Oxidative Stress in Kidney Transplantation
Causes, Consequences, and Potential Treatment

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Oxidative stress is a major mediator of adverse outcomes throughout the course of transplantation. Transplanted kidneys are prone to oxidative stress-mediated injury by pre-transplant and post-transplant conditions that cause reperfusion injury or imbalance between oxidants and antioxidants. Besides adversely affecting the allograft, oxidative stress and its constant companion, inflammation, cause cardiovascular disease, cancer, metabolic syndrome, and other disorders in transplant recipients. Presence and severity of oxidative stress can be assessed by various biomarkers produced from interaction of reactive oxygen species with lipids, proteins, nucleic acids, nitric oxide, glutathione, etc. In addition, expression and activities of redox-sensitive molecules such as antioxidant enzymes can serve as biomarkers of oxidative stress. Via activation of nuclear factor kappa B, oxidative stress promotes inflammation which, in turn, amplifies oxidative stress through reactive oxygen species generation by activated immune cells. Therefore, inflammation markers are indirect indicators of oxidative stress. Many treatment options have been evaluated in studies conducted at different stages of transplantation in humans and animals. These studies have provided useful strategies for use in donors or in organ preservation solutions. However, strategies tested for use in post-transplant phase have been largely inconclusive and controversial. A number of therapeutic options have been exclusively examined in animal models and only a few have been tested in humans. Most of the clinical investigations have been of short duration and have provided no insight into their impact on the long-term survival of transplant patients. Effective treatment of oxidative stress in transplant population remains elusive and awaits future explorations.

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INTRODUCTION

Kidney transplantation is the ideal treatment for patients with end-stage kidney disease (ESRD). Different pre-existing conditions such as diabetes mellitus and post-transplant complications can alter short-term and long-term survival of the allograft and the recipient. Oxidative stress, an imbalance between generation of oxidants and antioxidant defense system, is one of the major events which influence the allograft outcome during the peri-transplantation period. The inflammatory state plays an important role in causing oxidative stress, especially in ESRD, and among renal graft recipients.[1,2] The ESRD-associated oxidative stress,

ischemia-reperfusion, and immunosuppressive drugs are the main sources of reactive oxygen species generation after transplantation. Reperfusion injury is a common phenomenon in kidney transplantation and can cause allograft dysfunction during the first post-transplant week.[3]

The adverse effects of oxidative stress and inflammation on the kidney transplantation have been shown by experimental studies in animals, observational evidence from population-based studies, and a number of controlled clinical trials. In addition to adversely affecting the allograft function and structure, oxidative stress plays a major role in the pathogenesis of systemic inflammation, hypertension, cardiovascular disease, metabolic syndrome and neoplasm among other complication in transplant recipients.

In this review, the risk factors, biomarkers, treatment options, and management of oxidative stress in transplant patients are discussed. In preparation of this article, relevant articles on oxidative stress and transplantation, including those conducted on animals and human were reviewed. The studies were identified by searching the MEDLINE database. The following key words and subject terms were used in the search: *oxidative stress*, *kidney transplantation*, *ischemia-reperfusion injury*, *biomarkers*, *free radical production*, and *treatment of oxidative stress*.

RISK FACTORS

According to a number of studies, markers of oxidative stress are higher in chronic kidney disease (CKD) patients.[4,5] Patients with CKD have a high level of inflammation and oxidative stress, which is the main cause of cardiovascular morbidity and mortality in this population. Retention of water-soluble toxins as well as protein-bound toxins contributes to oxidative stress by promoting reactive oxygen species production. In addition, duration of dialysis treatment is associated with increased oxidative stress and cytokine levels in uremic patients.[6] Maintenance hemodialysis is not sufficient to adequately control these abnormalities. The levels of the pro-inflammatory proteins such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and C-reactive protein (CRP); oxidative stress markers; and plasma protein carbonyls were significantly elevated in ESRD patients before transplantation in comparison with healthy individuals, and all of these biomarkers significantly declined 2 months after transplantation.[7] Also, endothelial function is impaired both in hemodialysis patients and kidney transplantation recipients as compared with healthy controls.[8]

Data obtained from living donor transplant recipients show that improvement of oxidative stress parameters begins immediately after kidney transplantation and continues up to the 28th post-transplant day.[9] A complete remission is only possible when the kidney function becomes normal.[10] In effect, kidney transplant recipients are prone to reperfusion injury and demonstrate continual oxidative stress during the early phase of transplantation.[11-13] Recipients of deceased donors are at greater risk of developing reperfusion injury and oxidative stress-induced kidney injury. Brain death of the kidney allograft donor is associated with hemodynamic disturbances in systemic circulation and free radical formation that cause extensive damage to the donor's tissues. Reperfusion and oxidative injury can also occur during kidney preservation and correlates with the immediate and long-term kidney function. Reperfusion injury and the associated oxidative injury may also render the allograft prone to acute rejection.[14,15] In addition, ischemia episodes during transplantation procedure can contribute to reperfusion injury. In one study on rats, a dramatic decrease in tissue antioxidant defense capacity was observed during warm renal ischemia.[11] It should be noted that warm ischemia time is more critical for induction of oxidative stress in cadaveric donor organs. Finally, oxidative stress in kidney transplant recipients may be, in part, caused by the immunosuppressive therapy.[9] For instance, transplant recipients treated with immunosuppressive regimens containing cyclosporine A have been shown to exhibit oxidative stress as evidenced by elevation of malondialdehyde (MDA) after transplantation.[10]

