CORTICAL INFARCTION IN RABBITS WITH SERUM SICKNESS FOLLOWING CYCLOSPORIN A THERAPY

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Summary

Rabbits given acute serum sickness (ASS) and treated with Cyclosporin A (CyA) developed glomerular capillary thrombosis and cortical infarction. These lesions were associated with severe endothelial injury and platelet-fibrin-leucocyte thrombi. They were not seen in untreated rabbits with ASS, nor in normal rabbits given CyA alone in equivalent doses. A similar renal lesion has been reported in patients receiving CyA following bone marrow transplantation.

Introduction

Nephrotoxicity is an important complication of CyA which usually causes tubular damage and reversible renal failure in a dose-dependent manner [1, 2]. Another renal complication has been recently described in CyA-treated bone marrow recipients who developed a haemolytic-uraemic syndrome (HUS) with thrombocytopenia and fragmented red cells, associated with glomerular capillary thrombosis [2]. During experiments in which we examined the effect of CyA on ASS (immune complex) nephritis in rabbits [3], we noted that with a high dose of 25mg/kg/day of CyA some rabbits became oliguric. The most severely oliguric had macroscopic infarction of the renal cortex. In this paper we describe the glomerular and tubular lesions which are very similar to those seen in bone marrow recipients treated with CyA.

Materials and methods

Experimental model of ASS

Female NZW rabbits (1.8–2.5kg) were injected i.v. with 250mg/kg crystallised bovine serum albumin (BSA). BSA was radiolabelled with Na$^{125}$I by the chloramine-T method. BSA was given either alone or together with 5μg/kg of E. coli endotoxin (Difeo Labs, LPS.W, E. coli 0111:B4). BSA elimination was
followed, as previously described [3, 4]. Antigen elimination was considered complete when less than one per cent of the initial activity remained.

**Serology**

Precipitating antibody was measured by radial immunodiffusion of neat serum into agarose containing BSA. An enzyme-linked immunosorbent assay (ELISA) method was used to measure total immunoglobulin antibody activity (Ig) to BSA, and the IgM antibody fraction [4]. The amount of circulating immune complexes was measured by precipitation of serum globulin bound to BSA $^{125}$I, and their size was measured by ultracentrifugation of serum in 12–40 per cent sucrose gradients for 18 hours at 175,000g. Serum C$_3$ concentrations were measured by radial immunodiffusion.

**Proteinuria**

Proteinuria was measured by the Lowry method after TCA precipitation. Proteinuria greater than 150mg/100ml in a 24-hour volume was considered abnormal. An episode of 'significant proteinuria' was defined as two or more consecutive days of abnormal proteinuria.

**Haematuria, glycosuria and oliguria**

Haematuria and glycosuria were measured in fresh urine samples using 'Labstix' dip sticks (Ames, UK). A significant period of haematuria was defined as two or more days of 1+ or more haematuria. Oliguria was defined as a two day period (or longer) during which the mean daily urine volume was 50 per cent less than the mean of the previous four days.

**Histology**

Rabbits were killed on day 12 after BSA injection, or two to three days after immune elimination when elimination occurred after day 10. Whole sections of kidneys were fixed in 10 per cent buffered formal-saline. Sections were stained by the periodic acid-Schiff (PAS) reaction and with Martius Scarlet blue (MSB). Glomerular proliferation was scored on a scale of 0, +, 1 to 4+ and was considered present when the score was 1+. Cryostat sections were examined with immunofluorescence (IF) using fluorescein conjugated antisera to BSA, rabbit whole immunoglobulin (Ig), IgG, IgM, C$_3$, fibrinogen and albumin (Nordic Pharmaceuticals). Paraffin-embedded tissue sections were dewaxed, pronase-digested and examined for fibrinogen using a peroxidase-anti-peroxidase (PAP) technique. Renal tissue was processed for electron microscopy.

**Cyclosporin**

Cyclosporin A was dissolved in a solvent vehicle of Miglyol 812 and 100 per cent ethanol at a concentration of 100mg/ml and given by deep intramuscular injection.
Experimental protocol

Twenty-three rabbits were given BSA plus endotoxin but received no other parenteral treatment (untreated animals). Groups of rabbits receiving CyA (15 or 25mg/kg/day) were given BSA plus endotoxin or BSA alone (see Table I). Two groups of animals were used as controls. Twenty-one with ASS received equivalent volumes of solvent alone for the same period as the treated groups. Six normal rabbits were given CyA 25mg/kg for five days: in addition on day 0 three were given i.v. saline plus 5μg/kg endotoxin, three were given saline alone.

<table>
<thead>
<tr>
<th>Rabbits (numbers)</th>
<th>Treatment Dose mg/kg/day</th>
<th>Glomerular proliferation (% numbers)</th>
<th>Glomerular thrombi (% numbers)</th>
<th>Cortical infarction (% numbers)</th>
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<tr>
<td></td>
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<td>Days</td>
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<tr>
<td>BSA with endotoxin</td>
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<td>1) 12</td>
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<td>3) 32</td>
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<td>BSA no endotoxin</td>
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<td>Control</td>
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<td>42</td>
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Results

Clinical data

CyA inhibited the proteinuria normally occurring with ASS, but did not inhibit the haematuria (see [3]). Rabbits also became oliguric (30%) and developed glycosuria (44%), two features which do not normally occur in ASS. Using BSA alone the nephritis was usually mild, with slight proteinuria and haematuria but when such rabbits were given BSA and CyA the disease produced was as severe as in those which received BSA and endotoxin.
Serological data

CyA had no significant inhibitory effect on the humoral response as measured by (i) the time to BSA elimination; (ii) the magnitude or character of the antibody response; (iii) the amount or size of circulating immune complexes; (iv) the fall in C₃ at the time of immune elimination [3].

