Agents and Actions
vol. 6/5 (1976)
Birkhäuser Verlag, Basel

Anti-Inflammatory Drugs, Prostaglandins and Leucocyte Migration

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Abstract

The action of some non-steroidal acidic anti-inflammatory drugs, aspirin, phenylbutazone and indomethacin, in reducing leucocyte migration into the exudates of inert porous sponges implanted subdermally in the rat has been shown to be distinct from their effect in reducing the content of prostaglandins in the exudates. It is concluded that a component of the anti-inflammatory and antirheumatic actions of the drugs is concerned with a mechanism other than inhibition of prostaglandin biosynthesis.

Introduction

Non-steroidal anti-inflammatory drugs, including aspirin, indomethacin and phenylbutazone, share a number of actions on several aspects of inflammatory responses. They inhibit the activity of prostaglandin synthetase preparations in vitro [1], reduce the content of prostaglandins in developing inflammatory exudates in vitro [2] and suppress the emigration of leucocytes, in particular that of polymorphonuclear cells, into such exudates [3, 4]. In acute paw oedema reactions, such as that induced by carrageenan, there is evidence that the drugs preferentially affect phases of the developing inflammation associated with prostaglandin production and leucocyte emigration [5, 6]. It is possible to construct a unifying hypothesis to explain both the inter-relationship and sequence of these effects. The primary action of the drugs is to inhibit prostaglandin synthetase activity and hence to reduce the biosynthesis of prostaglandins in vivo. The main source of the prostaglandins in the later phases of carrageenan-induced paw oedema and in other models, such as carrageenan pleurisy, is the polymorphonuclear leucocyte which releases E-type prostaglandins during phagocytosis [7, 8]. The released prostaglandins not only enhance the impaired vascular permeability by potentiating the effects of other mediators [9, 10] but also sustain the inflammatory reaction by acting as chemotactic factors [11] and causing more leucocytes to accumulate in the inflamed site. A key stage in this chain of events in the ability of prostaglandins to function as leucotactic agents in vivo. There is general agreement that prostaglandins A_1, E_2 and $F_{2\alpha}$ are devoid of such activity in vitro at concentrations up to 100 μg ml^{-1} [12, 13] but it has been claimed that prostaglandin E_1 is chemotactic towards polymorphonuclear leucocytes at concentrations down to 10 ng ml^{-1} [14] which is within the concentrations of total prostaglandins present in inflammatory exudates of various types [15]. It has recently been shown [16] that freshly prepared solutions of prostaglandin E_1 are not chemotactic in vitro when tested at concentrations up to 100 μg ml^{-1} and that the appearance of such activity is associated with the age of the solution, being due to a chemical rather than a biological change in the molecule. Thus the association between inhibition of prostaglandin formation and reduced cellular migration becomes suspect. We have therefore studied the effects of some conventional antirheumatic drugs on these two activities in the exudates formed in porous inert sponges implanted subdermally in the rat [3]. In addition, the effects of 5,8,11,14-eicosatetraynoic acid (TYA), a specific inhibitor of prostaglandin production from arachidonic acid [17], and of a fraction isolated from normal human plasma, which does not inhibit prostaglandin synthetase activity (Table 1) but suppresses leucocyte emigration in the sponge system [3], were studied for comparison. A preliminary account of part of the work has been published elsewhere [18].

Materials and methods

The drugs used were of British Pharmacopoeial grade, the TYA was a gift from Dr K.J. Stone, Roche Products Ltd., and the human plasma fraction was prepared according to the directions of WALKER et al. [19]. The prostaglandin synthetase preparation was made from guinea-pig lung and assayed by standard methods [1, 20]. Female albino Wistar rats, 150–200 g, were obtained from Oxfordshire Laboratory Animal Colonies, Southern Ltd. The sponge implantation technique was that described previously [3] except that each animal received four sponges, soaked in 0.9% w/v NaCl solution, which were removed after 9 hours. The pooled exudate from three of the sponges was used to assay prostaglandins using the rat fundic strip method [21] after removal of any cellular material by centrifugation followed by extraction with ether. The polymorphonuclear and mononuclear cells were counted together in the exudate from the remaining sponge. The test substances were administered either orally or intraperitoneally (TYA) 1 hour before the sponge implantation at 0 hour, except for the plasma fraction which was given by intravenous injection at 0 hour. In further experiments some of the drugs were administered locally by being distributed in the solid form throughout dry sponges before these were implanted.