Diabetes mellitus is invariably associated with inflammation and oxidative stress. In a study, reported by Morales-Indiano and colleagues, the authors found that while the magnitude of inflammation and oxidative stress was similar among their diabetic and non-diabetic ESRD patients before transplantation, it was significantly greater after transplantation in their diabetic patients. This was associated with a poorer kidney allograft function in the diabetic recipients.[16] These results

suggest that impaired glucose metabolism (detected by elevated hemoglobin A1c) may adversely affect the long-term allograft function, in part, by promoting oxidative stress.[17]

A number of observational studies have shown that incidence of cardiovascular disease is greater in kidney transplant patients than in the general population, constituting the main cause of mortality in this population. The excessive risk for cardiovascular disease and oxidative stress in this population has been attributed to a high prevalence of traditional and nontraditional atherogenic risk factors before and after transplantation.[18]

Oxidative stress is one of the main contributors to cellular damage which is frequently associated with fragmentation and oxidation of DNA. To investigate the mechanism of cell damage, markers of nucleic acid oxidation such as 8-hydroxy-2'-deoxyguanosine (8-OHDG), were studied. A high degree of tubular DNA fragmentation is associated with oxidative stress in acute allograft rejection after kidney transplantation.[19]

In studies evaluating the incidence of malignancies after kidney transplantation, it has been shown that oxidative stress acts as a co-carcinogenic factor in development of squamous cell carcinomas in patients receiving immunosuppressive agents.[20] In fact, the incidence of skin cancer is significantly increased among transplant patients. The greater the burden of oxidative stress, the higher the risk of development of skin cancer.[21] In one study designed to evaluate the risk factors for malignancy, peripheral blood samples were used to measure biomarkers of oxidative stress in 116 white adult recipients of kidney or combined kidney-pancreas transplant. The mean plasma level of oxidative stress markers was significantly greater in transplant recipients than healthy controls. These findings suggest that an imbalance exists between pro-oxidant and antioxidant status in transplant recipients causing transplant recipients to be at higher risk of skin cancer.[22]

These findings show that several risk factors predispose this population to oxidative stress and its complications before and after transplantation.

EFFECTS OF OXIDATIVE STRESS

Reperfusion injury and reactive oxygen species play a detrimental role in the pathophysiology of acute allograft rejection and kidney function in early post-transplantation phase.[23] Elevated levels of oxidative stress were detected in deceased donor allografts with delayed graft function.[24]

Oxidative stress not only affects the early post-transplantation phase, but also graft and patient's long-term outcomes. Oxidative stress appears to play a role in chronic allograft nephropathy (CAN), a condition which presents with slow deterioration of allograft kidney function over periods of months to years following transplantation. In addition, oxidative stress and inflammation in reperfusion injury result in endothelial injury.[25] Arteriosclerotic lesions are common histological features of chronic allograft nephropathy. The association of CAN with arteriosclerosis and progression of kidney disease, which are, in part, driven by oxidative stress, point to the possible role of oxidative stress in the pathogenesis of CAN.[26] This supposition is supported by significantly higher MDA levels in patients with CAN compared to their counterparts with a similar degree of kidney failure.[27]

Products of intracellular lipid and protein oxidation such as MDA and carbonylated proteins have been shown to increase, and glutathione to decrease in CAN.[28] One year after transplantation, markers of oxidative stress, such as IL-6, MDA, heat shock protein 70, and transforming growth factor-β levels, were higher in patient with increased serum creatinine than those with normal serum creatinine levels.[29] In addition, compared to the healthy subjects, transplant recipients showed significantly higher CRP, TNF-α, and 8-isoprostaglandin F2α values, which were associated with atherosclerosis and unfavorable long-term outcomes.[30,31]

Oxidative stress increases the risk of cardiovascular disease in transplanted patients, and participates in atheroma plaque formation.[32,33] Patients with atheromatous plaques, vascular calcification, and carotid artery stenosis have a greater degree of hypercholesterolemia and lower plasma antioxidant activity (e.g. lower glutathione peroxidase activity).[33] Angiogenesis, on the other hand, is a known pathologic feature of inflammation, ischemia, and chronic inflammatory diseases, including allograft rejection. Chronic inflammation and oxidative stress have been shown to cause endothelial injury and dysfunction and to impair endothelial repair process.[34]

It is noteworthy that anti-rejection medications have different effects on oxidative stress and its complications such as CAN. Calcineurin-inhibitors

have been shown to induce oxidative stress. In contrast, mycophenolate mophetil ameliorates oxidative stress.[35]

Together, these findings illustrate the adverse effects of oxidative stress on allograft and patient outcome and the need for detecting and treating or preventing this condition.[36]

BIOMARKERS

Oxidation Products

Reactive oxygen species attack and modify various biological molecules such as lipids, proteins, and nucleic acids. The byproducts of these reactions can serve as biomarkers of oxidative stress.. In a study of porcine kidney transplantation, evaluating blood samples taken before and after reperfusion injury, the investigators demonstrated that both plasma carbonyl and 8-isporostane (product of protein and lipid damage by free radicals respectively) could be reliable biomarkers to predict the reperfusion injury.[37]

