Transpl Int (1996) 9 [Suppl 1]: S479–S482

R. Hower
Th. Minor
H. Schneeberger
J. Theodorakis
S. Rembold
W.-D. Illner
G. O. Hofmann
P. Fraunberger
W. Isselhard
W. Land

Assessment of oxygen radicals during kidney transplantation – effect of radical scavenger

R. Hower (✉) · H. Schneeberger · J. Theodorakis · S. Rembold · W.-D. Illner · G. O. Hofmann · P. Fraunberger · W. Land
Division of Transplant Surgery, Department of Surgery, University of Munich, Klinikum Großhadern, Marchioninistr. 15, D-81377 Munich, Germany,
Fax: + 49/89/700 41 60

Th. Minor · W. Isselhard
Department of Experimental Surgery, University of Cologne, Germany

Abstract In the present study, levels of free oxygen radicals, generated in the very early period of reperfusion during human kidney transplantation, were assessed by determination of malondialdehyde (MDA) levels using a high-pressure liquid chromatography (HPLC) method. Renal blood samples were obtained during reperfusion by intraoperative cannulation of the renal vein. Simultaneously, systemic MDA levels were determined. Furthermore, local and systemic levels of interleukin 6 (IL-6), tumor necrosis factor (TNF) receptors, p55 and p75, and vitamin E were measured. In a second group of patients, 500 mg of ascorbic acid were given prior to reperfusion. Renal MDA levels in the control group were always higher compared to systemic levels. IL-6 showed a marked increase shortly after reperfusion in the renal blood. In the scavenger group there was a diminution of these effects. TNF receptor levels and vitamin E remained largely unchanged. The results of this pilot study demonstrated clinically the moderate production of reactive oxygen species and the liberation of IL-6 shortly after reperfusion of human transplanted kidneys. Furthermore, the modulating effect of a radical scavenger on these effects was shown.

Key words Oxygen free radicals · Kidney transplantation · IL-6 · TNF receptors · Vitamin E · Radical scavenger

Introduction

The mechanisms of ischemia-reperfusion injury in the context of organ transplantation are of increasing importance, apart from immunological considerations [1, 2]. Ischemia of the donor organ after harvesting and during conservation, as well as the subsequent reperfusion may lead to endothelial injury [3]. Membrane lipids are peroxidated by the generation of free oxygen radicals, and these lipidperoxidate products can be measured as conjugated dienes or malondialdehyde (MDA) [4]. Furthermore, there is an expression of adhesion molecules and cytokine release with systemic inflammatory reactions. Recently, the question of whether these early local damages of the transplanted organ may lead to an increased immunogenicity and subsequently to an increased rate of rejection has been discussed [5]. In the present pilot study, indirect measurements of free oxygen radicals, cytokines, and vitamin E levels were done intraoperatively in the very early reperfusion period during human kidney transplantation. Furthermore, the effect of vitamin C as a radical scavenger was tested.

Patients and methods

The patients involved in this study consisted of 17 patients undergoing kidney transplantation at our center. They were randomly assigned to a control group ($n = 8$) or a vitamin C group ($n = 9$), receiving 500 mg ascorbic acid (Ascorvit) intraoperatively prior to reperfusion. Both groups received a standard immunosuppressive protocol with triple-drug induction therapy (cyclosporin A, azathioprine, steroids).