Figure 1. Glomerulus with multiple capillary thrombi, some of which are associated with polymorph infiltration (H & E x 25)

Figure 2. Necrotic glomerulus with thrombosis of afferent arteriole. Some of the surrounding tubules are also infarcted (H & E x 25)
Histology

Eighty-seven per cent of rabbits given BSA and endotoxin developed glomerular proliferation. Proliferation was less common in those given BSA alone. Treatment with CyA significantly reduced glomerular proliferation [3] but was associated with several new histological features which were more diffuse and severe in rabbits receiving 25mg/kg of CyA. These changes were not seen in untreated ASS or rabbits given CyA alone.

a) Glomerular capillary thrombi: Capillary loops were partially or totally occluded by amorphous, eosinophilic material and these areas were often infiltrated with polymorphs (Figure 1). In some cases capillary thrombi were associated with focal and segmental glomerular infarction (Figure 2) and necrosis of the afferent arterioles. The thrombi were PAS positive, and stained scarlet with MSB. IF examination showed strongly positive staining for BSA, but weak and equal staining for IgS, IgG, IgM, C3 and albumin. Fresh thrombi (one to two days old) stained strongly positive for fibrinogen by both IF and immunoperoxidase.

b) Tubular and interstitial injury: Rabbits developed foci of dilated tubules, in which the height of the tubular epithelium was greatly reduced, or tubular cells appeared to be entirely absent. Amorphous eosinophilic casts which appeared to consist of the shed tubular epithelium were often seen in the tubules. At its most severe there was total tubular necrosis. Severe macroscopic infarction was associated with interstitial haemorrhage.

Electron microscopy showed swelling and disruption of the endothelial cytoplasm. At times, when grossly swollen, endothelium was indistinguishable from degranulated (‘exhausted’) platelets. Sometimes the endothelium was raised off the basement membrane, and the expanded subendothelial space was filled with a loose amorphous material or occasionally electron dense material with the periodicity of fibrin. At times capillary loops were packed with degenerating cells including platelets, polymorphs, and mononuclear cells. Strands of fibrin ran through this cellular mass. At later stages, the loops were filled with an electron dense mass of amorphous material, which did not have the periodicity of fibrin. Glomerular infarction was associated with accumulation of polymorphs in the capillary lumen phagocytosing amorphous material. In areas of tubular infarction peritubular capillaries were occluded by platelet thrombi.

Discussion

In this new model of glomerular and tubular infarction the vascular injury is initiated when immune complexes are being rapidly formed, suggesting that the vascular damage and haematuria are initiated by an immune complex dependent mechanism. In contrast, we have suggested that the proteinuria and glomerular proliferation in unmodified ASS may be T-cell mediated [3]. Vascular damage and endothelial injury are features of ASS although the mechanism [3] causing
damage [6, 7] remains unknown. Vascular permeability increases and immune complexes are deposited in the vessel walls [5]. Arterial injury may progress to a proliferative and necrotic arteritis. However, severe endothelial injury is not a feature of ASS, and capillary thrombosis and glomerular infarction do not occur.

What is the pathogenetic mechanism of the new lesion that we have observed? There are several conditions with a similar appearance. Glomerular capillary thrombosis and cortical infarction may occur in rabbits following the Shwartzman reaction. The classic generalised Shwartzman reaction (GSR) follows two i.v. injections of endotoxin [6]. The first, or ‘priming’ dose, prepares the animal so that the intravascular coagulation triggered by the second dose initiates widespread thrombosis. Rabbits may be primed in other ways (e.g. pregnancy, pre-treatment with steroids), so that a single dose of endotoxin causes a GSR. Furthermore, if rabbits are primed for a GSR, then an infusion of immune complexes can replace endotoxin and trigger the reaction [7]. Although the pathogenesis of GSR has remained elusive, experiments suggest that the priming dose of endotoxin has a direct effect on the vascular endothelium.

It has been reported that some bone marrow recipients treated with CyA develop an HUS-like illness with glomerular and tubular infarction [2] similar to that described in our rabbits with CyA-treated ASS. Little is known of the pathogenesis of HUS, but recently Remuzzi et al [8] showed that in patients with HUS vascular synthesis of PGI₂ was reduced. Moreover, many of these patients lacked a prostacyclin stimulating factor (PSF) in their plasma which was necessary for synthesis of PGI₂ by vascular tissue. We have subsequently found that CyA therapy profoundly reduces PSF activity in plasma [9], but has no effect on platelets, or PGI₂ synthesis by aorta taken from CyA-treated animals.

From our observations and those in HUS and marrow transplantation, a unifying hypothesis can be made. Local production by vascular endothelium of PGI₂ limits excessive platelet aggregation and thrombus formation at sites of endothelial injury. In the absence of PSF, synthesis of PGI₂ is impaired and vascular injury can progress to capillary thrombosis and necrosis of the vessel wall. We suggest that ASS, graft-versus-host disease after marrow transplantation and HUS are three examples of different mechanisms of endothelial injury which, when they occur in the absence of PSF, result in the same final vascular injury.

Acknowledgments

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References

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6 Thomas L, Good RA. J Exp Med 1952; 96: 605