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COMMUNICATIONS

Inhibition of leucocyte migration by salicylates and indomethacin

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Migration of leucocytes into an inflamed pleural space (Di Rosa & others, 1972; Velo & others, 1973) or into subcutaneously implanted polyvinyl sponges (Ford-Hutchinson & others, 1975) is well documented. The response is characterized by an early influx of polymorphonuclear cells over the first 5 h and a late migration of mononuclear cells within 24 h. The ability of non-steroidal anti-inflammatory drugs (NSAID) to inhibit this migration of leucocytes is considered by some workers to be an important component of NSAID action (Smith & others, 1975) and others have shown that NSAID preferentially inhibit the mononuclear influx (Di Rosa & Willoughby, 1971). However, Vinegar (1974) has pointed out that the doses of some drugs required to inhibit leucocyte migration exceed those used therapeutically, and Meacock & Kitchen (1976) reported that, while they found indomethacin to show a preferential effect on mononuclear migration, this was not a general property of NSAID. Furthermore, the activity of salicylates in these cell migration models has been found to be inconsistent (Brune, 1977).

We have tested the effects of salicylates and indomethacin in two models of leucocyte migration: (1) the pleural space of rats which has been inflamed by carrageenan administered 24 h previously and (2) polyvinyl sponges subcutaneously implanted into rats 5 h previously.

Groups of 5 male Wistar rats (150–200 g) obtained from the Tuck colony were used. Initially, the ability of aspirin, sodium salicylate, octanoyl salicylic acid (8-SA) and indomethacin to inhibit oedema induced by subcutaneous injections of carrageenan (1 mg in 0·1 ml 0·9% w/v saline) into the right hind-paws was determined, by administering them suspended or dissolved in 0·5% (w/v) gum tragacanth, orally both at 24 h and at 1 h before the inflammatory stimulus (Ford-Hutchinson & others, 1976). In these conditions, the doses producing about 50% inhibition were sodium salicylate (200 mg kg^{-1}), aspirin (225 mg kg^{-1}, 8-SA (330 mg kg^{-1}), and indomethacin (10 mg kg^{-1}).

For the cell migration studies, carrageenan (1 mg in 0·1 ml sterile 0·9% w/v sodium chloride) was injected into the pleural space of rats under light ether anaesthesia and 24 h later the exudates were withdrawn using plastic pipettes. After each exudate was mixed with an equal volume of heparinized Isoton (Coulter Electronics), total leucocyte counts were made using the Coulter Counter and differential counts were recorded on smears fixed in methanol, stained with Giemsa stain and differentiated in double phosphate buffer (pH 6·8). In other rats, polyvinyl sponges were implanted simultaneously (Ford-Hutchinson & others, 1975) and total and differential leucocyte counts carried out, as described previously, 5 h after implantation.

Inhibition of pleural leucocyte infiltration. From a mean resting cell population in the pleural space of 4·5 ± 0·8 $\times 10^6$ leucocytes per rat (mean ± s.e.m.) (96% of which were mononuclear cells, mostly lymphocytes), carrageenan induced a migration, 24 h later, of 80 ± 2·5 $\times 10^6$ leucocytes per rat (40% of which were mononuclear cells). The salicylates and indomethacin produced dose-dependent inhibitions of this migration. The results for aspirin and indomethacin are shown in Fig. 1 and they compare well with those already reported for the two drugs in a dextran pleurisy model (Di Rosa, 1974). The results show that therapeutic doses of indomethacin (2·5 mg kg^{-1}) do not inhibit cell migration whereas those of aspirin (50 mg kg^{-1}) exert a significant inhibition. Differential counts made after administration of these two drugs (Fig. 2) indicate that doses of indomethacin above 2·5 mg kg^{-1} inhibit the migration of both polymorpho- and mono-nuclear cells (the former particularly at the highest doses used) whereas the lower doses of aspirin preferentially inhibit mononuclear cell migration. Results with sodium salicylate and 8-SA closely followed those obtained with aspirin.

These results were obtained during the winter months (January–March) but when the experiments were repeated in the summer months (June–August) all three salicylates were unexpectedly inactive at all doses used whereas indomethacin retained its inhibitory activity (Fig. 3). Further studies during the period October to November indicated a partial return of activity for the salicylates at that time of year. The results shown in

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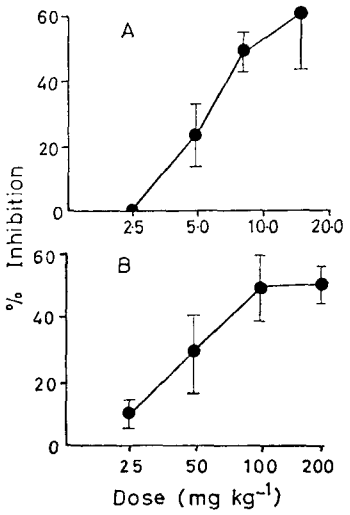

FIG. 1. Percentage inhibition (± s.e.m.) by indomethacin (A) and by aspirin (B) of cell migration into the pleural space of rats after intrapleural carrageenan given 24 h previously. Time of year was January to March.

Fig. 3 were obtained using doses of compounds previously found to be equi-active at all times of the year in producing about 50% inhibition of carrageenan paw oedema (anti-inflammatory activity). The reason for the loss of activity of the salicylates in the summer against leucocyte migration induced by intrapleural carrageenan has not so far been identified.

