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Antioxidative Status in the Early Post-Transplant Period: Cyclosporine A-Based vs Sirolimus-Based Regimens

Mohsen Nafar[1], Mehri Kadkhodaee[2], Mitra Mahdavi-Mazdeh[3*], Maryam Zahmatkesh[2], Behjat Seifi[2], Rana Ghaznavi[2]

[1]Urology and Nephrology Research Center (UNRC), Shahid Beheshti University of Medical Sciences, Tehran, I.R.Iran

[2]Department of Physiology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, I.R.Iran

[3]Department of Nephrology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, IR.Iran

Abstract

Background and Aims: It is renowned that the principal causes of oxidative stress will disappear after transplantation. However, it does not seem to improve totally, which may be due to an immunologic response to the graft or possibly immunosuppressive agents. This study was conducted to investigate the possible relation between oxidative stress and different immunosuppressive regimens (cyclosporine versus sirolimus based) in renal transplant patients.

Methods: Twenty-five renal transplanted patients with uneventful operation and stable function were randomly assigned to one regimen of immunosuppressive protocols cyclosporine (CsA) (Group A) or sirolimus (Group B) plus mycophenolate mofetil (MMF) and Steroids. Erythrocyte Glutathione (GSH), Superoxide dismutase activity, plasma malondialdehyde (MDA) and α-tocopherol were measured and compared.

Results: There were no significant changes of SOD activity in CsA group in different days. However, there was a significant reduction of SOD activity in sirolimus group 14 days after transplantation. Erythrocyte GSH did not show any significant changes between the groups. Plasma MDA and vitamin E level were not significantly different between the groups before and 48 hour after transplantation. Considerably higher MDA (1.82 ± 0.43 vs. 1.03 ± 0.12, μmol/l, $p<0.05$) and vitamin E levels (0.18 ± 0.008 vs. 0.12 ± 0.006 μmol/l, $p<0.05$) were seen 2 weeks after transplantation in sirolimus group compared to CsA group.

Discussion: The results of the present study showed that although sirolimus-treated recipients had decreased activity of SOD in 14th day of transplantation but no alteration of GSH during this period in comparison with cyclosporine-treated recipients was found. Increased level of plasma vitamin E on 14th day was possibly a secondary response to the change of MDA level.

Conclusions: It seems that sirolimus may not offer better protection against increased reactive oxygen species (ROS) production in early period of renal transplantation.

Keywords: Cyclosporine A, Sirolimus, Immunosuppressive Agent, Oxidative Stress

Introduction

It is renowned that the principal causes of oxidative stress, the imbalance between the formation of reactive oxygen species (ROS) and the power of the antioxidant defense mechanism in End Stage Renal Disease

*Correspondence:
Mitra Mahdavi-Mazdeh,
Emam Khomeini Hospital, Keshavarz Blv. Tehran, I. R. Iran.
Tel / Fax: +98-21-66581568
Email: mmahdavi@tums.ac.ir
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(ESRD) are multi factorial. On one hand uremic toxins, interactions between circulating mononuclear cells and bio-incompatible dialyzers or infections, are triggers and on the other hand these patients have reduced amounts of antioxidant substrates (1-3).

Although a lot of studies have shown that re-establishment of renal function by transplantation improves the oxidative stress but it is insisted on the fact that it can not be normalized totally (4-6). It seems that enhancement of oxidative stress could be due to an immunologic response to the graft or possibly immunosuppressive agents (4).

The main drug group (calcineurin inhibitors) in most immunosuppressive regimens, cyclosporine A (CsA), is a powerful stimulator of oxidative stress signaling, leading to TGF beta production, NO degradation, endothelial dysfunction, hypertension, post-transplant nephropathy and possibly playing an important role in Epstein-Barr virus (EBV)-related PTLD (7, 8). In spite of such side-effects, the general consensus is that calcineurin inhibitors (CNIs) are still needed to prevent rejection but some are concerned about the risk of progressive renal lesions caused by its nephrotoxicity (9).

Sirolimus (SRL) is a recently introduced drug from the class of immunosuppressants that inhibit the mammalian target of sirolimus, the kinase involved in regulation of cell growth and proliferation, and has been claimed to be renoprotective by trials and demonstrated that cyclosporine (CsA) could be withdrawn safely from a combined SRL, CsA, and corticosteroid regimen at 2 to 4 months after transplantation.(9, 10) It was also demonstrated that SRL in patients with biopsy proven chronic allograft nephropathy (CAN) can reduce in vivo, the expression of plasminogen activator inhibitor type 1 (PAI-1), a key mediator in the development and progression of interstitial fibrosis and glomerulosclerosis (11).

The present study was conducted to investigate oxidative stress status in different immunosuppressive regimens (cyclosporine versus sirolimus based) in early period of renal transplantation in patients with stable renal function and uneventful postoperative course.

