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A study of clofazimine in the rat

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Summary

1. Clofazimine, an anti-mycobacterial agent used in the treatment of leprosy, has been reported to be particularly effective in the treatment of acute erythema nodosum leprosum reactions.

2. The present experiments were designed to study in the rat this apparent anti-inflammatory activity.

3. In doses relatively non-toxic to the animals clofazimine inhibited both rat adjuvant arthritis and the inflammatory paw swelling following an adjuvant injection.

4. Clofamizine failed to inhibit both the primary antibody response to sheep erythrocytes and the tuberculin skin response.

5. It is concluded that clofazimine exhibits definite anti-inflammatory (but not immunosuppressive) activity, and that it should be tested in patients with rheumatoid arthritis.

Introduction

Clofazimine is a stable phenazine derivative, insoluble in water, but soluble in ethanol and organic solvents (Vischer, 1969). Given by mouth it is well absorbed (in rats and in man) and is stored in the reticulo-endothelial system and in fat until excreted slowly in an unchanged form. It produces an orange-coloured pigmentation of tissues.

Clofazimine has anti-mycobacterial properties and it has been reported to be of value in the treatment of leprosy (Browne & Hogerzeil, 1962 ; Williams, Mott, Wertlake, Rubio, Adler, Hill, Suarez & Knight, 1965 ; Atkinson, Sheagren, Rubio & Knight, 1967 ; Imkamp, 1968). Browne (1965) first noted that the drug had what appeared to be an anti-inflammatory action in acute lepromatous and erythema nodosum leprosum reactions. Other authors have confirmed this (Warren, 1968 ; Imkamp, 1968 ; Helmy, Pearson & Waters, 1972) and it appears that clofazimine can considerably reduce corticosteroid requirements in these patients.

If, as these reports suggest, clofazimine has, in addition to its antimicrobial properties, an anti-inflammatory (or even immunosuppressive) action, then there would be considerable interest in testing the drug in conditions such as rheumatoid arthritis, especially since it appears to be relatively non-toxic.

This paper describes experiments designed to examine the potential anti-inflammatory and immunosuppressive properties of clofazimine in the rat. The methods employed were: adjuvant-induced arthritis, the primary humoral antibody response to sheep erythrocytes, local sensitivity to tuberculin and local tissue swelling following an irritant injection.

Methods

Animals. Sprague-Dawley rats weighing 160–260 g were used. Each test group contained at least six rats.

Administration of test substances. Clofazimine ('Lamprene', Geigy Pharmaceuticals, Macclesfield, England) was dissolved in arachis oil (warmed to 50° C) and the concentration adjusted so that animals received their single daily dosage (by oral tube) in a volume of 1 ml or less. Control groups received a similar volume of arachis oil. 'Daily' administration consisted of five doses a week (omitting weekends).

Adjuvant arthritis. Adjuvant disease was induced by a single foot-pad injection (under ether anaesthesia) of 0·6 mg dried, heat-killed tubercle bacilli (Central Veterinary Laboratory, Weybridge), finely suspended in 0·1 ml heavy mineral oil (liquid paraffin, B.P.). Almost all control animals developed a widespread polyarthritis beginning about day +10. The mean 'joint score' (Currey & Ziff, 1968) on day +21 was used as an index of the severity of adjuvant disease in the different groups. (Regular scoring of the animals showed the pattern of evolution of the arthritis, but did not reveal any differences between the groups not reflected in the day +21 score).

Primary antibody responses. The same rats used in the adjuvant arthritis experiments also received, on day 0, an intraperitoneal injection of 1·0 ml of 1% washed sheep erythrocytes in physiological saline. Seven days later the animals were bled by orbital sinus puncture (under ether anaesthesia) and the haemagglutination titre (using a 1% suspension of sheep erythrocytes) was determined by the 'microtitre' method.

Tuberculin skin testing. On the last day of a course of treatment with either clofazimine or arachis oil, rats were injected with 0·05 ml Freund's complete adjuvant (3 mg tubercle bacilli per ml) into a hind foot pad. Eleven days later 0·02 ml of 1:10 strength protein purified derivative (PPD—Central Veterinary Laboratory, Weybridge) was injected into the pinna of one ear. A precision gauge ('dial gauge', Mercer, St. Albans) was used to determine the thickness of the ear immediately before, and 24 and 48 h after the injection.

Inflammatory paw swelling

Separate groups of rats received injections into both hind foot pads of 0·05 ml Freund's complete adjuvant (6 mg tubercle bacilli/ml). A precision gauge was used to measure the thickness of the feet, both before and 48 h after the injections. The difference between the two readings was used as an index of the degree of swelling.

Results

Adjuvant arthritis. Because of the fate of clofazimine after ingestion it seemed likely that any pharmacological actions might depend more on the total dose administered, rather than the daily dosage level. The drug was therefore administered in a fixed dose of (50 mg/kg)/day for a variable number of consecutive days (weekends excluded) ending with the last dose on the day on which Freund's adjuvant was injected. The results are shown in Table 1: 350 mg produced almost complete suppression, 200 mg and 100 mg produced partial suppression (although the mean score in these animals was not significantly different from that in the control group) while 50 mg produced no suppression of the arthritic signs.

