Serum sickness and acute renal failure after streptokinase therapy for myocardial infarction

K. A. DAVIES, P. MATHIESON, C. G. WINEARLS, A. J. REES & M. J. WALPORT
Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, England

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SUMMARY

A patient developed serum sickness and acute renal failure following therapy with streptokinase for myocardial ischaemia. There was a previous history of a cellulitic infection of the leg, and antibodies to streptokinase were measurable in a serum sample taken from the patient before therapy. A cryoglobulin was detected at the time of presentation with serum sickness. This contained polyclonal IgG (with anti-streptokinase activity), streptokinase, and C3. Circulating immune complexes were demonstrated by Clq-binding assay. Deposition of C3 was observed in skin and renal biopsies, and bound to erythrocytes. Renal histology, however, showed acute tubular necrosis, with no vasculitis or inflammatory cell infiltrate. This case provides an unusual example of the characterization of an immune complex comprising a specific antibody and an exogenous antigen, and has clinical implications for the use of streptokinase.

Keywords streptokinase complement immune complexes renal failure

INTRODUCTION

The benefits of thrombolytic therapy in acute myocardial infarction are now well established (Lipkin & Reid, 1988). Streptokinase, administered either intravenously or by the intra-coronary route, is one of the drugs most frequently employed for this purpose (Mason, 1981). Streptokinase is a non-enzymatic protein (47 kD) produced by group C streptococci, and the recommended initial dose in the UK is 250 000 IU, equivalent to 0.5 g protein. The reported frequency of allergic reactions to the drug varies between 1.7% and 18% (McGrath & Patterson, 1984) of which the majority is mild and self-limiting, taking the form of acute immediate-type hypersensitivity reactions (Sharma, Cella & Parisi, 1982).

We describe a patient with a delayed allergic reaction to streptokinase, administered as part of the therapy of acute myocardial ischaemia. He presented 8 days after streptokinase treatment with a serum-sickness type illness and acute renal failure. An IgG immune response to streptokinase and a cryoglobulin containing streptokinase, anti-streptokinase and C3, together with evidence of systemic complement activation were detected in the patient’s serum. Despite these findings, and the coincident development of acute renal failure, histological examination of renal tissue, obtained by percutaneous biopsy, revealed acute tubular necrosis, with no inflammatory cell infiltrate or vasculitis, although C3, IgA and IgM were detected in the mesangium by direct immunofluorescence. These observations have implications for the clinical use of streptokinase, as well as for an understanding of the pathogenesis of ‘immune complex disease.’

CASE REPORT

A 53-year-old caucasoid man presented to the Accident and Emergency Department of his local hospital with a short history of crushing central chest pain. The only relevant past history was of a severe cellulitic infection of his right leg 13 years previously. The history and ECG were consistent with a diagnosis of acute myocardial infarction, and he received thrombolytic therapy with 1.2 x 106 U of streptokinase (Hoechst, Hounslow, UK) by i.v. infusion into a peripheral vein over 1 h. The infusion was uneventful. The ECG reverted to normal, and there was only a small rise in cardiac enzymes; maximum CPK was 20% above the upper limit of normal. Plasma creatinine was 94 g/moles/l on admission and 85 g/moles/l on the third day, at which time he developed bilateral loin pain, fever, and a rash on his feet and shins, followed by arthralgia in his knees and elbows, and a falling urine output. He presented to Hammersmith Hospital on day 8.

Physical examination revealed him to be generally unwell, with a blood pressure of 140/95, with no postural hypotension, pulse 90 b/min, and temperature 38.2°C. The rash on his lower extremities was petechial and his knees and elbows were tender with a reduced range of movement, limited by pain. Urine
output during the first 24 h after admission was 25 ml. Urinalysis (Ames) revealed + + blood and + + protein, and urine microscopy showed numerous granular and cellular casts. Plasma creatinine was 460 μmol/l. Other relevant results were: sodium 135 mmol/l; potassium 4.0 mmol/l; urea 21.6 mmol/l; haemoglobin 13.4 g/dl; white cell count 12.2 × 10⁹/l (normal differential), platelets 243 × 10⁹/l; ESR 80 mm in the first hour; C-reactive protein 120 mg/l (normal <10 mg/l); creatine kinase 370 iU/l; anti-streptolysin O titre 200 iU/l. Anti-neutrophil cytoplasmic antibody was negative, and antibodies to GBM were not detected by radio-immunoassay. Skin biopsy showed a perivascular lymphocytic infiltrate, and there were deposits of C3 in dermal capillaries demonstrated by direct immunofluorescence. Renal biopsy revealed acute tubular necrosis with structurally normal glomeruli, and weak, granular, mesangial deposition of IgA, IgM and C3, but no IgG, was seen by direct immunofluorescence. No tissue was obtained for electron microscopy. There was no evidence of cholesterol emboli in either skin or renal biopsies.