Results and discussion

Table 1 shows that indomethacin caused a dose-dependent inhibition of the prostaglandin synthetase used in the present work. In contrast the human plasma fraction, in quantities, 0.1 ml, which were one tenth of the amounts found to produce at least a 50% reduction of either carrageenan-induced paw oedema or leucocyte infiltration in the sponge exudate [3] (see also Table 2) when administered intravenously to whole rats, had no effect. This in vitro finding is a more direct piece of evidence supporting the results of other work [22, 23] which strongly suggested that the anti-inflammatory activity of the active material in the human plasma fraction does not involve interference with the formation of prostaglandins in vivo.

Table 1
Effects of indomethacin and human plasma fraction on prostaglandin synthetase activity in vitro.

Drug	Concentration[1] ($\mu g\ ml^{-1}$)	Percentage inhibition of control preparation[2]
Indomethacin	0.1	12
	0.3	38
	1.0	98
Plasma fraction	0.1 ml	0

[1]) The figures for indomethacin are the concentration of the drug in the final reaction mixtures whereas that for the plasma fraction is the amount added.
[2]) Results represent the mean values from at least four separate experiments.

When the plasma fraction was administered systemically to rats with implanted sponges it did not affect the prostaglandin content of the exudate, as might be expected from the results in Table 1, but caused a significant reduction in the number of leucocytes in the 9-hour exudate (Table 2). In contrast the intraperitoneal injection of TYA caused a predictable decrease in the prostaglandin content, this effect increasing with increasing dose, but had no action on the extent of leucocyte migration. It must be concluded that these two effects, i.e. the presence of prostaglandins and the migration of leucocytes, are independent phenomena in the implanted sponge system since they are

Table 2
Effects of systemic administration of anti-inflammatory drugs on prostaglandin content and leucocyte migration in 9-hour sponge exudates in the rat.

Drug	Dose ($mg\ kg^{-1}$)	Mean inhibition (%) of control values[1]) Total prostaglandins	Total leucocytes
Plasma fraction (10)	1 ml	2	68[2])
TYA (5)	150	43[2])	6
TYA (5)	500	76[2])	1
Paracetamol (5)	200	41[2])	3
Aspirin (10)	200	86[2])	47[2])
Phenylbutazone (5)	100	98[2])	74[2])
Indomethacin (10)	3	97[2])	50[2])

[1]) In the sponge exudates of the corresponding control group of rats the mean prostaglandin content was 10 ng ml^{-1} and the total leucocyte count was 770×10^4 ml^{-1}. The numbers of rats in each group are given in brackets.
[2]) $p < 0.05$ from results of corresponding control group.

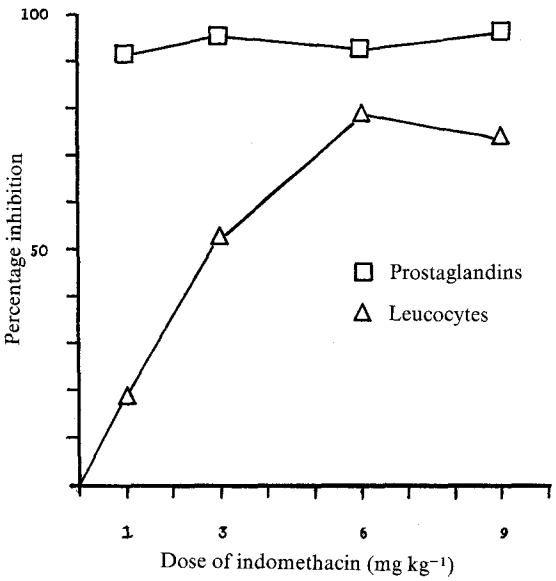
differentially affected by the two test materials. Furthermore reduction of prostaglandins in the sponge exudate seems to have little, if any, correlation with anti-inflammatory activity in vivo since it is not affected by the plasma fraction which is known to exert a wide variety of anti-inflammatory actions in animal models [24] but is significantly reduced by the administration of paracetamol, which is devoid of either experimental or clinical anti-inflammatory action [25, 26].

