Gastric irritancy of aspirin and its congeners: anti-inflammatory activity without this side-effect

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The acidity of the aspirin molecule (*o*-acetylsalicylic acid) is one factor in determining its *acute* gastric irritancy, i.e. in causing lesions to the gastric mucosa in rats and pigs (Rainsford, 1975a,b). Anderson (1963) stated that the gastric irritancy in guinea-pigs is associated with the presence of an unsubstituted carboxyl group in *o*-substituted benzoic acids, provided the compounds are readily absorbed.

In rats, the superimposition of stress (cold, restraint) greatly amplifies the acute irritant effect of aspirin on the gastric mucosa (Rainsford, 1975c) and affords a facile assay for determining the potential ulcerogenic activity of some non-steriodal anti-inflammatory drugs (Rainsford, 1975c; Rainsford & Whitehouse, 1976). In this report we present data indicating that certain phenyl acetates related to aspirin may display much less gastric irritancy and retain anti-inflammatory activity comparable with aspirin.

Gastric damage was assessed in groups of 4 to 10 Wistar rats of either sex (S.P.F and open colony), 150 to 230 g. The animals were starved and allowed free access to water for 24 h before administering the compounds orally as fine suspensions in water (Rainsford, 1975a). Gastric and intestinal damage was assessed in stressed (−15°, 45 min) and non-stressed rats (Rainsford, 1975c), using the procedures described previously (Rainsford, 1975a), 2 h after drug administration. A standard aspirin dosage was employed in each day's group of experiments to identify any daily variation in response.

Anti-inflammatory assays were performed in female Wistar rats since aspirin had greater activity against carageenan paw oedema (6 h max) in females than in males. Anti-inflammatory activity was assessed in the carageenan- and urate crystal-induced paw oedemas as used previously (Rainsford & Whitehouse, 1976).

The phenyl (-*o*-) acetates and their parent phenols were kindly donated by the Nicholas Research Institute, Slough (those designated 'AGN' in Table 1), and Carter Wallace Laboratories, Cranbury, New Jersey ('W' compounds), or synthesized from the corresponding phenols by reaction with acetic anhydride catalysed by pyridine (Simokoriyama, 1941), sulphuric acid (Stahmann, Wolff & Link, 1943) or perchloric acid (Whitehouse & Dean, 1965) or in aqueous alkaline solutions (Chattaway, 1931). The products had theoretical *o*-acetyl content and gave no colour reaction with ferric chloride (in ethanol). Other compounds were generously provided by Merck, Sharpe and Dohme Ltd (Flufenisal), and Winthrop Laboratories (benorylate).

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The following conclusions can be drawn from the experimental findings (Table 1):

(1) *o*-Acetylation of 2-hydroxybenzoic acids that were intrinsically irritant to the gastric mucosa (e.g. salicylic acid itself) enhanced this irritancy (compounds I, VI, VIII, XI), with the sole exception of compound X.

(2) *o*-Acetylation of two cresotinic acids (compounds IV, V) and an *o*-hydroxynaphthoic acid (XII) engenders gastric irritancy.

(3) On the other hand *o*-acetylation did not engender irritancy in some other phenols. These were either acids (IX, XIII) or lacking a free carboxyl group (compounds II, III, XIV, XVI).

(4) Of the ten 2-acetoxybenzoyl compounds with anti-oedemic activity (I to X), only five were definitely irritating to the gastric mucosa. There was, therefore, no general correlation between gastric irritancy and systemic anti-inflammatory activity in this series of phenyl acetates. Thus a potent irritant such as 2,6-diacetoxybenzoic acid (XI) exhibited no anti-oedemic activity. Conversely, certain non-irritant phenyl acetates exhibited anti-oedemic activity comparable with aspirin, e.g. 2-acetoxy, 5-(4′-fluorophenyl) benzoic acid (Flufenisal) (IX), aspirin methyl ester (II) and 2,3-diacetoxybenzoic acid (X). Benorylate (III) and 4-t-butyl-aspirin (VII) though non-irritant, were less potent than aspirin against the carrageenan-induced oedema.

(5) The isostere of the aspirin anion, 2-nitrophenyl acetate (XVI) showed no aspirin-like irritancy or anti-oedemic activity.

(6) The methyl esters of both aspirin and salicylic acid were much less irritant to the stomach than the parent acids.