On admission it was first thought likely that the patient had rapidly progressive glomerulonephritis, and he was treated with prednisolone 60 mg daily, cyclophosphamide 200 mg daily, and received two 4-l plasma exchanges. Following the results of the renal biopsy and the initial immunological investigations, therapy with cyclophosphamide and plasma exchange was discontinued, and the dose of prednisolone was reduced over 2 weeks. The patient's arthralgia resolved within 24 h of admission, following two doses of prednisolone, and his urine output returned to normal over 6 days, with a concomitant improvement in plasma creatinine (Fig. 1). He was discharged from hospital after 2 weeks, and at follow-up has remained entirely well.

**Materials and Methods**

**Demonstration of precipitating anti-streptokinase antibodies in serum**

Precipitating antibodies to streptokinase (Kabivitrum, Uxbridge, UK) were demonstrated in the patient's serum initially by simple diffusion in a 1% agarose gel and a quantitative precipitin test. Titration of antigen at a starting concentration of 20 000 U/ml (equivalent to 40 mg/ml) in the presence of constant antibody concentration enabled the establishment of optimal proportions for the reaction, and serial dilutions of peak sera in the same test system were used to calibrate a quantitative radial immunodiffusion assay. Anti-streptokinase activity was measured in serum obtained from the patient prior to the therapeutic infusion of the drug, and in all subsequent blood samples.

**Measurement of antibody affinity**

Binding affinity of anti-streptokinase antibodies in the patient's serum was estimated by incubation of serum with various dilutions of the antigen radiolabelled with ¹²⁵I to a specific activity of 1 μCi/μg, followed by precipitation at 50% saturated ammonium sulphate (Minden & Farr, 1973). Bound and free antigen concentrations were measured, and binding affinity calculated from a plot of the logarithmic transformations of Sip's equation.

**Preparation of a rabbit anti-streptokinase reagent**

An adult New Zealand white rabbit was immunized with streptokinase. An initial dose of 4 mg in Freund's complete adjuvant was administered subcutaneously, followed by two further doses of 500 mg, 1 and 2 weeks later, and a boost of 1 mg of the drug at 5 weeks. Following a test bleed, anti-streptokinase activity was confirmed as described above.

**Preparation of FITC-conjugated streptokinase**

Streptokinase was dissolved in 0.25 M sodium carbonate/0.1 M NaCl buffer, pH 9.0, at a concentration of 10 mg/ml; 2 ml streptokinase were incubated overnight with 1 mg FITC (Sigma, Poole, UK) at 4°C. Unlabelled fluorochrome was removed by separation on a PD10 column.

**Immunofluorescent staining of renal biopsy for streptokinase and anti-streptokinase**

Sections were incubated with FITC-streptokinase at concentrations of 0.25 to 500 μg/ml, for 1 h at room temperature, followed by extensive washing in PBS. Rabbit polyclonal anti-streptokinase (prepared as above) was used at dilutions ranging from 1:1 to 1:200 to probe for streptokinase in renal biopsy tissue. Incubation was performed overnight at 4°C, followed by incubation with FITC-conjugated swine anti-rabbit antibody (Dako).

**Assay for soluble immune complexes**

Circulating immune complexes in serum were detected using two radioligand binding assays. All incubations were performed in PBS/NaNO₃ 10 mm/BSA 0.2%EDTA 10 mm, pH 7.2. Polystyrene microtitre plates (Immulon II Removawells, Dynatech) were coated with either purified C1q or antibody to iC3b neoantigen (Cytotech) and incubated with test sera, at 1:10 dilution, overnight at room temperature. Following six washes
with buffer, plates were probed by incubation with a monoclonal antibody to C3dg (Clone 9, kindly donated by Professor P.J. Lachmann, MRC MIP Unit, Cambridge) radiolabelled with $^{125}$I, for 12 h at 4°C. Heat-aggregated IgG (prepared at 200 μg/ml) and BSA/anti-BSA immune complexes, prepared at 50-fold excess of antibody, both opsonised with complement by incubation in normal serum at 37°C for 30 min, were used as positive controls.