Reactive oxygen species degrade polyunsaturated fatty acids, forming MDA, a cytotoxic reactive aldehyde which is can be used as a biomarker to measure the level of oxidative stress in an organism. Studies in CKD patients have shown elevated MDA level after transplantation.[38] High plasma MDA concentration and low superoxide dismutase (SOD) activity were reported in transplant recipients before and 48 hours after transplantation in comparison with the corresponding values found in the healthy individuals. Significant reductions in MDA and hepatocyte growth factor (internal antioxidant) were seen on the 7th and 12th days post-transplantation. Direct correlation were observed between hepatocyte growth factor and serum creatinine level.[13] Higher MDA levels have also been found in post-transplant patients with skin cancer, indicating greater incidence of oxidative stress in these patients.[21]

Among oxidative products, carbonylated proteins are well known, as well as MDA, and are among the most commonly used markers of oxidative stress. Elevated levels of protein carbonyl have been shown in CKD and transplant patients, and a negative relation exists between carbonyl level and kidney function in CKD patients.[39] This product of protein oxidation declines 7 to 11 months after kidney transplantation; thus, it is a useful biomarker of oxidative stress and inflammation in the early phase of transplantation.[4]

As mentioned before, oxidative stress results in oxidation of lipids. Serial changes in lipid peroxide as an oxidative stress marker has been evaluated after kidney transplantation in several studies. The mean lipid peroxide concentration was increased significantly 5 days after transplantation and decreased after a year. The mean serum creatinine concentration correlated directly with lipid peroxide concentration in the 1st post-transplant week.[40] Lipofuscin is the end-product of lipid peroxidation. High serum level of lipofuscin after kidney transplantation is an indicator of severity of oxidative stress in this group of patients.[41]

The DNA is also damaged by reperfusion injury and subsequently produces molecules which can serve as markers of oxidative stress. Patients with acute cellular rejection have the highest number of tubular DNA fragmentations as compared with those experiencing chronic allograft nephropathy.[19] Purine nucleotides and oxypurines are products of adenine nucleotide degradation.[42] The plasma concentrations of hypoxanthine (ischemia marker) and inosine significantly increase immediately after total tissue reperfusion. To examine the extent of DNA damage via oxidative stress after kidney transplantation, the level of 8-OHDG, a byproduct of deoxyguanosine oxidation, was measured by enzyme-linked immunosorbent assay before and after reperfusion of the graft. Serum 8-OHDG level increased shortly after reperfusion and then decreased within 2 hours.[43] A faster rate of decline from the first peak of serum 8-OHDG correlated with a lower serum creatinine and reduced incidence of acute rejection. In addition, these biomarkers of oxidative stress may act as a predictor of graft prognosis.[43]

Oxidation of thymidine by hydroxyl radicals produces dihydroxy dihydrothymidine (thymidine glycol). Urine thymidine glycol can be used for detecting oxidative DNA damage. In early posttransplantation phase (up to 12 hours), the urinary excretion rate of thymidine glycol increases and reaches the maximum level within the first 48 hours.[44] Preliminary studies suggested oxidative metabolite of creatinine (5-hydroxycreatinine; also known as *creatol*) as an indicator of oxidative stress after kidney transplantation. Serum creatol closely correlates with serum creatinine concentration and the severity of oxidative stress.[45]

Nitric Oxide

Nitric oxide (NO) and carbon monoxide are two important endogenous signal transduction gases, which are produced by various cell types including endothelial cells and immune cells. Carbon monoxide is generated by heme oxygenase (HO)-1 and HO-2 isotypes, of which HO-1 is inducible, whereas HO-2 is constitutively expressed. Inflammatory cytokines and oxidative stress trigger HO-1 expression to produce carbon monoxide and biliverdin, a potent antioxidant molecule. Nitric oxide is produced from L-arginine by 3 different NO synthases (NOS), which include endothelial, neuronal, and inducible NOS isotypes, the former two of which are constitutively expressed, whereas inducible NOS expression is induced by inflammatory cytokines. Once induced, the inducible NOS generates massive quantities of NO that rapidly reacts with reactive oxygen species, simultaneously formed by leukocytes producing peroxynitrite, which is a highly cytotoxic reactive nitrogen molecule. Expression of these enzymes was examined in acute kidney allograft rejection. Increased level of NO, as a result of inducible NOS activity, as well as induction of HO in the kidney allograft, has been shown in acute rejection. Excessive unregulated production of NO can cause cytotoxicity through different pathways.[46] Nitric oxide improves renal blood flow via vasodilation. Later in reperfusion phase, accumulation of nitrogenous free radicals causes tissue injury and impairs renal blood flow.[47]

Oxidative stress plays a major role in acute renal allograft rejection, and in one study, NO level measurements 30 minutes after graft reperfusion and on days 1, 5, and 10 post-transplantation were used for predicting acute kidney allograft rejection. There was at least 30% increase in NO level during episodes of acute allograft rejection.[48]

In observational trials measuring serum free radicals and NO in living and cadaveric transplant recipients, the amount of these markers were significantly increased after reperfusion. Elevation of NO in the peripheral blood of transplant recipients occurred 4.8 ± 1.2 hours after reperfusion and showed free radical-mediated reperfusion injury in kidney transplantation.[49]

Inflammatory Biomarkers

Inflammation is another phenomenon that usually occurs after transplantation and can be the cause or consequence of oxidative stress. Therefore, inflammatory markers can be used as surrogates for detecting oxidative stress. Serum concentrations of high-sensitivity CRP, TNF-α, and 8-isoprostaglandin F2α (inflammatory marker) were measured in 15 patients with chronic kidney failure, 15 transplanted, and 15 healthy controls. The control group had significantly lower levels than the other groups.[3] Interestingly, markers of tubular injury, inflammation, and oxidative stress were higher among recipients of kidneys from donors after cardiac death than the recipients of living donor kidneys with minimal ischemia.[50]

