Case Report

Coexistent membranous nephropathy and ANCA-positive crescentic glomerulonephritis in association with penicillamine

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Introduction

D-penicillamine is an effective disease-modifying drug in rheumatoid arthritis, but adverse effects are common and often lead to the cessation of treatment with this agent [1]. A variety of autoimmune disorders may be induced by the drug, either associated with organ-specific autoantibodies (e.g. myasthenia gravis or pemphigus vulgaris) or with non-organ-specific autoantibodies (e.g. systemic lupus erythematosus) [2,3]. Renal injury is seen in up to 30% of patients treated with penicillamine, typically causing proteinuria without impairment of renal excretory capacity [4]. The commonest histological finding is membranous nephropathy, but in a minority of cases there will be minimal-change nephropathy or the presence of electron-dense mesangial deposits [4].

The prognosis of penicillamine nephropathy is considered generally very good, with complete resolution typically occurring when the drug is stopped, and deterioration of renal function being exceptional. A small number of cases of crescentic glomerulonephritis associated with D-penicillamine therapy have been reported in recent years [5–7], and this may sometimes occur in the context of penicillamine-induced lupus [8]. Antineutrophil cytoplasm autoantibodies (ANCA) have been reported in one case [6]. We report a patient who developed proteinuria whilst taking D-penicillamine for rheumatoid arthritis. Proteinuria increased despite a reduction in penicillamine dosage, and he subsequently developed rapidly progressive renal failure associated with circulating ANCA. Renal biopsy showed both membranous nephropathy and a necrotizing crescentic glomerulonephritis. Despite intensive immunosuppression he subsequently developed full-blown systemic vasculitis.

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Case report

Case history

The patient, a white male, was given a diagnosis of rheumatoid arthritis in 1980 at the age of 39 years after typical joint symptoms of 3 years' duration, with rheumatoid nodules on both elbows, radiological erosions, and a strongly positive rheumatoid factor. Initially he was treated with non-steroidal anti-inflammatory drugs, but in February 1989 he was started on D-penicillamine because of inadequate control of the arthritis. The drug was introduced at 250 mg daily, increasing after 6 months to 500 mg daily. There was an excellent effect on his arthritis, and complete resolution of the rheumatoid nodules. Urinalysis and full blood counts were monitored monthly and remained normal until April 1994. At that time proteinuria (+ on dipstick analysis) was first noted. In May 1994 proteinuria persisted: 24-h urine protein excretion was 1.66 g. Blood pressure was 150/80 mmHg. Plasma creatinine concentration at this time was 81 μmol/l, a midstream specimen of urine showed no abnormality, and full blood count was normal. Penicillamine was continued at a reduced dosage (250 mg daily) for a further month. In June 1994, 24-h urine protein excretion had increased to 2.7 g and penicillamine was stopped. Two weeks later the patient became generally unwell with fever, vomiting, and malaise. Blood pressure had risen to 185/110, plasma creatinine was 324 μmol/l and he was referred to us for further investigation and management.

On admission, he was febrile (37.8°C), pale and unwell. Blood pressure was 210/115, there was no oedema and no other abnormal physical finding. Urinalysis showed + + + + blood and + + + protein. Microscopy of the urinary sediment revealed numerous crenated red blood cells, numerous granular casts and a few red cell casts. Further investigations showed haemoglobin 10.4 g/dl. White blood count 11.6 × 10⁹/l (neutrophils 9.51 × 10⁹/l, eosinophils 0.16 × 10⁹/l), platelets 352 × 10⁹/l, ESR 110 mm/h, fibrinogen > 10 g/l, plasma creatinine 358 μmol/l, and C-reactive protein 110 mg/l. ELISA for antiglomerular basement

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membrane (GBM) antibody was negative. ANCA was strongly positive by indirect immunofluorescence on alcohol-fixed normal human neutrophils, with a p-ANCA pattern. Subsequent antigen-specific ELISAs showed that the specificity of the ANCA was for myeloperoxidase (MPO), with no binding to either proteinase 3 or lactoferrin. Ultrasound scanning showed two normal sized, unobstructed kidneys. After control of his blood pressure, he underwent percutaneous renal biopsy.