The absence of gastric damage in animals treated with 100 mg kg^{-1} aspirin methyl ester (methyl 2-acetoxybenzoate, II) or 2,3-diacetoxybenzoic acid (X) was confirmed by light microscopic examination of these tissues (formalin fixed and stained with either haematoxylin and eosin, the periodic acid-Schiff stain reaction or Alcian Blue, pH 2·5). Absence of lesions was also noted microscopically and visually 2 h after oral administration of 200 and 50 mg kg^{-1} of aspirin methyl ester (II) to stressed and non-stressed animals, although some oedema was observed associated with extensive gastric swelling at the higher dose.

Table 1. *Gastric irritancy and anti-inflammatory activity of some phenyl acetates and the corresponding phenols in rats.*

Compound	Anti-oedemic* activity of phenyl acetate	Gastric irritancy (L.I.**) of phenyl acetate		Gastric irritancy (L.I.**) of (desacetyl) phenol	
		Alone	With cold stress	Alone	With cold stress
I. 2-Acetoxybenzoic acid (aspirin)	++	17·3	37·8	2·8	10·6
II. Methyl 2-acetoxybenzoate (m.p. 49°)	++	0	0	0	0
III. 4-Acetamido, 2-acetoxybenzoate (Benorylate)	+	0	0	N.D.	N.D.
IV. 2-Acetoxy, 3-methylbenzoic acid (AGN 357)	+	2·4	6·8	0	0
V. 2-Acetoxy, 6-methylbenzoic acid (AGN 833)	++	15·7	28·7	0	0
VI. 2-Acetoxy, 5-chlorobenzoic acid (W-2080)	+++	10·7	32·3	6·5	4·0
VII. 2-Acetoxy, 4-t-butylbenzoic acid (AGN 918)	+	0	0	0	0
VIII. 2-Acetoxy, 3-phenylbenzoic acid (AGN 608)	+	3·6	N.D.	0	7·1
IX. 2-Acetoxy, 5-(4′-fluorophenyl) benzoic acid (Flufenisal)	++§	0	0	0	0
X. 2,3-Diacetoxybenzoic acid (m.p. 164°)	++	0	0	7·5	7·0
XI. 2,6-Diacetoxybenzoic acid (m.p. 113°)	0	15·7	28·1	7·1	10·3
XII. 1-Acetoxy, 2-naphthoic acid (m.p. 151°)	0	0	8·1	0	0
XIII. 4-Acetoxybenzoic acid (m.p. 188°)	0	0	0	0	0
XIV. 2-Acetoxybenzamide (m.p. 139°)	0	0	0	0	0
XV. *N*-Acetyl, 4-aminophenyl acetate (AGN 1782)	0	0	6·75	0	3·0
XVI. 2-Nitrophenyl acetate (m.p. 41°)	0	0	0	0	N.D.

Notes: * Anti-inflammatory potency determined by inhibition of paw oedema, elicited in the subplantar surface of rear paw with 1 mg Na carrageenan in 0·1 ml saline as measured with a micrometer screw gauge 3 and 7 h after drug administration and scored + = 60–80%; ++ = 40–60%; +++ ≤40% of control values. Compounds were administered orally in 1% acacia at 1·1 mmol kg^{-1} (equivalent to 200 mg kg^{-1} aspirin). Only 5-chloroaspirin (VI) showed activity against the slow-developing paw oedema-elicited with crystalline monosodium urate monohydrate measured 6 h after injecting the crystal (3 mg per paw). § = tested at 200 mg kg^{-1}.

** L.I. = lesion index calculated from the number and severity of lesions (Rainsford, 1975a) 2 h after administration of 100 mg kg^{-1} compounds as suspensions in H_2O. Data on number of lesions parallels the damage assessed by lesion index.

N.D. = Not determined.

Further comparisons of II with aspirin, administered orally to starved rats, showed:

(*a*) rapid absorption to give comparable blood concentrations of total salicylates (measured spectrofluorimetrically);

(*b*) after 7 daily doses (125 mg kg^{-1} a day), II caused no gastric damage at all to either normal or (adjuvant arthritic hooded rats while aspirin gave lesion indices of 13·2 (normal) or 13·6 (arthritic);

(*c*) intragastric aspirin aggregated the gastric mucus and also caused its sloughing away from the mucosa within 10 min but II did not affect the mucus coat.

The irritancy of some of the phenyl acetates outside the stomach (i.e. in a non-acidic environment with no parietal cells) was also assessed by the oedemic swelling they elicited in the rear paws of female (180 g) rats, (paw swelling measured 2 and 6 h after injecting 20 μmol of finely ground compound dispersed in 0·25% w/v gum acacia in saline). Non-acidic 2-acetoxybenzoyl compounds (II, III and XIV) and 2-nitrophenyl acetate were non-irritant in the paw. The two acidic derivatives of aspirin with anti-inflammatory activity, which were non-irritant in the stomach (IX, X), were also less irritating than either aspirin or methyl salicylate in the paw.

