J. Pharm. Pharmacol. 1984, 36: 171–174
Received July 22, 1983

Studies into the mode of action of non-steroidal anti-inflammatory drugs using a model of facsimile synovium

A. D. SEDGWICK*, Y. M. SIN, A. R. MACKAY, A. AL-DUAIJ AND D. A. WILLOUGHBY

Department of Experimental Pathology, St Bartholomew's Hospital Medical School, London EC1, UK.

The effect of non-steroidal anti-inflammatory drugs on inflammation produced in the 6-day old air pouch by carrageenan or calcium pyrophosphate crystals has been examined. The system is a very reproducible way of studying the cellular and vascular components of inflammation. Non-steroidal drugs have different profiles of activity when tested on the inflammation produced with the two irritants. In general, however, the tested compounds showed similar activity on individual models.

Many therapeutic agents have been designed with the object of reducing the inflammation seen in human synovitis. Vane (1971) suggested that acidic anti-inflammatory drugs (NSAID) owe their activity to inhibition of prostaglandin synthetase. This resulted in the development of many compounds capable of blocking this enzyme activity. It is becoming increasingly evident that the mechanism of action of NSAIDs is not solely dependent on the blockade of prostaglandin synthetase. To elucidate the detailed mode of action of these drugs, animal models of inflammation are necessary. In rheumatoid disease inflammation is primarily seen in synovial cavities of affected joints. There is, therefore, a need for models of inflammation similar to human synovitis. Our recent observation that the simple injection of air into the dorsal surface of rodents can produce a tissue with features of synovium (Edwards et al 1981) has led us to suggest that this tissue may be useful for examining anti-inflammatory drug activity.

MATERIALS AND METHODS

Animals

Male Wistar rats, 200–250 g, were used. For each experiment not less than 6 rats were used per observation. Experiments were repeated 3 times.

Air cavities

Simple air cavities were produced using the method of Edwards et al (1981). Rats were injected with 20 ml of air into the subcutaneous tissue of the back. Subsequently 10 ml of air was injected into the same cavity every 3 days to keep the cavity open. Six days after initial air injection animals were re-anaesthetized and various irritants introduced using a sterile syringe and needle. Groups of animals were killed at 2, 4, 6 and 24 h after irritant injection. The total leucocyte number and the volume of exudate were measured. Smears of exudate cells were stained with May Grunwald Giemsa stain and differentially counted.

* Correspondence.

Irritants

Carrageenan, Viscarin 402 (lot 272500) was kindly provided by Marine Colloids Springfield USA. This was suspended in sterile 0·9% NaCl (saline) at a concentration of 10 mg ml^{-1} for injection. The suspension was stirred continually at 37 °C so that the injected mixture was homogeneous. Each animal was injected with 1 ml of the suspension.

Calcium pyrophosphate dihydrate crystals

These were prepared by the method of Denko & Whitehouse (1976). The crystals were suspended in sterile saline and ultrasonicated at 15 000 Hz for 5 min at room temperature (20 °C). The crystals were checked by polarized light microscopy before use. Crystal suspensions were stirred continually at 37 °C before injection. Each animal was given 10 mg of crystals suspended in 5 ml of saline. As this irritant was suspended in a volume of 5 ml it was necessary to subtract this volume from that of the harvested exudate.

Drugs

Indomethacin, benoxaprofen, piroxicam and aspirin were administered orally suspended in water to animals, 24 and 1h before irritant injection.

RESULTS

Inflammation produced in the 6 day old air pouch was found to be very reproducible, whether car-

rageenan or CPPD crystals were used as the irritant. Fig. 1A–D shows the results of experiments where the total number of leucocytes in response to irritants were compared in normal and NSAID-treated animals. In control animals carrageenan produced a substantial influx of leucocytes into the cavity. The maximum influx (approx. 300 × 10^6 cells) occurred between 4–24 h after carrageenan injection. Aspirin, benoxaprofen, piroxicam and indomethacin at the higher doses used significantly inhibited total leucocyte influx between 4–6 h after carrageenan injection. After 6 h no significant reduction could be detected with any of these compounds. With CPPD crystals maximum influx could be detected at 6 h (approx. 110 × 10^6 cells) after this time the response was observed to plateau. NSAID treatment of animals before crystal injection caused some inhibition of cell influx between 4–6 h but maximum reduction was detected with all compounds 24 h after irritant injection. Differential counts made on exudate smears showed no significant changes in the ratio of polymorphonuclear leucocytes to mononuclear cells in response to either irritant injection following NSAID treatment (Table 1).