Materials and Methods

The study group consisted of 25 renal transplanted patients of live donors in postoperative period. Recipients were randomly assigned to one of the two regimens of immunosuppressive protocols (Group A: CsA, mycophenolate mofetil (MMF), Steroids, Group B: Sirolimus, MMF, Steroids). Mean age of group A (13 patients) and B (12 patients) were 44.5±11.7 years and 44.5±14.5 years respectively. All the patients had an uneventful postoperative course according to their serum creatinine (Cr) as a measure of stable renal function. Exclusion criteria for patients were cardiac diseases, diabetes, hepatitis, and infectious diseases at the time of study and administration of antioxidant drugs such as vitamin E, C or HMG CoA reductase inhibitors. They were followed till the time of dismissal.

Venous blood was sampled before and after 48 hours and 2 weeks (the latest time that it was planned to have the patients admitted) of transplantation with heparin as an anticoagulant. Erythrocyte Glutathione (GSH), Superoxide dismutase activity, plasma malondialdehyde (MDA) and α-tocopherol were measured. General biochemical tests, cell blood count (CBC), liver functions tests (LFT) and creatinine (Cr) was used as measures of stable renal function. The study was performed in accordance with the regulations of the Ethical Committee of Urology and Nephrology Research Center (UNRC).

Glutathione assay: Reduced GSH was assayed according to the Tietze method in fresh heparinized whole blood samples. The continuous reduction of 5, 5 ´ (E-dithiobis-(2-nitrobenzoic acid)) was measured at 412 nm. The value for each sample was read from the standard curve and was expressed in mg/mg Hb.

Malondialdehyde: Malondialdehyde, as marker of lipid peroxidation was measured in fresh plasma

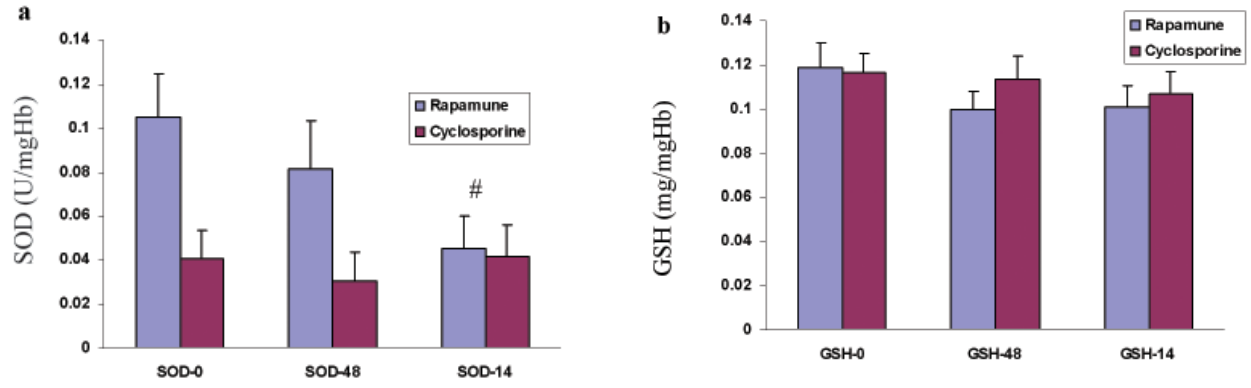

Figure 1. Alterations in SOD activity (a) and GSH (b) in two groups at before (0), 48 hour after transplantation (48) and two weeks after transplantation (14). The data are presented as mean ± SEM.
# p<0.05 compared to before transplantation

samples according to the Esterbauer and Cheeseman method (10). MDA reacts with thiobarbituric acid (TBA) and produces a pink pigment that has a maximum absorption at 532 nm.

Vitamin E determination: Vitamin E extraction from plasma was carried out according to the method of Arnaud. At first, 100 mL of ethanolbutylated hydroxytoluene and a-tocopherol acetate (50 mmol/L; Sigma/Aldrich, Steinheim, Germany) was added to 200 mL of plasma samples, and 250 mL of the sample was transferred into a 10 mL test tube. Samples were centrifuged at 7000 g for 5 min and 250 mL of the upper layer was transferred to another test tube and dried under nitrogen gas. The residue was redissolved in 200 mL of mobile phase (methanol) by mixing it for 2 min, and 50 mL was immediately injected into the chromatograph. A carefully measured quantity of α-tocopherol acetate as an internal standard was introduced into each standard and sample, and the ratio of analyte to internal standard peak area served as the analytical parameter.

Statistics

The results are expressed as mean ± standard error of the mean (SEM). The statistical significance was determined using the paired and unpaired t-test. Repeated measurement statistical analysis was used for comparison of means in different days.

The null hypothesis was rejected at the 0.05 level of significance. The SPSS 11.0 software (Chicago, IL, USA) was used for data analysis.

Results

Biochemical measurements

All patients showed significant improvement of plasma levels of urea nitrogen and creatinine within first week after renal transplantation.

Erythrocyte GSH and SOD activity

Figure 1a shows the erythrocyte SOD activity. There were no significant changes of SOD activity in cyclosporine group in different days. But there was a considerable reduction of SOD activity in sirolimus group, 14 days after transplantation compared to before transplantation (Fig1a).

