Oxidative Stress Status in Renal Transplant Recipients

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Abstract

Objectives: Despite the demonstration of oxidative stress in patients with end-stage renal disease, the oxidative status during and after a renal transplant are not completely understood. Hepatocyte growth factor is reported to act as an endogenous factor against oxidative stress. The aim of this study was to evaluate the pattern of changes in plasma oxidative status and hepatocyte growth factor levels in living-donor renal transplant recipients during the early phase after transplant.

Materials and Methods: Nineteen patients who underwent a renal transplant were included. All were on cyclosporine-based immunosuppression. Plasma levels of malondialdehyde, ferric reducing activity, hepatocyte growth factor, vitamin E, erythrocyte glutathione, and superoxide dismutase activities were determined before, and on the second, seventh, and 12th days after the transplant.

Results: High malondialdehyde concentration and low superoxide dismutase activity were seen before and 48 hours after transplant compared with healthy subjects. Significant reductions in plasma ferric reducing activity, malondialdehyde, and hepatocyte growth factor were seen on the seventh and twelfth days after transplant, compared with the before-transplant data. Direct correlations were found between hepatocyte growth factor levels and ferric reducing activity of plasma as well as hepatocyte growth factor and creatinine or uric acid.

Conclusions: Renal transplant recipients display persistent oxidative stress during the early phase of transplant. The pattern of oxidative changes should be considered for appropriate time, dosage, type, and the duration of antioxidant therapy in these patients.

Key words: Kidney, Reactive oxygen species, Hepatocyte growth factor, Living-donor.

Ischemia-reperfusion accompanied during renal transplant induces oxidative stress (1). Although there are improvements in metabolic disturbances after a renal transplant, but the oxidative status during and after transplant is not completely understood (2). Previous studies have reported the measurements of serum antioxidant levels following transplant. Some studies demonstrated increased activities of enzymatic antioxidants (2, 3), while other studies reported a decrease in enzymatic and nonenzymatic antioxidant defense mechanisms (4-6). In some cases, antioxidant activities did not show any alteration (7). Because these studies used different experimental conditions, it is plausible that these varying experimental conditions were responsible for the inconsistent results. It should also be noted that measurement of the total antioxidant capacity in biological samples would allow detection of the contribution of still unknown antioxidants, and the synergism between antioxidants (8-10).

Understanding the pattern of oxidative changes after transplant will lead to clinical strategies to combat oxidative injuries. Therefore, in this study, the pattern of changes in plasma oxidative status was evaluated by measurement of erythrocyte glutathione (reduced glutathione), superoxide dismutase activity, plasma malondialdehyde, ferric...
reducing activity of plasma, and vitamin E before the transplant, and after including the second, seventh, and 12th days in living-donor kidney transplant recipients.

There are some reports of hepatocyte growth factor protection against oxidative stress (11). Its blood level increases in patients with acute renal rejection after renal transplant (12, 13). Some studies have shown that hepatocyte growth factor is qualified as a candidate for renotropin. Thus, in the present study, alteration of hepatocyte growth factor level also was evaluated at the same time intervals.

Patients and Methods

Nineteen patients (9 females; mean age, 41.05 ± 11.9 years; body mass index, 22.6 ± 2.35) (Table 1) who had undergone a living-donor renal transplant were enrolled in this study. The model is based on controlled, living-unrelated donor nephrectomy (14, 15).

All patients were on cyclosporine-based immunosuppression with normal graft function; mean plasma creatinine was 123.76 ± 27.4 µmol/L. Diabetics, patients with dehydration, ischemic or infectious complications, multiorgan transplants, those with a history of earlier renal transplant, and patients treated with vitamin supplements were excluded. Recipient body mass index was abstracted from height and weight recorded at the time of transplant or registration.

