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Relationship between the anti-inflammatory and irritant properties of inflammatory exudate

D. C. ATKINSON AND R. HICKS

Pharmaceutical Research Laboratories, Reckitt and Colman Pharmaceutical Division, Hull, and Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford 7

Summary

1. Using the rat paw oedema assay procedure a comparison was made between the anti-inflammatory and irritant properties of the inflammatory exudate obtained from polyester sponges implanted subcutaneously in adrenalectomized rats. Where necessary, comparison was also made with a known counter-irritant, carrageenin.

2. A significant correlation between the anti-inflammatory and irritant properties of sponge exudate was observed when each parameter was determined in relation to dose. A similar result was obtained with carrageenin.

3. A comparison of the two activities of sponge exudate samples harvested at various times following sponge implantation did not give a significant correlation although in each case significant anti-inflammatory activity was accompanied by marked irritation.

4. Time-effect curves of the two activities of sponge exudate showed a significant correlation over the time period examined. However, no such correlation was obtained using carrageenin.

5. Both activities of sponge exudate were retained following dialysis.

6. These findings are discussed in relation to a counter-irritant mode of action for sponge exudate.

Introduction

The mechanism by which irritants exert a systemic anti-inflammatory effect is unknown. In the past various hypotheses have been put forward to explain this so-called ' counter-irritant ' effect, many of which have been subsequently disproved. However, one possible explanation which has received much attention recently is that first suggested by Laden, Blackwell & Fosdick (1958). According to their hypothesis at an inflammatory site a factor or factors are produced which then enter the blood stream and can thus exert an inhibitory effect on inflammation in a distant part of the body. This explanation received support when it was shown that inflammatory exudate, obtained from polyester sponges implanted subcutaneously in adrenalectomized rats, exerted anti-inflammatory effects (Robinson & Robson, 1964, 1966, 1967 ; Billingham, Robinson & Robson, 1969a & b).

However, results obtained by Atkinson, Boura & Hicks (1969) suggested that the anti-inflammatory activity of sponge exudate may be mediated through a counter-irritant mechanism. Contrary to the observations of Robinson & Robson (1966),

exudate was markedly irritant. Furthermore, this material exerted its anti-inflammatory action following systemic but not local administration ; in the latter case a significant pro-inflammatory effect was obtained. The purpose of our study was to establish whether or not a correlation exists between the anti-inflammatory and irritant properties of sponge exudate. Demonstration of such a relationship would support an irritant mode of action.

A preliminary communication of this work was presented to the British Pharmacological Society (Atkinson, 1970).

Methods

Animals

Male albino Sprague-Dawley rats (Carworth Farm/Elias strain) were used. Animals used in the preparation of sponge exudate weighed 140–250 g and in tests for biological activity 120–180 g.

Collection and preparation of sponge exudate

The methods used closely followed those of Robinson & Robson (1966) as described by Atkinson *et al.* (1969). Dialysed material was prepared by taking up 10 g of freeze-dried exudate in 50 ml of cold normal saline, which was then poured into a length of dialysis tubing, and finally dialysed against 5 l. of normal saline at 2° C for 66 h, the saline solution being changed at 12 h intervals. The contents of the dialysis sac were then freeze-dried.

Evaluation of biological activity

Anti-inflammatory activity was determined using the carrageenin rat paw oedema test (Winter, Risley & Nuss, 1962), as described by Atkinson *et al.* (1969). Modifications of the test, when introduced, are referred to under the respective experimental headings. The substances under test were administered intraperitoneally in a 10 ml/kg dose volume, control animals receiving similar injections of the vehicle, Water for Injection, B.P.

Irritant activity was assessed in other animals by measuring the increase in paw volume following subplantar injection of 0·1 ml of a solution or suspension of the substances under investigation.

To test the relationship between the anti-inflammatory and irritant activities correlation coefficients were calculated, and where necessary, their distribution was normalized using Fisher's z transformation.

