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Prostaglandins and the anti-inflammatory activities of aspirin and sodium salicylate

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Acetylsalicylic acid (aspirin) and sodium salicylate are equally effective in reducing the swelling in the carrageenan-induced paw test and the accumulation of leucocytes into the inflammatory exudate produced by the subcutaneous implantation of polyvinyl sponges in the rat. Aspirin but not sodium salicylate caused a significant reduction in the potentiation of paw oedema found after the concurrent administration of carrageenan and arachidonic acid. Some implications of these findings are discussed.

Aspirin inhibits the activity of prostaglandin synthetase *in vitro* and reduces the biosynthesis of prostaglandins in a variety of systems (Vane, 1971). It has been proposed that this enzyme inhibition explains many of the therapeutic effects, such as analgesia, antipyresis and the antirheumatic properties both of aspirin and of other non-steroidal anti-inflammatory drugs (Vane, 1973). Evidence in support of this hypothesis is of two general kinds. Firstly, the prostaglandins have been implicated either as mediators or modulators of pain, fever and inflammation. Secondly, it has been reported that there is a good correlation between the potencies of anti-inflammatory drugs as inhibitors of prostaglandin synthetase *in vitro* and their peak concentrations found in plasma after therapeutic dosage (Moncada, Ferreira & Vane, 1973). One drug of particular interest is sodium salicylate which is apparently as effective as aspirin against experimental inflammation and in the treatment of rheumatoid arthritis (Collier, 1969) but has little, if any, inhibitory activity against prostaglandin synthetase *in vitro* (Vane, 1971). In the present work we have compared the effects of aspirin and sodium salicylate on two models of inflammation in the rat, i.e., carrageenan-induced paw oedema and the accumulation of leucocytes into the inflammatory exudates of subdermally implanted inert sponges. The interactions of the two drugs on an inflammation specifically modulated by prostaglandins *in vivo* was studied using the potentiation of the carrageenan-induced paw swelling by arachidonic acid.

MATERIALS AND METHODS

Female albino Wister rats (Oxfordshire Laboratory Animal Colonies, Southern Ltd.) 150–200g, were used for anti-inflammatory testing. Carrageenan was a gift from Viscarin Marine Colloids, acetylsalicylic acid and arachidonic acid (Grade 1) were obtained from the Sigma Chemical Co. and the sodium salicylate was of British Pharmacopoeial grade. The methods used for the carrageenan paw odema test and its potentiation by arachidonic acid were those of Winter, Risley & Nuss (1962) and Lewis, Nelson & Sugrue (1974) modified according to the directions of Smith, Ford-Hutchinson & others (1974). The implanted sponge technique was that described by

Ford-Hutchinson, Smith & others (1975), except that polymorphonuclear and mononuclear leucocytes were counted together. The aspirin and sodium salicylate were administered orally as either solutions or suspensions in 1·5 ml distilled water in doses of either 50 or 200 mg kg^{-1} body weight. In the paw oedema experiments they were given 1 h before either the carrageenan or carrageenan-arachidonic acid mixture and in the sponge experiments immediately before implantation, the sponges being removed after 9 h. In the corresponding control groups each rat received orally an equal volume of water at similar times.

RESULTS

The results for the carrageenan-induced paw oedema (Fig. 1) show that the 50 and 200 mg kg^{-1} doses of aspirin and sodium salicylate produced almost identical reductions in the development of the paw swelling. At 3 h the values found after the 50 mg doses were significantly different ($P < 0{\cdot}01$) from the corresponding control values and the 200 mg doses caused a further significant decrease ($P < 0{\cdot}001$). Similar results were observed in the sponge experiments (Table 1).

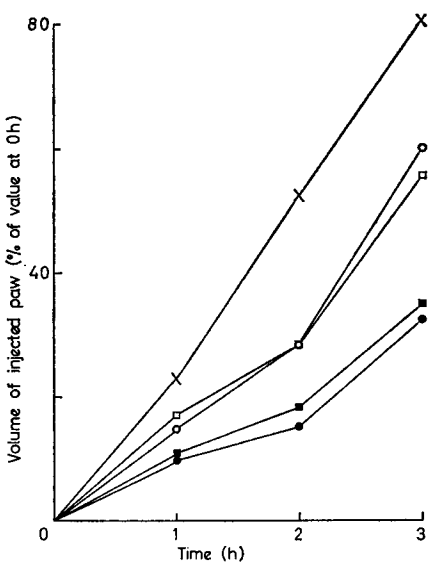

FIG. 1. Effects of aspirin and sodium salicylate in carrageenan-induced paw oedema in the rat. Results calculated as volume of paw as percentage of corresponding volume at 0 h. They are given as means for the following groups, each of 5 animals: ×——× saline control, □——□ aspirin 50 mg kg^{-1}, ○——○ sodium salicylate 50 mg kg^{-1}, ■——■ aspirin 200 mg kg^{-1}, ●——● sodium salicylate 200 mg kg^{-1}. In this and the subsequent figure the values between the various groups have been analysed by the *t*-test. A statistically significant difference ($P < 0{\cdot}01$) at one or more of the time intervals studied has been taken to represent a significant effect of the particular treatment.