Histological findings

The renal biopsy of both cortex and medulla contained 20 glomeruli: 15 showed varying stages of focal and segmental fibrinoid necrotizing glomerulonephritis, the remaining five showed only mild and non-specific increase in cellularity. Six tufts showed proliferation of the parietal epithelial cells in Bowman's space, with both fibrous and cellular crescents (Figure 1). Silver stain revealed cross-cut 'spikes' in several regions. There was much parenchymal loss and scarring with fibrosis, tubular atrophy, and diffuse, mild inflammation.

Direct immunofluorescence showed brilliant granular positivity with IgG and C3 (Figure 2) but negative staining with IgA, IgM, and C1q. Formaldehyde-fixed tissue was processed for electron-microscopy. There were numerous intramembranous immune deposits, localized to the epithelial side of the basement membrane (Figure 3). The findings were of a stage II membranous glomerulonephritis with a coexistent necrotizing crescentic glomerulonephritis.

Management and progress

The patient was treated with prednisolone 60 mg/day and cyclophosphamide 200 mg/day, both taken orally.
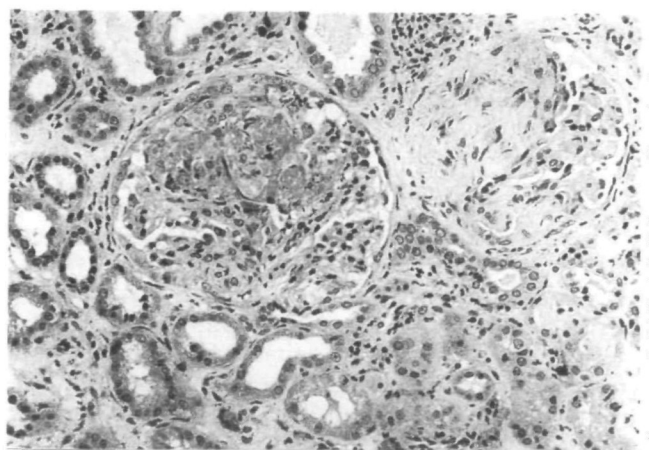

Fig. 1. Glomerulus with segmental solidification and fibrinoid necrosis with neutrophils, karyorrhectic debris and parietal cell proliferation amounting to a cellular crescent. A second glomerulus has segmental scarring indicating previous active disease (H&E ×350).
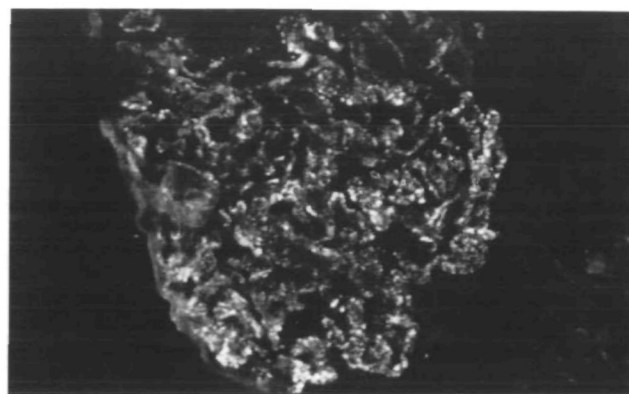

Fig. 2. Brilliant granular immunofluorescence of glomerular basement membranes with antibodies to IgG (×500).
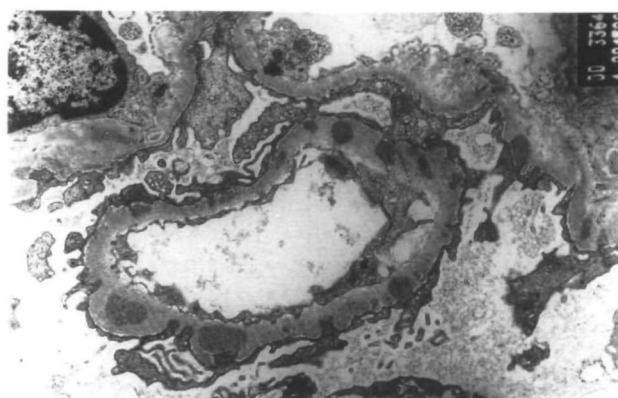

Fig. 3. A capillary loop of the glomerulus with expanded basement membrane and electron-dense deposits localizing to the epithelial side of the membrane (×8100).

