Oxidative stress occurs when there is an overproduction of reactive oxygen species and/or a decline of antioxidant defenses. Oxidative stress is a significant burden to patients with chronic kidney disease (CKD), because of their declining renal function, and this can be worsened following renal replacement therapy. Previous studies have used in vivo circulating biomarkers to assess the burden of oxidative stress of CKD patients. For example, an increased oxidation of lipids, proteins, and nucleic acids, especially in the vascular wall, play critical roles in the early stages of atherogenesis in uremia patients. The pathogenesis of oxidative stress in uremia patients is complex, and includes several factors. Uremia- and dialysis-associated factors, including priming of leukocytes, impairment of antioxidant defense, exposure to dialysate endotoxins, use of bioincompatible hemodialysis dialyzer membranes or peritoneal dialysis solutions, and even intravenous iron supplementation, may contribute to the increased oxidative stress of CKD patients. The consequences of oxidative stress, such as atherosclerosis, amyloidosis, and anemia are discussed in detail. Several therapeutic strategies, including dietary administration of antioxidant vitamins (vitamin E and vitamin C), combination of antioxidants, the use of vitamin E-coated dialysis membranes and electrolyte-reduced water, appear to ameliorate the long-term complications of oxidative stress in uremic patients. Two initial randomized clinical studies have suggested that antioxidant therapy with N-acetylcysteine or vitamin E may improve the cardiovascular outcome of dialysis patients. Adequately powered randomized controlled trials must be performed in larger patient cohorts with longer follow-up to definitively demonstrate that correction of oxidative stress is beneficial for outcome of patients with chronic kidney disease.

Key Words: oxidative stress, chronic kidney disease, antioxidant therapy

Introduction

Cardiovascular (CV) disease is the leading cause of death in patients with chronic kidney disease (CKD). Recent studies have focused on the development new therapies to reduce the CV mortality and morbidity of CKD patients. Well-known CV risk factors, such as hypertension, diabetes, and hyperlipidemia, are strongly associated with poor outcome, but there are non-traditional risk factors for CV disease in CKD patients, such as oxidative stress. Oxidative stress, which results from an over-production of reactive oxygen species (ROS) and/or a reduction in antioxidant defense capacity, is well-documented in uremic patients and may be associated with dialysis-related complications, such as endothelial cell dysfunction and accelerated atherosclerosis. In this review article, we will present evidence that increased oxidative stress is a significant burden to patients with uremia, and that the measurement of oxidative stress biomarkers allows for better management of oxidative stress in CKD patients.

What is Oxidative Stress

Oxidative stress arises from a disturbance of the balance between free radical production and antioxidant defense. This imbalance can lead to oxidation of bio-molecules and result in structural and functional modifications of these molecules (6, 19, 25). Oxidant production mainly occurs in the mitochondria, and mitochondrial cytochrome oxidase enzymes, such as cytochrome P450, account for production of ~90% of the oxygen metabolized in mammalian cells. Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is another important source of oxidants, and generates ROS in phagocytes and endothelial cells. Phagocytes can utilize high levels of oxygen to produce ROS as a host defense mechanism against invading pathogens via a respiratory burst. The enzymes involved in the respiratory burst include NADPH oxidase, superoxide dismutase.
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(SOD), nitric oxide synthase (NOS), and myeloperoxidase (6, 25). The most potent ROS, superoxide anion (O$_2$•$^-$), is metabolized by SOD to hydrogen peroxide (H$_2$O$_2$), and then converted to the highly reactive hydroxyl radical (OH$^-$) by ferrous iron (Fe$^{++}$). O$_2$•$^-$ also reacts with nitric oxide (NO) to yield peroxynitrite (ONOO$^-$. H$_2$O$_2$ and chloride (Cl$^-$) are metabolized by myeloperoxidase to hypochlorous acid (HOCl) in activated phagocytes (25).

At present, four different pathways of oxidative stress have been identified: (i) classical oxidative stress, (ii) chlorinated stress, (iii) nitrosative stress, and (iv) carbonyl stress (Fig. 1). The antioxidant defense system in human cells consists of antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase, and non-enzymatic antioxidants. Non-enzymatic antioxidants are classified as hydrophilic (ascorbic acid, uric acid, bilirubin, albumin, and flavonoids) or lipophilic (alphatocopherol, ubiquinol, and carotenoids) (13, 14, 25).

