SERUM ALPHA-2-MACROGLOBULIN, ANTITRYPSIN AND ANTICHYMOTRYPSIN ACTIVITIES IN PATIENTS RECEIVING TREATMENT WITH CYCLOSPORINE

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ABSTRACT

Cyclosporine has been reported to function as an inhibitor of the chymotrypsin like activity of proteasome. We hypothesized that the administration of an exogenous proteinase inhibitor may affect the activities of the naturally occurring serum anti proteinases. The aim of this study was to observe the pattern of alteration of serum alpha 2 macroglobulin (AMG), alpha 1- antitrypsin (AT) and alpha 1-antichymotrypsin (ACT) activities in renal transplant patients receiving the immunosuppressive drug, cyclosporine. Patients (97) who had received a single renal allograft were inducted into the study. Subjects were on a twice-daily dosage of cyclosporine capsules. Trough (Co) and two-hour post dose (C 2) cyclosporine levels were regularly estimated and all patients had stable creatinine levels. In 5 newly transplanted patients, antiproteinase activities were estimated weekly over a 4-week period as their cyclosporine doses were gradually tapered. Average serum activities of ACT and AMG in the transplant group were significantly less than in the control group ($p < 0.002$ and $p < 0.003$ respectively). AT and ACT activities fell gradually over 4 weeks. AMG activities showed a biphasic pattern, initially falling by almost 50% in the second week, increasing marginally in the third week and decreasing to less than 50% of the activities observed in the first week. Serum antiproteinase activities of serum alpha 2 macroglobulin (AMG), alpha 1- antitrypsin (AT) and alpha 1-antichymotrypsin (ACT) were found to be altered in renal transplant patients receiving cyclosporine.

KEY WORDS

Alpha-2-macroglobulin, alpha-1-antitrypsin, alpha antichymotrypsin, C2, cyclosporine.

INTRODUCTION

Cyclosporine is a most widely used immunosuppressive agent to prevent rejection in patients with renal transplants. Although acute and chronic toxicity problems exist, cyclosporine has reduced the frequency of acute rejection episodes and improved short term renal allograft survival (1,2,3,4). It was reported that cyclosporine acts as a non competitive inhibitor of the chymotrypsin-like activity of the 20S proteasome in vitro and that it suppresses lipopolysaccharide-induced NF-kappa B degradation and p105 processing in vivo (5).

We hypothesized that cyclosporine treatment would alter the activities of the naturally occurring anti - proteinases in blood , since cyclosporine would act as an exogenous source of anti – proteinase activity. We selected alpha 2 macroglobulin (AMG), alpha 1- antitrypsin (AT) and alpha 1-antichymotrypsin (ACT) as representatives of the major anti - proteinases in the blood.

The effect that cyclosporine has on serum activities of AT, ACT and AMG are presented for the first time in this study. The serum proteinase inhibitory activities were monitored in a few patients who were initially put on cyclosporine therapy immediately after a renal transplant and followed up over a period of 4 weeks as the cyclosporine dose was tapered. The study also evaluated whether the dose of cyclosporine and the measured blood levels of cyclosporine as evidenced by C0 (trough) and C2 (2 hour post dose values) affected serum proteinase inhibitor levels.

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MATERIALS AND METHODS

Subjects

Patients (n=97), attending the Department of Nephrology at Kamineni Hospitals Ltd, Hyderabad, who had received a single renal allograft were inducted into the study. Subjects were on a stable twice-daily dosage of cyclosporine capsules with demonstrated stable trough (Co) and two-hour post dose (C2) cyclosporine levels and stable creatinine levels. Patients had no history of cardiac, renal or hepatic disease according to clinical and biochemical investigations carried out. Serum chemistry testing included electrolytes, blood urea and creatinine, total bilirubin, alkaline phosphatase, aspartate transaminase, total protein, albumin and lipid profile (total cholesterol, high density lipoprotein, triglycerides). Plasma glucose and HbA1c was determined in all patients. A 24-hour urine specimen was also obtained for calculation of creatinine clearance and urinary proteins. Patients with diabetes mellitus were found to be medically stable as indicated by glycosylated hemoglobin A1c measurements between 6.2 % and 7.6 %. The demographic profiles of the renal transplant recipients are given in Table 1. Co and C2 were measured in all patients by Cobas Integra 400 using an EMIT based kit. C2 was measured according to the protocol described by us earlier [6].

