Transpl Int (1998) 11 [Suppl 1]: S 125–S 129

S. Simic-Ogrizovic
T. Simic
Z. Reljic
S. Markovic
R. Blagojevic
D. Radivojevic
V. Lezaic
Lj. Djukanovic
J. Mimic-Oka

Markers of oxidative stress after renal transplantation

S. Simic-Ogrizovic (✉) · R. Blagojevic · D. Radivojevic · V. Lezaic · Lj. Djukanovic
Department of Nephrology,
Institute of Urology and Nephrology,
University Clinical Center of Serbia,
Pasterova 2, Beograd, Yugoslavia

T. Simic · Z. Reljic · J. Mimic-Oka
Institute of Biochemistry,
University Clinical Center of Serbia,
Beograd, Yugoslavia

S. Markovic
Clinical Biochemistry Laboratory,
University Clinical Center of Serbia,
Beograd, Yugoslavia

Abstract An increased degree of oxidative stress (OS) in chronic renal failure (CRF) and a possible role of free radicals in CRF have already been described. However, data on OS after renal transplantation are scarce. The aim of the present study was to estimate the degree of OS in renal transplant patients. The study included four groups: 1) 15 haemodialysis patients (HD group), 2) 11 renal transplant patients with stable function (SF group), 3) 12 renal transplant patients with chronic biopsy-proven rejection (CR group), and 4) 10 healthy controls (C group). Markers of OS (malondialdehyde and thiol group levels) and antioxidant activity (glutathione peroxidase and Cu,Zn-superoxide dismutase) were determined in plasma and in red blood cells of all examined individuals. After successful renal transplantation a significant improvement, but not normalization, of antioxidant enzyme activities accompanied by significantly reduced lipid peroxidation were found. In the CR group the degree of OS was increased, and our results suggest that OS may be a relevant pathophysiological factor for CR development.

Key words Renal transplantation · Oxidative stress

Introduction

Kidney transplantation has emerged as the treatment of choice for patients with end-stage renal disease. After successful renal transplantation, the entire restoration of different renal functions and normalization of many metabolic disorders can be expected [1]. However, even with major improvements in surgical techniques, preservation techniques and preservation solutions, every transplantation starts with the inevitable insult of graft ischaemia and reperfusion and consequent oxidative damage [2–4]. The role of free radicals in ischaemia-reperfusion injury immediately after kidney transplantation has been extensively studied [5–7]. On the other hand, there are few reports about the existence of oxidative stress in renal transplant patients with long-term graft survival.

Chronic rejection (CR) is the single most important cause of long-term graft failure. The aetiology and pathophysiology of CR are incompletely understood, but most likely involve both immune and nonimmune factors [8]. Allograft recipients with CR, like other chronic renal failure (CRF) patients, suffer characteristic CRF complications. However, data related to both oxidative stress and its relationship with renal dysfunction in these patients are rather scarce. To the best of our knowledge, there has been no reported study in which the total plasma and red blood cell antioxidant status was examined in renal transplant recipients with stable function (SF) and patients with CR.

In order to investigate the degree of oxidative stress in patients after renal transplantation, in this study markers of oxidative stress (malondialdehyde, MDA, and thiol group levels) and antioxidant activity (glutathione peroxidase, GPx, and Cu,Zn-superoxide dismutase, SOD) were studied in red blood cells and plasma of patients on maintenance haemodialysis (HD), renal transplant recipients with SF and renal transplant patients with CR.

Table 1 Clinical characteristics of the renal transplant recipients

	SF group	CR group
Blood pressure		
· Systolic (mm Hg)	140.0 ± 21.32	155.83 ± 19.75
Diastolic (mm Hg)	87.2 ± 13.48	99.16 ± 12.21*
Serum creatinine (μmol/l)	138.72 ± 21.50	376.08 ± 184.09*
Proteinuria (g/24 h)	0.29 ± 0.20	2.52 ± 1.13*

* $P < 0.05$

Materials and methods

Patients and controls

The study group comprised 38 renal patients, 15 HD patients (HD group, 8 males and 7 females, 46.7 ± 10.7 years old) and 23 renal transplant patients. Dialysis patients were dialysed with cuprophan membrane dialyzers three times a week for 4 h under the same conditions. Of the 23 transplant recipients, 11 had SF (SF group, 5 males and 6 females, 45.72 ± 10.28 years old) and 12 showed the clinical and morphological characteristics of CR (CR group, 10 males and 2 females, 37.68 ± 4.25 years old). The clinical characteristics of the patients are presented in the Table 1. The mean time of survival after renal transplantation was 5 ± 0.5 years. Plasma concentration of endogenous creatinine (Cr) was used as the measure of renal function. All transplanted patients were on triple immunosuppressive therapy (prednisone + azathioprine + cyclosporine).

