MAO-B (Heikkila et al 1984b). The discrepant findings may indicate a species difference in the ability of MMPP to be converted to a neurotoxic metabolite. Perhaps in the experiments of Wilkening et al (1986), in monkeys MMPP underwent enzymatic or non-enzymatic dehydration, to form TMMP. Currently there is no evidence that mice or monkeys would more accurately predict neurotoxic potential of MPTP-like compounds in man.

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Gastric ulcerogenicity of non-steroidal anti-inflammatory drugs in mice with mucosa sensitized by cholinomimetic treatment

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A novel technique is described for the assay of acute gastric irritancy of non-steroidal anti-inflammatory drugs (NSAIDs) in mice in which (a) the gastric mucosa is sensitized to the irritant actions of the drugs by co-administration of bethanechol chloride to increase acid and pepsin production, and (b) the area and number of haemorrhagic lesions in the glandular mucosa is measured quantitatively by visual image analysis. The technique has been used to assess the acute gastric irritancy of 20 NSAIDs in mice. In relation to published values for their acute and chronic anti-inflammatory activities, drugs with low relative gastric irritancy (e.g. carprofen, chloroquine, diclofenac, fenbufen, tenoxicam, tilomisole) were differentiated from the drugs of higher relative irritancy.

Various approaches are used in the assay of acute gastric irritancy of ulcerogenicity of non-steroidal anti-inflammatory drugs (NSAIDs) in laboratory animals, each technique having merits and disadvantages (Rainsford & Whitehouse 1977; Menassé 1979; Ghanayem & Ahmed 1982; Dearden & Nicholson 1984; Rainsford 1981, 1984, 1985a, 1987a, b; Whitehouse et al 1984; Szabo et al 1985). That most frequently used is the counting or grading of haemorrhagic lesions visible about 1–6 h after oral or parenteral administration of NSAIDs to fasted rats (Menassé 1979; Rainsford 1984). This method, while simple, yields data with high error and variability (Rainsford 1987b). To improve the sensitivity of lesion detection and reduce data error, while at the same time employing systems relevant to the therapeutic situation, some authors have subjected rats to cold, restraint, or inflammatory stress conditions to enhance the gastric irritancy of NSAI drugs (Shriver et al 1977; Rainsford 1978, 1981, 1987b; Dearden & Nicholson 1984; Whitehouse et al 1984). However, these procedures expose animals to stressful situations.

An approach to overcome these is to mimic the stressful reactions in the stomach by means of a cholinomimetic agent thereby enhancing mucosal sensitivity to the gastric irritancy of drugs such as NSAIDs. Muscarinic agents (e.g. bethanechol chloride) stimulate the secretion of acid and pepsin (Magee et al 1985) which have actions in NSAID- and stress gastric ulcerogenesis (Rainsford 1978, 1987b). Hence, in the present study the gastric irritancy of a range of NSAI drugs in mice treated with the muscarinic agent, bethanechol chloride, has been evaluated. The method used mice for improved economy (cf. rats) and their gastric lesions were quantified by magnified image analysis.

Methods

Animal procedures. The studies were in female MF1

mice (Central Animal Services, University of Cambridge), 25–30 g, that had beeen fasted for 24 h. Cholinergic stimulation was by i.p. administration of bethanechol chloride (carbamyl β-methyl choline chloride: Sigma) prepared in sterile pyrogen-free saline (0·15 M NaCl) immediately before use. In preliminary dose-response studies, an optimal dose of 5 mg kg^{-1} (in 0·1 mL saline) produced statistically significant enhancement of gastric lesions caused by aspirin (200 mg kg^{-1} p.o.), azapropazone (200 mg kg^{-1} p.o.), indomethacin (15 mg kg^{-1} p.o.) and oxaprozin (200 mg kg^{-1} p.o.) without producing lesions when given alone. This dose also produced a three-fold increase in titratable acid over 1 h. The NSAIDs were given orally as 0·5 mL aqueous suspensions (prepared by homogenization immediately before dosing) followed by the bethanechol chloride. Animals were killed 2 h later by CO_2 asphyxiation and cervical dislocation.

