Proc EDTA–ERA (1985) Vol 22

FACTOR VIII-RELATED ANTIGEN LEVELS IN RENAL ALLOGRAFT RECIPIENTS TAKING CYCLOSPORIN A

Z Brown, *G H Neild, J J Willoughby, N V Somia, J S Cameron

*Guy's Hospital, *Institute of Urology, London, United Kingdom*

Summary

Very high plasma concentrations of factor VIII-related antigen (FVIII RAg) were found in renal allograft recipients during periods of nephrotoxicity induced by Cyclosporin A. Significantly raised values of FVIII RAg were found in eight patients at the time of toxicity (5.9±1.36IU/ml) which were reduced when patients were no longer toxic (3.1±1.1IU/ml). In addition we studied eight patients in whom nephrotoxicity never occurred. These findings are further evidence that toxic doses of Cyclosporin A are associated with vascular injury.

Introduction

Differentiation between rejection and nephrotoxicity can be difficult in patients receiving Cyclosporin A [1]. The mechanism of acute nephrotoxicity is unknown but it is reversible and dose-dependent. Cyclosporin A has been associated with vascular injury and this effect may relate to its nephrotoxicity. Shulman [2] reported a haemolytic uraemic syndrome (HUS) following bone marrow transplantation. Renal allograft recipients, receiving high doses of Cyclosporin A, may develop glomerular capillary thrombosis [3] or a renal arteriolar vasculopathy, similar to the arteriolar lesion associated with severe hypertension or HUS [4]. Neild et al [5] have demonstrated an enhancement of vascular injury by Cyclosporin A in experimental acute serum sickness in rabbits.

Endothelial cells are the major site of synthesis of the plasma protein, factor VIII-related antigen (FVIII RAg). Increased blood factor VIII RAg have been found in diseases associated with vascular injury [6]. To examine whether Cyclosporin A is implicated in renal vascular injury, we measured plasma concentrations of factor VIII RAg in renal allograft recipients during periods of nephrotoxicity.

Patients and methods

Patients The patients were selected from a group of 60 consecutive renal allograft recipients who were immunosuppressed with Cyclosporin A and pred-

nisolone. Their clinical details and the immunological status of donor and recipient have been reported [7].

Immunosuppression Cyclosporin A (Sandimmun, Sandoz Ltd) was given orally as a single daily dose in the following regime: 14mg/kg/day for 14 days, then 12mg/kg/day until day 30, 10mg/kg/day until day 60, 8mg/kg/day until day 90, then 6mg/kg/day. Prednisolone was given orally at a starting dose of $15mg/m^2$ tapering to a dose of $10mg/m^2$ at six months. Whole blood Cyclosporin A ($\mu g/L$) was measured by radio-immunoassay (RIA), using the Sandoz RIA kit. Cyclosporin A was given at 6.00pm, and blood levels were measured the following morning.

Methods For this study Cyclosporin A was measured at weekly intervals and subsequently every two to four weeks at clinic attendances. Aliquots of serum and citrated plasma were stored at $-70°C$.

Factor VIII RAg was measured in citrated plasma by Laurell 'Rocket' immuno-electrophoresis using rabbit antibody to human FVIII RAg (Atlantic Antibodies) and standardized against the ninth British standard for factor VIII (National Institute for Biological standards and control, Hampstead).

C-reactive protein was determined by single radial immunodiffusion using goat anti-human C-reactive protein antibody and standardized using Calibrator 7 (Atlantic Antibodies). The lower limit of detection was $6\mu g/ml$.

Factor VIII-coagulant activity (FVIII-C) was assayed by the one stage technique using factor VIII deficient plasma (Immuno Ltd).