A control group of ten healthy volunteers from the laboratory staff comparable in sex and age with the renal patients was selected. All participants gave their informed consent to participation in the study.

Laboratory methods

Blood sampling

Peripheral venous blood (5 ml) was collected for analysis in tubes over trace element-free heparin in the morning after an overnight fast and immediately prior to dialysis in patients who were receiving HD.

Isolation of plasma and erythrocytes

Plasma was separated by low speed separation at 4 °C. The packed red cells were washed in cold saline until the supernatant was clear. After separation by centrifugation at 4000 rpm at 4 °C for 5 min, the cells were lysed with distilled water (1:5 v/v) and three freeze-thaw cycles. The supernatant solution, obtained by centrifugation of cell lysates for 20 min at 10000 *g*, was used for the biochemical assays. Plasma and erythrocytes were stored at −70 °C until used in the biochemical assays.

Measurement of lipid peroxidation

The lipid peroxidation level in plasma was monitored by determining the end product of lipid peroxidation, MDA, by the commonly used thiobarbituric acid (TBA) method [9]. Red blood cell lipid peroxidation was evaluated by determining the MDA-TBA conjugate concentration according to the method of Stoks and Dormandy [10]. Plasma and erythrocyte MDA values were calculated using the extinction coefficient of the MDA-TBA complex at 532 nm = 1.56×10^5 and are expressed as micromoles per litre.

Measurement of thiol groups

The plasma concentration of thiol groups, the most important chain-breaking antioxidant, was determined according to the method of Jocelyn [11], and is expressed as millimoles per litre. The concentration of nonprotein thiol groups (GSH) in red blood cells was estimated in protein-free supernatant according to the method of Ellman [12].

Enzyme assays

GPx was measured by the coupled assay procedure of Gunzler et al. [13]. One unit of GPx activity is reported as micromoles of NADPH oxidized per minute, assuming 6.22×10^3 to be the molar absorbance of NADPH at 340 nm. SOD activity was assayed according to the method of Misra and Fridovich [14], based on the ability of SOD to inhibit autooxidation of epinephrine at alkaline pH (pH 10.2). One unit of SOD activity was defined as the amount of enzyme which inhibited the oxidation of epinephrine by 50% under the assay conditions.

Serum creatinine and protein in urine

Serum Cr was determined using the Jaffe method with modifications and protein in urine using the Ponse-S method.

Statistical analysis

Data are expressed as means ± SD. Statistical significance was evaluated using the paired Student's *t*-test. Differences were considered significant at $P < 0.05$. Correlation between the parameters tested was evaluated by regression analysis.

Results

The plasma concentrations of the markers of oxidative stress are given in Table 2. The plasma MDA levels of the HD group were significantly higher and the thiol levels and GPx activity were significantly lower than those of the SF group. There was no significant difference in plasma SOD activity between the HD group and the SF group.

The results presented in Table 2 also show that the plasma MDA levels of the SF and CR groups were significantly higher (290–219%) and the thiol levels of the same groups were significantly lower (10.3–31.6%) than the levels of the control group. Plasma GPx activity in the CR group was significantly lower (24.7%) and plasma SOD activity was significantly higher (49%) than in the control group. There was no significant difference between the SF group and the control group in antioxidant enzyme activities. In addition, the plasma

Table 2 Markers of oxidative stress in plasma of haemodialysis patients and renal transplant recipients. The values are means ± SD; the percentage values are with respect to the control group (*CR group* transplant recipients with chronic rejection; *HD group* haemodialysis patients, *SF group* transplant recipients with stable function; *GPx* glutathione peroxidase, *GSH* glutathione, *MDA* malondialdehyde, *SH* thiol, *SOD* superoxide dismutase)