Blood samples (4 mL) were obtained before the transplant, and then on the second, seventh, and 12th days after the transplant. Samples were collected from fasting patients in heparinized tubes and were centrifuged (3000 g at 0-4°C for 8 min). The plasma was removed, aliquoted, and stored at -70°C. None of the patients required dialysis during the posttransplant course of the study. Because anemia is a driving factor for increased oxidative stress, hemoglobin levels were measured during each sampling, and the values of glutathione and superoxide dismutase were expressed as U/g Hb.

Measurement of erythrocyte glutathione
Reduced glutathione was assayed according to the Tietz method in fresh, heparinized, whole blood samples. First, 200 µL of whole blood was mixed with the precipitating solution and after adding DTNB solution (5, 5'-Dithiobis 2-nitrobenzoic acid), its continuous reduction was measured at 412 nm. The value for each sample extracted from the standard curve is expressed as g/g Hb (16).

Measurement of erythrocyte superoxide dismutase activity
Erythrocyte superoxide dismutase activity was measured in fresh blood samples according to the method of Paoletti and associates (17). In this assay, nicotinamide adenine dinucleotide phosphate oxidation is linked to the availability of superoxide anions in the medium. The value of each sample is expressed as U/g Hb.

Measurement of plasma malondialdehyde
Malondialdehyde was measured in fresh plasma samples according to the method of Esterbauer and Cheeseman (18). Malondialdehyde reacts with thiobarbituric acid and produces a pink pigment, which has a maximum absorption at 532 nm. The value of each sample was expressed as µmol/L.

Measurement of plasma vitamin E
Plasma vitamin E levels were measured using reversed-phase high-performance liquid
chromatography (HPLC) and ultraviolet (UV) detection. Vitamin E extraction was done according to Arnaud (19). First, 100 µL of ethanol-butylated hydroxy toluene and α-tocopherol acetate (50 µM; Sigma/Aldrich, Germany) were added to 200 µL of plasma samples. Samples centrifuged at 7000 g for 5 minutes and dried under nitrogen gas. The residue was redissolved in 200 µL of mobile phase (methanol) by mixing for 2 minutes, and 50 µL were immediately injected into the chromatograph.

Measurement of ferric reducing ability of plasma

The ferric reducing activity assay was done according to the method of Benzie and Strain (20). In this assay, using the antioxidants in a sample reduces ferric ion in a complex with tripyridyltriazine to a colored ferrous complex. A plasma sample was stored at -70°C until use. Ten microliters of plasma was added to 1.8 mL of a freshly prepared ferric reducing activity of plasma solution, and the absorbance was measured at 593 nm.

Measurement of plasma hepatocyte growth factor

Plasma hepatocyte growth factor level was determined using enzyme-linked immunosorbent assay (ELISA) kits (R and D Systems, Inc., Minneapolis, MN, USA). This assay uses the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody, specific for hepatocyte growth factor, had been precoated onto a microplate. Standards and samples were pipetted into the wells, and any hepatocyte growth factor present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for hepatocyte growth factor was added to the wells. After a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells, and color develops in proportion to the amount of hepatocyte growth factor bound in the initial step. The color development is stopped, and the intensity of the color is measured.

Statistical analysis

Results are expressed as the mean ± SEM. The data were analyzed by the Kruskal-Wallis test; when the analysis showed significant differences among groups, means were compared with the Mann-Whitney U test. Pearson correlation coefficient was used to determine association between the changes in hepatocyte growth factor and oxidative variables. The null hypothesis was rejected at the .05 level of significance. Statistical analyses were performed with SPSS software for Windows (Statistical Product and Service Solutions, version 11.0, SSPS Inc, Chicago, IL, USA).

Results

Biochemical measurements

Plasma levels of urea nitrogen and creatinine improved significantly in all patients, notable within first week after renal transplant (P < .05 for all, data not shown). Some of these data are summarized in Table 1.

