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The effect of desferrioxamine on antigen-induced inflammation in the rat air pouch

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The effect of desferrioxamine, a relatively specific iron chelating drug, has been examined in an allergic air pouch model of inflammation in the rat. The model has both an acute and chronic phase and allows quantitative measurements of the cellular and exudative response within the pouch fluid and the tissue response in the membrane that develops around the preformed cavity. Desferrioxamine given as a single bolus injection, directly into the cavity, stimulated the acute inflammatory phase, increasing both the exudative and leukocyte response, in a dose-dependent fashion. In marked contrast, repeated injections during the acute to chronic phase or during the established chronic reaction, led to a reduction in both leukocyte numbers within the cavity and the amount of granulation tissue surrounding it. The exudative response was, however, unaltered. These results are discussed in relation to the potential role of iron in promoting an acute and chronic inflammatory reaction.

Desferrioxamine, a sideramine, is a relatively specific iron chelating drug with a stability constant for trivalent ferric iron of 10^{31}. We, and others, have previously speculated that iron, particularly in a divalent ferrous state, may promote inflammation by its ability to (i) induce the peroxidation of membrane phospholipids (Tappel 1975; Blake et al 1981; Halliwell 1981) and (ii) catalyse the formation of the toxic hydroxyl radical from less active oxygen metabolites, released from neutrophils and pre-sensitized monocytes (Wills 1965; Kaschnitz & Hatefi 1975: McCord & Day 1978; Blake et al 1981). Trivalent ferric iron, in the form of ferric chelates, may also aggravate synovial inflammation by its ability to stimulate collagenase and prostaglandin (PGE_2) release from synovial cells (Okazaki et al 1981).

On the basis of these suggestions we previously examined the effect of desferrioxamine in three relatively simple animal models of inflammation and noted a 'biphasic' effect (Blake et al 1983). High doses of desferrioxamine (>200 mg kg^{-1}) reduced acute inflammation induced in the rat foot pad, when stimulated by monosodium urate or carrageenan, whilst lower doses exacerbated it. In guinea-pigs, in which Glyn-Dumonde mono-arthritis had been induced with bovine gamma globulin, desferrioxamine again had a biphasic effect; with a single dose exacerbating the acute phase of this model, and repeated dosing reducing the chronic phase.

All of these models have limitations as quantitative assessment of leukocyte numbers, the extent of the exudate reaction or degree of the tissue reaction are impossible to make. We have therefore examined the effect of desferrioxamine in the allergic air pouch model of inflammation in rats where these parameters of inflammation can be independently and accurately quantified (Tsurufuji et al 1982; Ohuchi et al 1982; Yoshino et al 1984).

The studies reported here were designed to (1) test if desferrioxamine is also pro- and anti-inflammatory in this model, (2) establish if either of the effects were dose-dependent, (3) examine the effect of single or repeated dosing and (4) see if the drug equally affected the leukocyte response and exudative or tissue reaction.

Materials and methods

Animals and model. Allergic inflammation was induced in young male Sprague Dawley rats as described by Yoshino et al (1984). In brief, rats were sensitized to bovine serum albumin (BSA), emulsified in Freund's complete adjuvant (FCA). Thirteen days after immunization, air was injected subcutaneously into the dorsum and 24 h later the pouch was challenged with BSA in a solution of carboxymethyl cellulose (CMC) in 0·9% NaCl (saline), supplemented with antibiotics (penicillin G potassium and dihydrostreptomycin sulphate) as a single antigenic challenge. Five days after the first challenge, BSA in saline was injected into the pouch as an antigenic rechallenge.

Drug treatment. Desferrioxamine mesylate BP (Desferal) was generously provided by Ciba Laboratories, Horsham, UK, and injected as Table 1. This outlines our experimental design which was selected to study: (i) The effect of single, but variable doses of desferrioxamine on acute inflammation; (ii) the effect of repeated but fixed dosage of desferrioxamine on the acute to chronic phase of inflammation; (iii) the effect of single and repeated doses of desferrioxamine (fixed dose) on the chronic phase of inflammation; (iv) the effect of repeated and variable doses of desferrioxamine on the chronic phase of inflammation.

Assessment of inflammatory activity includes measurement of the entire exudate fluid volume. The number of leukocytes within the exudate fluid were counted with a haemocytometer and the pouch wall was excised and weighed. The data are parametric and were analysed between groups by Student's *t*-test.