In contrast, only aspirin produced an effect (Fig. 2) on the enhanced response to carrageenan caused by the concurrent administration of arachidonic acid. The potentiating effect of the prostaglandin precursor on the paw swelling over the period 15 to 60 min was reduced by a significant ($P < 0{\cdot}01$), and almost equal, extent by both dose levels of aspirin but was not affected by the administration of sodium salicylate.

Table 1. *Effects of aspirin and sodium salicylate on leucocyte migration into the 9 h sponge exudate.* Results given as means with s.d. and expressed as counts × 10^4 per ml of exudate. Each group contained 6 rats.

Treatment	Dose (mg kg^{-1})	Number of cells
Control		1158 s.d. 144
Aspirin	50	813 s.d. 200 *
Sodium salicylate	50	863 s.d. 187 *
Aspirin	200	597 s.d. 194 **
Sodium salicylate	200	599 s.d. 101 **

* $P < 0.01$ compared to control value.
** $P < 0.001$ compared to control value.

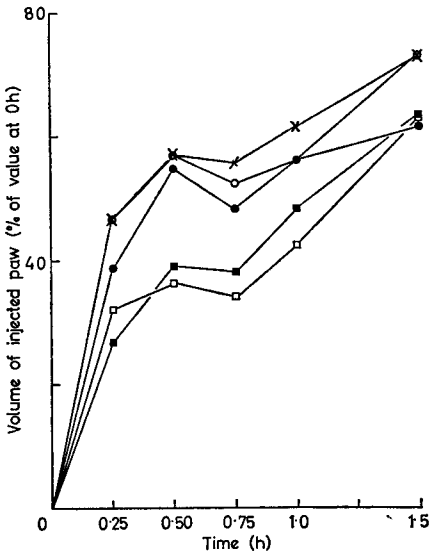

FIG. 2. Effects of aspirin and sodium salicylate in carrageenan-induced paw oedema in the rat potentiated by arachidonic acid. Results calculated and expressed as in FIG. 1 for the following groups, each of 5 rats; ×——× saline control, □——□ aspirin 50 mg kg^{-1}, ○——○ sodium salicylate 50 mg kg^{-1}, ■——■ aspirin 200 mg kg^{-1}, ●——● sodium salicylate 200 mg kg^{-1}.

DISCUSSION

The inhibitory action of aspirin and aspirin-like drugs on prostaglandin production has been demonstrated in a number of biological preparations and laboratory species (Ferreira & Vane, 1974). There appears to be a good correlation between the *in vitro* inhibition of prostaglandin synthetase and the *in vivo* anti-inflammatory activity in tests such as the carrageenan-induced rat paw oedema (Flower, Gryglewski & others, 1972). The free, i.e. non protein-bound, concentrations of aspirin in human plasma after the oral administration of therapeutic doses are more than sufficient to induce prostaglandin synthetase inhibition (Ferreira & Vane, 1974). It has also been shown that prostaglandins of the E series are able to sensitize blood vessels to the permeability-increasing effects of other mediators locally released by carrageenan. Removal of endogenous prostaglandins has therefore been proposed as a mechanism to explain the anti-oedema effects of aspirin-like drugs (Moncada & others, 1973).

The results of the present work appear to support this mechanism of action for a pirin but not for sodium salicylate. Equivalent doses of aspirin not only inhibit the development of the paw swelling in the carrageenan test (Fig. 1) but also reduce he potentiation of paw oedema induced over the first two hours by the concurrent administration of arachidonic acid (Fig. 2). However, in the latter reaction the effects of the 50 and 200 mg kg^{-1} doses of aspirin were identical whereas in the carrageenan-induced paw oedema test the higher dose produced a significantly greater effect. This finding suggests that there may be more than one component in the anti-carrageenan activity of aspirin in the rat since the 50 mg kg^{-1} dose appears to be fully capable of blocking prostaglandin synthesis in the paw after the local administration of a substantial quantity (100 μg) of the prostaglandin precursor. An allied and pertinent query is why the relatively small doses (600 mg) of aspirin used to produce analgesia in man are not sufficient to exert antirheumatic effects via inhibition of prostaglandin formation. It is known (Boardman & Hart, 1967) that aspirin in sufficient dosage exerts a clinical antirheumatic action distinct from its analgesic effect and that the *in vitro* inhibitory effects of aspirin on prostaglandin production are elicited by the concentrations attained after analgesic doses. Indeed a striking effect on the thrombin-induced production of prostaglandin in human blood platelets was found in subjects who took single doses of 600 mg of aspirin (Smith & Willis, 1971).