Thus, oxidative stress has positive and negative consequences in healthy humans. On the positive side, oxidative stress is an important component of several host defense mechanisms, such as hydrolysis of invading microorganisms and denaturation of foreign antigens. On the negative side, oxidative...
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stress can be harmful because it damages healthy cells and tissues. These injuries are mainly mediated by peroxidation of lipid membranes, oxidation of proteins or DNA, and disruption of cytokines function and the NO system (6).

Biomarkers of Oxidative Stress in CKD

Direct measurement of ROS in vivo is difficult because of its low concentration, highly reactive nature, and short half-life. Thus, measurement of the oxidative byproducts of different ROS reaction pathways and of antioxidant levels is often performed. Oxidative damage to cellular constituents, such as membrane lipids, proteins, and nucleic acids, are indications of oxidative damage, and chemical alterations of these compounds can lead to structural and functional modifications. Biomarkers of different oxidative pathways are increasingly used to assess the in vivo significance of oxidative stress associated with diverse diseases, including uremia (25, 47). Table 1 summarizes the most commonly used circulating biomarkers of oxidative stress in CKD patients. In addition to serving as biomarkers of oxidative stress, some of these oxidation byproducts, such as oxidized low density lipoprotein (LDL), reactive carbonyl compounds, advanced glycosylation end product (AGE), and oxidized thiol compounds, can potentially contribute to the pathogenesis of CV disease, inflammation, and other complications of uremia (43, 58, 59). Clinical studies of these biomarkers in patients with uremia have indicated that CKD patients are under oxidative stress.

Several biomarkers require further discussion. Lipid peroxidation products, such as malondialdehyde (MDA), have been reported to be higher in hemodialysis (HD) patients than healthy subjects (29, 44). Witko-Sarats et al. identified the presence of advanced oxidation protein products (AOPPs) in the plasma of uremic patients (58, 59), the first time that oxidative stress was determined to result from direct damage to proteins. This study also confirmed that AOPP levels increased as renal function declined. Moreover, AGES are generated from carbonyl intermediates by glycoxidation of free carbohydrates or by lipoxidation to form malondialdehyde-lysine (59). In dialysis patients, AGES have been associated with atherosclerosis and dialysis-associated amyloidosis (25). Our previous work showed that 8-hydroxydeoxyguanosine (8-OHdG) concentrations were highest in long-term HD patients, somewhat lower in CKD patients who were not on HD, and lowest in healthy individuals. The levels of cellular 8-OHdG are a consequence of cellular redox status, ROS production, and antioxidant defense mechanisms. We suggest that the 8-OHdG content of lymphocyte DNA may be a reliable, steady-state marker for oxidative DNA damage in CKD patients (50, 51, 52, 53).

Mechanisms of Oxidative Stress in CKD

Mounting evidence suggests that CKD patients are under oxidative stress, as indicated by observations that (i) circulating biomarkers (lipids, proteins, and nucleic acids) are at higher concentrations in CKD patients; (ii) decreases in antioxidant defense capacity, due to failure of ROS clearance, occurs during renal function decline; (iii) some oxidative stress markers, including AGE, occur in the atherosclerotic lesions of uremic patients. Increased ROS generation and decreased antioxidant capacity have been implicated in the progression in CKD. Increases in circulating oxidative stress biomarkers have been reported in patients with CKD stage 3, so oxidative stress seems to happen early during the course of renal decline (42). Furthermore, dialysis therapy, either HD or peritoneal dialysis (PD), may impose an additional oxidative stress on uremic patients possibly due to the use of bioincompatible dialysis membranes or PD solutions (50, 52). The pathogenesis of oxidative stress in CKD patients is complex and includes uremia-related factors and dialysis-related factors.

