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Comparison of the effect of phenylbutazone, desonide and cyclophosphamide on four types of experimental pleurisy

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The action of phenylbutazone, a non-steroid anti-inflammatory drug, desonide, a corticosteroid, and cyclophosphamide, an immunosuppressant agent, was studied on four types of experimental pleurisy: carrageenan-pleurisy in rats; passive reversed Arthus pleurisy in rats; *Bordetella pertussis*-delayed hypersensitivity pleurisy in rats and PPD (purified protein derivative)—delayed hypersensitivity pleurisy in guinea-pigs. For each compound, the action on the exudate and on the number of the different categories of leucocytes in the inflammation focus was evaluated. In carrageenan-inflammation, phenylbutazone reduced the oedema and the number of neutrophils and macrophages. Its favourable effect on exudative events in Arthus—and *B. pertussis*—reactions was not accompanied by high modifications at the cellular level. With the exception of PPD-pleurisy, desonide reduced the three other reactions. Its action related to the exudate and the various leucocyte types, except in the Arthus reaction in which only the number of neutrophils was decreased. The effect of cyclophosphamide was mainly in *B. pertussis* pleurisy in which it resulted in a decrease of oedema and a reduction in the number of mononuclears. For each compound, correlations between the effect on exudative and cellular phenomena are discussed.

Immunological reactions to immune complexes on the one hand and the phenomena of cell immunity on the other play a predominant role in the various rheumatic conditions, in particular rheumatoid arthritis (Clot & Sany 1975). To examine more closely the mechanisms of action of existing anti-inflammatory and antirheumatic agents and also to discover new types of compounds, attempts have been made to devise experimental models that reproduce these reactions. The pleural cavity of animals provides a means of making a complete study of the different inflammatory parameters (Willoughby et al 1977). Following the non-immune acute inflammation due to carrageenan in this model (Di Rosa et al 1971a), research was directed at pleurisy where immune complexes and cell immunity play a determining role. Yamamoto et al (1975b), for example, induced an Arthus reaction in the pleural cavity of the rat. Also, two types of delayed hypersensitivity pleurisy were created: that to PPD (protein purified derivative) in the guinea-pig (Apicella & Allen 1969; Leibowitz et al 1973; Yamamoto et al 1975a), and that to *Bordetella pertussis* in the rat (Dieppe et al 1976).

We have examined the effects of a non-steroid anti-inflammatory drug (NSAID), phenylbutazone; a corticosteroid, desonide (desfluorotriamcinolone acetonide) and an immunosuppressant drug, cyclophosphamide on all four types of pleurisy described. Special attention was paid to the compounds' effect on the volume of exudate and on the numbers of the various types of leucocytes at the focus of inflammation.

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MATERIAL AND METHODS

Animals

Male Sprague Dawley rats and Dukkin Hartley guinea-pigs of either sex were used.

Induction of pleurisy

The animals were anaesthetized with ether. The intrapleural injection was given by the intercostal route on the animal's right flank after incising the skin and the underlying muscles. A 20/8 needle 9 mm long and with a blunt end was used. After death the volume of exudate was removed and measured and the pleural cavity washed with 199 medium (Pasteur Institute) to collect the remaining white cells. The total number of leucocytes was counted by Coulter Counter (Model ZF—Coultronics) and the different categories of white cells were ascertained after suitable staining.

Carrageenan-induced inflammation in the rat

Rats of 250 g had 0·15 ml of a 1% carrageenan (Marine Colloids) solution in 0·9% NaCl injected

into the pleural cavity and 6 h later they were killed. Neutrophil and eosinophil polynuclears, macrophages and lymphocytes were identified and counted.

Reversed passive Arthus reaction in the rat

Rats of 250 g had 0·2 ml of rabbit antibovine albumin serum (Difco—the lyophilized antibodies were solubilized in 2 ml of 0·9% NaCl) injected into the pleural cavity, 20 min later 25 mg kg^{-1} of bovine albumin (Merck) was administered intravenously and the animals were killed 6 h after the intrapleural injection. In this model we distinguished neutrophils, eosinophils and mononuclears.

Delayed hypersensitivity reaction to Bordetella pertussis *in the rat*

An homogeneous suspension was prepared containing 50% *B. pertussis* suspension (Perthydral, Pasteur Institute; 5×10^9 killed organisms ml^{-1}) + 50% Freund complete adjuvant (Difco). Rats of 300–350 g were sensitized by injection of 0·2 ml of this mixture intramuscularly into each thigh. Six days after sensitization, 0·1 ml of the *B. pertussis* suspension was injected into the cavity. The rats were killed after 48 h.