	Control group	HD group	SF group	CR group
MDA (μmol/l)	0.21 ± 0.07 100%	1.43 ± 0.39*[c] 681%	0.82 ± 0.33*[a] 390%	0.67 ± 0.17*[a] 319%
SH group (mmol/l)	0.57 ± 0.08 100%	0.29 ± 0.08*[c] 51%	0.46 ± 0.06*[a b] 81%	0.39 ± 0.09*[a] 68%
GPx (U/l)	375 ± 39.1 100%	56.4 ± 7.48*[c] 15%	368.3 ± 48.0*[b] 98%	282.7 ± 95.5*[a] 75%
SOD (U/l × 10^3)	31.0 ± 2.0 100%	44.2 ± 11.8 142%	41.4 ± 11.3 133%	46.2 ± 10.9*[a] 149%

* $P < 0.05$; [a] vs control group, [b] vs CR group, [c] vs SF group

Table 3 Markers of oxidative stress in red blood cells of haemodialysis patients and renal transplant recipients. The values are means ± SD; the percentage values are with respect to the control group (*CR group* transplant recipients with chronic rejection; *HD group* haemodialysis patients, *SF group* transplant recipients with stable function; *GPx* glutathione peroxidase, *GSH* glutathione, *MDA* malondialdehyde, *SH* thiol, *SOD* superoxide dismutase)

	Control group	HD group	SF group	CR group
MDA (μmol/l)	3.0 ± 0.8 100%	12.7 ± 3.0*[b] 423%	8.0 ± 3.0*[a] 266%	9.5 ± 4.2*[a] 316%
GSH (mmol/l)	1.58 ± 0.22 100%	2.71 ± 0.28*[b] 171.5%	1.5 ± 0.34 95%	1.84 ± 0.59 116%
GPx (U/l)	12.3 ± 1.7 100%	13.4 ± 1.3 109%	15.7 ± 4.5*[a] 127%	20.0 ± 7.7*[a] 162%
SOD (U/l × 10^3)	851 ± 107 100%	492 ± 126*[b] 58%	750 ± 142 88%	800 ± 115 94%

* $P < 0.05$; [a] vs control group, [b] vs SF group

Table 4 Correlation between markers of oxidative stress in plasma and in red blood cells and serum creatinine levels. Values are correlation coefficients, *r* (*GPx* glutathione peroxidase, *GSH* glutathione, *MDA* malondialdehyde, *SH* thiol, *SOD* superoxide dismutase)

Plasma		Red blood cells	
MDA (μmol/l)	0.177	MDA (μmol/l)	0.763*
SH group (mmol/l)	−0.809*	GSH (mmol/l)	0.581
GPx (U/l)	−0.074	GPx (U/l)	0.379
SOD (U/l × 10^3)	0.043	SOD (U/l)	0.442

* $P < 0.05$

thiol level and GPx activity of the CR group was significantly lower than the levels of the SF group.

Markers of oxidative stress in red blood cells of the controls and patients are given in Table 3. The MDA and GSH levels in red blood cells of HD patients were significantly higher and SOD activity was significantly lower than in red blood cells of the SF group. There was no significant difference in GPx activity in red blood cells between the HD group and the SF group.

The results presented in the Table 3 also show that the MDA levels (166% and 216%) and GPx activities (27% and 62%) in the SF group and the CR group red blood cells were significantly higher than in the control group. There were no significant differences between the SF group, the CR group and the control group in red blood cell GSH levels and SOD activities. In addition, there were no significant differences between the SF group and the CR group in the levels of markers of oxidative stress in red blood cells.

The results presented in the Table 4 show a statistically significant negative correlation between plasma thiol levels and serum Cr levels ($r = -0.809$, $P < 0.05$). There was also a statistically significant positive correlation between red blood cell MDA levels and serum Cr levels ($r = 0.63$, $P < 0.05$). There was no statistically significant correlation between other markers of oxidative stress and serum creatinine.

Discussion

The generation and harmful effects of reactive oxygen radicals (ROS) in transplanted organs has been demonstrated experimentally [15] and in clinical kidney transplantation [6, 7]. In all of these studies plasma levels of MDA, as a marker of lipid peroxidation, have been shown to be increased immediately after reperfusion of a transplanted kidney. Several studies have also shown an enhanced consumption of the antioxidant plasma capacity in organ recipients [7]. Antioxidant vitamin supplementation [4] and human recombinant SOD therapy [16] have been used in an attempt to improve antioxidant capacity and graft survival in human kidney recipients. However, the existence of oxidative stress in long-term graft survival patients has not been extensively studied.