| Table 1. Circulating biomarkers of oxidative stress in chronic kidney disease patients |
|---------------------------------|-------------------------------------------------|
| **Lipids** | Malondialdehyde (MDA) |
| | Lipid hydroperoxides |
| | Oxidized low-density lipoprotein (ox-LDL) |
| | Exhaled alkanes |
| | Advanced lipoxidation end products (ALEs) |
| **Arachidonic acid derivatives** | F2 isoprostanes |
| | Isolevuglandins |
| **Carbohydrates** | Reactive aldehydes |
| | Advanced glycosylation end products (AGEs) |
| **Amino acids** | Cysteine |
| | Homocysteine |
| | Isoaspartate |
| | Nitrotyrosine |
| **Proteins** | Thiol oxidation |
| | Carbonyl formation |
| | Advanced oxidation protein products (AOPPs) |
| | Amine oxidation |
| **Nucleic acids** | 8-Hydroxy 2'-deoxyguanosine (8-OHdG) |
Uremia-Related Factors

Ward and McLeish (56) showed that the leukocytes of uremia patients were primed for superoxide anion production in response to phorbol myristate acetate (PMA). Moreover, additional studies have shown that the resting serum levels of superoxide anion were higher in HD patients than in healthy controls (8). Moreover, several lines of evidence suggest that antioxidant enzymes are gradually altered as renal function declines, and are profoundly impaired in patients with uremia. Investigators have also found that CKD was associated with low concentration of serum selenium and lower platelet glutathione peroxidase (GPx) activity (6). Similarly, Ceballos-Picot et al. (7) demonstrated lower serum levels of glutathione and plasma GPx activity in patients undergoing renal failure. CKD is also associated with profound disturbance of the nitric oxide system, because it increases the concentration of endogenous NOS inhibitors (2).

Dialysis-Related Factors

The bioincompatibility of HD systems plays a pivotal role in ROS production. In particular, two major components of the HD system contribute to oxidative stress: the dialyzer membrane and the trace amount of endotoxin in the dialysate (10, 31, 56). Similarly, it has been well documented that the low pH and high osmolality of glucose-based PD solutions, when biologic functions with lactate, have a negative impact on diverse biologic functions of peripheral phagocytes in PD patients. Peripheral phagocyte oxygen metabolism is augmented due to activation by contact with the peritoneum when using conventional, bioincompatible dialysates (50). An increase in oxidative metabolism leads to over-production of H$_2$O$_2$ and O$_2$•$^-$. Moreover, certain HD modalities that use highly permeable membranes induce losses of solutes, waste products, some anti-oxidants, and trace elements. The losses of anti-oxidants during HD appear particularly relevant for hydrophilic and unbound small molecules, such as vitamins. This loss may be the cause of the observed impairment of enzymatic antioxidants that occurs during uremia.

Consequences of Oxidative Stress in CKD

The most significant consequence of oxidative stress in CKD patients is that it promotes the progression of long-term complications, such as atherosclerosis, amyloidosis, and anemia.

Atherosclerosis

Uremia predisposes patients to accelerated atherosclerosis and is often associated with increased oxidative stress, and reduced vascular bioavailability of NO. In a pilot study, Steinberg et al. first demonstrated that the atherogenicity of LDL can be modified by oxidative stress (49). These authors found that oxidized LDL can be taken up by scavenger receptors and potentiate the transformation of monocytes to foam cells, proliferation of vascular smooth cells, and production of inflammatory cytokines. In patients with higher oxidative stress, the superoxide anion produced via NADPH oxidase can rapidly react with NO (40, 41), resulting in reduced NO availability, endothelial cell dysfunction, and subsequent atherosclerosis. Maggi et al. (32) reported that LDL isolated from uremia patients is more susceptible to in vitro lipid peroxidation than the LDL from healthy subjects, suggesting that oxidative stress may potentiate atherosclerosis in CKD patients. Cristol et al. (10) confirmed the results of Maggi et al. (32) by showing that LDL isolated HD patients was more susceptible to ex vivo oxidation than the LDL from healthy subjects (37, 38).

Amyloidosis

In the presence of oxidative stress, ROS can directly modify the function of proteins via the formation of oxidized amino acids. ROS can also react with other substrates to form potent pro-oxidant molecules, such as AGEs. Miyata et al. demonstrated that AGEs occur in beta2-microglobulin deposits of long-term HD patients (34, 35), suggesting that oxidative stress promotes amyloidosis due to protein denaturation.

Anemia

Membrane lipids are highly susceptible to damage in the presence of oxidative stress, and lipid peroxidation can lead to profound modification of cellular structures and function. Previous studies indicated that the serum of HD patients has higher levels of MDA in erythrocytes and a severe deficiency in vitamin content (57), possibly explaining the shortened lifespan of erythrocytes in CKD patients. In fact, a previous study showed that anti-oxidant therapy improved anemia in CKD patients and experimental animals with CKD, and reduced the requirement for erythropoiesis-stimulating agents (ESAs) in CKD patients (12, 27).