Results

Dose-effect relationships of the anti-inflammatory and irritant activities

The possible correlation between anti-inflammatory and irritant activity was investigated by comparing dose-effect relationships for each activity of exudate and a known irritant, carrageenin. Anti-inflammatory activity was assessed by administering various doses of test materials 1 h before induction of paw oedema by a subplantar injection of 1% carrageenin. The percentage inhibition of paw swelling was calculated from measurements made 3 h later. Irritant activity was assessed by

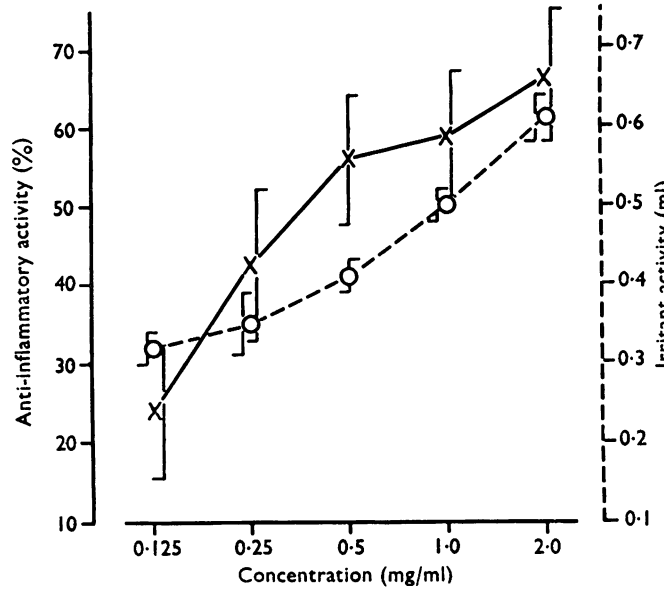

FIG. 1. Dose-response relationship of anti-inflammatory (×——×) and irritant (○--○) properties of carrageenin. Results expressed as mean values of ten observations ± standard error.

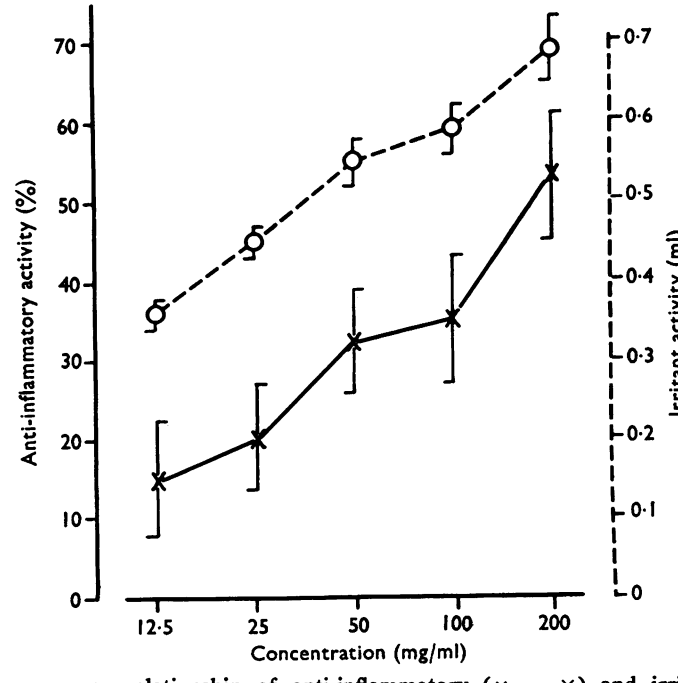

FIG. 2. Dose-response relationship of anti-inflammatory (×——×) and irritant (○--○) properties of sponge exudate. Results expressed as mean values of eight–ten observations ± standard error.

measurement of paw oedema at 4 h after administration of the same concentrations of test materials examined for anti-inflammatory activity. Thus both types of activity were measured at the same time following administration.

The results were as illustrated in Figs. 1 and 2. The correlation coefficients for sponge exudate and carrageenin were 0·98 ($P<0·01$) and 0·88 ($P<0·05$) respectively, indicating a significant correlation between the two types of activity for both agents.