Results

The effect of single, but variable doses of desferrioxamine on the acute inflammatory phase of inflammation induced by a single antigenic challenge is shown in Table

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Table 1. Experimental design. All animals were sensitized by intradermal injection of BSA in Freund's complete adjuvant at Day-14 and an air pouch formed at Day-1. Desferrioxamine, when given at day 0, was injected into the pouch along with 4 ml of 2% CMC solution containing 2 mg of BSA (antigen). On subsequent occasions desferrioxamine was made up in 1 ml of saline. 1 ml of saline alone acted as control. Group numbers allow comparison with Table 2.

Group	Dose (mg/rat)	No. of rats	Day(s) of drug injection	Day(s) of challenge	Day of death
1A	0	6	0	0	1
1B	0·4	6	0	0	1
1C	4	6	0	0	1
1D	40	6	0	0	1
2A	0	5	0	0	1
2B	40	5	0	0	1
2C	0	5	0, 1, 2, 3, 4, 5	0	6
2D	40	5	0, 1, 2, 3, 4, 5	0	6
3A	0	5	5	0, 5	6
3B	40	5	5	0, 5	6
3C	0	5	5, 6, 7, 8, 9	0, 5	10
3D	40	5	5, 6, 7, 8, 9	0, 5	10
4A	0	5	5, 6, 7, 8, 9	0, 5	10
4B	0·8	5	5, 6, 7, 8, 9	0, 5	10
4C	4	5	5, 6, 7, 8, 9	0, 5	10
4D	20	5	5, 6, 7, 8, 9	0, 5	10

2 (group 1A–1D). The drug stimulated both the accumulation of exudative pouch fluid and the number of polymorphonuclear leukocytes assessed 24 h later in a dose-dependent fashion. Both indices of acute inflammation were significantly altered by 4 mg ($P < 0·05$) and 40 mg ($P < 0·025$) of desferrioxamine per rat.

The effect of repeated, but fixed, dosage of desferrioxamine on inflammation, again induced by a single antigenic challenge, is shown in Table 2 (group 2A–2D). To supplement and allow comparison with experiment 1 (group 1A–1D) a single dose of desferrioxamine was also given and the animals killed at 24 h. Desferrioxamine (40 mg) again stimulated the accumulation of the pouch fluid and increased leukocyte numbers ($P <$ 0·01). In contrast, when given at this dosage (40 mg/rat) but repeatedly over five days, significantly decreased both the leukocyte numbers and the wet weight of granulation tissue in comparison with rats receiving saline.

To establish the effect of desferrioxamine during the chronic phase after a repeat injection of antigen, desferrioxamine (40 mg/rat) was injected into the pouch immediately after the rechallenge and then over 4 days. Animals were killed at 24 h and at 6 days after this second challenge (Table 2, group 3A–3D). In contrast to experiments 1 and 2, desferrioxamine did not significantly worsen the inflammatory reaction 24 h after the rechallenge. However, as in experiment 2, its repeated administration significantly decreased both leukocyte numbers and the weight of granulation tissue. The volume of the exudate remained unaltered.

Experiment 4, was constructed to establish if this selective anti-inflammatory effect during the late chronic phase was also dose-dependent. Table 2 (group 4A–4D) details the results. As in experiment 3, a significant reduction in both granulation tissue weight and the number of leukocytes was observed following the repeated administration of desferrioxamine over 4 days following an antigenic *re*-challenge. The effect was dose-dependent reaching significance at 20 mg/rat. Again the exudate volume remained unaltered.

Discussion

The chronic allergic air pouch model we have used for this study is an example of connective tissue activation and granulation tissue formation in response to repeated immunological stimulation (Yoshino et al 1984 in the press). The method is specific, in so far as it depends upon the presence of hypersensitivity before there are many major changes, and sensitive, in that it

Table 2. Effect of desferrioxamine on exudate production, leukocyte migration and granulation tissue formation in antigen-induced inflammation in the rat air pouch.