Table 1. Ratio of polymorphonuclear leucocytes to mononuclear cells at different times after irritant injection into 6 day old air pouch.

Irritants	Cell types	Cell ratio (%) Time (h) 2	4	6	24
Carrageenan	Polymorphonuclear leucocyte	90	98	95	74
	Mononuclear cell	10	2	5	26
Calcium pyrophosphate crystals	Polymorphonuclear leucocyte	74	97	93	85
	Mononuclear cell	26	3	7	15

Fig. 2A–D show the results of experiments where the volume of exudate i.e. oedema was measured in response to irritant injection. Carrageenan induced greater exudation than CPPD crystals, the response to carrageenan reaching maximum at 24 h (approx. 2 ml) whereas the response to CPPD reaches maximum at 4–6 h (approx. 0·5 ml). In general a similar pattern of reduction in exudate volume was found using all the NSAIDs and these results corresponded to the ability of these compounds to reduce leucocyte influx. Aspirin and indomethacin (Fig. 2B, D) were found to have some activity at reducing exudate formation 24 h after irritant injection.

DISCUSSION

Various animal models have been used to examine the mode of action of non-steroidal anti-

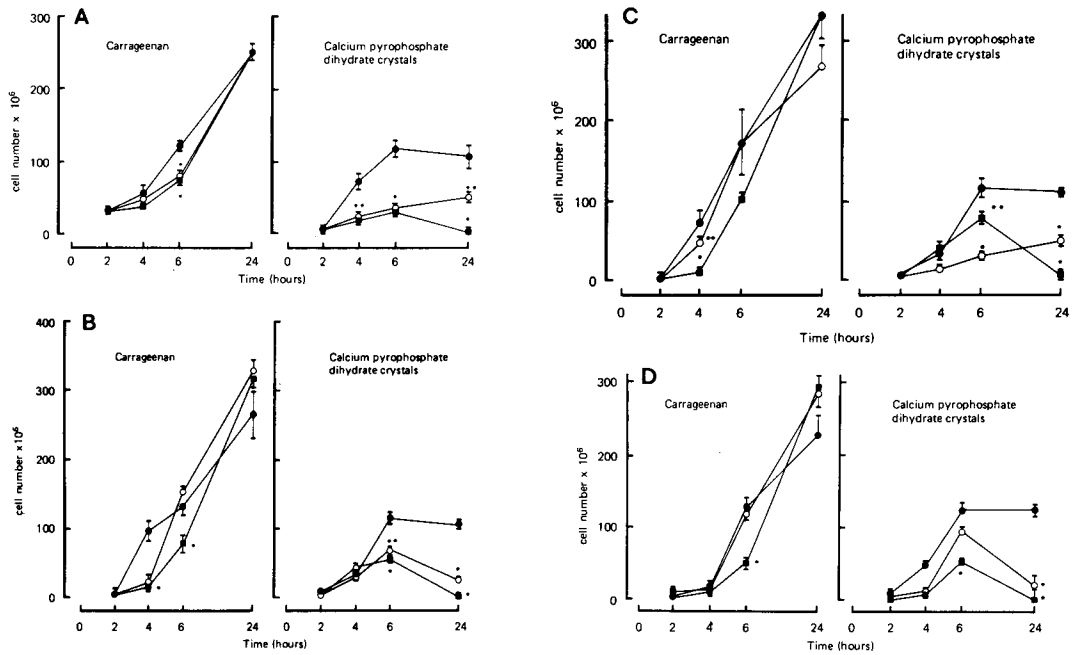

FIG. 1. Total leucocyte number in response to various irritants following treatment with (A) indomethacin (1, 3 mg kg); (B) benoxaprofen (10, 25 mg kg^{-1}); (C) piroxicam (0·5, 5 mg kg^{-1}); (D) aspirin (100, 200 mg kg^{-1}); ●—● = control, ○—○ low dose, ■—■ high dose; $*P < 0{\cdot}001$, $**P < 0{\cdot}01$.