**Demonstration and characterization of a cryoprecipitate**

Five-millilitre samples of serum from the patient were incubated at 4°C for 5 days. A cryoprecipitate (protein concentration 370 μg/ml) was detectable in the serum sample obtained on day 9 following therapy with streptokinase. A trace of cryoprecipitate was detected 24 h after the first plasma exchange (concentration < 20 μg/ml), and none in subsequent serum samples. The cryoglobulin was dissolved in 0.2 M sodium acetate buffer, pH 4.2, and extensively dialysed against PBS for further purification. Cryoglobulin samples were tested in an Ouchterlony plate assay against polyclonal anti-mu, anti-gamma, anti-lambda, anti-kappa and anti-alpha reagents (Dako), polyclonal sheep anti-C3, and dilutions of streptokinase. The molecular composition of the cryoprecipitate was analysed by electrophoresis on a 12.5% SDS–PAGE. Immunoblotting of the cryoglobulin run on an SDS gel was performed on to nitrocellulose filters. These were probed with either the patient’s own serum, measured to have maximal anti-streptokinase activity (at 1:20 dilution), or with a 1:20 dilution of the rabbit anti-streptokinase reagent in tris 50 mm/NaCl 150 mm/BSA 1.5%, pH 6.9, for 4 h at room temperature. Hybridization of antibody to the membrane was identified by probing with $^{125}$I-labelled staphylococcal protein A.

**Complement assays**

**Antigenic complement.** Plasma C3 and C4 levels were measured using single radial immunodiffusion in 1-2% CFDagarose gels containing antibody (polyclonal goat anti-human C3, or sheep anti-human C4 (Serotec, Oxford, UK)). CH$_{50}$ was estimated using a standard haemolytic plate assay.

**Enumeration of erythrocyte CR1 (CD35).** C3dg and C4d. Mean numbers of antigen sites on erythrocytes were measured using radio ligand-binding assays, as previously described (Hogg et al., 1984). Monoclonal antibodies to CR-1 was E-11 (Hogg et al., 1984) (donated by Dr Nancy Hogg, ICRF, Lincoln’s Inn Fields, London), to C3dg was Clone 9 (Lachmann et al., 1980) (donated by Professor P. J. Lachmann), and to C4d was T2.C5.12 (donated by Professor G. D. Ross, Chapel Hill, NC). Radiolabelling was performed using Iodogen (Fraker & Speck, 1978) to a measured specific activity of 1-2 μCi/μg.

**RESULTS**

**Anti-streptokinase activity**

Precipitating antibodies to streptokinase were detected at a titre of 1:8 before challenge with the drug. At the time of presentation with serum sickness and renal failure, 1 week after therapy, the titre had risen 32-fold, falling rapidly coincident with plasma exchange, immunosuppression, and recovery of renal function (Fig. 2). The binding affinity of the antibody present in the patient’s serum on day 8 was $1.62 \times 10^{11}$ l/mol.

**Immune complexes**

Peak levels of soluble immune complexes by both the C1q and iC3b ligand-binding assay were detected in samples taken at the time of presentation with acute serum sickness. With therapy, and clinical improvement, levels fell, as shown in Fig. 2.

**Cryoprecipitate**

Maximal concentration of cryoglobulin was detected on day 9 following streptokinase therapy—corresponding to the peak of the patient’s illness. Precipitation lines were observed when the solubilized cryoglobulin was tested against anti-gamma, anti-kappa, anti-lambda, and anti-C3 antibodies, and against streptokinase. These findings suggested that the cryoprecipitate contained polyclonal IgG with anti-streptokinase activity, and C3. The presence of IgG in the cryoprecipitate was confirmed by SDS–PAGE. In addition, a second, well-defined band approximating to a molecular weight of 48 kDa was observed. We suspected that this might indicate the presence of streptokinase (M, 47.4 kDa) in the cryoglobulin (Fig. 3a). Immunoblotting of the cryoglobulin run on SDS–PAGE, with the patient’s own serum known to have maximal anti-streptokinase activity, confirmed hybridisation to the 48-kDa band and a number of smaller polypeptides, in a pattern similar to that seen using purified streptokinase on the same gel (Fig. 3b). In order to confirm that this material was streptokinase, the polyclonal rabbit anti-streptokinase reagent was used to probe the Western blots; results are shown in Fig. 3c, confirming the presence of streptokinase in the cryoprecipitate. No streptokinase was detectable in corresponding serum samples obtained from the patient on days 8 or 9, or subsequently.