In conclusion, studies in the rat indicate that the *o*-acetyl group of aspirin is not a toxophore *per se*, though it may enhance gastric irritation by substituted salicylic (e.g. cresotinic) acids. Gastric irritancy is primarily associated with the carboxylic acids group in aspirin itself but not perhaps in certain acidic derivatives, notably 2-acetoxy, 5-(4′-fluorophenyl) benzoic acid and 3-acetoxyaspirin (X). Esterification of the aspirin molecule is one way to attenuate gastric irritancy while retaining oral anti-inflammatory activity.

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Coprecipitates of trifluoperazine embonate and polymers: duration of action by intramuscular route

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Nylon microcapsules of trifluoperazine embonate, prepared by the interfacial condensation of hexane diamine and sebacyl chloride, show activity over two or more weeks after injection intramuscularly as suspensions into beagles (Florence, Jenkins & Loveless, 1973). Provided that biologically acceptable encapsulating agents can be found the results with the microencapsulated drug suggest that such formulations would be an approach to the preparation of prolonged acting parenterals. Not all polymers can be formed by interfacial condensation procedures and thus cannot readily produce conventional microcapsules. In a programme of work aimed at producing injections with activity over several weeks we have prepared polymer-drug mixtures by a simple process of coprecipitation, and tested the resulting products in beagles. One of these preparations employing polymethylmethacrylate has shown duration of activity equivalent to that of the conventional nylon microcapsule formulation. Although polymethylmethacrylate is not a bio-degradable polymer, its physicochemical properties are well characterized. It is one of a series of polyacrylates being studied in our laboratories. Its use here is simply to demonstrate the prolongation of release possible with coprecipitation techniques. The combination may have a use in experimental animal studies.

Poly DL-aspartic acid was prepared from DL-aspartic acid by thermal polymerization at 160–180° following the method of Neri, Antoni & others (1973). The intrinsic viscosity of two samples in dimethylformamide (DMF) was 13–14 ml g^{-1}. While no molecular weights can be deduced from these data others have found molecular weights in the range 5000 to 15 000 after similar preparation techniques (Alexander & Lundgren, 1966). Polymethylmethacrylate (PMMA) was BDH material of 'high molecular weight'. Coprecipitates of trifluoperazine embonate and the two polymers were prepared by dissolving drug and polymer in DMF and adding the solution to a rapidly stirred volume of water. Both polymers and drug are insoluble in water. The drug-polyaspartic acid system was prepared by dissolving 0·4 g drug and 2 g polymer in 60 ml DMF. The solution was poured into 250 ml water and the resulting precipitate dried in an oven under vacuum at 30° ± 1° over P_2O_5. The dried product was ground and ball milled for 1 h. Microscopy showed that the number mean diameter was 2 μm (range 1–25 μm). Similar methods were employed for the polymethylmethacrylate product but in addition to the 5:1 polymer: drug ratio a preparation with a 10:1 polymer: drug ratio was obtained. Particle size distributions of the products were very similar. The formulations were tested in beagle dogs (10–17 kg) using the subcutaneous apomorphine challenge test. Doses of 5 mg kg^{-1} were administered by deep intramuscular injection into the thigh, in the form of suspensions of the coprecipitate particles in sesame oil.

Fig. 1 summarizes the results. Return of the measured response to 60% of the control value is a reasonable measure of duration. Examination of Fig. 1 shows that a solution of the drug in polyethylene glycol allows the response to return to 60% in 6 days; the polyaspartic acid preparation returns to 60% values at 11 days and is equivalent to the PMMA preparation in which the drug; polymer ratio is 1:10. When the drug comprises 16% of the formulation the PMMA preparation equalled the performance of the best nylon 6:10 preparations previously reported (Florence, Jenkins & Loveless 1976). This PMMA formulation is effective in producing 100% inhibition of the response to the apomorphine challenge at 3 days whereas the other preparations begin to show a decreased effectiveness at this time.

Dissolution rates of drug were measured by stirring the powdered material in buffer at pH 7·4; results in Fig. 2 are compared with dissolution from drug particles precipitated from DMF without polymer. These results suggest that reduction of dissolution below a certain value (which appears to be about 4×10^{-8}M min^{-1}) diminishes the biological effectiveness of the formulation. Obviously if release of drug is too slow effective concentrations of drug are not achieved because of the relatively rapid metabolism of the drug species. A satisfactory *in vitro* model would require simulation of removal of drug from the cir-

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