Pro-Oxidant Effect of Intravenous Iron in CKD Patients

The use of intravenous (IV) iron therapy, as a key component to enhance the efficacy of erythro-
poiesis-stimulating agents (ESAs) in CKD patients, has received increasing attention in recent years. IV iron therapy helps to reduce the requirement for ESAs, increases the hemoglobin levels of dialysis patients, and improves the cost-effectiveness of ESA treatment (27).

However, iron is a cellular transition element, and its ionic forms can participate in one-electron transfer reactions, so it can also generate free radicals. In the presence of iron, reactive hydroxyl radicals can be formed via the Fenton reaction and the iron-catalyzed Haber-Weiss reaction (15, 30). Druke et al. reported a relationship of common carotid artery intima-media thickness, AOPP, and annual IV iron dose administered to patients undergoing conventional HD (11). It is speculated that long-term IV administration of iron might increase oxidative stress and potentially cause harm to patients with renal disease. Thus, IV iron may worsen the cardiovascular outcome of dialysis patients. In fact, some in vivo studies demonstrated that administration of IV iron to HD patients increased the levels of oxidative byproducts, such as AOPP (11), MDA (28), carboxylated fibrinogen (33, 55), and esterified F₂-isoprostanes (48), and also reduced the levels of plasma antioxidants, such as SOD and GPx (28). However, large randomized and prospective studies are needed to determine whether IV iron therapy actually exacerbates oxidative stress in CKD patients.

Recently, our prospective, randomized, controlled study demonstrated that IV iron exacerbates oxidative damage to peripheral blood lymphocyte DNA in HD patients (26). In this study, we investigated the effect of a single dose and of multiple doses of IV iron on the levels of lymphocyte 8-OHdG in order to evaluate the effect of an imbalance of ROS production and antioxidant defense on DNA damage. Our results indicated that IV iron therapy increased intracellular ROS production by circulating lymphocytes, and decreased total plasma antioxidant capacity (i.e. decreased the levels of vitamin C and vitamin E, and increased the ratio of oxidized glutathione/reduced glutathione) in HD patients. Furthermore, we demonstrated that IV iron therapy exacerbates lymphocyte 8-OHdG generation in HD patients, especially those with serum ferritin levels above 500 µg/L. Our study was too short to assess the long-term consequences of IV iron-induced oxidative DNA damage. Clearly, large, randomized, long-term, prospective studies are needed to assess the safety and clinical role of IV iron therapy.

**Management of Oxidative Stress in CKD Patients**

Clinical data that support the association of oxidative stress, atherosclerosis, and CV disease in CKD patients are limited. In a study of an apolipoprotein-E deficient murine model of CKD-accelerated atherosclerosis, Ivanovski et al. demonstrated that treatment with N-acetylcysteine (NAC), a precursor to the anti-oxidant glutathione, decreased the expression of aortic nitrotyrosine (a marker of nitrosative oxidative stress), and reduced atherosclerotic plaque progression (24). In a similar uremic model, Phan et al. reported that the reduction of atheromatous plaque by sevelamer, a phosphate-binding drug given to prevent hyperphosphatemia, was associated with decreased expression of aortic nitrotyrosine (45). Bro et al. showed that accelerated atherosclerosis in uremia could be prevented by renin-angiotensin system inhibition, or markedly reduced by receptor for advanced glycation end products (RAGE) blockade, probably through anti-inflammatory and antioxidative effects (4, 5). In an animal model of arterial injury, Miyata et al. showed that OPB-9195 (2-iso-propyllidenehydrazono-4-oxo-thiazolidin-5-acylacetonilde) inhibited neointimal formation (34). Other studies of rats with CKD showed that administration of the antioxidant or renin-angiotensin system inhibitor attenuated cardiomyocyte/capillary mismatch and myocardial fibrosis (1, 46). Taken together, these studies suggest a causal relationship between oxidative stress and CV disease in experimental animals with CKD.