The three non-steroidal anti-inflammatory and antirheumatic drugs tested, aspirin, phenylbutazone and indomethacin, all caused significant decreases both in the prostaglandin content and in the leucocyte content of the sponge exudate. If it were not for the plasma fraction and TYA results it could be argued that these two actions might be related. However, when the effects of varying doses of indomethacin were studied (Figure) it became obvious that the drug, in doses of 1 mg kg^{-1} body weight and above, produced an almost complete, i.e. over 90%, reduction in the prostaglandin content of the sponge exudate whereas its inhibitory action on leucocyte migration was dose-dependent over the range 1 to 6 mg kg^{-1}. Thus at a 1 mg kg^{-1} dose this inhibition was 18% rising to 52% at 3 mg kg^{-1} and to 78% at 6 mg kg^{-1}.

A similar lack of correlation between the two inhibitory effects was observed when small amounts of the drugs were applied locally, i.e. when distributed throughout the sponges before implantation (Table 3). Although phenylbutazone and indomethacin caused significant reductions of both prostaglandin formation and leucocyte emigration, TYA, paracetamol and aspirin affected only the former.

Conclusions

The results of the present work show firstly, that prostaglandin formation and leucocyte migration in the implanted sponge exudates are independent phenomena and secondly, that some typical and conventional anti-inflammatory drugs, aspirin, phenylbutazone and indomethacin, inhibit both aspects by different mechanisms. These findings support earlier proposals [27–29] that aspirin possesses an anti-inflammatory action other than an inhibition of prostaglandin synthetase activity. They also serve to extend the argument to include indomethacin which is not only a much more potent inhibitor of the enzyme activity than is aspirin [2] but is widely regarded as a specific



Dissociation between inhibitory effects of indomethacin on prostaglandin content and leucocyte migration in 9-hour sponge exudate in the rat. Each point represents the mean value from a group of 5 animals, results expressed as in Table 2.

Table 3
Effects of local administration of anti-inflammatory drugs in 9-hour sponge exudates. Results expressed as in Table 2.

Drug	Dose (mg persponge)	Mean inhibition (%) Total prostaglandins	Total leucocytes
TYA (5)	1.0	86[1])	0
Paracetamol (5)	0.5	43[1])	0
Aspirin (10)	0.5	84[1])	2
Phenylbutazone (5)	0.5	96[1])	72[1])
Indomethacin (10)	0.001	68[1])	35[1])

[1]) $p < 0.05$ from results of corresponding control group.

inhibitor. There have been indications from other workers that indomethacin has effects not explicable as resulting solely from inhibition of prostaglandin synthetase. Thus it has been found that the anti-inflammatory activity of the drug was only partially abolished in rats maintained on a diet deficient in essential fatty acids [30]. It and aspirin also fail to block papillary meoisis in the rabbit eye, this being an inflammatory response supposedly produced by the release of prostaglandins into the aqueous humour [31]. If aspirin-like drugs affect such an important aspect of inflammation as leucocyte migration into inflammatory exudates and there is no causal connection between such migration and prostaglandin content then the simple correlation of anti-prostaglandin synthetase activity and anti-inflammatory activity in this group of drugs cannot be justified. It is suggested that future research on the mechanism of the anti-inflammatory action of such drugs be directed to studies of their effects on leucocyte migration in vivo.

Acknowledgment

We are grateful to the National Research Development Corporation and the King's College Hospital and Medical School Research Committee for financial support.

Received 20 February 1976.

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