Forty-three post-transplant patients were compared with 50 healthy individuals on account of inflammatory markers including CRP, IL-6, TNF-α, and pregnancy-associated plasma protein A, before and after transplantation. Levels of inflammatory and oxidative stress markers were higher in the transplanted patients than in the control group, but they improved slowly following kidney transplantation.[51] Marked release of IL-6 and a modest release of IL-8 in the first 30 minutes of graft reperfusion in human living donor kidney transplantation were identified by another group of investigators.[52] However, one study demonstrated that inflammatory markers (IL-6, TNF, IL-1b, and CRP) were at their maximum levels 4 hours after transplantation, but then declined to or even below their preoperative levels on the 4th day posttransplantation.[53]

Inflammation can exacerbate CAN, and serum level of CRP, IL-6, IL-10, TNF-α and its soluble receptor, soluble-IL-2R, and IL-4 are markedly elevated in CAN.[54] The peak level of these markers in the immediate postoperative period influences the long-term graft and patient survival,[55] and there is a significant relation between levels of these markers and the transplant kidney function.[21]

Endogenous Antioxidant Molecules

Oxidative stress triggers upregulation of various antioxidant enzymes and scavenger molecules such as superoxide dismutase, catalase, glutathione, and glutathione peroxidase. Therefore, elevation of these molecules can be used as a surrogate for assessing oxidative stress in reperfusion injury. Catalase activity correlates with the severity of oxidative stress. A few minutes after total tissue

reperfusion, catalase activity increases.[42] Elevated levels of reactive oxygen species within 48 hours after transplantation resulted in high levels of glutathione reductase and a marked decrease in plasma and erythrocyte glutathione peroxidase.[56] Measurement of glutathione peroxidase and copper-zinc SOD in plasma and erythrocytes of patients before and after transplantation has shown increased level of these makers in CKD patients, with modest improvement in early post-transplantation phase. There is a high level of oxidative stress in chronic rejection suggesting that oxidative stress may play an important role in the pathogenesis of biopsy-proven chronic rejection.[38]

In animal transplantation models, superoxide dismutase and glutathione reductase are depleted at the cold ischemic phase, because of their reduced biosynthesis and excessive free radicals generation.[57,58] Following kidney transplantation, plasma glutathione peroxidase activity increases, and after approximately 3 months, it approaches the normal levels. An inverse correlation between creatinine level and plasma glutathione peroxidase activity is observed in patients after kidney transplantation. Monitoring of plasma glutathione peroxidase activity may be a useful additional marker of the graft function.[59,60]

Manganese SOD activity has been shown to diminish in both human and rats with chronic allograft rejection. The reduction of manganese SOD occurred rapidly in renal ischemia-reperfusion, suggesting that loss of manganese SOD activity leads to further renal injury.[61]

The free radical-mediated injuries are mitigated by metallothionein, a cysteine-rich low-molecular-weight protein that binds redox-active metals such as zinc, copper, selenium, and xenobiotics such as cadmium, mercury, silver, arsenic, and heavy metals, and it scavenges reactive oxygen species. Metallothionein level, as a biomarker of antioxidant activity, was evaluated in the plasma of 11 patients before and 48 hours, 1 week, and 2 weeks after kidney transplantation. Metallothionein plasma concentration was lower in transplanted patients after 48 hours and partially recovered 1 and 2 weeks later.[62]

Xanthine oxidoreductase and its active forms, xanthine dehydrogenase and xanthine oxidase, generate antioxidants, such as uric acid, and simultaneously produce free radicals. There is an association between graft function and xanthine metabolizing enzymes. It seems that these parameters are lower in patients with early graft function than those with delayed graft function.[63]

Newer Biomarkers

Severe reperfusion injury is a risk factor for delayed graft function. Nuclear magnetic resonance spectroscopy-based metabolomics are used to establish reperfusion-specific metabolic markers of oxidative stress both in the blood and in kidney tissue. Some of the immunosuppressant drugs can also alter urine metabolomic pattern by inducing oxidative stress, which can be detected by nuclear magnetic resonance spectroscopy.[64] These markers have been shown to be related with the pathologic changes of the allograft and could be used as a predicting factor in diagnosis of delayed graft function.[65] While the application of the omics technologies can identify novel and promising biomarker candidates for early assessment of reperfusion injury, the clinical utility of these markers requires validation in clinical trials.[66] The plasma level of polyunsaturated fatty acids, as a marker of adaptation to ischemia, falls with cold ischemia and the level of allantoin, a marker of oxidative stress, rises after reperfusion and correlates with cold ischemia time.[65]

Low serum level of sulfatide, a major component of lipoproteins, is correlated with a high incidence of cardiovascular diseases in hemodialysis patients. After transplantation, there is a slow and gradual increase in sulfatide level towards normal values over the first post-transplant year, suggesting a close correlation between serum sulfatide and kidney function. This rise in sulfatide level parallels the decline in MDA, improvement in platelet function, and cardiovascular disease risk reduction.[67]