In 5 randomly selected patients, serum levels of AT, AMG and ACT were measured from the first post transplant day after they received cyclosporine on a twice-daily oral dose regimen. The dose of cyclosporine was tapered over a period of 4 weeks from 400 to 375, 350 and 325 mg/day uniformly in all patients. Since there is a large variation in the absorption and metabolism of orally administered cyclosporine, Co and C2 blood levels of cyclosporine were estimated in these patients, over a period of 4 weeks on a weekly basis. In all the 5 patients, serum creatinine clearance improved. None of the patients had any signs of acute cyclosporine toxicity, infection or post-operative complications during this period. The Institutional review board of Kamineni Hospitals Ltd approved the study. The control group (n=20) comprised of healthy volunteers.

Measurement of antiproteinase activities

Bovine trypsin and chymotrypsin were from Worthington Biochemicals, Freehold, NJ, USA. Casein (purified) and N - acetyl L-tyrosine ethyl ester (ATEE) were from Sigma Chemicals, USA. All other reagents were analytical grade commercial chemicals. The modified Kunitz caseinolytic method (7) was used for estimating antitryptic and antichymotryptic activities in the serum samples. One unit of

inhibitory activity was defined as the amount that caused suppression of caseinolytic activity by one absorbance unit. Estimation of AMG was based on the esterolytic activity of bound alpha chymotrypsin (8).

Statistics

Results are expressed as mean $\pm$ SEM. Results from patients and controls were compared by independent samples t-test using SPSS package, version 10. The effect of the dose of cyclosporine and the blood levels of cyclosporine (C0 and C2) on the antiproteinase activities were studied by a Pearson's correlation matrix and significance was calculated. All analysis were performed with 1-tailed tests. Statistical significance was defined as p less than 0.05.

RESULTS

The transplant group had patients who had undergone renal transplantation between 3 months to 4 years earlier at the time of this study (Table 1).

Table 1
Demographic profile of patients in renal transplant group

Parameter	Details
Number of patients	97
Males/ Females	81/16
Age (Years)	21 - 47
Duration of transplant (months)	3 - 58
Glycated hemoglobin	6.2 – 7.6 %
Serum Creatinine	0.9 – 1.8 mg/dl

Average serum activities of ACT and AMG were significantly less than in the control group ($p < 0.002$ and $p < 0.003$ respectively). Average serum activity of AT in the transplant group was greater than in the control group ($p < 0.05$) (Table-2). Activities of AMG, AT and ACT did not correlate significantly with blood levels of cyclosporine as evinced by Co, C2 or with the dose of cyclosporine received.

Serum activities of AMG, ACT and AT were measured in 5 newly transplanted patients after starting them on cyclosporine. Over a 4 week duration, in 4 of the patients, the average serum activities of AMG and ACT were less than that of the control group. In all the 5 patients, the average serum activity of AT was greater than that in the control group (Table-3). This indicates that antiproteolytic activities in the serum began to alter within 4 weeks after the patients were placed on

TABLE 2

Serum activities of antitrypsin, antichymotrypsin and alpha-2 macroglobulin in controls and in patients on immunosuppression following renal transplant. Results are reported as mean $\pm$ SEM.