Erythrocyte GSH did not show any significant changes following transplantation. The level of GSH was not significantly different between groups in different days after transplantation (Fig1b).

Plasma MDA and vitamin E

MDA was not significantly different between groups, before and 48 hour after transplantation (Fig 2a). Considerably higher MDA level (1.82 ± 0.43 vs. 1.03 ± 0.12, µmol/l, p<0.05, fig 2a) was seen 2

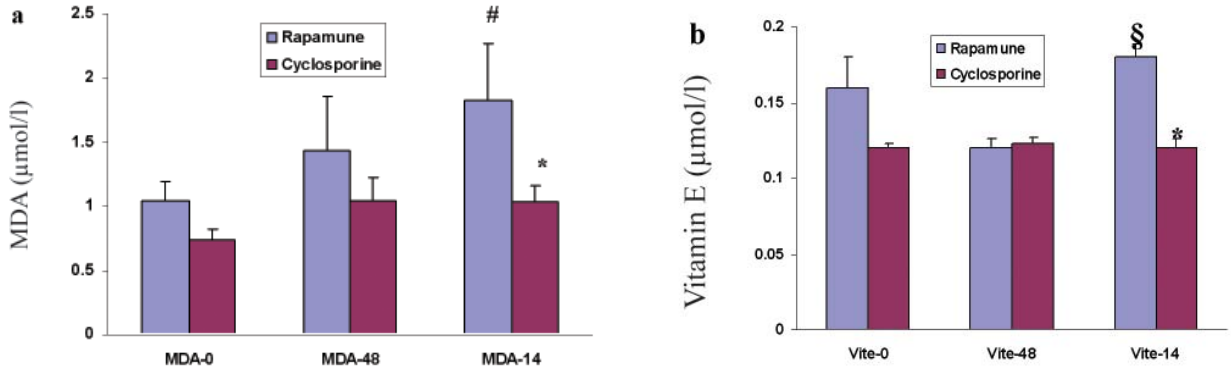

Figure 2. Alterations in MDA (a) and plasma vitamin E (b) in two groups before transplantation (0), 48 hour after transplantation (48) and two weeks after transplantation (14). The data are presented as mean ± SEM.
* p<0.05 compared to Sirolimus.
# p<0.05 compared to before transplantation.
§ p<0.05 compared to 48 hour after transplantation.

weeks after transplantation in SRL group compared to CsA group. There was also a significant increase in MDA level in SRL group in comparison with before transplantation data.

Plasma vitamin E levels are presented in figure 2b. There was a significant difference in plasma vitamin E level (0.18 ± 0.008 vs. 0.12 ± 0.006 µmol/l, p<0.05, fig 2b) between two groups at 2 weeks after transplantation. There was also a significant difference in plasma vitamin E level of SRL group (0.18 ± 0.008 vs. 0.12 ± 0.006 µmol/l, p<0.05, fig 2b) compared to 48 hour after transplantation.

Discussion

Among the many processes involved in atherogenesis, oxidative stress and modification of low density lipoprotein has been assigned a major role which may be affected by the immunosuppressive regimen used (12).

Since before transplantation, a higher SOD activity was detected in SRL-treated recipients compared to CsA group, we then compared the trend of changes in two treated groups. SOD activity showed a downward trend in SRL-treated recipients which caused in a significant difference on 14th days after transplantation compared to before transplantation data. However, CsA-treated recipients did not show the same results. It was interesting that although statistically insignificant, MDA level increased in sirolimus-treated patients. It is well recognized that vitamin E is the second line of antioxidative defense mechanism; thus we cannot expect to see a major change immediately after transplantation. Moreover, there are studies showing no changes for vitamin E, despite considerable changes in MDA and GSH levels in patients on hemodialysis (3, 13). Probably, increased level of plasma vitamin E on 14th day was a secondary response to the change of MDA level. It may also be linked to higher incidence of hypercholestolemia in sirolimus-treated recipients.

Although we hypothesized that possible renoprotective effect of continuous sirolimus exposure may be due to positive consequences on oxidative stress, we did not observe significant decrease in superoxide or GSH. McGrath et al did not find difference in vitamin E level between two different immunosuppressive regimens (20 on cyclosporin, 20 on azathioprine/prednisolone) and SOD was reduced in both transplant groups compared to controls mainly due to better graft function (12). Jobs et al detected a strong increase in superoxide levels that was not restricted to the endothelium cell layer but was observed throughout

the vascular wall in male Wistar rats administered sirolimus, and suggested that it may contribute to sirolimus-induced vascular dysfunction (14).

Our study had certain limits and constraints. First, indicators of oxidative stress were defined based on measurements during hospital admission and they were not been reevaluated after their discharge. Second, the number of patients was not so high which may affect on the power of our results.

Conclusions

It seems that sirolimus may not offer more protection against increased reactive oxygen species (ROS) production in early period after renal transplantation. In order to find the time of positive impact of sirolimus it is valuable to conduct studies after this time and with higher number of patients.

Acknowledgement

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Conflict of Interest

None declared.

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