Accumulation of advanced glycation end products such as N epsilon-carboxymethyllysine in chronic kidney failure induces oxidative stress and inflammation. Carboxymethyllysine homeostasis is regulated by megalin-mediated endocytosis and lysosomal degradation in proximal tubules. Accumulation of glycation end products in endothelium contributes to the cardiovascular complications. In biopsy specimens of chronic allograft nephropathy cases, tubular deposition of carboxymethyllysine was inversely associated with the degree of tubular atrophy and interstitial

fibrosis, a phenomenon which might be due to reduced megalin and megalin-mediated proximal tubular epithelial uptake. In contract, glomerular carboxymethyllysine deposition was positively associated with transplant dysfunction. Furthermore, glomerular deposition of carboxymethyllysine could play a pathophysiological role in chronic allograft injury, and as such it may be a reliable marker of oxidative stress.[68]

In patients with allograft nephropathy (CAN), impaired tissue oxygenation and oxidative stress causally related. To assess tissue oxygen bioavailability, blood oxygen level-dependent magnetic resonance imaging is usedin which deoxyhemoglobin is utilized as an endogenous contrast agent.Using this technique, a significant increase in medullary and cortical oxygen bioavailability has been detected in allografts with CAN pointing to reduced oxygen uptake. This was associated with significant increase in serum and urine hydrogen peroxide and serum heat shock protein 27 levels, denoting increased burden of oxidative stress in patients with CAN. Taken together these observations illustrate the association between intrarenal oxygenation and oxidative stress in CAN.[69]

Galectin-3 is a lectin with a variety of functions such as promoting neutrophil adhesion, inducing oxidative stress, mastocyte migration and degranulation, and producing pro-inflammatory cytokines. Lesser amount of reactive oxygen species was detected in galectin-3 knockout mice than the wild type mice in response to reperfusion injury; therefore, galactin-3 may be used as a marker of free radical injury.[70]

TREATMENT

Treatment Options in Oxidative Stress

Oxygen free radicals are generated during the reperfusion of ischemic organs. Experimental studies have demonstrated that the damage produced by reperfusion can be prevented by a free radical scavenger. Transplant recipients have elevated levels of oxidative stress, which has prompted the idea that supplementary antioxidants may be beneficial.

Ischemia Times and Donor's Condition

Some of the detrimental donor-related or transplantation-related factors like factors related to brain death (eg, hemodynamic instability, systemic release of cytokines, and reperfusion injury and ischemia times during surgery) are known to enhance immunogenicity and reactive oxygen species production.

In donors with cardiac death, where there is relatively higher incidence of graft dysfunction than donors with brain death, an important option to reduce ischemia-induced injuries is shortening of both cold and warm ischemia times by proper perfusion of organ after cardiac death and extracorporeal membrane oxygenation.[71] Even in donation after cardiac death, successful kidney transplantation has been performed by using a noninvasive load-distributing-band chest compression device to maintain adequate perfusion.[59,72] Data suggest that a cold ischemia time shorter than 18 hours does not significantly affect graft survival.[73]

Dopamine stimulates HO-1, which helps to attenuate oxidative stress and protect the organ from reperfusion injury and inflammation. Catecholamine treatment in the brain-dead organ donors lowers the risk of rejection and results in a better long-term graft survival by modulating cytokine production and preventing cold-induced damage and free radical production.[74]

In animal studies of autotransplanted kidneys, sildenafil application before ischemia, resulted in higher levels of NO and renal vascular flow and also lower post-transplant reperfusion- and warm-ischemia damage; hence, it can be used as a preventive option.[75,76] Also, long-term sildenafil administration in hypertensive rats attenuates endothelial dysfunction and reduces renal oxidative stress and renal macrophage accumulation.[77] Furthermore, pretreatment of rat donor with allopurinol attenuates ischemia-induced damages and leads to lower serum creatinine values and better survival and renal histology after transplantation.[78]

Vitamins

By scavenging reactive oxygen species, antioxidant compounds can attenuate oxidative stress and lipid peroxidation. Several animal and human studies have suggested that antioxidant vitamins such as vitamin C (ascorbic acid) and vitamin E (α-tocopherol) might reduce oxidative stress caused by reperfusion injury and calcineurin inhibitor nephrotoxicity. Five patients supplemented with vitamin C (500 mg per day), vitamin E (500

mg per day), or both in the first 3 months after kidney transplantation exhibited more than 20% reduction in serum creatinine levels. Interestingly, serum creatinine level was increased more than 50% by discontinuing the vitamins. The role of vitamins is more important especially in grafts donated from deceased donors.[79]

In a model of dog autotransplantation, ascorbic acid administration 3 days after transplantation led to higher levels of antioxidant enzymes (SOD and glutathione peroxidase) which may play a role in attenuating reperfusion injury.[73] Intravenous administration of multivitamins 1 hour before kidney transplantation in a pig model was shown to diminish plasma MDA 2 hours after the procedure.[80] Even after a single dose of vitamin C (2 g), lipid peroxidation was reduced after transplantation.[81] On the other hand, in a randomized placebo-controlled trial, the effect of 400 IU/d of vitamin E, 500 mg/d of vitamin C, and 6 mg/d of β-carotene was evaluated for 6 months in 10 kidney transplant recipients treated with cyclosporine A as a part of their immunosuppressive therapy. Trough level of cyclosporine decreased by 24% and glomerular filtration rate improved by 12% without any changes in markers of oxidative stress (MDA) or plasma antioxidant enzymes.[82]

Niacin, an antioxidant and anti-inflammatory agent attenuates oxidative stress, inflammation, proteinuria, and hypertension in rats with chronic kidney failure.[83] Niacin also improves lipid metabolism in rat models of chronic kidney disease.[84] Melatonin is another potent antioxidant and anti-inflammatory product. Its production is impaired in chronic kidney failure which may in part contribute to the associated oxidative stress. Melatonin administration has been shown to reduced oxidative stress (MDA levels) and renal inflammation in rats with renal mass reduction.[85]