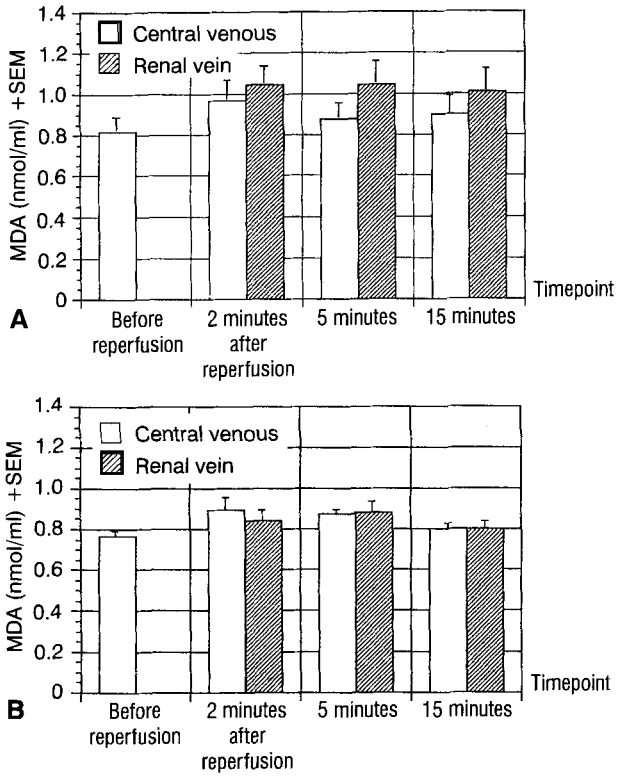

Fig. 1 Malondialdehyde (MDA) levels in **A** control group and **B** vitamin C group intraoperatively after kidney transplantation. Renal blood samples were obtained by cannulating the renal vein

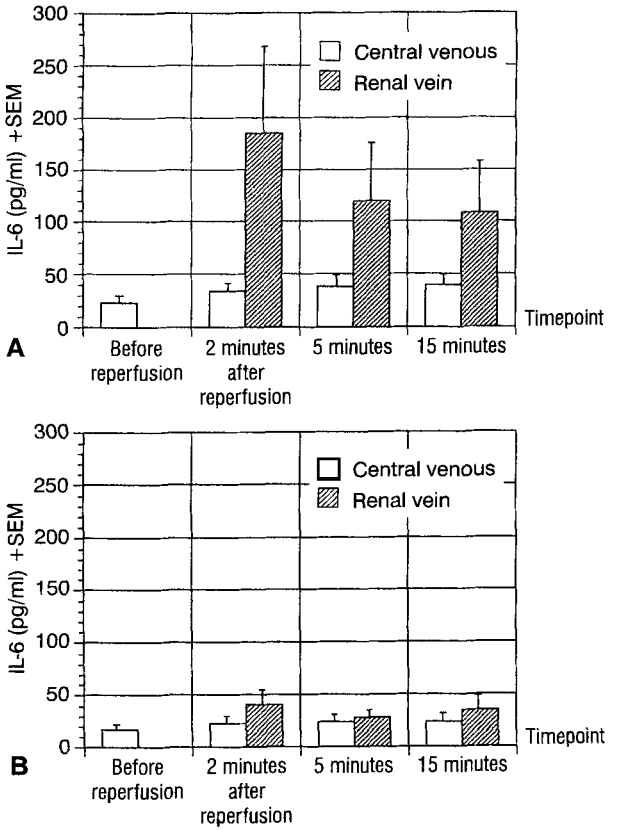

Fig. 2 Interleukin 6 (IL-6) levels in **A** control group and **B** vitamin C group intraoperatively after kidney transplantation

Table 1 Demographic data of the donors and recipients included in the pilot study. There were no significant differences between the groups (*ATG* antilymphocyte globulin, *HD* hemodialysis, *TX* transplant, *ATN* acute tubular necrosis)

	Control group ($n = 9$)	Vitamin C group ($n = 8$)
Donor data		
Age (years) 48 ± 5	37 ± 8	
Sex	4M/5F	5M/3F
Last creatinin level	0.8 ± 0.1 mg/dl	1.0 ± 0.2 mg/dl
Recipient data		
HLA-A mismatch	88 %	62.5 %
– B	77 %	62.5 %
– DR	44 %	25 %
Antibodies	0 %	0 %
ATG treatment	5	4
Cold ischemia time (h)	20.8 ± 3	27.1 ± 3
Early function parameters		
Amount of HD until TX function	7	2
Days until drop in creatinin	14	7
ATN	55 %	25 %

Concerning demographic data, there was no significant difference in donor age, last creatinin level or systolic blood pressure. Cold ischemia time was also comparable in both groups (Table 1). Using the amount of hemodialysis required till transplant function and days up to the first drop in creatinin as early indicators of transplant function, there was a trend for earlier function in the vitamin C group (Table 1).