Delayed hypersensitivity reaction to PPD in the guinea-pig

Groups of guinea-pigs, homogeneous in sex and weight, chosen so that the weight reached 500 g at the time of challenge were given 0·5 ml of a suspension of 80 mg *Mycobacterium tuberculosis* (previously mixed in a mortar) in a mixture of 10 ml Freund incomplete adjuvant + 10 ml buffer phosphate solution 0·2 M (pH = 7) into the muscles of one thigh. 5 to 7 weeks after sensitization, 10 μg of PPD (Pasteur Institute) in 0·1 ml was injected into the pleural cavity. The guinea-pigs were killed after 48 h.

Drugs: Phenylbutazone (Expandia), cyclophosphamide (Lucien) and desonide (Expandia). In the rat the products were administered orally in distilled water with the addition of Tween 80. In the guinea-pig the compounds were injected subcutaneously in propyleneglycol.

RESULTS

Carrageenan-induced pleurisy (Table 1)

Phenylbutazone and desonide markedly reduced the volume of exudate and, to a lesser extent, the total number of white cells. The action of phenylbutazone was directed at the macrophages (especially) and the neutrophils. Desonide reduced the number of macrophages, neutrophils and lymphocytes; with cyclophosphamide, only the number of macrophages was significantly lowered.

Arthus pleurisy (Table 2)

The pleural exudate was lowered by desonide and phenylbutazone. Desonide reduced the total number of white cells and neutrophils; phenylbutazone slightly lowered the number of mononuclears. Cyclophosphamide showed no significant modification of any of the inflammation states.

Bordetella pertussis *pleurisy* (Table 3)

The three compounds reduced the volume of exudate. The total number of white cells was lowered by cyclophosphamide and desonide, but whereas desonide reduced significantly all classes of white cells, cyclophosphamide affected only the mononuclears. With phenylbutazone there was only a small decrease of mononuclears. Although during treatment with desonide the hypersensitivity reaction of the controls was less than that of the other two experiments, it was markedly dissociated from the slight inflammation obtained after 48 h with the

Table 1. Carrageenan pleurisy in the rat. Exudate volume and differential white cell count ($\times 10^6$). Means are followed by standard errors. In brackets number of animals used. Compounds were administered by mouth 18 and 1 h before the intrapleural injection of carrageenan.

Groups	Doses mg kg^{-1}	Exudate volume (ml)	No of total leucocytes	No of neutrophils	No of eosinophils	No of macrophages	No of lymphocytes
Controls	—	2·2 ± 0·2 (13)	220·3 ± 9·0	178·4 ± 8·9	1·1 ± 0·7	35·2 ± 3·8	5·5 ± 1·4
Phenylbutazone	50 (×2)	1·2 ± 0·1 (13) **	162·4 ± 12·8 **	138·0 ± 11·3 *	1·6 ± 0·5	17·9 ± 1·8 **	4·9 ± 1·5
Desonide	0·25 (×2)	0·9 ± 0·1 (9) **	150·0 ± 16·0 **	129·0 ± 14·4 **	0·7 ± 0·2	18·7 ± 2·9 **	1·5 ± 0·5 *
Cyclophosphamide	5 (×2)	1·9 ± 0·2 (13)	204·1 ± 16·7	179·6 ± 16·5	1·0 ± 0·3	19·4 ± 2·3 **	4·1 ± 1·0

* $P < 0·05$ ** $P < 0·01$ compared with controls.

Table 2. Reversed passive Arthus pleurisy in the rat. Exudate volume and differential white cell count ($\times 10^6$). Compounds were given by mouth 18 and 1 h before experiment.

Groups	Doses mg kg^{-1}	Exudate vol. (ml)	No. of total leucocytes	No. of neutrophils	No. of eosinophils	No. of mononuclears
Controls	—	1·4 ± 0·1 (14)	82·2 ± 5·9	58·0 ± 5·3	0·7 ± 0·2	23·4 ± 2·0
Phenylbutazone	50 (×2)	0·7 ± 0·03 ** (13)	69·0 ± 4·4	52·0 ± 4·1	0·3 ± 0·1	16·7 ± 1·2 **
Desonide	0·25 (×2)	0·6 ± 0·1 ** (14)	66·5 ± 4·7 *	41·0 ± 3·5 *	0·3 ± 0·2	25·1 ± 1·5
Cyclophosphamide	5 (×2)	1·3 ± 0·1 (14)	72·3 ± 3·0	50·9 ± 4·3	0·3 ± 0·1	21·1 ± 2·1

* $P < 0.05$ ** $P < 0.01$ compared with controls.