Figure 4 shows his early progress. At 3 months, cyclophosphamide was stopped and azathioprine 100 mg daily substituted. At this time plasma creatinine was 134 μmol/l, CRP 4 mg/l, ESR 20 mm/h, ANCA positive (anti-MPO ELISA titre 34% of reference positive). Two weeks later, the patient became generally unwell with culture-negative fever (up to 40°C), nausea and vomiting, bilateral scleritis and a generalized vasculitic rash. Renal function deteriorated over the next few days, to a peak plasma creatinine of 781 μmol/l. He was treated with i.v. methylprednisolone and pulse i.v. cyclophosphamide. He developed a deep venous thrombosis and was anticoagulated: this precluded further tissue biopsies. His rash and scleritis rapidly resolved, and his renal function improved (plasma creatinine 190 μmol/l). His treatment was maintained with frequent pulses of cyclophosphamide and maintenance oral prednisolone. At most recent follow-up he was well, but with persisting hypertension. His plasma creatinine has fallen further to 121 μmol/l.

Discussion

This patient developed proteinuria after taking D-penicillamine for 5½ years. Patients with typical penicillamine nephropathy, usually due to membranous
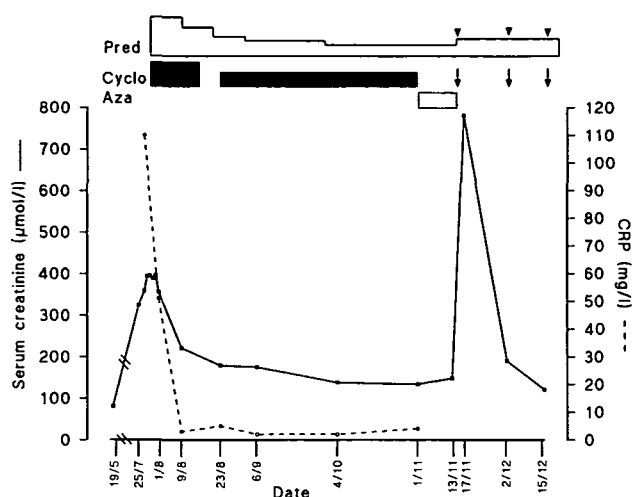
Fig. 4. Patient's progress from diagnosis: serum creatinine (μmol/l) against time, with C-reactive protein (mg/l) when available. Immunosuppressive drugs: Pred, prednisolone, oral dosage starting at 60 mg/day, arrowheads indicate intravenous pulses of methyl-prednisolone, 1000 mg each. Cyclo, cyclophosphamide, oral dosage starting at 200 mg/day, arrows indicate intravenous pulses of cyclo-phosphamide, first pulse 800 mg, subsequent pulses 500 mg each. Aza, azathioprine, oral dosage 100 mg/day.

glomerulonephritis, tend to develop proteinuria within 12 months of starting the drug, but onset as late as 5 years after initiation of penicillamine therapy has been well described [4]. In our patient, plasma creatinine was initially normal, but soon after stopping the drug he developed an acute nephritic syndrome and rapidly progressive renal failure associated with the presence of ANCA. Renal biopsy showed coexistent membranous glomerulonephritis and crescentic necrotizing glomerulonephritis. Initially, he responded well to immunosuppression, with induction of a clinical remission, but when the immunosuppression was reduced he rapidly relapsed with full-blown systemic vasculitis. Intensification of his immunosuppression again induced a good response.

D-penicillamine has been implicated in the development of numerous autoimmune conditions [2]. Renal disease is one of the most common, the typical histological pattern being membranous nephropathy and the prognosis being excellent when the drug is stopped [4]. Occasional cases of crescentic glomerulonephritis have been reported in association with penicillamine therapy, sometimes together with pulmonary haemorrhage (Goodpasture's syndrome) [5,9,10]. The association appears to be with the drug itself rather than an effect of underlying rheumatoid arthritis, since cases have also occurred when penicillamine has been used for the treatment of Wilson's disease [9] and primary biliary cirrhosis [11]. Immunofluorescence on renal tissue has usually been negative, indicating pauci-immune crescentic glomerulonephritis, or renal-limited vasculitis, of the type that is typically associated with ANCA of anti-MPO type [12].