Erythrocyte glutathione and superoxide dismutase activity

Erythrocyte glutathione was not significantly different between controls (80.85 ± 3.59 g /g Hb) and patients (80.71 ± 4.15 g /g Hb) before transplant. The level of glutathione did not show any significant changes after transplant (Figure 1a). Figure 1b shows the erythrocyte superoxide dismutase activity. Before transplant, there was approximately a 46% lower level of erythrocyte superoxide dismutase activity.

![Figure 1](image-url)
(280 ± 28 vs 530 ± 22 U/g Hb) (P < .05) when compared with controls. Although after 1 week, there was about 28% increase in superoxide dismutase activity (370 ± 40 vs 280 ± 28 U/g Hb) compared to before the transplant, but it was still significantly lower than controls (370 ± 40 vs 530 ± 22 U/g Hb) (P < .05).

**Plasma malondialdehyde and vitamin E**
Malondialdehyde level was higher before transplant than it was for controls (1.33 ± 0.15 vs 0.55 ± 0.052, µmol/L) (P < .05) (Figure 2a). The same results were seen 48 hours after transplant. On the seventh and 12th days after transplant, malondialdehyde significantly decreased (0.68 ± 0.06 and 0.84 ± 0.13 vs 1.33 ± 0.15; µmol/L) (P < .05) (Figure 2a) about 49% and 37% respectively, when compared with the before transplant data. Plasma vitamin E levels are presented in Figure 2b. There was a significant reduction (19%) in vitamin E the second day after transplant (27.68 ± 1.94 vs 34.19 ± 1.66 µmol/L) (P < .05) (Figure 2b) when compared with the before operation data. However, vitamin E was increased, so there was no significant difference between the seventh and 12th days after transplant compared with controls and before transplant data.

**Plasma ferric reducing activity, hepatocyte growth factor, and uric acid**
Plasma ferric reducing activity was significantly higher before transplant (1378.81 ± 102.5 vs 722.51 ± 50.6, µmol/L) (P < .05) (Figure 3a) when compared with controls. Ferric reducing activity of plasma significantly decreased on the second, seventh, and twelfth days after transplant (861.81 ± 67.29, 844.18 ± 43.20, 870.25 ± 84.38 µmol/L) (P < .05) (Figure 3a) compared with the before transplant data; there was no significant difference in comparison to controls (Figure 3a).

Hepatocyte growth factor changes were similar to the pattern seen for ferric reducing activity of plasma (Figure 3b). Plasma hepatocyte growth factor was significantly higher before transplant (847.12 ± 69.38 vs 198.13 ± 19.06, pg/mL) (P < .05) (Figure 3a) when compared with controls. Hepatocyte growth factor significantly decreased in second, seventh, and twelfth days after transplant (409.56 ± 29.64, 335.12 ± 30.40, 296.53 ± 25.93 pg/mL) (P < .05) (Figure 3b) compared to that before transplant data and to controls. The level of uric acid reduced significantly after transplant in comparison to levels before the operation (420.5 ± 15.4 vs 322.9 ± 22 µmol/L) (P < .05) (Figure 3c). There also were significant differences in uric acid on the seventh and twelfth days after transplant compared with controls and before transplant data.

![Figure 2. Alterations in plasma malondialdehyde (a) and vitamin E (b) in the controls, before the transplant, and then the second, seventh, and 12th days after transplant. The data are presented as mean ± SEM. *P < .05 compared to the controls; †P < .05 compared to before the transplant.](image-url)
after transplant compared to levels before the operation.

**Correlations of hepatocyte growth factor and other variables**

There was a significant correlation ($r=0.52; P < .05$) between hepatocyte growth factor and creatinine changes in transplant patients. There also was a significant correlation ($r=0.41; P < .05$) between hepatocyte growth factor and uric acid changes in transplant patients. In the patients, hepatocyte growth factor was significantly and directly correlated with ferric reducing activity of plasma ($r=0.83; P < .01$) (Figure 4) but not with superoxide dismutase levels. There were no statistically significant relations between hepatocyte growth factor and malondialdehyde levels.