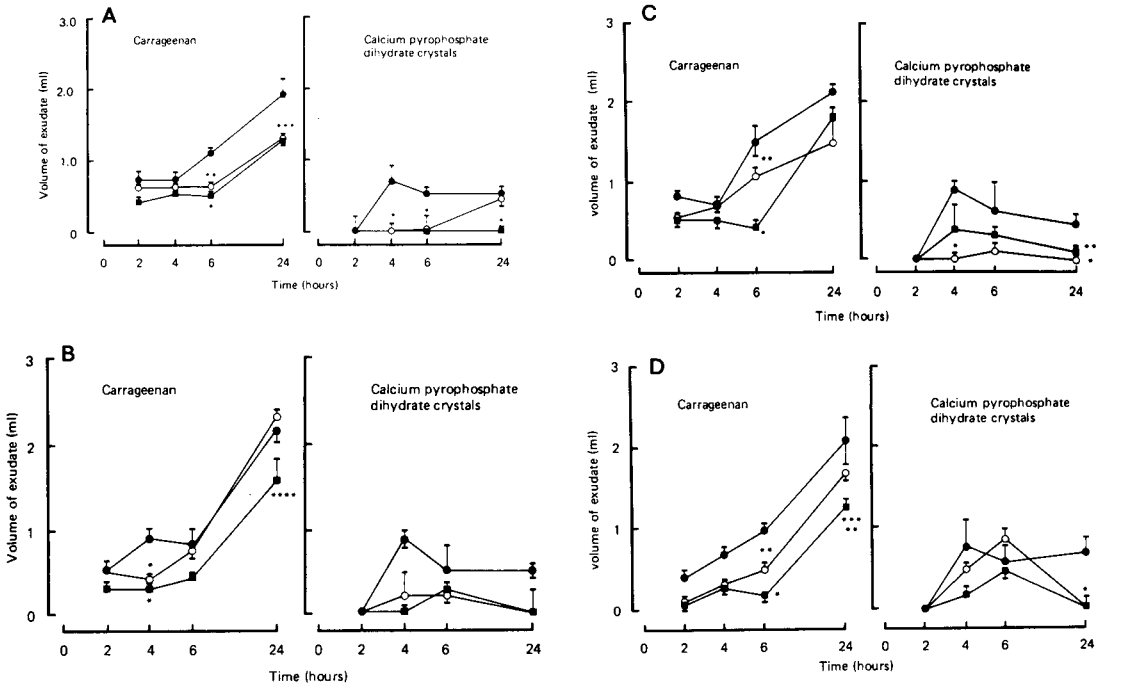

FIG. 2. Volume of exudate in resonse to various irritants following treatment with (A) indomethacin (0·1, 3 mg kg^{-1}, ***P < 0·02, **P < 0·01, *P < 0·001); (B) benoxaprofen (10, 25 mg kg^{-1}, *P < 0·01, ****P 0·05); (C) piroxicam (0·5, 5 mg kg^{-1}, *P < 0·001, **P < 0·01); (D) aspirin (100, 200 mg kg^{-1}), *P < 0·001, **P < 0·01, *****P < 0·1); ●—● control, ○—○ low dose, ■—■ high dose.