The renal vein of the donor organ was cannulated intraoperatively and blood samples were taken at 2, 5, and 15 min after the onset of reperfusion of the transplanted kidney. Concomitantly, blood samples were taken from the central line before reperfusion and at the same timepoints following reperfusion. The plasma was seperated by centrifugation and immediately stored at – 70 °C until analysis.

Lipid peroxidation was assessed by measuring MDA levels. MDA-thiobarbituric acid adducts were determined by high-pressure liquid chromatography (HPLC), according to Wong et al. [6]. Levels of interleukin-6 (IL-6) and tumor necrosis factor (TNF) receptors, p55 and p75, were measured by ELISA. Vitamin E was measured by HPLC. Statistical analysis was performed by the nonparametric Mann-Whitney *U* test.

Results

Systemic and renal MDA levels in the control group showed an increase shortly after reperfusion (Fig. 1 A). MDA levels in the renal vein, as a marker of lipid per-

Table 2 Intraoperative values of tumor necrosis factor (TNF) receptors, p55 and p75, and of vitamin E (mean ± SEM)

	Baseline	2	5	15
TNF receptor p55 (ng/ml)				
Control central venous	25.5 ± 2.5	26.1 ± 2.5	26.3 ± 2.5	22.9 ± 1.4
Vitamin C central venous	24.5 ± 3.6	23.2 ± 3.5	22.8 ± 3	19.7 ± 3.3
Control renal vein		24.4 ± 2.7	28.0 ± 2.8	25.9 ± 2.5
Vitamin C renal vein		21.2 ± 2.3	20.5 ± 2.7	16.8 ± 3.1
TNF receptor p75 (ng/ml)				
Control central venous	17.7 ± 2.0	15.9 ± 1.6	17.4 ± 2.1	18.0 ± 2.0
Vitamin C central venous	18.0 ± 1.5	16.8 ± 1.5	17.0 ± 1.2	18.3 ± 0.9
Control renal vein		17.3 ± 1.3	17.4 ± 0.9	18.4 ± 0.7
Vitamin C renal vein		16.4 ± 1.6	17.3 ± 1.9	17.4 ± 1.7
Vitamin E (μg/mg chol.)				
Control central venous	5.27 ± 1.3	6.09 ± 0.2	5.31 ± 0.6	5.77 ± 0.8
Vitamin C central venous	6.11 ± 0.9	5.52 ± 0.8	6.04 ± 0.8	5.82 ± 0.7
Control renal vein		7.10 ± 1.2	5.87 ± 0.3	6.19 ± 0.4
Vitamin C renal vein		6.31 ± 1.0	5.10 ± 0.9	5.98 ± 0.7

oxidation, showed, for example, after 2 min of reperfusion an increase of 30 % compared with the systemic baseline value. MDA levels in the renal vein were always higher than systemic values, but without definite significance. Treatment with vitamin C prior to reperfusion resulted in a slight increase in MDA levels mainly in renal venous blood samples (Fig. 1 B). IL-6 values peaked in the renal blood of the control group 2 min after reperfusion. This peak was not seen in the treatment group (Fig. 2). There were no differences in the levels of the two TNF receptors, p55 and p75, in either group (Table 2). The levels of α-tocopherol (vitamin E), as a marker of endogenous scavenger pool, also showed no significant difference in either group (Table 2).

Discussion

Transplantation of a solid organ is linked regulary with processes of ischemia-reperfusion, resulting in a so-called reperfusion injury [7]. Free oxygen radicals, generated during reperfusion [8], may damage the microvascular endothelium, leading to increased immunogenicity and finally to an increased incidence of acute and chronic rejection, as discussed earlier by Land et al. [5].

The production of free oxygen radicals, indirectly measured by MDA levels, as the key event of reperfusion injury [9] was shown by our group clinically for the first time in transplanted kidneys intraoperatively. Renal MDA levels in the control group were always higher compared to systemic levels, indicating local release at the site of reperfusion. The peak in IL-6 2 min after reperfusion in the venous renal blood may be caused by local injury following reperfusion, thus IL-6 is a marker after trauma or injury [10].