Renal function Plasma urea and creatinine, plasma biochemistry and haematological indices were monitored. Renal biopsies were performed routinely after one week, one month, one year and for episodes of renal dysfunction. Renal dysfunction was defined as either an episode of deteriorating renal function with a rise in plasma creatinine of more than 20 per cent above the preceding baseline value, or an inappropriately high plasma creatinine which fell by 20 per cent or more with the correct treatment. Episodes of dysfunction which were considered to be due to rejection were treated with pulses of 1g of methylprednisolone on three consecutive days. Episodes of dysfunction which were thought to be due to nephrotoxicity were treated by a reduction in the dose of Cyclosporin A of 2mg/kg/day every seven days.

Controls Control groups were (1) 21 healthy volunteers, (2) 10 patients on haemodialysis, (3) 19 transplant recipients who were maintained on prednisolone and azathioprine, including seven with plasma creatinines $>200\mu mol/L$ and deteriorating function, (4) eight patients receiving prednisolone and Cyclosporin A who had neither rejection nor toxicity. In addition we examined six plasma samples from five patients, within two days of a biopsy proven diagnosis of rejection occurring after day seven.

Statistics Comparison of different groups was made by the Wilcoxon rank sum test.

Results

Our assessment of nephrotoxicity was based on clinical and morphological findings and the response to a reduction in the Cyclosporin A dose, as previously reported [7]. During the time of this study we did not use the Cyclosporin A levels in our clinical management and were using higher doses than at present. Several of our patients remained in a nephrotoxic state for a longer period than we would now permit.

Plasma concentrations of Factor VIII RAg were measured retrospectively on serial samples from eight patients who had episodes of nephrotoxicity lasting at least 22 weeks and who never had evidence of rejection. Their factor VIII RAg levels at the beginning of their nephrotoxic period were 5.9±1.36IU/ml (mean ± SEM), which occurred 5.8±2.4 weeks (mean ± SEM) after transplantation. The mean FVIII RAg level was significantly reduced to 3.09±1.1IU/ml, 44±11 weeks later, when they were either less toxic or considered to be no longer toxic. They were compared with the following groups: (1)normal controls; (2) uraemic controls; (3) transplant recipients receiving prednisolone and azathioprine with creatinine concentrations ranging from 80 to 550μmol/L (205± 73); (4) transplant recipients with stable function on prednisolone and Cyclosporin A (i) during weeks five to six, and (ii) after week 37. These data and the renal function at the time of study are summarized in Table I.

Factor VIII-C was not significantly raised in any group (Table I). In our normal population C-reactive protein values were <6.0μg/ml. We considered levels >18μg/ml as being raised. C-reactive protein was raised in the first week following transplant surgery [8]; it was not subsequently raised in any of our patients who were nephrotoxic. We have examined six samples taken within two days of a diagnosis of rejection. Rejection occurred at day 16±4 (mean ± SEM), range days 9–21; C-reactive protein was raised in all cases 43.5±19μg/ml (mean ± SEM), range 30–83. In these samples the mean factor VIII RAg was 2.8±0.8IU/ml (mean ± SEM), range 1.5–4.

Discussion

Our study shows abnormally high levels of factor VIII-related antigen in renal allograft recipients during periods of cyclosporin A nephrotoxicity. Raised plasma levels are indicative of vascular injury [6]. Both FVIII RAg and FVIII-C are acute phase reactants, FVIII-C is not synthesized by the endothelium. Hence, in cases of vascular injury circulating levels of FVIII RAg would be high without a parallel increase of FVIII-C.

Factor VIII-coagulant activity (VIII-C) was not significantly raised in any of our patients. We do not believe that we have simply measured an acute phase protein response, since levels of the acute phase protein C-reactive protein were not raised in either of the Cyclosporin A treated groups. Raised levels of FVIII RAg have been reported in uraemia [9], although this may reflect only an