**Complement assays**

Antigenic levels of C3 and C4 levels were normal throughout, but CH$_{50}$ values of 33% normal human serum (NHS) and 39%
NHS were observed on days 8 and 9 (at the peak of the illness), returning to levels within the normal range 10 days later. Levels of erythrocyte complement receptor type 1 (CR1(CD35)) measured using the monoclonal antibody E11 were normal (498 molecules/cell) and did not change throughout the illness and recovery period, but both C3dg and C4d were detected on the surface of erythrocytes obtained from the patient on day 8 and day 10 of the illness. The numbers of C3 molecules detected were 309 and 180 on day 8 and day 10, respectively, while 407 and 162 molecules of C4 were detected (normal levels: C3, <200 molecules/RBC; C4, <400 molecules/RBC). On day 15, 65 molecules of C3 and 93 molecules of C4 were found, and on day 42 of the illness no significant complement fixation could be detected.

**Immunofluorescent staining of renal biopsy**

Neither streptokinase nor anti-streptokinase were detectable in the patient’s renal biopsy by indirect immunofluorescence.

**DISCUSSION**

Circulating immune complexes, detected as a cryoglobulin, comprising anti-streptokinase, streptokinase and C3, were demonstrated in the serum of a patient, coincident with development of a vasculitic rash and serum sickness. These have not been described previously, although there is one report of the formation of an uncharacterized cryoglobulin in a patient who developed an allergic reaction to streptokinase (Totty et al., 1982). The formation of specific anti-streptokinase antibodies after therapeutic administration of the drug is well described (Gross & Harth, 1962; Mavor et al., 1969; Flute, 1973; McGrath et al., 1985). However, our patient is unusual in that IgG anti-streptokinase antibodies were detectable in his serum before initiation of therapy, the antibody titre rising rapidly after treatment, prior to the development of serum sickness. He had a past history of severe cellulitis, suggestive of streptococcal infection. There are close antigenic similarities between group A streptococci, which include the common human pathogen *Streptococcus pyogenes*, and group C streptococci from which streptokinase is produced (Read & Zabriskie, 1981). It is probable that he was pre-sensitized by a previous streptococcal infection, resulting in the formation of antibodies cross-reactive with streptokinase. He than developed a vigorous secondary immune response when that antigen was administered exogenously.

There are several case reports of allergic reactions to streptokinase therapy which have resulted in a serum sickness type of illness (Mavor et al., 1969; Totty et al., 1982; Alexopoulos, Raine & Cobbe, 1984; Noel et al., 1987). Transient mild renal impairment has been reported, usually attributed to an ‘immune complex nephritis,’ but this diagnosis has not been confirmed by renal biopsy in any of these cases. Although the patient described here had evidence of mesangial complement

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**Fig. 3.** (a) SDS–PAGE showing the presence of IgG and streptokinase in the cryoprecipitate. Lane a, purified streptokinase preparation; lanes b and c, purified cryoglobulin; lane d, purified IgG; (b) Western blot of patient’s cryoprecipitate run on SDS–PAGE-probed with autologous anti-streptokinase followed by ¹²⁵I-staphylococcal protein A. Lanes a and c, purified streptokinase; lane b, cryoglobulin; (c) Western blot probed with polyclonal rabbit anti-streptokinase followed by ¹²⁵I-staphylococcal protein A. Lane a, cryoglobulin, lane b, purified streptokinase.
deposition in his renal biopsy, there was no evidence of IgG deposition, and the histological picture was compatible with a diagnosis of acute tubular necrosis, with morphologically normal glomeruli and no evidence of cholesterol embolization. One possible explanation for these findings may be that ischemic renal damage resulted from local vasospasm. The clinical course of his myocardial ischaemia was extremely mild, and at no time did he develop systemic hypotension which could have precipitated acute tubular necrosis.