Table 3. *Bordetella pertussis* pleurisy in the rat. Exudate volume and differential white cell count ($\times 10^6$). Compounds were administered by mouth 24 and 2 h before the intrapleural challenge and then 2 and 24 h after it.

Groups	Doses mg kg^{-1}	Exudate vol. (ml)	No. of total leucocytes	No. of mononuclears	No. of neutrophils	No. of eosinophils
Controls	—	3·4 ± 0·3 (41)	205·0 ± 10·0	116·0 ± 5·0	76·0 ± 4·0	13·0 ± 2·0
Phenylbutazone	50 (×4)	1·9 ± 0·2 (41) **	181·0 ± 9·0	97·0 ± 6·0 *	69·0 ± 5·0	15·0 ± 2·0
Controls	—	1·3 ± 0·1 (19)	148·0 ± 10·0	83·0 ± 6·0	59·0 ± 5·0	6·0 ± 1·0
Desonide	0·25 (×4)	0·4 ± 0·03 (18) **	88·4 ± 6·7 **	47·3 ± 4·0 **	40·2 ± 3·0 **	0·9 ± 0·3 **
Controls	—	5·3 ± 0·5 (19)	239·0 ± 11·0	129·0 ± 5·0	99·0 ± 7·0	11·0 ± 3·0
Cyclophosphamide	5 (×4)	1·8 ± 0·3 (20) **	145·0 ± 12·0 **	55·0 ± 5·0 **	84·0 ± 8·0	6·0 ± 1·0

* $P < 0.05$ ** $P < 0.01$ compared with controls.

Table 4. PPD pleurisy in the guinea-pig. Exudate volume and differential white cell count ($\times 10^6$). Compounds were injected s.c. 24 and 2 h before the intrapleural challenge; and then 2 and 24 h after it.

Groups	Doses mg kg^{-1}	Exudate vol. (ml)	No. of total leucocytes	No. of mononuclears	No. of neutrophils	No. of eosinophils
Controls	—	4·0 ± 0·6 (34)	91·5 ± 8·4	75·5 ± 5·9	14·6 ± 3·0	1·4 ± 0·3
Phenylbutazone	50 (×4)	2·9 ± 0·4 (31)	75·0 ± 6·6	64·1 ± 5·8	9·4 ± 1·8	1·5 ± 0·3
Desonide	0·25 (×4)	3·2 ± 0·4 (30)	77·5 ± 5·9	61·6 ± 5·1	15·1 ± 3·1	0·8 ± 0·2
Cyclophosphamide	5 (×4)	2·8 ± 0·5 (30)	58·0 ± 5·6 **	45·0 ± 3·7 **	12·1 ± 3·5	0·9 ± 0·2

* $P < 0.05$ ** $P < 0.01$ compared with controls.

same dose of antigen in the non-sensitized animals (Dieppe et al 1976; Tarayre et al 1977).

PPD pleurisy (Table 4)

Despite a reduction of the volume of exudate by cyclophosphamide and phenylbutazone, the level of significance was not reached but the total number of white cells and mononuclears after cyclophosphamide fell significantly. Desonide did not modify any parameter significantly.

DISCUSSION

Experimental inflammatory models are usually concerned with the skin or the plantar pad. The use of the pleural cavity permits easy evaluation of the exudate and the cells, but it does not respond in the same way as the other two tissues. For instance, absorption by the lymphatic system plays an important part, particularly in delayed hypersensitivity reactions (Apicella & Allen 1969). Moreover, some mediators that are phlogogenic when injected into the skin or

foot, cause no reaction in the pleural cavity (Vinegar et al 1976). The inflammation of delayed skin hypersensitivity to PPD in the guinea-pig is usually read 24 h after the challenge. Histological examination shows at that time the classical picture of a preponderance of mononuclears over polynuclears (Martins & Raffel 1964; Turk et al 1966). In preliminary tests in which the guinea-pigs were killed 24 h after the intrapleural challenge by injection of PPD, we found a volume of exudate similar to that obtained at the end of 48 h, but polynucleate were more numerous than the mononucleate cells. These results, which differ from those of Leibowitz et al (1973) and Yamamoto et al (1975a), induced us to kill our animals 48 h after the challenge, at a time where we obtained a classic differential white cell count of the focus of delayed hypersensitivity. With *B. pertussis* pleurisy, Dieppe et al (1976) showed that the reaction was also maximum 48 h after challenge.