In order to determine how long the effect of clofazimine persisted after administration, further groups of rats were given the drug in a dose of 50 mg/kg daily for seven days, then challenged with Freund's adjuvant at varying intervals thereafter. The results are shown in Table 2. Suppression was still apparent at two

TABLE 1. *Suppression of adjuvant arthritis by clofazimine: effect of dosage. The drug was administered daily (excluding weekends), the final dose being given on the day of adjuvant injection*

No. of rats	Clofazimine treatment	Total dose (mg/kg)	Mean joint score on day +21	(S.D.)
12	Arachis oil 1 ml×4 or 7	0 (control)	15·9	(9·9)
6	50 mg/kg×7	350	0·7*	(1·6)
6	50 mg/kg×4	200	8·3	(9·1)
6	50 mg/kg×2	100	9·7	(7·5)
6	50 mg/kg×1	50	15·2	(14·4)

*Significantly different from control at 5%.

TABLE 2. *Suppression of adjuvant arthritis by clofazimine: persistence of effect*

No. of rats	Clofazimine treatment	Total dose (mg/kg)	Interval between last dose of clofazimine and adjuvant injection	Mean joint score on day +21	(S.D.)
6	Arachis oil 1 ml×7	0 (control)	0	16.7	(12·2)
6	50 mg/kg×7	350	0	0·7*	(1·6)
6	50 mg/kg×7	350	2 weeks	2·0*	(4·0)
5	50 mg/kg×7	350	4 weeks	13·2	(7·9)
6	50 mg/kg×7	350	6 weeks	7·0	(6·4)

*Significantly different from control at 5%.

TABLE 3. *Suppression by clorfazimine of inflammatory foot swelling 48 hours after an irritant injection (Freund's complete adjuvant). Clofazimine was administered daily (excluding weekends), the last dose being given on the day on which adjuvant was injected*

No. of rats	Clofazimine treatment	Total dose (mg/kg)	Mean increase in paw thickness at 48 h (mm)	(S.D.)
8 (16 paws)	Arachis oil 1 ml×7	0 (control)	3·523	(0·953)
8 (16 paws)	50 mg/kg×7	350	2·118*	(0·754)
8 (16 paws)	100 mg/kg×7	700	2·032*	(0·848)

*Significantly different from control at 5%.

weeks, but after four and six weeks the relatively small numbers of rats tested and the variation between animals produced somewhat anomalous figures which do no more than suggest that there was partial suppression after these intervals.

Inflammatory paw swelling. The results of the experiment in which 0·05 ml of Freund's complete adjuvant was injected into each hind foot pad, and the increase in paw thickness determined after 48 h, are shown in Table 3. Pre-treatment with clofazimine 350 mg/kg produced a 40% reduction in paw swelling. Double this dose of clofazimine produced a reduction of swelling which was not significantly greater than that achieved with the smaller dose.

Primary humoral antibody response. The result of the sheep erythrocyte haemagglutination test is shown in Figure 1. Pre-treatment with clofazimine 350 mg/kg and 700 mg/kg produced no significant suppression of this primary antibody response.

Tuberculin skin test. The results of the tuberculin skin test experiment are shown in Table 4. Not only did clofazimine fail to suppress the tuberculin response, but there is a suggestion that the animals receiving the larger dose may actually have shown a slightly enhanced response, particularly at 48 hours.

Toxicity. In one experiment a group of 6 rats all died unexpectedly during ether anaesthesia 18 days after receiving a course of 550 mg/kg clofazimine. The reason for this is unknown. However, it is felt that the cause is unlikely to have

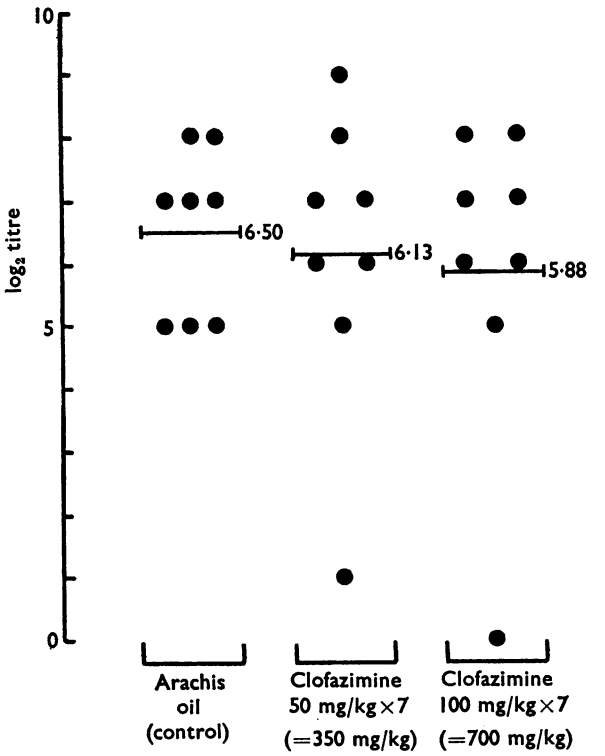

FIG. 1. Failure of clofazimine to suppress a primary humoral antibody response. Rats were challenged with 1 ml of 1% sheep erythrocytes (i.p.) and bled 7 days later. The drug was administered daily (excluding weekends), the final dose being given on the same day as the sheep erythrocyte injection. Eight animals in each group.