Specimen preparation and visual image analysis. Stomachs were clamped at the oesophago-gastric junction and pylorus with Spenser-Wells forceps, injected through the non-glandular stomach with 2 mL 4·0% v/v formaldehyde in saline and the inflated stomachs allowed to fix in-situ under this slight positive pressure for ca. 15 min to flatten the rugal folds of the mucosa. After dissection the freed stomachs were opened along the lesser curvature and washed free of food and debris with formal-saline solution. The pyloric region, non-glandular mucosa and blood vessels adherent on the serosal side, were dissected away leaving the fundus and antrum which was subsequently fixed in formal-saline for at least 2 h. The stomach was then cut in two, the serosal surface dried quickly with paper tissues and mounted serosal surface downward on glass microscope slides on a bed of quick-setting cyanoacrylate ester adhesive (Super Glue 3, Loctite UK, Welwyn Garden City, UK). The tissues were cleared in glycerol for at least 24 h, which was essential for good visualization of the haemorrhagic lesions.

Image analysis was by an AMS 40-10 system (Analytical Measuring Systems, Saffron Walden, Essex, UK). The specimens (in Petri dishes with glycerol) were illuminated above and below the tissue with a cold light source (Flexilux 150 HL Universal) coupled to a Fibreoptic System (Schölly Fibreoptic AG, CH 8600, Dubendorf, Switzerland).

The area of haemorrhagic lesions relative to the total area of the stomach was determined after first adjusting the contrast image intensity to illuminate the lesions. The total area of the glandular region of the stomach was then illuminated and the area thus recorded. The per cent area of lesions was then calculated

$$\text{Per cent area} = \frac{\text{Area of lesions (in mm}^2\text{)}}{\text{Total area of glandular mucosa}} \times \frac{100}{1}$$

The number of gastric lesions was determined as described by the manufacturer's instructions using the 'end point' procedure.

Drugs. The following compounds were generously donated: aspirin (Monsanto Ltd, Melbourne, Australia), azapropazone (A. H. Robins Co. Ltd, Horsham, UK), BW755c (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline (Wellcome Research Laboratories, Beckenham, Kent, UK), carprofen and tenoxicam (Roche Products Ltd, Welwyn Garden City, UK), diclofenac sodium and phenylbutazone (Ciba Geigy Pharmaceuticals Ltd, Horsham, UK), diflunisal, indomethacin and sulindac (Merck, Sharp & Dohme Research Laboratories, Rahway, USA), fenbufen (Lederle Laboratories, Division Cyanamid of GB Ltd, Gosport, UK), fenclofenac (Reckitt & Colman Ltd, Kingston-upon-Hull, UK), nabumetone (Beecham Pharmaceuticals Ltd, Gt Burgh, Epsom, Surrey, UK), naproxen (Syntex Corporation, Palo Alto, USA), oxaprozin, tilomisole (Wy-18,251) and Wy 41,770 (Wyeth Laboratories Inc, Philadelphia, USA), proquazone (Sandoz A. G., Basel, Switzerland), and piroxicam (Pfizer Inc, Groton, USA). Chloroquine diphosphate was purchased from Sigma UK.

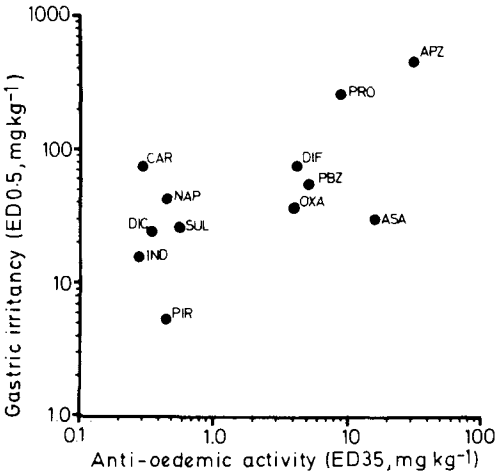

FIG. 4. Relation of the gastro-irritant effects of NSAIDs in mice to the anti-inflammatory effects in the carrageenan paw swelling assay in rats. A poor correlation is evident with the logarithmically transformed data (correlation coefficient r = 0·565, t = 0·783, P = 0·55, not significant at 5% level, d.f. = 11). The correlation is even lower (r = 0·367, t = 1·174, P = 0·21) with the non-transformed data. Thus in view of the poor correlation between these two parameters it is justified to segregate data of the groups of drugs lying either within or outside the range of effects shown by standard ulcerogenic agents such as aspirin, indomethacin and pheylbutazone. Abbreviations: APZ azapropazone, ASA aspirin, CAR carprofen, DIC diclofenac, DIF diflunisal, IND indomethacin, NAP naproxen, OXA oxaprozin, PBZ phenylbutazone, PIR piroxicam, PRO proquazone, SUL sulindac.