TABLE I. Clinical and haematological data

		n	FVIII RAg (IU/ml)	FVIII C (IU/ml)	Creatinine (μmol/L)	CyA levels (μg/L)	CyA dose (mg/kg/day)	CRP (μg/ml)
i	Cyclosporin A + prednisolone toxic	8	5.9±1.36	1.55±0.6	215±54	1280±196	9.8±1.9	6.77±1.8
ii	Cyclosporin A + prednisolone ex-toxic	8	3.09±1.10	1.55±0.6	152±52	599±254	4.1±0.9	<6.0
i	Cyclosporin A + prednisolone non-toxic	8	2.01±0.26	1.4±1.1	182±54	559±208	9.5±0.6	8.9±3.7
ii	Cyclosporin A + prednisolone non-toxic	8	1.0±0.25	–	137±25	405±98	5.18±0.6	<6.0
	Prednisolone + azathioprine	19	1.69±0.37	1.24±0.5	205±73	–	–	6.2±0.46
	Uraemic patients	10	1.89±0.38	–	1069±164	–	–	8.75±4.76
	Normal controls	21	0.99±0.17	0.97±0.3	97±14	–	–	<6.0
	Acute rejection	5	2.85±0.82	–	740±479	1009±479	10.75±0.92	43.5±19.2

n=number of patients.

All results are shown as mean ± SEM

Cyclosporin A levels are whole blood values measured by radio-immunoassay.

CRP=C-reactive protein values

acute phase protein response, rather than vascular injury. The nephrotoxic patients in our study were not uraemic, and their factor VIII RAg levels were significantly higher than the uraemic controls.

Cyclosporin A has been associated with vascular injury, evidence from both animal and human studies suggesting that it may be involved in initiating or enhancing the vascular injury [1–4]. It may enhance platelet-endothelial interaction [4] and this might lead to endothelial injury. Cyclosporin A has also been associated with an increased incidence of thrombosis and platelet hyperaggregability [10]. However, we cannot exclude the possibility that cyclosporin A may have a direct affect on the endothelium either causing abnormal stimulation or a toxic injury.

The factor VIII RAg levels may be raised due to chronic vascular rejection. None of our 60 transplant recipients, including the nephrotoxic patients, who have received Cyclosporin A and who have been studied prospectively have had clinical and morphological evidence of chronic rejection within the first year [7]. However, within our prednisolone and azathioprine group there were seven patients who had slowly and progressively deteriorating renal function. Their levels of factor VIII RAg were no higher than those on similar therapy with normal function.

Patients were not routinely studied during episodes of acute rejection. However, we found raised levels of C-reactive protein in all patients studied at the time of rejection. It is likely that one single measurement of a high level of factor VIII RAg reflects only endothelial injury, but the simultaneous measurement of C-reactive protein may permit one to distinguish rejection (with raised C-reactive protein) from nephrotoxicity (with normal C-reactive protein).

In conclusion, we have found that Cyclosporin A-toxicity leads to enhanced production of factor VIII RAg by the vascular endothelium. Serial measurements of factor VIII RAg may be a useful guide in the management of nephrotoxicity.

Acknowledgment

We thank the Leukaemia Research Fund and special trustees of Guy's Hospital for financial support. Our thanks to Jane Needham and Cecilia Gillespie for technical assistance, and to Dr Sam Machin for valuable discussions.

References

1 Klintmalm GBG, Iwatsuki S, Starzl TE. *Lancet 1981; i:* 470
2 Shulman H, Striker G, Deeg HJ et al. *N Engl J Med 1981; 305:* 1392
3 Neild GH, Reuben R, Hartley RB, Cameron JS. *J Clin Pathol 1985; 38:* 253
4 Mihatsch MJ, Thiel G, Spichtin HP et al. *Transplant Proc 1983; 15:* 2821
5 Neild GH, Ivory K, Williams DG. *Br J Exp Pathol 1983; 65:* 133
6 Kahaleh MB, Osborn I, LeRoy EC. *Annal Int Med 1981; 94:* 482
7 Taube DH, Neild GH, Williams DG et al. *Lancet 1985; ii:* 171
8 Maury CPJ, Teppo A-M. *Clin Nephrol 1984; 22:* 284
9 Deykin D. *Kidney Int 1983; 24:* 698
10 Vanrenterghem Y, Roels L, Lerut T et al. *Lancet 1985; i:* 999