	Control Group (n=20)	Transplant Group (n=97)
Alpha-2 macroglobulin (mg%)	244.83 $\pm$ 11.03	196.7 $\pm$ 9.55*
Antichymotrypsin (U/ml)	204.72 $\pm$ 10.28	166.50 $\pm$ 5.75**
Antitrypsin (U/ml)	104.50 $\pm$ 6.34	184.09 $\pm$ 6.42***

*p<0.003; **p<0.002; ***p<0.05

TABLE 3

The 4 weeks average serum concentrations of C2, AMG, ACT and AT in 5 patients are presented. Activities were measured while the patients received tapering doses of cyclosporine (400 mg / day to 325 mg/day).

Patient No.	C2 Ng/ml	AMG mg%	ACT U/ml	AT U/ml
1	2912	173	191	194
2.	2334	379	227	254
3.	2260	168	150	138
4.	1617	158	145	180
5.	1387	176	182	194

immunosuppressive therapy. Although table 2 indicates the general pattern of change in antiproteinase activities, it is possible that certain individuals (like patient 2 in Table 3) respond differently. In this patient, AMG activity was greater than the average AMG levels in the control group (p< 0.05).

The patterns of change of AMG, ACT and AT in the newly transplanted patients varied with C2 levels. In patients in whom the weekly average C2 levels were greater than 2300 ng/ml, AT activities fell gradually over 3 weeks to 53% and increased sharply in the fourth week to 110% when compared to the first week activity. ACT activities fell gradually over 4 weeks. AMG activities showed a biphasic pattern, initially falling to 61% in the second week, increasing to 79% in the third week and decreasing to 55% of the activities observed in the first week.

In patients in whom the weekly average C2 levels were less than 2300 ng/ml, AT and ACT activities fell to 71% and 74% respectively at the end of 4 weeks. AMG activities decreased to 83% in the second week, rose to 110% in the third week and fell to 89% of the activity in the first week. Hence, patients who achieved higher C2 activities had greater variation in ACT and AMG activities.

No significant correlation was observed between serum antiproteinase activities and the C0 or C2 levels of cyclosporine in any of the 5 patients.

The daily dose of cyclosporine received by the patient was correlated to serum activities of antiproteinases. Serum AMG levels correlated significantly with the dose of cyclosporine received in 3 patients who had C2 levels greater than 2300ng/ml. (r=0.86, p<0.013). In 2 patients who had lower C2 levels, the correlation of AMG activities to dose of cyclosporine was not significant

Similarly, in patients with C2 levels greater than 2300ng/ml, ACT correlated well with the dose (r=0.70, p<0.05). However, no significant correlation was obtained between AT activity and C2 or the dose of cyclosporine administered.

DISCUSSION

The importance of proteolytic enzymes and their inhibitors in organ transplantation can be demonstrated by the fact that cyclosporine itself functions as an antiproteinase.

AMG functions as an extra cellular carrier of diverse cytokines and growth factors including transforming growth factor – beta 1 and 2, interleukins IL –4, 6, 8, 10, platelet derived growth factor, nerve growth factor, insulin like growth factor, tumor necrosis factor alpha, fibroblast growth factors, vascular endothelial growth factor, ciliary neutrophilic factor, neutrophin 3, activin, inhibitin, definisin, growth hormone and amyloid (9,10). AT has been shown to have anti-apoptotic and anti-inflammatory effects which can contribute to the delayed type of protection associated with ischemic preconditioning in the kidney during an ischemic / reperfusion injury (11). Hence alterations of the antiproteinase activities could play an important role in the mechanism of cyclosporine action.

There is a large degree of variation among patients in the absorption and metabolism of orally administered cyclosporine (12). Patients who achieved higher C2 activities showed greater alterations in antiproteinase activities. The initial increase of AMG and ACT activities in patients achieving higher C2 levels may be significant in the mechanism of the acute nephrotoxicity seen in some patients. However, our study period of 4 weeks gives us preliminary data only.

In conclusion, serum antiproteinase activities were found to be altered in renal transplant patients receiving cyclosporine. The variations were greater in patients who had high blood

levels of cyclosporine (C2). Further studies are required to understand the clinical significance of these alterations.

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