**Discussion**

In this study, we evaluated plasma hepatocyte growth factor changes in parallel with total plasma antioxidant capacity, as well as single antioxidants, in about 2 weeks. The patients showed an oxidative stress before transplant that was demonstrated by higher malondialdehyde and lower superoxide dismutase activity when compared with healthy subjects. Almost, the same results were seen 48 hours after transplant. The lower superoxide dismutase value in patients is indicative of oxidative stress, which is present in end-stage renal disease. Davenport and associates (21) reported that the superoxide dismutase levels decreased in patients who had just received a transplant and received cyclosporine.

Fernandez and associates (3) and Dasgupta and associates (22) have also reported that kidney patients who underwent a renal transplant showed higher malondialdehyde levels compared with a control group. This agrees with the findings of the present study. A significant reduction of malondialdehyde levels and a recovery of plasma vitamin E level on the seventh and 12th days after transplant suggest an improvement in antioxidant defense system compared with the before transplant data. In our study, the level of glutathione did not change significantly throughout the study. This agrees with the Zachara and associates (23) study. They showed no significant changes of red blood cell glutathione level after transplant. In the present study, recovery of plasma vitamin E level was detected after 1 week. One explanation may be a possible vitamin E mobilization from organs such as the liver in response to oxidative stress, as reported by Elsayed and associates (24).

In addition, uric acid possesses significant antioxidant activity (25) that may lead to major variations of the internal redox state. Compared with controls, the plasma ferric reducing activity was significantly higher before the transplant. In this case, an increase in uric acid modifies plasma antioxidant activity (26), and reduction of plasma ferric reducing activity after transplant may be due to uric acid reduction.

Presence of low superoxide dismutase, 12 days after transplant, indicates a persistent source of reactive oxygen species production or oxidative stress during early phase of the transplant. A possible reason may be the cyclosporine-based treatment, because cyclosporine is reported to increase glomerular synthesis of reactive oxygen species (27).

There are some reports of hepatocyte growth factor endogenous protection against oxidative stress (11). In the present study, plasma hepatocyte growth factor was significantly higher before the transplant.
when compared with the controls. A decrease in plasma hepatocyte growth factor after the transplant was seen. However, Cheng and associates (28) observed an increase in plasma hepatocyte growth factor in 7 patients after transplant. The main difference was that their patients underwent deceased-donor renal transplant. Pratschke and associates (29) reported a difference in functional output between organs from deceased-donor and living sources. It was suggested that elevation of serum hepatocyte growth factor may have a renal origin that aims to protect kidneys from necrosis in patients who undergo a deceased-donor renal transplant (28).

In the present study, a significant correlation between plasma hepatocyte growth factor and creatinine, uric acid, or ferric-reducing activity may suggest the presence of a relation between the renal function and plasma oxidative status, with the hepatocyte growth factor production. Kitta and associates (11) reported that hepatocyte growth factor protects cardiac myocytes against oxidative stress. Their findings support the hypothesis that hepatocyte growth factor is an endogenous cardioprotective factor against oxidative stress.

In the present study, low superoxide dismutase levels throughout the study indicate a persistent oxidative stress that suggests the need for a specific or suitable antioxidant therapy in these patients. However, antioxidant supplementation may affect adequacy of immunosuppression that was reported by Blackhall and associates (30). In most cases, antioxidant is administered before measuring the patient’s needs, and its effects are not monitored. Choosing an appropriate antioxidant is important. Because a low superoxide dismutase level persists after 12 days posttransplant, a superoxide dismutase mimetic may have beneficial effect than other antioxidants. There also have been reports of a pro-oxidant effect of prolonged supplementation of vitamins (31). Therefore, the duration of antioxidant therapy is another factor that should be considered.

For a conclusion about a possible relation between hepatocyte growth factor and redox status of these patients, more-extensive studies must be performed. However, these results suggest persistent oxidative stress in these patients. The pattern of oxidative changes should be considered for appropriate time, dosage, type, and the duration of antioxidant therapy in these patients.

References