The counting of the cells in the focus of inflammation is a step forward in the study of any inflammatory reaction (Willoughby et al 1977). With the histological technique we used we were able to distinguish the macrophages from the lymphocytes only in carrageenan-induced pleurisy. Classically, we found a preponderance of polynuclear neutrophils in carrageenan inflammation and the Arthus reaction. On the other hand, although mononuclears constitute most of the cell population in the PPD pleurisy after 48 h, *B. pertussis* pleurisy appears to be a less pure model of delayed hypersensitivity, the neutrophils being numerous and in addition we found eosinophils. Although the preponderance of the characteristics of cell immunity was well demonstrated in this reaction (Rowley et al 1959; Gruenewald et al 1961; Levine & Gruenewald 1962; Willoughby 1966; Arrigoni-Martelli et al 1976; Dieppe et al 1976), it does not exclude humoral phenomena from also playing a part. We found numerous analogies with the hypersensitivity to tetanus toxoid induced in the peritoneal cavity of the mouse by Speirs & Speirs (1974).

Determination of the different classes of white cells in the focus of inflammation makes it possible to attempt to correlate for each test the effect of compounds on the volume of exudate and on the cell phenomena.

At the dose studied, the effect of phenylbutazone on the volume of exudate was marked in carrageenan-induced pleurisy. It was accompanied by a clear reduction in the number of white cells, particularly the percentage of mononuclears. The effect of NSAIDs on the different types of white cells in carrageenan inflammation is varied (Di Rosa et al 1971b; Meacock & Kitchen 1976; Perper 1978). The mononuclears are few in number in this reaction. Meacock & Kitchen (1979) demonstrated that a new NSAID, benoxaprofen, lowered the mononuclears without reducing the exudate. Moreover, cyclophosphamide, which reduced the number of mononucleated cells in a similar manner to phenylbutazone, caused no decrease of the exudate volume. It may well be, in line with the view of Blackham & Owen (1975) and Vinegar et al (1976), that the neutrophils are in a preponderance in this inflammation. For Vinegar et al (1976), in particular, the action of the non-steroid agents is directed more at the liberation of certain mediators or lysosomal enzymes by the neutrophils than at their diapedesis. With Arthus and the *B. pertussis* pleurisy, the marked reduction of the exudate volume obtained with phenylbutazone was accompanied in both cases only by a very slight decrease in the number of mononucleated cells. In the PPD reaction there was a non-significant anti-exudative tendency but no effect on the cells. Therefore, if we except carrageenan pleurisy where the reduction of some classes of white cells (particularly neutrophils) perhaps plays a part in the anti-inflammatory action of phenylbutazone, the effect on the number of leucocytes in the other models appears less important.

The lack of significant activity of desonide, at the dose employed, on PPD pleurisy in the guinea-pig, is probably linked with the resistance of this species to corticosteroids (Claman 1972). On the other hand, in the three other types of inflammation, desonide's action on the exudate was always accompanied by a fall in the number of cells. According to Vinegar et al (1976), inhibition of the migration of the neutrophils during carrageenan pleurisy is the principal action of the corticosteroids in this reaction. Moreover, preliminary results in our laboratory with dexamethasone on Arthus and *B. pertussis* pleurisy show, equally and in an even more marked manner than with desonide, there was a decrease in the number of total leucocytes accompanying the reduction of exudate. This inhibition by corticosteroids on the number of white cells in the focus of inflammation does seem to be an essential factor in their anti-inflammatory action. With desonide, this reduction affected both mononuclears and polynuclears, except in Arthus pleurisy where only the neutrophils were affected.

Whereas cyclophosphamide produced no inhibition of the volume of exudate and little or no effect

on cell phenomena in carrageenan and Arthus pleurisy, the decrease of oedema and mononuclears was marked in *B. pertussis* inflammation. An identical but less important phenomenon (we did not reach the level of significance for reduction of the exudate) was also found in PPD pleurisy. It is therefore tempting to attribute an important role to the action of the alkylating agent on the cells in mitosis (Gordon et al 1969; Van Putten & Lelieveld 1970)—the target cells here being the lymphocytes and/or macrophages of the focus of inflammation—in the reduction of the two delayed-hypersensitivity reactions.

Therefore, the correlation between anti-exudative effect and reduction of number of white cells in the focus of inflammation is most marked with desonide. It appears on the other hand that decrease of the mononuclears could play a part in the inhibition of some reactions of delayed hypersensitivity by cyclophosphamide. With phenylbutazone, the effect on the oedema seems to be independent of the number of cells in the three immunological pleurisies studied. The importance of the reduction of neutrophils in its anti-inflammatory action in carrageenan pleurisy remains to be defined.

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