Uniquely, our patient had *both* membranous nephropathy and crescentic glomerulonephritis together with ANCA of anti-MPO specificity, and subsequently developed full-blown systemic vasculitis. The clinical course was aggressive, requiring intensive immunosuppression to gain control of the disease. It is possible, of course, that the penicillamine was not implicated in the causation of the disease. The development of proteinuria may have been the first sign of the onset of coincidental ANCA-positive systemic vasculitis. However, we find this implausible as it would imply that the co-existing membranous glomerulonephritis had been clinically silent, since monthly urinalysis had previously been negative. Rather, we suspect that our patient developed membranous glomerulonephritis as a late consequence of penicillamine therapy, and that soon after this he developed ANCA-positive small-vessel vasculitis, initially confined to the kidney but subsequently causing generalized systemic vasculitis. It is conceivable that the membranous lesion led to the exposure of neoantigens in the kidney and the induction of an autoimmune nephritis. Such a mechanism has been proposed for the rare association between penicillamine and anti-GBM antibodies [13,14]. Anti-GBM antibodies were not present in our patient.

Hydralazine, a drug with a similar ability to cause autoimmune disease, has also previously been associated with ANCA-positive systemic vasculitis, presenting with a characteristic pattern of autoantibodies. Such patients have anti-MPO antibodies and antinuclear antibodies, and the large majority also have anti-lactoferrin antibodies (Short *et al.*, unpublished observations). Others have reported antielastase antibodies [15,16]. Antibodies against double-stranded DNA are also found. This pattern of autoantibodies is not seen in primary systemic vasculitis. In the present patient the autoantibody specificity was restricted to MPO.

Several putative mechanisms for the capacity of penicillamine to induce autoimmunity have been proposed. The drug could act as a hapten, binding to autoantigens and leading to a breakdown in self-tolerance [17]. However, no evidence of anti-penicillamine antibody activity has been found. In rodents, penicillamine acts as a polyclonal B cell activator [18]. In one susceptible animal, the Brown Norway rat, polyclonal B cell activation by mercuric chloride induces necrotizing vasculitis and anti-MPO antibodies [19,20], and similar observations have recently been made after treatment with penicillamine (Qasim *et al.* unpublished observations). This animal model is thought to result from preferential activation of the Th2 subset of helper T lymphocytes [21,22] and it is possible that a similar mechanism could underlie the development of some autoimmune conditions in man. Penicillamine also inhibits the fourth component of complement C4 *in vitro* at concentrations similar to those achieved *in vivo* [23] and may impair the role of the classical pathway of complement in disposing of immune complexes. Penicillamine nephropathy, like idiopathic membranous nephropathy, is more common

in individuals whose MHC type includes HLA-DR3 [24]. The genes encoding C4 are contained within the MHC, and haplotypes bearing DR3 are commonly associated with null alleles for C4, leading to a lower level of circulating C4 protein: thus it is possible that DR3-positive individuals are relatively C4 deficient, and a deleterious effect of penicillamine on remaining C4 function could induce disease.

It has been suggested that penicillamine is more likely to induce toxic effects in individuals who have a low sulphoxidation capacity (tested using oral carbocysteine) [25]. Certain drugs which can induce autoreactivity have been shown to have an inhibitory effect on DNA methylase, resulting in hypomethylation of DNA, which is particularly evident in T lymphocytes [26]. Methylation is an important mechanism involved in gene regulation and a breakdown of such regulation could lead to a loss of immunological tolerance and the development of autoreactivity. It is not yet clear whether penicillamine has such an effect on human DNA.

In conclusion, we report a unique case in which penicillamine therapy for rheumatoid arthritis was complicated by the development of coexistent membranous nephropathy and ANCA-positive crescentic glomerulonephritis, with an aggressive clinical course and subsequent development of full-blown systemic vasculitis. Whilst a causative relationship with penicillamine cannot be proved, this drug may have the potential to induce systemic vasculitis as well as other autoimmune conditions.

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