Free radicals are well-known risk factors of cardiovascular disease in kidney transplant recipients and CKD patients. Progression of atherosclerosis is mediated by increased lipid and lipoprotein oxidation and endothelial damage and dysfunction. Different results were reported by the two randomized clinical trials evaluating the effect of oral vitamin E supplementation on clinical endpoints in patients with mild-to-moderate renal insufficiency and for hemodialysis patients. Daily supplementation with 400 IU of vitamin E for 4.5 years in patients with renal insufficiency showed no significant change in the rate of mortality related to myocardial infarction, stroke, or cardiovascular. In contrast, a 50% risk reduction in the cardiovascular events was seen with 800 IU vitamin E daily supplement in transplant recipients.[86]

Thiamine deficiency is a potential risk factor for delayed graft function in transplanted patients. Acute tubular necrosis caused by reperfusion injury plays a major role in the pathogenesis of delayed graft function, and thiamine supplementation may attenuate reperfusion injury.[87]

Management of hyperhomocysteinemia by folic acid and vitamin B12 in kidney transplant recipient may result in cardiovascular protection and better graft function by improving endothelial function, limiting oxidative stress, and reducing prothrombotic status.[88] There is a decline in CRP levels 3 months after treating hyperhomocysteinemia with these agents.[89] Folic acid supplementation has been shown to attenuate carotid intima-media thickness after kidney transplantation.[90] Folic acid supplementation for 12 weeks as well as vitamin B6 supplementation for 6 months has been shown to significantly lower homocysteine level in transplanted patients with stable graft function.[91,92]

Coenzyme Q10 is a lipid-soluble substance that is present in most eukaryotic cells and plays an essential role in the mitochondrial electron transport chain. In a study of 11 transplanted patients, treatment with Coenzyme Q10 for 4 weeks resulted in a significant attenuation of oxidative stress as assessed by measurements of MDA, SOD, glutathione peroxidase, and the basic parameters of lipid metabolism.[93]

Anticoagulation Treatment

Coagulation is a key phenomenon in organs from the deceased donors and a significant cause of reperfusion injury. Inhibition of thrombin is an effective therapy against reperfusion injury and results in reduced chronic graft fibrosis, with a significantly positive effect on graft survival. In a study conducted in pigs, treatment with melagatran (a thrombin inhibitor) before warm ischemia or in the preservation solution demonstrated improved graft survival and reduced renal fibrosis.[94]

Antihypertensive Agents and Statins

Carvedilol is an antihypertensive drug with

potent anti-oxidant properties. Treatment with carvedilol (30 minutes before surgery and 12 hours after reperfusion) in the rat transplant model has been reported to significantly lower plasma creatinine levels after reperfusion injury, suggesting improvement in kidney function. Histopathological analysis revealed decreased reperfusion injury-induced damage in the kidney.[95] In a study of a small cohort of patients with chronic graft rejection, treatment with carvedilol improved lipid profile and reduced lipid oxidation, but it did not alter the course of the chronic rejection.[96] In addition, carvedilol has been shown to attenuate cyclosporine-induced oxidative stress.[97,98]

Nebivolol is a β1-receptor blocker that raises NO availability and exerts antioxidant, anti-apoptotic, and anti-inflammatory effects. Nebivolol administration for 15 days before ischemia-reperfusion in rats has been reported to improve kidney function and attenuate inflammation and apoptosis after renal reperfusion injury.[99]

Administration of angiotensin II type 1 receptor antagonist, losartan, in transplant patients significantly reduces the plasma TGF-β and uric acid levels and lowers proteinuria.[100] Treatment with the angiotensin II type 1 receptor antagonist, irbesartan, 1 day before and for 21 days after transplantation, concurrently with cyclosporine, has been shown to improve markers of oxidative stress in rats.[101] Likewise, the angiotensin-converting enzyme inhibitor, ramipril, attenuates cyclosporine-induced oxidative stress in human kidney transplant recipients.[102]

Addition of diazoxide to a rat kidney preservation solution has been reported to significantly attenuate the rise in MDA level, enhance SOD activity, reduce oxidative stress-mediated injury, and decrease cell apoptosis in the kidneys during hypothermic preservation.[103] Treatment with nicorandil (a potassium channel opener and vasodilator drug used to treat angina pectoris) has been shown to dose-dependently reduce urinary β2-microglobulin level and lower the severity of acute tubular damage in a rat model of reperfusion injury.[104]

Statins inhibit synthesis of isoprenoids which are involved intra-cellular trafficking of proteins and cell signaling, events that are critical in immune cell activation. Consequently, they can exert anti-inflammatory effects and attenuate oxidative stress by limiting activation of nicotinamide adenine dinucleotide phosphate oxidase and production of reactive oxygen species. Via these pleotropic effects, statins may attenuate progression of chronic allograft nephropathy. In this context, statins have been shown to increase the level of the potent antioxidant enzyme, glutathione peroxidase, in transplanted patients during the first 6 months after transplantation without affecting serum creatinine or glomerular filtration rate.[105] In addition, several large randomized trials have shown that statins modulate cell proliferation and inflammation and that long-term (2 years) statin therapy following kidney transplantation can slow down the rate of decline in kidney function.[106]