Effect of sponge implantation time

Billingham (1968) reported that the anti-inflammatory potency of sponge exudate, as measured against carrageenin induced rat paw oedema, varied according to its time of collection. It was, therefore, decided to evaluate the irritant activity of exudate obtained at various times following sponge implantation and to compare it with the corresponding anti-inflammatory activity. Exudate was obtained on days 1, 2, 3, 4, 6 and 8 following implantation. The anti-inflammatory and irritant activities were obtained using 10% solutions of freeze-dried material.

The results, illustrated in Fig. 3, showed some degree of correlation as for all samples examined significant anti-inflammatory activity was associated with marked irritation. However, the quantitative changes in both activities were not significantly related (correlation coefficient 0·63; $P>0·05$).

Time-effect relationships of the anti-inflammatory and irritant activities

To investigate the possible correlation between the anti-inflammatory and irritant activities with respect to time, a comparison of the time-effect curves of these activities was undertaken for both carrageenin and sponge exudate. The irritant activity was assessed by measuring the increase in paw volume at 2, 3, 4, 5 and 6 h following subplantar injection of a 0·05% suspension of carrageenin, or 10% solution of a 4-day sponge exudate. The anti-inflammatory activity was measured by

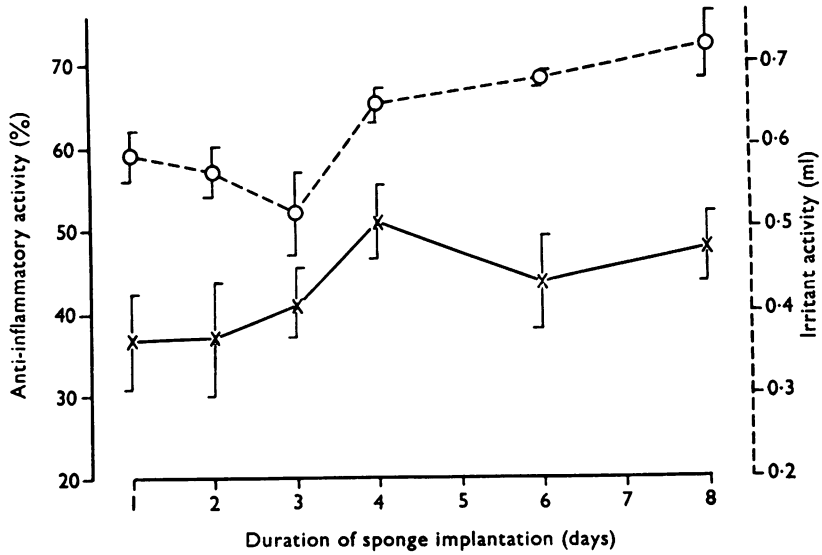

FIG. 3. Effect of duration of sponge implantation on the anti-inflammatory (×——×) and irritant (○- - -○) potency of sponge exudate (10% concentration used for each determination). Results expressed as mean values of ten observations ± standard error.