Animal studies and limited clinical studies suggest that increased oxidative stress aggravates CV risk in the presence of uremia. Thus, it is a logical hypothesis that CKD patients may benefit from antioxidant therapy. Although a number of small clinical studies have reported that the administration of antioxidants, such as vitamin E and vitamin C, reduces the levels of biomarkers of oxidative stress in uremia patients, there are limited interventional clinical trials of the effect of antioxidant treatment aimed on attenuation of CV disease in CKD patients (9, 20). The Secondary Prevention with Antioxidants of Cardiovascular disease in End-stage renal disease (SPACE) trial was the first randomized, placebo-controlled study to demonstrate a reduction of combined CV events in dialysis patients given an antioxidant (3). In this study, 196 HD patients with established CV disease were randomly assigned 800 IU of vitamin E per day or a placebo. Compared with patients given the placebo, the vitamin E-treated patients had significantly fewer CV events, but there was no significant difference in overall survival of the two groups.

The use of vitamin E-coated dialysis membranes seems to have great potential, and deserves further investigation. A vitamin E-coated dialysis membrane is composed of a multilayer membrane with lipo-
soluble alpha-tocopherol on the blood surface side that allows direct free radical scavenging. Miyazaki et al. (36) reported that vitamin E-coated cellulose dialysis membrane prevented the impairment of endothelial function induced by HD. Our study also demonstrated that use of a vitamin E-bound dialysis membrane reduced the oxidative stress of HD patients, as indicated by a decline of lymphocyte 8-OHdG levels and the preservation of plasma vitamin E levels (51). These beneficial effects were attributed to a decrease in ROS generation by activation of phagocytes during their contact with the vitamin E-coated membrane. Nevertheless, when compared with a biocompatible polysulfone membrane, patients treated with a vitamin E-bound membrane had similar levels of oxidative stress markers (14). Thus, it is not clear whether vitamin E-coated membranes would be superior to biocompatible membranes in clinical practice.

Another randomized controlled study of long-term HD patients evaluated the effects of antioxidant therapy with NAC. The results indicated 40% fewer CV events in the treatment group than in the placebo group (54). As with the SPACE trial (3), these results support the beneficial effects of anti-oxidant therapy on CV disease in patients with CKD. However, both of these prospective clinical trials had small sample sizes and only short-term follow-up. The long-term effects of antioxidants on the oxidative stress status of CKD patients are not well understood. Moreover, detailed information about the specific pharmacokinetics and pharmacodynamics of each antioxidant (16, 17, 18) in the milieu of uremia are needed. It appears that use of a combination of antioxidants directed against intracellular and extracellular ROS might provide CKD patients with protection against CV disease. In Anti-Oxidant Therapy in Chronic Renal Insufficiency (ATIC) Study (39), investigators reveal that in non-diabetic CKD patients who had well-controlled blood pressure, 18 months of treatment with an oxidative stress-lowering strategy consisting of pravastatin, vitamin E and homocysteine-lowering therapy resulted in a significant reduction in common carotid intima-media thickness and a significant improvement in brachial artery flow-mediated dilatation and urinary albumin excretion. These results suggest that active treatment strategy might safely reduce the burden of CV events in CKD patients. Recently, the use of electrolyte-reduced water (ERW) obtained by electrolysis for dialysate is an alternative approach to improving oxidative stress in HD patients. ERW treatment administration can partially restore antioxidant status and effectively palliate HD-evoked oxidative stress, such as lipid peroxidation, hemolysis, and overexpression of proinflammatory cytokines in HD patients (21, 22, 23). Further studies evaluating this novel strategy to reducing oxidative stress are definitely needed.

Conclusion

Taken together, existing pre-clinical and clinical studies strongly suggest that oxidative stress plays an important role in the setting of CKD. Thus, the presence of oxidative stress, uremic status, type of dialysis system, and the concomitant use of drugs are all important factors related to the condition and outcome of patients with CKD. Oxidative stress is also associated with other co-morbid conditions, such as anemia, malnutrition, inflammation, and atherosclerosis. Therapeutic strategies, including exogenous administration of antioxidant vitamin E, vitamin C, combination of antioxidants, the use of vitamin E-coated dialysis membranes and ERW, may ameliorate the long term complications of oxidative stress in CKD patients. Large, adequately powered, randomized controlled trials with long-term follow-up are needed to definitively establish that correction of oxidative stress is beneficial to patient outcome.

References