The nonresponse of TNF receptors in this very early phase of reperfusion could be explained by the time course of production of this cytokine. In other settings of reperfusion, levels are measurable about 30–60 min after the onset of reperfusion [11].

Vitamin E, as a lipophil vitamin and an indicator for endogenous antioxidative capacity [12], may be a parameter that is too weak for this early phase of reperfusion injury. Application of the radical scavenger, vitamin C, resulted mainly in a moderate reduction in locally measured MDA and IL-6 levels at the transplanted kidney site. These statistically nonsignificant but measurable differences showed a slight trend for a diminution in acute injury in the very early period of kidney transplantation. Application of a multivitamin mixture and measurement of MDA levels in the later time course after kidney transplantation have shown similar results [13].

Thus, our data confirmed clinically earlier observations concerning the possible role of antioxidants in the elimination of reperfusion injury after transplantation [14]. Further studies may be indicated for the registration of microvascular injury and its prevention.

References

1. Koyama I, Bulkley GB, Williams GM, Im MJ (1985) The role of oxygen free radicals in mediating the reperfusion injury of cold-preserved ischemic kidneys. Transplantation 40: 590–595
2. Schneeberger H, Schleibner S, Illner WD, Messmer K, Land W (1993) The impact of free radical-mediated reperfusion injury on acute and chronic rejection events following cadaveric renal transplantation. Clin Transplant 219–232
3. Andreoli SP, McAteer JA (1990) Reactive oxygen molecule-mediated injury in endothelial and renal tubular epithelial cells in vitro. Kidney Int 38: 785–794
4. Paller MS, Hoidal JR, Ferris TF (1984) Oxygen free radicals in ischemic acute renal failure in the rat. J Clin Invest 74: 1156–1164
5. Land W, Schneeberger H, Schleibner S, Illner WD, Abendroth D, Rutili G, Arfors KE, Messmer K (1994) The beneficial effect of human recombinant superoxide dismutase on acute and chronic rejection events in recipients of cadaveric renal transplants. Transplantation 57: 211–217
6. Wong SHY, Knight JA, Hopfer SM, Zaharia O, Leach CN, Sunderman FW (1987) Lipoperoxides in plasma as measured by liquid-chromatographic seperation of malondialdehyde-thiobarbituric acid adduct. Clin Chem 33: 214–220
7. Parks DA, Bulkley GB, Granger DN (1983) Role of oxygen free radicals in shock, ischemia, and organ preservation. Surgery 94: 407–411
8. Granger DN, Höllwarth ME, Parks DA (1986) Ischemia-reperfusion injury: role of oxygen derived free radicals. Acta Physiol Scand 548: 47–63
9. Parks DA, Granger DN (1988) Ischemia-reperfusion injury: a radical view. Hepatology 8: 680
10. Schlüter B, König B, Bergmann U, Müller FE, König W (1991) Interleukin 6 – a potential mediator of lethal sepsis after major thermal trauma: evidence for increased IL-6 production by peripheral blood mononuclear cells. J Trauma 31: 1663–1670
11. Bittermann H, Lazarovich H, Kinarty A, Cohen L, Lahat N (1990) The profile of cytokines and cytokine inhibition during splanchnic artery occlussion (SAO) shock. Circ Shock 31: 25
12. Defraigne JO, Pincemail J, Franssen C, Meurisse D, Defechereux T, Philippart C, Serteyn D, Lamy M, Deby C, Limet R (1993) In vivo free radical production after cross-clamping and reperfusion of the renal artery in the rabbit. Cardiovasc Surg 1: 343–349
13. Rabl H, Khoschsorur G, Colombo T, Petritsch P, Rauchenwald M, Költringer P, Tatzber F, Esterbauer H (1993) A multi-vitamin infusion prevents lipid peroxidation and improves performance of transplanted kidneys in humans. Kidney Int 43: 912–917
14. Rangan U, Bulkley GB (1993) Prospects for treatment of free radical-mediated tissue injury. Br Med Bull 49: 700–718