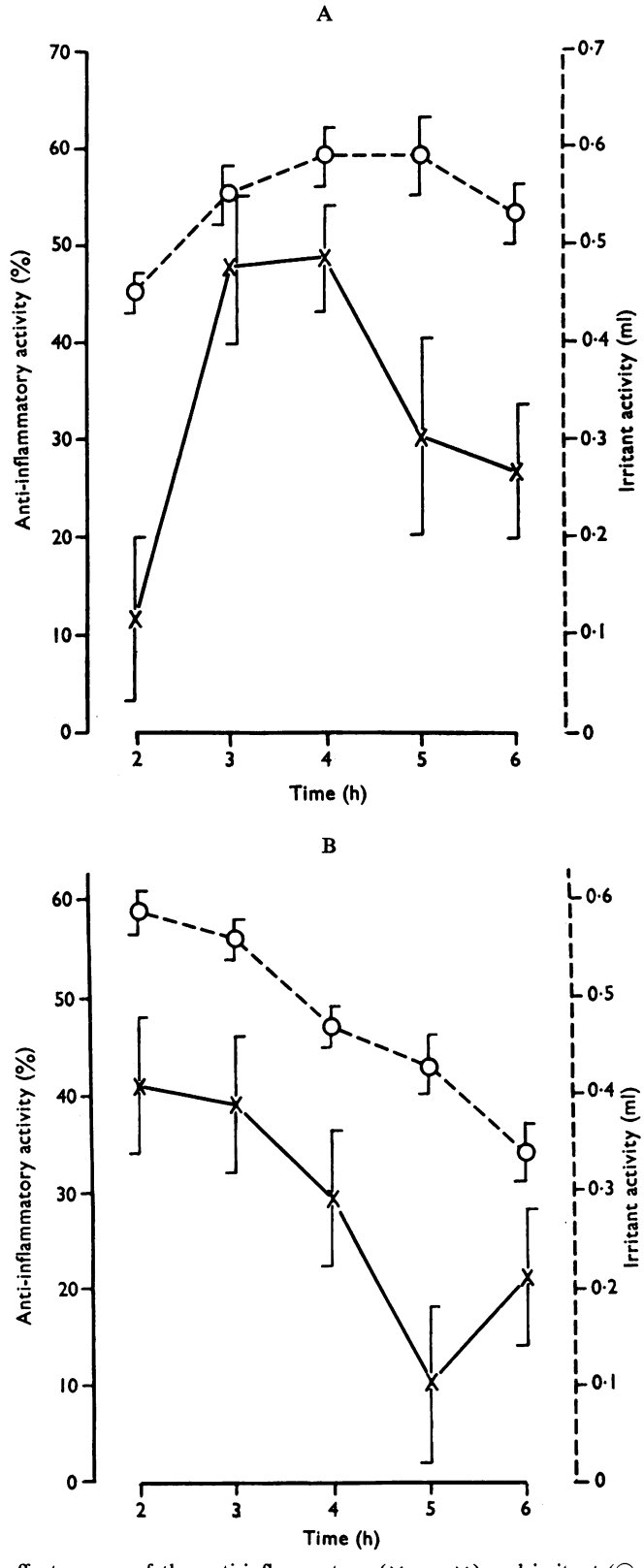

FIG. 4. Time-effect curves of the anti-inflammatory (×——×) and irritant (○ - - ○) activities of A carrageenin (0·05%) and B sponge exudate (10%). Results expressed as mean values of ten–twenty observations ± standard error.

injecting intraperitoneally doses of either 0·05% carrageenin or 10% sponge exudate at various time intervals before the subplantar injection of carrageenin, and by measuring the resultant increase in paw volume after 2 h instead of the normal 3 hours. Separate control animals were used for the determination of activity at each time interval.

The results obtained for carrageenin and sponge exudate are shown in Fig. 4A and B, respectively. Over the time period investigated there was no significant correlation between the irritancy and anti-inflammatory activities of carrageenin (correlation coefficient 0·78; $P>0·05$) although the peak of both activities occurred at a similar time. However, in the case of sponge exudate, a significant correlation was obtained (correlation coefficient 0·96; the 95% confidence limits calculated by Fisher's z transformation were 0·41–1·00).

Effect of dialysis

The anti-inflammatory activity of sponge exudate has been shown to be non-dialysable (Robinson & Robson, 1966; Billingham *et al.*, 1969a). It was, therefore, of interest to see if the irritant activity was also non-dialysable. The anti-inflammatory and irritant activities were assessed as before and the results obtained are shown in Table 1. Both activities were retained within the dialysis sac, the potency in each case being virtually unchanged.

