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D-penicillamine and immune complex deposition

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SUMMARY Dense, granular immunoglobulin deposits have been identified at the epidermo-dermal junction in 4 out of 10 patients who developed toxic reactions to D-penicillamine therapy for rheumatoid arthritis. Three of 4 patients developing a lupus-like syndrome while on penicillamine had similar findings on skin biopsy. Serum immunoglobulin and complement levels decreased significantly in patients treated with penicillamine. It is suggested that, in addition to penicillamine nephropathy, other side effects of this drug may be related to widespread deposition of immune complexes.

Patients with rheumatoid arthritis derive benefit from treatment with D-penicillamine (Multicentre Trial Group, 1973; Huskisson, 1976a). This improvement takes some months to occur and is accompanied by a fall in the erythrocyte sedimentation rate (ESR) and rheumatoid factor titre, and improvement in the systemic features of the disease. These actions distinguish D-penicillamine from the anti-inflammatory drugs and suggest a more fundamental mode of action (Huskisson, 1976b). Serious complications of therapy sometimes occur and may affect a number of tissues. They include rashes, mouth ulcers, thrombocytopenia, leucopenia, bone marrow aplasia, and nephropathy, and they may occur separately or in combination. In addition there have been isolated case reports of a lupus-like syndrome developing in patients taking D-penicillamine (Harpey *et al.*, 1971; Camus *et al.*, 1974).

The presence and nature of circulating immune complexes in patients with rheumatoid arthritis has been investigated by several workers (Onyewotu *et al.*, 1974; Roberts-Thomson *et al.*, 1976), and the effects of D-penicillamine on these complexes have also been studied (Mohammed *et al.*, 1976). Alteration in the nature of the complexes has been found, and there is also evidence that nephropathy induced by the drug is due to immune complex deposition (Jaffe *et al.*, 1968; Bacon *et al.*, 1976).

The present study reports on the immunological changes in patients taking D-penicillamine for rheumatoid arthritis; 4 further cases of a lupus-like syndrome are also recorded. Evidence for immune deposits in the skin of these and other patients has been sought in an attempt to correlate immune complex deposition at this site with other toxic effects of penicillamine in addition to the nephropathy

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Methods

Four separate studies are reported. In the first study skin immunofluorescence in 3 groups of patients was compared. Ten patients who had never been treated with penicillamine or similar drugs acted as the control group (group 1). Patients taking D-penicillamine were divided into 2 groups: those who had developed no toxic reactions (group 2) and those developing serious side effects limited to 1 tissue (group 3). These reactions included severe late onset rashes (3) thrombocytopenia (2), aplastic anaemia (1), and proteinuria (4). The groups were well matched, and the duration and dose of D-penicillamine therapy were similar in groups 2 and 3. None of the treated patients had been taking penicillamine for less than 6 months.

Skin biopsies were taken from normal unexposed forearm skin with a 3 mm punch. The tissue was snap-frozen in liquid nitrogen and 5 μm cryostat sections were cut and air dried. Sections were stained with fluorescein conjugated antihuman IgA, IgG, and IgM (Wellcome Reagents, Beckenham). Antihuman C3 was obtained from Meloy, Virginia, USA. The monospecificities of the antisera were confirmed by immunoelectrophoresis against whole human sera. The fluorescein to protein molar regions of the antisera were determined spectrophotometrically and found to be between 2·7 and 4·0. The stained sections

were examined on a microscope with an HBO 200 mercury vapour ultraviolet light source. An FITC 3 interference filter, a BG 12 filter, and a K530 barrier filter were used.

In the second study 4 patients who developed systemic lupus erythematosus while receiving penicillamine were documented and had skin biopsies for immunofluorescence.

17 patients with penicillamine-induced nephropathy represent a third study, and the changes in serum immunoglobulins in 20 patients treated for 6 months with penicillamine a fourth.

Results

The results of the skin biopsies in the 3 groups of patients in the first study are shown in Table 1. None of the patients in the control group or those without toxic reactions to D-penicillamine were positive. In group 3, consisting of patients with toxic reactions, immunoglobulins and complement deposits were seen in 4 out of 10.

The clinical and immunological findings in the 4 patients developing a lupus-like syndrome are shown in Table 2. All had classical rheumatoid arthritis prior to treatment with D-penicillamine, with erosive disease and positive rheumatoid factor. All developed a positive or increased titre of ANF, and DNA binding was high in 3. All 4 patients had responded well to the drug and had been on it for a long time prior to the development of the syndrome. An increase in joint pain, of a burning quality, in the absence of joint swelling occurred in all cases. All 4 patients quickly recovered from this and from the other clinical features when D-penicillamine therapy was stopped. Three of these 4 patients had deposits of immunoglobulins and complemen in the skin.

The abnormalities seen on the skin biopsy were of 2 types. One consisted of a dense granular deposit of IgM along the epidermal dermal junction. This deposit was similar to that seen on the basement membrane of the kidney in immune complex disease. It was also seen in systemic lupus erythematosus, but in that condition the deposits were more often IgG. The other feature seen in 3 cases was the adherence of IgG to the epidermal cell nuclei.