Group	Dose (mg/rat)	Day of death	Exudate volume (ml)	No. of cells ($\times 10^8$)	Granulation tissue wet wt (g)
1A	0	1	6·35 ± 0·24	2·64 ± 0·15	—
1B	0·4	1	7·35 ± 0·62	3·09 ± 0·39	—
1C	4	1	7·78 ± 0·31**	3·73 ± 0·50**	—
1D	40	1	8·13 ± 0·47***	4·00 ± 0·36***	—
2A	0	1	7·58 ± 0·25	2·97 ± 0·10	—
2B	40	1	8·87 ± 0·26***	4·18 ± 0·17***	—
2C	0	6	10·48 ± 0·70	0·22 ± 0·02	2·28 ± 0·12
2D	40	6	8·84 ± 0·84	0·07 ± 0·001***	1·67 ± 0·13***
3A	0	6	12·80 ± 0·61	1·42 ± 0·10	—
3B	40	6	12·65 ± 1·26	1·48 ± 0·17	—
3C	0	10	12·69 ± 2·08	0·34 ± 0·02	3·44 ± 0·06
3D	40	10	16·16 ± 0·93	0·16 ± 0·01***	2·84 ± 0·12***
4A	0	10	11·16 ± 2·50	0·37 ± 0·04	2·86 ± 0·06
4B	0·8	10	11·00 ± 0·64	0·30 ± 0·06	2·71 ± 0·22
4C	4	10	11·67 ± 1·17	0·29 ± 0·07	2·53 ± 0·12
4D	20	10	11·13 ± 1·78	0·24 ± 0·02*	2·26 ± 0·20**

The values are the means ± s.e.m. of 5 or 6 rats. (*$P < 0·05$, **$P < 0·025$, ***$P < 0·01$) significance values relate to comparison with controls as indicated.

needs continuing antigenic rechallenge to maintain the chronic inflammatory reaction. A single antigenic challenge induces acute inflammation with a predominant polymorph infiltrate during the first 48 h. Later samples show a low grade mononuclear response persisting for 5–7 days. Repeated challenge produces chronic inflammation with an accentuated mononuclear response.

The experiment reported here shows that desferrioxamine affects both the acute and chronic phase of inflammation in this model, but in different ways. In the acute phase, desferrioxamine (assessed 24 h after the single antigenic challenge) produced dose dependent *enhancement* of the accumulation of the pouch fluid and the number of leukocytes in the exudate. However, its repeated administration for 5 days after the single challenge suppressed leukocyte cell numbers and the formation of granulation tissue, though no effect of the drug on exudative production was observed. Similar selective anti-inflammatory effects were observed during the established chronic reaction.

This acute exacerbation might be explained by a direct irritant effect of desferrioxamine. This is, however, unlikely as in previous studies where the drug was given intraperitoneally (away from the site of inflammation) the acute exacerbation was also observed (Blake et al 1981). No peritoneal irritation was observed on simple inspection. Had 'microscopic' irritation been present—then as a counter irritant, the inflammation at a distant site should be suppressed. An alternative explanation that we favour relates to the known damaging effects of reactive oxygen species on the neutrophil membrane. Scavengers of superoxide and hydrogen peroxide can increase neutrophil longevity. This effect has been attributed to an intereaction of superoxide and hydrogen peroxide to form the toxic hydroxyl radical—an intereaction dependent upon an iron catalyst (McCord et al 1980). Desferrioxamine, by binding the iron necessary for this reaction, might well increase the neutrophil life span and thereby increases neutrophil numbers. Such a phenomenon may increase the inflammatory reaction (as observed) by allowing the controlled synthesis and release of myeloperoxidase, neutral proteases and other hydrolytic and digestive enzymes to occur more effectively.

A variety of mechanisms might explain the anti-inflammatory effect observed during the chronic phase. Iron is essential for the activity of proline hydroxylase and is a cofactor in collagen synthesis (Hunt et al 1979). Considerable evidence suggests that cardiac and hepatic fibrosis occur in patients with iron overload (Buja & Roberts 1971; Witzelben & Wyatt 1961; Jacobs 1977). Long term chelation therapy with desferrioxamine is shown to halt the progress of hepatic fibrosis (Barry et al 1974). Therefore the inhibition of the formation of granulation tissue by desferrioxamine, which we have observed in this experiment seems to show the inhibitory effect of the drug on collagen synthesis in a chronic inflammatory reaction. The modest drop in the white cell count, could be produced (i) by a tendency to *increase* the count by increasing white cell survival (see above) coupled with, (ii) a decrease in the amount of chemo-attractant, which would tend to *decrease* it. Superoxide derived from neutrophils reacts with a component of plasma to form a chemotactic product for neutrophils, whilst the hydroxyl radical may generate a similar factor derived from arachidonic acid (Petrone et al 1980; Perez & Goldstein 1979).

Desferrioxamine will modify an inflammatory reaction in two distinct ways. It exacerbates acute inflammation but suppresses some aspects of the chronic response. We would suggest that these iron chelation experiments provide a novel insight into mechanisms of inflammation and a greater understanding of the subtleties of these mechanisms might allow the development of clinically useful anti-inflammatory drugs.

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