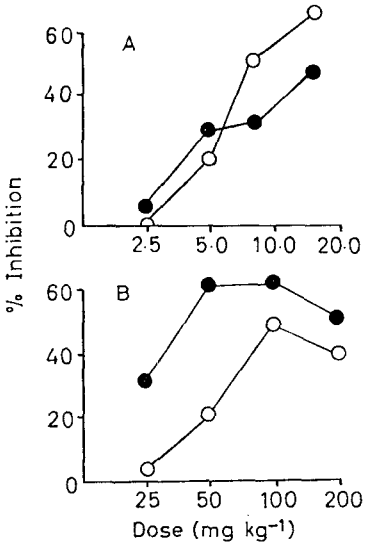

FIG. 2. Percentage inhibition by indomethacin (A) and by aspirin (B) of migration of polymorpho- (O) and mono-nuclear cells (●) into the inflamed pleural space of rats. Time of year was January to March.

Inhibition of leucocyte infiltration into sponges. When tests were carried out to determine the inhibitory activity on leucocyte migration into implanted polyvinyl sponges, a similar result to that found in the pleural space was obtained. In untreated rats, the mean leucocyte count (±s.e.m.) in the sponges at 5 h was $72 \pm 3{\cdot}3 \times 10^4$ and 10 mg kg^{-1} indomethacin consistently reduced this value by 80–90% (mean value, $10 \pm 2{\cdot}9 \times 10^4$ cells). The three salicylates were again ineffective in the period June to August, just as they were against leucocyte infiltration into the pleural space.

Whereas indomethacin in the dose range of 5–20 mg kg^{-1} inhibited leucocyte migration in both models tested at all times of the year, the three salicylates produced results which varied with the time of year. This seasonal variation may well explain some of the conflicting results reported in the literature (Brune, 1977). When the salicylates were effective in both models (January to March), they preferentially inhibited the migration of mononuclear cells in contrast to indomethacin which inhibited both polymorpho- and mononuclear cells.

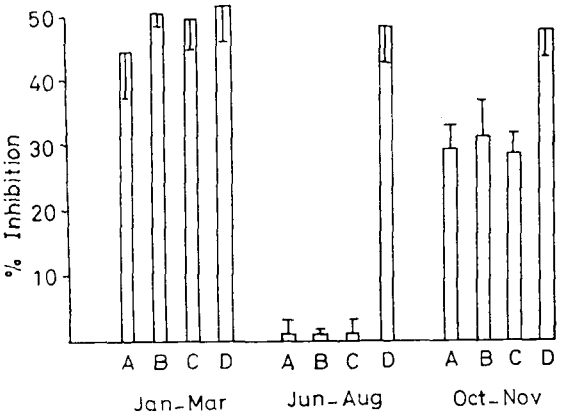

FIG. 3. Percentage inhibition (± s.e.m.) by sodium salicylate (A, 200 mg kg^{-1}), aspirin (B, 225 mg kg^{-1}), 8-SA (C, 330 mg kg^{-1}) and indomethacin (D, 10 mg kg^{-1}) at different times of the year of cell migration into the inflamed pleural space of rats. There is a highly significant loss of activity of the salicylates in the summer months.

Differences in drug performance have previously been noted by other workers. For example, Bonta & others (1977) found that the depressed response to carrageenan in the paws of rats fed on a diet deficient in essential fatty acids was further depressed by aspirin but not by indomethacin. Yet another difference between the two types of drug has been reported by Hayden & others (1978) who found gastric lesions could be induced in rats with aspirin given orally whereas with indomethacin the lesions arose whatever route was used.

Differences in activation of complement-derived chemotactic factors (Di Perri & Auteri, 1974) may be part of the explanation for the differences seen in the inhibition of leucocyte migration. The inhibitory action of aspirin (possibly on C1 esterase) may be by-passed when carrageenan activates complement via the alternate pathway in the pleural model whereas that of indomethacin may not be so by-passed.

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Alterations in the vascular compartment with acute ethanol treatment

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While investigating *in vivo* effects of ethanol on rat plasma, we became concerned about possible changes in the vascular compartment of treated animals. We therefore investigated the effects of three doses of ethanol on extravasation of large molecules from the vascular to the peritoneal compartment of rats. Ethanol, especially by intraperitoneal injection, effected considerable changes in the protein and Evans Blue content of the two fluid compartments, suggesting a shift in molecules from the blood to the peritoneal compartment, but only partially as a result of the local irritant or osmotic properties of ethanol.

Male Sprague Dawley rats (250–300 g) (Holtzman) were housed singly for one week before use in a room maintained on a 12:12 light/dark cycle and at 21°. Evans Blue was injected intravenously via the tail vein (2·5 g kg^{-1}). Ethanol (10 or 20% solution) was injected intraperitoneally or given intragastrically. Control rats received the equivalent volumes of saline. In most experiments, animals were decapitated and blood was collected into heparinized tubes. Haematocrits were rapidly determined by means of an International microcapillary centrifuge and reader. Blood was centrifuged at 8000 *g* to separate the cellular elements, and plasma was immediately separated and frozen at −20°. In some experiments, animals were killed by cervical dislocator (Wausau, WI 54401) and the peritoneal fluid was examined.

Peritoneal fluid was collected according to Dolphin, Elliott & Jenner (1976). Two ml of heparinized saline was introduced through a small slit made through the peritoneal wall. The hole was kept closed while the body was rocked gently from side to side to mix the peritoneal contents. The abdominal wall opening was then enlarged and the peritoneal fluid removed.

Evans Blue in plasma and in the exudate was measured spectrophotometrically. Absorbance of exudates was read at 605 nm after dilution (2·5×) with water

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