inflammatory drugs (NSAID). Such models as paw oedema, experimental pleurisy and sponge implants have been widely used, each method having its advantages and disadvantages. Paw oedema (Winter et al 1962), for example, is a useful measure of anti-oedema activity of drugs but does not allow for the detailed examination of the effects on cells. Experimental pleurisy (Willoughby 1975), on the other hand, allows examination of both fluid and cellular parameters but is both time-consuming and technically more difficult to perform. Polyester sponges (Higgs et al 1979) have the further disadvantage of producing foreign body reactions. We have found that the injection of air into the dorsal surface of rats leads to the formation of an air pouch with lining cells closely resembling synovial cells of both Type A and Type B (Edwards et al 1981). Intra-articular injections in small rodents have been used to study synovial cell response to injury, however these are fraught with hazard and often inadvertently produce varying amounts of non-specific damage. The air pouch permits the easy study of injury in synovial-like tissue. We have previously shown that according to the age of the pouch, it progressively develops phagocytic and fibroblast-like lining cells, followed by organized vasculature and finally acts as a mechanical barrier which retains the products of the inflammatory response (Sedgwick, Sin, Edwards and Willoughby, in the press). The 6 day old air pouch would seem to be an ideal model to test for anti-inflammatory activity as it mimics so closely the human synovium. The present results have confirmed the use of this model for studying both the effects of drugs on the cellular and oedematous components of inflammation. The added advantages of this model are its reproducibility and technical ease in induction as well as the results obtained being a more true reflection of what may be going on in synovial-like tissue.

The literature contains a vast amount of work which has been carried out to elucidate the mechanism of action NSAIDs, however it still remains unclear. Most studies which have examined leucocyte migration in-vivo using animal models have concentrated on examining the inflammatory response at single time intervals after irritant injection. As inflammation is a dynamic process, the ratio of polymorphonuclear leucocytes (polymorphs) to

mononuclear cells and the concentration of various mediators varying with time (Di Rosa et al 1971a) it is possible that the activity of drugs on leucocyte migration may be missed unless full time course studies are conducted. The present results show the importance of studying the response of different non-steroidal anti-inflammatory drugs at various time intervals particularly where different irritants are used.

The experiments reported here show that NSAIDs can reduce the migration of leucocytes in response to complement dependent i.e. carrageenan or complement independent, i.e. CPPD (Di Rosa et al 1971b; Willoughby et al 1975) irritants. The correlation between leucocyte inhibition and inhibition of oedema formation supports the concept that NSAIDs may be acting by inhibiting oedema formation rather than direct action on cell movement (Ammendola et al 1975; Blackham & Owen 1975).

The six day air pouch would seem to be a useful model to be used in the search for new anti-inflammatory agents. It has the advantage that the lining cells of the air pouch closely resembles synovium and it is easy to harvest exudate and cells and to perform analyses upon them. Thus in conclusion, this type of model may be of value in our further understanding of the mode of action of non-steroidal anti-inflammatory drugs.

Acknowledgement

We are grateful to the Arthritis and Rheumatism Council for their support.

REFERENCES

Ammendola, G., Di Rosa, M., Sorrentino, L. (1975) Agents Actions 5: 250–255

Blackham, A., Owen, R. T. (1975) J. Pharm. Pharmacol. 27: 201–202

Denko, C. W., Whitehouse, M. W. (1976) J. Rheumatol. 3: 54–62

Di Rosa, M., Giroud, J. P., Willoughby, D. A. (1971a) J. Pathol. 104: 15–29

Di Rosa, M. Papadimitriou, J. M., Willoughby, D. A. (1971b) Ibid. 105: 239–256

Edwards, J. C. W., Sedgwick, A. D., Willoughby, D. A. (1981) J. Pathol. 134: 147–156

Higgs, G. A., Flower, R. J., Vane, J. R. (1979) Biochem. Pharmacol. 28: 1959–1961

Vane, J. R. (1971) Nature (London) New Biol. 231: 232–235

Willoughby, D. A. (1975) Ann. Rheum. Dis. 34: 471–478

Willoughby, D. A., Dunn, C. J., Yamamoto, S., Capasso, F., Deporter, D. A., Giroud, J. P. (1975) Agents Actions 5: 35–39

Winter, C. A., Risley, E. A., Nuss, G. W. (1962) Proc. Soc. Exp. Biol. (NY) 111: 544–547