The third study was of 17 patients who developed significant proteinuria while receiving penicillamine. This developed after a mean of 9·5 months of treatment (4–15) and varied in amount from 0·9 to 21G daily. Three patients became nephrotic. Renal biopsy carried out in 3 patients showed deposits of IgG and complement on immunofluorescent staining and typical dense deposits on electron microscopy.

Penicillamine therapy was continued in 10 patients, none of whom were nephrotic, whose maximum 24-hourly urine protein output varied between 1·1 and 7·3g. No deterioration of renal function occurred in any of these patients, and there was a gradual diminution in proteinuria. The mean duration of proteinuria in 7 patients who continued taking penicillamine and who have been followed up for a sufficient period of time was 14·3 months (5 to 30), compared to 15·3 in a group who stopped.

The results of the serological tests in the fourth study are shown in Table 3. There was a fall in the titre of rheumatoid factor, immunoglobulin, and complement levels, but no significant change in ANF titre.

Table 1 *Immunofluorescent skin staining in patients with rheumatoid arthritis (group 1, never on penicillamine; group 2, on penicillamine without any side effects; group 3, on penicillamine causing toxic reactions)*

Group	*Number*	*Mean age*	*Sex* (F:M)	*Mean disease duration (yr)*	*Number with positive immunofluorescent staining*	*Type of staining*	
						Immunoglobulin at epidermal-dermal junction	*Epidermal cell nuclei*
1	10	66·3	8:2	9·75	0	0	0
2	10	57·8	8:2	13 1	0	0	0
3	10	58·3	5:5	8·0	4	4	2

Table 2 *Details of the 4 patients developing a 'lupus-like syndrome' on penicillamine*

Case no.	*Age*	*Sex*	*Disease duration (years)*	*Duration of therapy (months)*	*ANA*	*DNA binding (%)*	*Clinical problems*
1	47	F	5	24	1/1000	51	Depression, purpuric rash, arthralgia
2	43	M	4	26	1/1000	11	Proteinuria, arthralgia, depression
3	35	F	8	48	1/1000	29	Lymphadenopathy, fever, weight loss, proteinuria
4	30	F	14	25	1/1000	91	Depression, rash, pneumonitis, arthralgia

Table 3 *Serological findings before and after 6 months' therapy with D-penicillamine in 20 patients with rheumatoid arthritis. Statistical analysis by Student's t test; NS=not significant*

Test	*Mean values*		
	Before therapy	*After therapy*	*P*
IgG (GMS/L)	17·39	15·1	<0·02
IgA (GMS/L)	3·07	2·45	<0·02
IgM (GMS/L)	2·0	1·48	<0·01
Latex (>1:40)	17+ve	13+ve	<0·01
SCAT (>1/32)	18+ve	7+ve	<0·001
ANF (>1/100)	6+ve	8+ve	NS
C_3 (mgm/100 cm^3)	145·1	123·3	<0·01

Discussion

D-penicillamine is known to be an effective but potentially toxic form of treatment for rheumatoid arthritis (Multicentre Trial Group, 1973; Huskisson, 1976a). A large number of patients have been treated with the drug in the unit, and a few have experienced side effects of the type documented elsewhere.

For the purpose of the first study a small number of patients with toxic reactions was matched with groups who had no toxicity, or were not on the drug. The skin biopsy findings of dense immune deposits at the epidermal junction and immunoglobulins attached to epidermal cell nuclei seem to correlate with the occurrence of toxicity.

To isolated reports of a lupus-like syndrome occurring in patients on D-penicillamine (Harpey *et al.*, 1971; Camus *et al.*, 1974) the present study adds 4 further cases. In 3 of these patients DNA binding was abnormally high, which is unusual in most other drug-induced lupus syndromes (Hughes, 1973). The clinical features were typical of SLE, and the patients quickly recovered when the drug was discontinued. Three of these 4 patients also had positive immunofluorescence on skin biopsy.

The nephropathy of D-penicillamine is thought to be due to immune complex deposition (Jaffe *et al.*, 1968; Bacon *et al.*, 1976). Our studies lend support to this view, a new finding being that the proteinuria will subside in spite of continued therapy. This would also be compatible with an immune complex phenomenon.

The serological findings show that treatment with D-penicillamine is associated with a decrease in latex titre and immunoglobulin levels, including IgA, which did not alter in other studies (Bluestone and Goldberg, 1973). Complement levels were also significantly affected, though they did not fall below the lower limit of normal. The reasons for these changes are unknown but would again be compatible with the deposition of complexes in tissue including the skin.

These findings suggest that immunoglobulin deposits may occur in the skin and other tissues of patients taking D-penicillamine, and that this deposition may correlate to some extent with the occurrence of tissue reactions.

In addition to the nephropathy other side effects of D-penicillamine, including rashes and the lupus syndrome, could be related to immune complex deposition. The lupus-like syndrome could reflect the extreme end of the spectrum of toxic reactions to the drug associated with the disappearance of immunoglobulin and complement from the serum and the appearance of complexes in target tissues.

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