In the present study, plasma and red blood cell markers of oxidative stress and antioxidant capacity in kidney recipients 5 years after transplantation were determined and compared with those in CRF patients on HD and healthy subjects. Our results confirm previous reports that oxidative stress is increased in CRF patients [17] with increased plasma and red blood cell lipid peroxidation (MDA levels), and with decreased levels of chain-breaking antioxidants (protein thiol groups) and unbalanced (extracellular as well intracellular) antioxidant enzyme activity. In contrast to HD patients, allograft recipients with SF showed improved antioxidant enzyme activities as a result of a raised level of extracellular GPx activity.

These results provide indirect confirmation of the findings of Yoshimura et al. [18] that extracellular GPx originates from renal proximal tubular cells. The increase in antioxidant enzyme activities in plasma and red blood cells of transplant patients with SF was accompanied by significantly reduced lipid peroxidation when compared with patients undergoing regular HD. However, the evaluation of lipid peroxidation showed that recipients with SF still had significantly higher plasma and red blood cell MDA levels, than healthy individuals. The plasma concentrations of protein thiol groups in renal recipients with SF did not reach the control group levels, which is additional proof of increased oxidative stress in these patients. These results agree with the experimental and clinical findings of Suleymanlar et al. [19]. Based on these findings, it may be concluded that renal transplant recipients, despite better antioxidant capacity, show increased production of free radicals.

The leading cause of late kidney allograft failure is CR which has been defined as progressive functional deterioration occurring months or years after transplantation. Morphologically it is characterized by vascular obliteration resembling atherosclerosis, atrophy and interstitial fibrosis [20].

The results presented here show that renal graft dysfunction is associated with enhancement of oxidative stress. In the patients with CR we found a significant correlation between the lowering of protein thiol levels, increasing red blood cell MDA contents and serum Cr concentrations. These results agree with those of our previous studies, showing a significant correlation between the degree of oxidative stress and renal functional deterioration [17, 21]. However, no such correlation was found between serum Cr concentrations and other parameters tested.

Patients who had developed CR also had significantly lower plasma GPx activities in comparison with both SF patients and healthy individuals. Decreased plasma GPx activity was most probably a consequence of impaired production of extracellular GPx in long-surviving grafts with dysfunction. These results are in agreement with those of Cristol et al. [22] who also found increased consumption of chain-breaking antioxidants in CR and reduced plasma GPx activities during CR. These results may be relevant for determining the implications of oxidative stress in CR. Oxidative stress is a situation in which an overloading of "oxidants" or free radicals damages or destroys a cell. The assumption is that there is a natural balance between toxic oxidants and protective oxidant defences, and that under certain circumstances, too many oxidants are generated for the antioxidant defences to handle. Thus, any imbalance between the oxidant stress and antioxidant capacity may have deleterious effects on the structure and function of various cellular and noncellular components.

Experimental results also suggest that oxidative stress may impair various immune functions, such as lymphocyte activation by mitogens, natural killer cell activation and lymphocyte-mediated cytotoxicity [23]. Recent evidence also indicates that oxidative stress may enhance apoptosis of immunocompetent cells [24], and in vitro studies suggest that ROS may activate nuclear factor $\varkappa$B (NF-$\varkappa$B). These effects of oxidative stress are all relevant for the immunopathogenesis of CR [25]. However, the underlying mechanisms have not yet been identified. The formation of highly reactive free radicals is a consequence of a variety of biochemical reactions. The oxidative stress we observed may have been a consequence of the effects of the drug used to prevent graft rejection. It seems reasonable to assume that in renal transplant recipients free radical overloading can be attributed to cyclosporine-induced lipid peroxidation [26].

In conclusion, this study provided evidence that renal transplant recipients, and more particularly those with chronic graft dysfunction, show enhanced oxidative stress. We speculate that oxidative stress may play an important pathophysiological role in the development of graft dysfunction in renal transplant patients, and may possibly increase their risk of future renal transplant loss.