A further difficulty is the equipotency of aspirin and sodium salicylate as anti-rheumatic and anti-inflammatory agents *in vivo* compared to their different activities as inhibitors of prostaglandin synthetase *in vitro*. Clinical experience has shown that aspirin and sodium salicylate are equally effective when given in adequate chronic doses in the treatment of acute rheumatic fever and rheumatoid arthritis (Woodbury, 1965). Aspirin is usually preferred because it is more conveniently administered in a palatable form and is a more powerful analgesic. Sodium salicylate and aspirin are equipotent in a number of experimental oedemas of the foot and ankle in rodents, including those induced by yeast, formalin and dextran, and in the adjuvant arthritis test in the rat (Collier, 1969). The present work (Fig. 1 and Table 1) shows this similarity also extends to the carrageenan-induced paw oedema and the accumulation of leucocytes in the implanted sponges in the rat. Willis, Davison & others (1972), using the carrageenan-induced air bleb technique, have also shown that both drugs are equally effective. In contrast to these *in vivo* findings salicylate is inactive or only very weakly active, as an inhibitor of prostaglandin synthesis *in vitro* (Vane, 1971; Willis & others, 1972).

Several suggestions have been advanced to explain this discrepancy. One is that different tissues may contain isoenzymes of prostaglandin synthetase which not only produce varying patterns of prostaglandins from different substrates but have differing sensitivities to various inhibitors (Vane, 1971). Thus salicylate could inhibit a prostglandin synthetase in a tissue affected by an inflammatory process but need not affect the enzyme activity in another tissue, such as the spleen. However, Willis & others (1972) examined the effects of aspirin and sodium salicylate both on the prostaglandin content of carrageenan-induced air bleb exudate in the intact rat and on the activity of prostaglandin synthetase in a broken cell suspension prepared from the same exudate. The two drugs produced a diminution of prostaglandin production *in vivo* but only aspirin inhibited the enzyme activity *in vitro*.

It has also been proposed that sodium salicylate is converted *in vivo* to an active metabolite, i.e., one which is both anti-inflammatory and inhibits prostaglandin synthetase (Willis & others, 1972). A major metabolite of salicylate in the rat is gentisic acid (Quilley & Smith, 1952) and this is not only ineffective in various experimental inflammatory tests (Adams & Cobb, 1967) but has been stated (Bywaters, 1963) to be useless as a therapeutic agent. A second metabolite, salicyluric acid, has been shown to be inactive in a passive Arthus reaction in the rat whereas sodium salicylate possesses anti-inflammatory activity in this test (Ungar, Damgaard & Hummel, 1952). The remaining metabolites are glucuronic acid conjugates of salicylic acid plus traces of gentisyl glucuronides. These substances do not appear to have been tested either as anti-inflammatory agents or as inhibitors of prostaglandin synthetase. In the whole animal, aspirin is rapidly hydrolysed to salicylate and subsequently gives rise to the same pattern of metabolites (Levy & Leonards, 1966). The proposal of Willis & others (1972) therefore implies that both aspirin itself and one of its metabolites, either a salicylate glucuronide or unknown substance, act independently as inhibitors of prostaglandin biosynthesis.

A third explanation is that a component of the anti-inflammatory activity of aspirin, sodium salicylate and other non-steroidal drugs is exerted through a mechanism not involving prostaglandins. One possibility is an interference with the migration of blood leucocytes into inflammatory exudates. It is becoming clear that invasion of an inflamed area by polymorphonuclear and mononuclear leucocytes is an important factor in prolonging inflammation. As soon as leucocytes have accumulated and deteriorate a vicious circle may develop due to the release of lysosomal enzymes which can degrade macromolecules and liberate mediators, including prostaglandins, and also chemotactic factors (Cochrane & Janoff, 1974; Vogt, 1974). Although it has been reported (Di Rosa, Sorrentino & Parente, 1972) that aspirin and similar drugs affect only the migration of monocytes, the work of Ford-Hutchinson & others (1975) shows that aspirin, phenylbutazone and indomethacin inhibit the migration of both polymorphonuclear and mononuclear cells into the exudates of implanted sponges. The results of the present work (Table 1) show that aspirin and sodium salicylate have similar actions in the same system. It has been claimed that prostaglandins are chemotactic and may act as mediators in leucocyte emigration (Kaley & Weiner, 1971). However, McCall & Youlten (1973) found that only PGE_1 possesses chemotactic properties for polymorphonuclear leucocytes, the maximal response being at a concentration of 1 μg ml^{-1}. In contrast, the concentrations of all prostaglandins in the exudates formed over 24 h in the carrageenan-induced air bleb in the rat did not exceed 24 ng ml^{-1} (McCall & Youlten, 1974). Other data have been reviewed by Ferreira & Vane (1974) who have stated that there is no conclusive evidence that prostaglandins are leucotactic in an inflammatory process and by Ward (1974) who concluded that the reports of leucotactic activity of the prostaglandins have been remarkable for the low levels of such activity found.

It is suggested that an important component of the experimental anti-inflammatory action of sodium salicylate and aspirin is an interference with cellular infiltration (see Vinegar, Macklin & others, 1974). The mechanism of this action does not involve a primary effect on prostaglandin synthetase and the *in vivo* effects of sodium salicylate on the prostaglandin content of inflammatory exudates (see Willis, & others, 1972) is secondary to an initial action on leucocyte emigration.

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