The development of a leucocytoclastic vasculitis following streptokinase therapy has also been described previously (Noel et al., 1987). The skin biopsy from our patient showed evidence of perivascular lymphocytic infiltration and C3 deposition in dermal capillaries. The low CH50 measurements observed at the time of presentation on day 8 with acute serum sickness, as well as the observed deposition of C3dg and C4 on the erythrocyte surface, are both indicative of systemic complement activation. The results of the immune complex assays suggest that complement-fixing complexes had formed in the patient's circulation, with maximal immune complex levels occurring at the time of presentation with serum sickness, and a fall in circulating immune complexes following plasma exchange. The observations of this patient have both clinical relevance, and more general implications relating to the pathogenesis of immune complex disease. He exhibited signs and symptoms of serum sickness with a vasculitic rash, had circulating immune complexes and evidence of systemic complement activation, with mesangial deposition of immunoglobulin in the kidney, but no evidence of cellular infiltration. Granular deposits of immunoglobulin in glomeruli have traditionally been equated with the deposition of circulating immune complexes; but it is apparent that this is not the only mechanism, or the one most likely to be associated with glomerular injury. It has always been a paradox that i.v. injection of pre-formed immune complexes in animals localized only transiently in glomeruli, resulting in minimal injury (Striker, Mannik & Tung, 1979; Couser & Salant, 1980), whereas in situ formation of apparently similar amounts of complexes, resulted in nephritis (Mauer et al., 1973; Yamamoto and Wilson, 1986). Granular deposits of immunoglobulin formed after immunization with a foreign protein localize most easily to glomerular capillaries, either when animals are injected with antigens that have a natural affinity for these vessels, such as cationized ferritin (Border et al., 1982), or when antigen is administered to animals that produce only low-avidity antibodies, capable of dissociation and reassociation with antigen after deposition in glomeruli. Clearly either circumstance might result in local formation and persistence of immune complexes. Recently it has been shown that injection of antibodies to antigens expressed on the surface of glomerular cells also causes granular deposits of immunoglobulin (Brentjens & Andres, 1989), providing a third mechanism whereby such deposits might be formed.

Streptokinase has a2-mobility on an electrophoretic strip, is not known to have any intrinsic nephrotoxic properties, and would not be expected to display any affinity for glomerular basement membrane. The anti-streptokinase IgG formed by the patient was of high affinity and experimental serum sickness is generally associated with the production of low-affinity antibodies (Devey & Steward, 1980). These factors may explain why there was no evidence of glomerular deposition of immunoglobulin, or inflammation in the kidney; however, it begs the question of why the patient developed renal failure. A further possibility is that immune complexes were transiently deposited in the kidney, but had cleared by the time of the renal biopsy. However, given that a cryoglobulin was present in serum at the time that the kidney was biopsied, this explanation seems less probable.

ALLergic reactions to streptokinase in humans have been reported with a frequency of 1-7 to 18% (Dykiewicz et al., 1986). As well as serum sickness, descriptions of generalized immediate-type hypersensitivity reactions (including urticaria, angioedema, bronchospasm and anaphylaxis), and delayed cutaneous lymphocytic reactions have been reported (Kakkar et al., 1969; Kohnert et al., 1976; Six, Marbert & Walter, 1976; Baumgartner & Davis, 1982; Marbert, Eichlisberger & Duckert, 1982; Nelson & Timmis, 1984). It has been suggested that streptokinase skin tests performed 20 min before therapy may be of value in identifying patients at risk of immediate-type allergic reactions to the drug (Dykiewicz et al., 1986). Such a test proved to be a sensitive and specific indicator of elevated IgE levels to streptokinase, but it was of no value in detecting elevated IgG levels or in predicting delayed allergic reactions to the drug as described in the present report.

With the increasing use of streptokinase, the frequency of both immediate and delayed hypersensitivity reactions may well increase. The possibility that a previous severe streptococcal infection might induce pre-sensitization to the drug, resulting in the formation of IgG antibodies, circulating immune complexes and serum sickness, raises the question whether alternative thrombolytic or other treatment should be considered in patients with a history of such an infection. While measurement of IgG anti-streptokinase levels may be useful in monitoring patients after therapy, at present, no rapid test for IgG anti-streptokinase antibodies is available to assess immunity before treatment. Thrombolytic therapy may be life-saving in acute myocardial infarction or pulmonary embolism, and streptococcal infections of the skin and naso-pharynx are very common. On the basis of current evidence, it might therefore be reasonable to consider an alternative thrombolytic agent in patients in whom there is a history suggestive of previous severe streptococcal infection.

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