TABLE 4. *Failure of clofazimine to suppress the 24 and 48 h skin response to tuberculin (PPD). Each rat received 5 daily doses of either clofazimine or arachis oil and on the day of the last dose each was injected into a foot pad with Freund's complete adjuvant. Eleven days later tuberculin skin testing was carried out in an ear pinna (see* **Methods**)

No. of rats	Clofazimine treatment	Total dose (mg/kg)	Swelling in mm (S.D.) (24 h)	(48 h)
6	Arachis oil 1 ml × 5	0 (control)	0·627 (0·114)	0·737 (0·079)
6	100 mg/kg × 5	500	0·673 (0·053)	0·925 (0·193)
6	50 mg/kg × 5	250	0·627 (0·104)	0·871 (0·134)

been clofazimine toxicity, as no significant mortality occurred amongst treated animals in subsequent experiments in which this dosage was employed. Formal toxicity studies were not performed, and body weight changes under the conditions of these experiments clearly result from the interaction of a number of variables. Doses of clofazimine which protected against adjuvant arthritis also conferred an advantage in mean body weight (compared with the control group) by the end of the experiment. Blood counts performed 18 days after adjuvant injection showed no changes attributable to pretreatment with clofazimine 550 mg/kg.

Discussion

These results establish that clofazimine can protect against adjuvant arthritis when given in a dose that is well tolerated by rats. This dose also produced inhibition of inflammatory paw swelling, while larger doses failed to inhibit either the primary humoral antibody response to sheep erythrocytes or the skin response to tuberculin. Thus clofazimine probably inhibits adjuvant arthritis by virtue of some interruption in the chain of inflammatory events rather than a true immunosuppressive effect.

The experiments have shown that the effects of the drug persist for at least two weeks, probably longer. The probability that the pharmacological action of clofazimine (apart from its anti-mycobacterial activity) may depend on its slow accumulation in the tissues, makes it difficult to study further its mode of action by giving short 'pulses' of treatment at different stages during the course of adjuvant disease. As the tubercle bacilli used to induce adjuvant arthritis were dead it seems highly unlikely that the anti-mycobacterial properties of the drug could account for the observed inhibition.

Vischer (1969) has studied the anti-inflammatory action of clofazimine in rats: a reduction of approximately 30% in the cotton pellet granuloma required 10 × 200 mg/kg, while a similar reduction in the bradykinin oedema reaction in the rat paw required 1 × 200 mg/kg. In the 48 h response to Freund's complete adjuvant studied in the present experiments, the paw swelling presumably results from both tissue oedema and cellular infiltration. The present data provided two clues that clofazimine may modify different components of inflammation in different ways: the first was the failure to obtain a straightforward dose/response relationship in the paw swelling experiments, and the second was the slight tendency for clofazimine treatment actually to augment swelling in some tuberculin skin tests. Various lines of enquiry are being followed in an effort to elucidate this.

Conalty & Jackson (1962) and Conalty (1966) have studied the fate of ingested clofazimine in rodents. The unchanged drug can be identified in tissue sections.

On a dose of (50 mg/kg)/day clofazimine appears first in inclusions in macrophages, later as crystals in macrophages and also as free extra-cellular crystals. The intense concentration of the drug in macrophages is accompanied by significant depression of phagocytic function (Conalty, Barry & Jina, 1970). The main accumulation of clofazimine is in the reticulo-endothelial system, but all the tissues are discoloured and the fat depots particularly take on a mahogany/orange tint. Patients taking the drug develop some reversible pigmentation and the skin of rats receiving the doses used in these experiments showed obvious discolouration, which began to appear within 24 h of the first dose.

Ten years of testing clofazimine in leprosy has shown it to be a drug relatively free of toxicity (apart from skin pigmentation). Its apparent anti-inflammatory and steroid-sparing action in erythema nodosum leprosum, its definite anti-inflammatory effect in laboratory animals, and now the finding that it can inhibit rat adjuvant arthritis, suggest that this drug should be tested in inflammatory diseases such as rheumatoid arthritis. It might be argued that the dosage range used in these experiments was greatly in excess of that used clinically. This is true if the daily doses are compared, but if, as seems possible, this pharmacological action depends on the total load of the drug accumulated in the tissues, then these experimental dosage regimens may be more comparable to those used clinically. Considered in this light there would seem to be a strong case to be made for testing this drug in rheumatoid arthritis.

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