Discussion

These results support our earlier proposal that sponge exudate exerts its anti-inflammatory action through a counter-irritant mechanism (Atkinson *et al.*, 1969). The strong correlation between the dose-response curves for both activities of sponge exudate, together with the similarly shaped time-effect curves for these activities, is further evidence that the two properties cannot be dissociated. Sponge exudate at least appears to have similar properties to a known irritant, carrageenin, and, in fact, the correlation between the two activities is stronger in the former case. However, the results obtained with sponge exudate harvested at different times following sponge implantation do not directly support this relationship, but are not

TABLE 1. *Effect of dialysis on the anti-inflammatory and irritant properties of sponge exudate*

(A) Anti-inflammatory activity

Pretreatment	Dose (i.p.)	Mean increase in paw volume due to carrageenin ±S.E. (ml)	% Inhibition of controls
Controls (Water for Injection, B.P.)	10 ml/kg	0·70±0·05	
Normal exudate	1 g/kg	0·39±0·03	45
Dialysed exudate	1 g/kg	0·37±0·03	47

(B) Irritant activity

Treatment	Concentration (dose vol. 0·1 ml)	Mean increase in paw volume ±S.E. (ml)
Controls (Water for Injection, B.P.)		0·21±0·02
Normal exudate	100 mg/ml	0·62±0·02
Dialysed exudate	100 mg/ml	0·62±0·02

Each result is the mean of ten observations. Differences between mean values for normal and dialysed exudates calculated using the Student's t test were not significant ($P>0·05$).

inconsistent with the general principle. The retention of both activities following dialysis of sponge exudate provides further indication that these are closely associated with one another and suggests that both are associated with high molecular weight proteins.

Examination of the dose-response relationship for each activity for carrageenin and sponge exudate reveals that the same degree of irritation produced by different irritants, as assessed by measurement of paw oedema, does not give rise to the same level of systemic anti-inflammatory activity. It is noteworthy that the anti-inflammatory activity of carrageenin in relation to its irritancy is greater than for sponge exudate in which the presence of an anti-inflammatory factor had been suspected. It would be expected that the inflammatory reaction to sponge implants will vary qualitatively with time with corresponding changes in the irritant activity of sponge exudate. Thus one would not expect a close correlation with the associated anti-inflammatory activity.

The variations of anti-inflammatory activity with duration of sponge implantation do not agree with those described by Billingham (1968). He showed that at day 1 there was no significant anti-inflammatory activity, greatest activity at day 4 and a significant decline by day 8. In our investigation significant activity was present for each sample of exudate, there being no significant decline at day 8 compared with day 4. These discrepancies may be explained by the fact that Billingham (1968) administered the exudate by the subcutaneous rather than the intraperitoneal route.

The above experiments have assumed that inflammation following subplantar injection is qualitatively similar to peritoneal inflammation, an assumption which may not necessarily be correct. However, there may be a quantitative relationship between the two types of inflammation at different doses. The relationship between peritoneal inflammation and anti-inflammatory activity for sponge exudate is at present under investigation.

The hypothesis that anti-inflammatory factors are present in inflammatory exudate was based upon experiments which indicated that such material was not in itself irritant (DiPasquale & Girerd, 1961 ; Robinson & Robson, 1966). In contrast to this situation previous results clearly demonstrated that sponge exudate was irritant (Atkinson *et al.*, 1969), and our results show that this irritancy is closely associated with its anti-inflammatory activity. This fact must now cast doubt on the validity of the evidence for the presence of a specific anti-inflammatory factor in sponge exudate.

The results presented above do not necessarily conflict with the original hypothesis of Laden *et al.* (1958), as it is still possible that such factors are produced at inflammatory sites but that these do not enter the exudate in significant concentrations. It is feasible that they may be produced and carried away in blood vessels surrounding the affected area. If this is the case it is unlikely that the physiological role of any such factors is to terminate eventually the inflammatory process at the site producing them, as suggested by earlier workers (Rindani, 1956 ; Laden *et al.*, 1958; DiPasquale & Girerd, 1961 ; Robinson & Robson, 1964). Rather these factors would exert a physiological restraint on the general involvement of tissues in inflammatory processes. Resolution of the inflammatory process may instead be mediated by release of endogenous catecholamines (Spector & Willoughby, 1960, 1963, 1964).

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