigs given sustained-release or enteric-coated aspirin tablets. The predominent localization of damage in the fundic and antral regions noted here is in agreement with the observations of gastric ulceration, presumably due to aspirin, in man (Menguy, 1972; MacDonald, 1973). In all cases the presence of gastric ulcers was confirmed histologically. The lesions and ulcers in the fundic mucosa of animals dosed with aspirin tablets had the typical appearance of gastric ulcers seen in man (Menguy, 1972). In many animals extensive round (inflammatory) cell infiltration was evident near the erosions and ulcers.

The 'non-soluble' aspirin tablet preparations given as tablets all appeared equally as effective in causing lesions to the gastric mucosa, with the exception of the enteric-coated preparation which produced slightly fewer gastric mucosal lesions (Table 1). Although sustained-release and enteric-coated preparations have been claimed to produce less-damage than aspirin B.P. tablets or other formulations of aspirin (Wood & others, 1962; Treadwell, Carroll & Pomare, 1973; Corrigan & others, 1977), the results (Table 1) do not support these claims. The differences may be due to the fact that in most of the human studies the ^{51}Cr-blood loss technique was used for comparing the effects of the various tablet preparations and this could have led to inaccurate estimates of the true extent of gastric damage (Rainsford, 1975a). In contrast to the effects observed with tablets, administration of suspensions or solutions (i.e. aspirin with equimolar $NaHCO_3$) of aspirin, did not result in gastric ulceration *per se* but only haemorrhagic erosions (Table 1). This suggests that the presence of whole tablets (regardless of the formulation) in the stomach is a major factor in the development of gastric ulcers. All tablet preparations produced mucosal lesions of comparable number and severity (Table 1) and have the same propensity to cause ulcer development.

While aspirin B.P. tablets had disintegrated by 2 h after the final dose, undissolved tablets or fragments of tablets of both enteric-coated and sustained-release formulations of aspirin were present in the stomach. The presence of whole tablets and fragments of tablets appeared, in many animals, to be associated with lesions and ulcers. Tablets of the sustained-release preparation were also found in the upper intestine accompanied by a large quantity of bile of 'foaming' appearance 2 h after the final dose. Luminal distension in the region of the intestine near the tablets was also observed. Some of the bile had refluxed into the stomach of animals given tablets but not suspensions or solutions of aspirin. The presence of bile in the stomach is probably an added factor in the pathogenesis of asprin-induced ulceration (Reynolds, 1974).

In conclusion, the results show that the presence of whole tablets of aspirin in the stomach appears to be a principal cause of chronic gastric ulceration in the pig in which effects are produced which closely resemble the pathological signs of ulceration *per se* as observed in man (Menguy, 1972). Equimolar aspirin plus sodium bicarbonate mixtures, or 'soluble' aspirin preparations, while essentially being non-ulcerogenic, are in other respects no less damaging to the stomach than suspensions of the drug. These results demonstrate the importance of considering the formulation and the dosage form of aspirin and other anti-inflammatory drugs in the detection of their potential gastric ulcerogenic activity.

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Reduced sensitivity to L-tryptophan and *p*-chloroamphetamine in streptozotocin-diabetic rats

R. G. MACKENZIE*, M. E. TRULSON, *Program in Neuroscience, Department of Psychology, Princeton University, Princeton, New Jersey 08540, U.S.A.*

Rats made diabetic by injections of streptozotocin exhibit normal brain concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) despite a reduction of approximately 30% in the concentration of brain tryptophan (Curzon & Fernando, 1977; MacKenzie & Trulson, 1978). The reduction in brain tryptophan in diabetic rats is due to decreased uptake of the amino acid by brain as evidenced by a 200–300% greater accumulation of tryptophan in various brain regions in normal rats 1 h after a systemic load of L-tryptophan (50 mg kg^{-1} i.p.) (MacKenzie & Trulson, unpublished). However, the 5-HT and 5-HIAA produced from the accumulated tryptophan is only 20–50% greater in normal rats (MacKenzie & Trulson, unpublished) because the higher tryptophan concentrations attained in the normal rats probably saturate tryptophan hydroxylase (Fernstrom & Wurtman, 1971) which would attenuate the impact of greatly increased precursor concentrations on 5-HT metabolism. Therefore, although diabetes sharply restricts the accumulation of tryptophan by brain, it was not clear whether this restriction had any functional significance vis-à-vis the activation of 5-HT receptors. To test this question, normal and diabetic rats were submitted to drug treatments known to induce a behavioural syndrome that specifically reflects the activity in central 5-HT mediated synapses (Jacobs, 1976). Two drug treatments capable of inducing the syndrome are systemic loads of L-tryptophan preceded by pargyline to inhibit monoamine oxidase and injections of *p*-chloroamphetamine (PCA), a potent 5-HT releaser (Trulson & Jacobs, 1976; Trulson, Eubanks & Jacobs, 1976).

Since it had been reported that diabetes does not significantly affect the brain uptake of amphetamine (Marshall, Friedman & Heffner, 1976) but severely restricts the central accumulation of tryptophan, we predicted a shift to the right of the dose-response curve for the induction of the syndrome by tryptophan in diabetics, but no difference between normal and diabetic animals when the syndrome was induced by PCA.

Behavioural observations were made with rats placed in pairs in round plastic buckets (20 cm high × 35 cm in diameter) with metal screen lids and wood shavings covering the floor. At low drug doses diabetic were paired with normal animals, at high doses only diabetics were run and were thus paired with other diabetics. Following administration of the drugs, the rats were examined for signs of the behavioural syndrome consisting of resting tremor, rigidity or hypertonicity, hindlimb abduction, Straub tail, lateral head weaving and reciprocal forepaw treading (for a detailed description of these characteristics, see Jacobs, 1976). If at least four of these six signs were observed the syndrome was rated as present.

Female Sprague-Dawley rats (250–300 g) were made diabetic by injections of streptozotocin (75 mg kg^{-1}, i.p.) dissolved in citrate buffer pH 4·5 to 75 mg ml^{-1}. Controls received equivolume injections of buffer alone. Diabetes was verified by polydipsia, polyuria, and glucosuria. Two weeks after injections of streptozotocin or buffer, the rats were submitted to one of two drug treatments consisting of either pargyline (50 mg kg^{-1}, i.p.) as hydrochloride followed 30 min later by one of the following doses of L-tryptophan (50, 75, 100, 125, 150, 200, 300, 450 or 600 mg kg^{-1}, i.p.) or one of the following doses of PCA (2·5, 5, 7·5, 10, 12·5, 15 or 17·5 mg kg^{-1}, i.p.). The rats were observed for signs of the syndrome for 1 h after injection of L-tryptophan or PCA. Estimates of the ED50 for each drug were obtained by probit analysis (Bliss, 1952). Differences between

* Correspondence.