N-Acetylcysteine and Glutathione System

N-acetylcysteine treatment has been reported to lower markers of oxidative stress without significantly affecting histopathologial lesions following reperfusion injury in rats.[107] N-acetylcysteine also attenuates lipid peroxidation and increases glutathione levels after induction of ischemia-reperfusion in rats.[75] Co-administration of N-acetylcysteine and NO donor (sodium nitroprusside and phosphoramidon) has been found to lessen renal reperfusion injury in donor kidneys destined for transplantation.[76] N-acetylcysteine improves early outcomes of deceased donor kidney transplantation by attenuating oxidative stress.[24] Treatment with N-acetylcysteine, which serves as a precursor for production of reduced glutathione, showed no significant change in the plasma redox parameters of transplant patients with stable kidney function. However, N-acetylcysteine administration improved estimated glomerular filtration rate and increased high-density lipoprotein cholesterol, which positively correlated with the glutathione peroxidase activity.[106] In addition, cyclosporine-induced nephrotoxicity is significantly ameliorated by N-acetylcysteine. [108]

Selenium

As an essential constituent of glutathione peroxidase and related enzymes, selenium plays a major role in redox reactions. In fact, selenium supplementation for 3 months in transplant recipient patients has been shown to normalize glutathione system and low-density lipoprotein cholesterol level.[60]

Insulin

Ischemia-reperfusion and hyperglycemia are the main inducers of oxidative stress and have a major role in the pathophysiology of tissue injury in transplant recipients. In cadaveric kidney allograft recipients treated with intravenous insulin to maintain blood glucose below10 mmol/L, plasma total radical-trapping antioxidant values were significantly higher than the control group at days 1 and 4 after transplantation.[109]

Apotransferrin

Redox-active iron is released into the circulation in response to renal ischemia-reperfusion. By avidly binding the elemental iron, apotransferrin lowers the circulating redox-active iron and protects against renal reperfusion injury via inhibition of oxidative stress and inflammation. Apotransferrin could be used in the treatment of acute kidney failure, as seen after transplantation of ischemia-induced organ damage.[110]

Preservation Solution

Adding antioxidants to preservation solutions may be a proper strategy to protect organs from oxidative stress and minimize cold storage-induced organ damage. Preservation of kidney tissue before transplantation has a major role in the outcome of organ. Histidine-tryptophan-ketoglutarate (HTK) solution results in better survival, lower rate of initial nonfunctioning, and decreased free radicals. [111]

Addition of selenium to the preservation solution protects kidneys against oxidative stress during warm and cold ischemia.[112] Lifor is an artificial preservative solution that contains nutrients, growth factors, and a nonprotein oxygen and nutrient carrier. This solution lowers both warm and cold renal ischemia-reperfusion in comparison with the University of Wisconsin (UW) solution as assessed by the release of lactate dehydrogenase.[113] Adding selected flavonoids to UW or Euro-Collins solution attenuates cold-storage-induced injury demonstrated by less lipid peroxidation.[114] Lidoflazine, the calcium entry blocker, improves preservation properties of the UW solution in rat model. [115] Adding selenium to the reperfusion solution (HTK, custodiol) leads to decreased MDA concentration within 2 hours after transplantation.[116] Addition of p38 mitogen-activated protein kinase inhibitors to UW solution in pig renal preservation solution resulted in lesser TNF-α level and lesser apoptosis.[117] Also, adding mitoquinone, a mitochondria-targeted antioxidant, to UW preservation solution decreases oxidant production by about 2-fold, completely prevents mitochondrial dysfunction, and significantly improves cell viability and/or renal morphology.[118] These represent potentially helpful strategies to improve transplantation outcomes.

Comparing UW and HTK preservation solution, kidneys preserved by HTK produces highest ROS values.[119] However, perfusion with HTK prior to storage in UW may improve the results which are reflected by reduction of free radicals.[111]

In rats the capacity of intravenous infusion of DHL-HisZn, a new α-lipoic acid derivative, in inhibiting reactive oxygen species generation and preventing renal reperfusion injury was examined. Pretreatment with DHL-HisZn before ischemia-reperfusion induction, decreased reperfusion-induced tissue injuries, serum creatinine, blood urea, and MDA levels in the kidneys of rats with renal reperfusion injury.[120]

Inhibition of thrombin during preservation of graft in pig autotransplant model preserved kidney function, protecting against chronic inflammation and oxidative stress. Thus, anticoagulant could be a critical treatment for improving kidney quality for transplantation.[121]

When L-carnitine is added to Belzer solution for preserving kidney, less reperfusion injury and oxidative stress injury is detected. Furthermore, decreased lipid peroxidation, inducible nitric oxide synthase expression, and free radical generation are observed.[122,123]

Nitric Oxide System

Depletion of NOS cofactor, tetrahydrobiopterin, and accumulation of NOS inhibitor, asymmetrical dimethylarginine, or uncoupling of the normal homodimeric state of endothelial NOS results in conversion of NOS isoforms from the NO-producing to superoxide-producing enzymes. Accordingly, the effect of the NOS cofactor tetrahydrobiopterin was evaluated on early rejection in a rat kidney transplantation model. In allograft models, tetrahydrobiopterin precursor (sepiapterin) led to a noticeable decrease in superoxide production and was accompanied by a reduction in peri-arterial

macrophage infiltration and an increase in NO production, but these effects were not observe in the isografts transplantation.[124] In a randomized double-blind study, L-arginine (the substrate for NO production by NOS) supplementation improved kidney function in kidney transplant recipients.[125]