References

1. Wanner C, Bartens W, Galle J (1995) Clinical utility of antilipidemic therapies in chronic renal allograft failure. Kidney Int 48 [Suppl 52]: S60–S62
2. Lehr HA, Messmer K (1996) Rationale for use of antioxidant vitamins in clinical organ transplantation. Transplantation 62: 1197–1199
3. Paller MS, Hoidal JR, Ferris TF (1984) Oxygen radicals in ischemic acute renal failure in the rat. J Clin Invest 74: 1156–1164
4. Rabl H, Khoschsorur G, Colombo T, Petritsch P, Rauchenwald M, Koltringre P, Tatzber F, Esterbauer H (1993) A multivitamin infusion prevents lipid peroxidation and improves transplantation performance. Kidney Int 43: 912–917
5. Menger MD, Lehr HA, Messmer K (1991) Role of oxygen radicals in the microcirculatory manifestation of ischemia/reperfusion injury. Klin Wochenschr 69: 1050–1053
6. Princemail J, Defraigne JO, Franssen C (1993) Evidence for free radical formation during human kidney transplantation. Free Radic Biol Med 15: 343–347
7. Davenport A, Hopton M, Bolton C (1995) Measurement of malondialdehyde as a marker oxygen free radical production during renal allograft transplantation and the effect of early graft function. Clin Transplant 1995 9: 171–175
8. Massy ZA, Guijarro C, Kasiske BL (1995) Clinical predictors of chronic renal allograft rejection. Kidney Int 48 [Suppl 52]: S85–S88
9. Jentzsch AM, Bachmann H, Furst P, Biesalki HK (1996) Improved analysis of malondialdehyde in human body fluids. Free Radic Biol Med 20: 251–256
10. Stocks J, Dormandy TL (1971) The autoxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol 20: 95–110
11. Jocelyn PC (1987) Spectrophotometric assay of thiols. Methods Enzymol 143: 44–67
12. Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 74: 443–450
13. Gunzler WA, Kremers H, Flohe L (1974) An improved coupled test procedure for glutathione peroxidase in blood. Z Klin Chem Klin Biochem 12: 444–448
14. Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247: 3170–3175
15. Demibras A, Bozoklu S, Ozdemir A, Bilgin N, Naberal M (1993) Effect of alpha tocopherol on the prevention of reperfusion injury caused by free oxygen radicals in the canine kidney autotransplantation model. Transplant Proc 25: 2274–2276
16. Land W, Sheenberg H, Schleibner S (1994) The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. Transplantation 57: 211–216
17. Mimic-Oka J, Simic T, Ekmescic V, Dragicevic P (1995) Erythrocyte glutathione peroxidase and superoxide dismutase activities in different stages of chronic renal failure. Clin Nephrol 44: 44–48
18. Yoshimura S, Suemizu H, Nomoto Y (1996) Plasma glutathione peroxidase deficiency caused by renal dysfunction. Nephron 73: 207–211
19. Suleymanlar G, Suleymanlar I, Shapiro JI, Chan L (1994) Possible role of lipid peroxidation in cyclosporine nephrotoxicity in rats. Transplant Proc 26: 2886–2889
20. Fellstrom BC, Larson E (1993) Pathogenesis and treatment perspectives of chronic graft rejection. Immunol Rev 134: 83–98
21. Mimic-Oka J, Simic T, Djukanovic L (1996) Alteration in plasma antioxidant capacity in various degrees of chronic renal failure. Nephrol Dial Transplant 11: A135
22. Cristol JP, Maggi MF, Vela C, Descomps VB, Mourad G (1994) Lipid metabolism and oxidative stress in renal transplantation: implication for chronic rejection. Transplant Proc 28: 2820–2821
23. Burdon RH (1994) Free radicals and cell proliferation. In: Rice-Evans C, Burdon RH (eds) Free-radical damage and its control. Elsevier, Amsterdam, pp 155–180
24. Butke TM, Sanstrom PA (1994) Oxidative stress as a mediator of apoptosis. Immunol Today 15: 7–10
25. Paul LC (1995) Chronic renal transplant loss. Kidney Int 47: 1491–1499
26. Wang C, Salahudeen AK (1994) Cyclosporine nephrotoxicity: attenuation by an antioxidant-inhibitor of lipid peroxidation in vitro and in vivo. Transplantation 58: 940–946