Other Agents

L-carnitine is an endogenous mitochondrial membrane compound, which could effectively protect reperfusion injury in the kidney. In fact, addition of L-carnitine to the culture medium12 hours before reperfusion injury has been shown to inhibit hydrogen peroxide-induced injuries, intracellular reactive oxygen species generation, and lipid peroxidation in a concentration-dependent manner in cultured human proximal tubular epithelial cell line. This effect was mediated by promoting endogenous antioxidant molecules such as glutathione peroxidase, catalase, and SOD. In addition, L-carnitine reduced DNA fragmentation and caspase-3 activity.[126]

Taurine is a potent free radical scavenger that attenuates oxygen free radical-induced damage, and thereby can be potentially useful in a variety of kidney diseases. This organic acid can prevent FK506-induced kidney toxicity by attenuating production of reactive oxygen species, in vitro.[127] Administration of taurine to donors before nephrectomy has been shown to protect the kidney grafts from injury and improve graft function by limiting oxidative stress in the rat transplantation model.[128]

A non-enzymatic, reactive oxygen species-related pathway has been suggested to produce 8-isoprostaglandin F2α, which is an indicator of oxidative stress. Nonselective (acetylsalicylic acid) or selective cycloxygenase-1 inhibitor has been shown to completely abolish the 8-isoprostaglandin F2α and prostaglandin F2α production during reperfusion injury in kidneys. Also, it is assumed that fish oil could decrease or suppress oxidative stress and prostaglandin F2 production and as such could be useful in attenuating reperfusion injury. To test this hypothesis in a randomized trial, 22 kidney transplant patients were given either fish oil dietary supplementation, 6 g/d (720 mg of decosahexaenoic acid and 1080 mg of eicosapentaenoic acid) or placebo for 6 months. Contrary to expectations, omega-3 fatty acids supplementation raised plasma 8-isoprostane levels, whereas placebo treatment lowered it.[129] However, long-term omega-3 supplementation has been shown to attenuate upregulation of pro-oxidant, pro-inflammatory, and profibrotic pathways and decrease tubulointerstitial fibrosis in rats with CKD induced by subtotal nephrectomy.[130]

Comparison of the antioxidant capacity of anesthetic induction agents, propofol and thiopentone, has shown that propofol counteracts oxidative stress more efficiently by decreasing formation of a major F(2)-isoprostane in transplant patients.[131]

Pre-transplantation treatment with rabbit antirat thymocyte immunoglobulin in a rat model of reperfusion injury has been shown to reduce the infiltration of macrophages, CD4+ and CD8+ T cells, and leukocyte function-associated antigen-1-positive cells in the allograft after cold ischemia. However, pretreatment with rabbit anti-rat thymocyte immunoglobulin had no effect on either granulocyte infiltration severity of oxidative stress.[23]

The dietary supplementation with a carotenoid, astaxanthin, has shown promising results as an antioxidant and anti-inflammatory agent against cardiovascular disease in kidney transplant recipients. In a study of 66 kidney transplant recipients enrolled to receive either 12 mg/d of astaxanthin or placebo for 1 year, the group given astaxanthin showed lower plasma isoprostanes reflecting its efficacy as an antioxidant agent.[132]

Tempol, a superoxide dismutase mimetic compound, has been shown to reduce plasma MDA levels and enhance SOD activity of the kidney tissue in rats with subtotal nephrectomy. However, it failed to suppress oxidative stress and actually accelerated the deterioration of kidney function and structure.[133] This was attributed to increased production of hydrogen peroxide which is a potent activator of nuclear factor kappa B, which is the master regulator of genes encoding numerous pro-inflammatory cytokines and pro-apoptotic factors.

Plants

Nigella sativa is an annual flowering plant, native to south and southwest Asia, whose protective effect against ischemia-reperfusion damage to various organs has been previously documented. Co-administration of *Nigella sativa* with induction of reperfusion injury in rat model was effective

in reducing blood urea and creatinine levels and decreasing the tubular necrosis score. *Nigella sativa* treatment markedly reduced oxidative stress index and total oxidant status levels and increased total antioxidant capacity in both kidney tissue and blood.[134]

Ligustrazine, the key component of the Chinese herb *chuanxiong*, has been reported to protect murine kidney from warm reperfusion injury.[135] Administration of ligustrazine in the study animals significantly reduced myeloperoxidase activity, lowered MDA level, and raised SOD activity.[135]

Diet

Mediterranean-type diet seems to enhance the body's capacity to contain reactive oxygen species.[136] In fact, a prospective study of 160 adult kidney allograft recipients showed that Mediterranean diet reduced the risk of metabolic syndrome after a 1 year of follow-up.[137]

CONCLUSIONS

Oxidative stress is a common cause of allograft damage after transplantation; patients with reperfusion injuries, which can induce oxidative stress, are prone to acute allograft rejection, delayed graft function, chronic allograft nephropathy, and endothelial dysfunction. Chronic kidney failure, inflammation, diabetes mellitus, reperfusion injury, and immunosuppressive agents are among many risk factors of oxidative stress. There are various markers in blood, urine, and kidney tissue that can be used as indicators of oxidative stress status. Due to the fact that oxidative stress can affect short-term and long-term survival of both graft and patient, different prophylactic and therapeutic approaches have been employed to reduce oxidative stress, ranging from decreasing ischemic time to changes in composition of preservation solution and use of a variety of free radical scavengers.

CONFLICT OF INTEREST

None declared.

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