Oxidative Stress in Uremia: The Role of Anemia Correction

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Patients with chronic kidney disease (CKD) are prone to develop cardiovascular disorders. Numerous reports have shown the association between uremia and oxidative stress, which increases patients' risk for cumulative injury to multiple organs. Anemia is a common and disabling feature of CKD and seems to be a main cause of oxidative stress; correction of anemia represents an effective approach to reduce oxidative stress and, consequently, cardiovascular risk. There is increasing evidence that correction of anemia with erythropoiesis-stimulating agents could protect from oxidative stress in patients with CKD and ESRD. However, iron deficiency frequently complicates anemia in patients with CKD, and ferrous iron cation is a co-factor that is needed for hydroxyl radical production, which can promote cytotoxicity and tissue injury. This has raised a justifiable concern that prescription of intravenous iron may exacerbate oxidative stress and, hence, endothelial dysfunction, inflammation, and progression of cardiovascular disease, which are widely known consequences of CKD. Correction of anemia represents an effective approach to reduce oxidative stress and, consequently, cardiovascular risk. Iron deficiency is a common cause of resistance to erythropoiesis-stimulating agents, and the overall risk-benefit ratio favors use of intravenous iron to treat iron deficiency in patients with CKD. Consecutive or combined treatment with intravenous iron and erythropoiesis-stimulating agents clearly is beneficial for patients with CKD and iron deficiency, and anemia and could contribute to prevent the risk for cardiovascular events in these patients.

Oxidative Stress: Definition and Components

Oxidative stress can be considered an imbalance between reactive oxygen species (ROS) production and antioxidant defense. This imbalance can lead to the oxidation of molecules, resulting in tissue damage. The "oxidant condition" mainly depends on the oxidative processes inside the organism (1). Alterations in mitochondrial enzyme complex cytochrome oxidase accounts for an important part of oxidative processes, because the mitochondria handles 90% of total oxygen in a human. A fraction of oxygen that is metabolized in the mitochondria can leak through the electron transport chain, forming reactive oxygen intermediates and oxygen free radicals such as superoxide anions and hydrogen peroxide. These ROS can diffuse out of the mitochondria, being an important source of oxidative stress (1,2). Another source of ROS is the NAD(P)H oxidase, which is important in endothelial and phagocytic cells. In addition, xanthine oxidase is a main source of oxygen species in occlusion-reperfusion situations. On the other side, a number of enzyme activities such as superoxide dismutase, catalase, glutathione (GSH) reductase (GRed), and GSH peroxidase (GPx) are determinants of antioxidant defense, leading to ROS clearance and buffering (1,3,4). Reduced GSH is a primary antioxidant that has been proposed as a major scavenger of ROS.

Levels of GSH are maintained in the cells by the GPx/GRed system. GPx catalyzes the reduction of H_2O_2 to H_2O, which is coupled to the oxidation of GSH to its disulfide form, GSSG. The reduction of GSSG to GSH is coupled to the oxidation of NADPH to $NADP^+$ through GRed. Erythrocytes play a key role in the maintenance of both systemic and local redox balance, as a result of their ability to recycle GSH formation through the GPx/GRed system. Therefore, evaluation of GSH system parameters in erythrocyte is considered a reliable method to study redox status (4).

Oxidative Stress and Cardiovascular Disease

ROS are part of the unspecific defense system of an organism. However, ROS also may affect cells of the host organism, in particular at sites of inflammation, which plays a role in a variety of renal diseases, such as glomerulonephritis, acute or progressive renal failure, or tubulointerstitial nephritis (1,3), contributing to proteinuria. ROS also are considered to contribute to the pathogenesis of ischemia-reperfusion injury (5).

From a vascular point of view, many studies have shown that atherosclerosis and risk factors for the development of the disease are associated with an exaggerated production of ROS. Atherosclerosis involves the participation of several cell types and processes, such as endothelial dysfunction, oxidation, inflammation, and fibrinolytic imbalance (6). ROS are determinants for the oxidation of LDL, which are taken up by macrophages, leading to the formation of foam cells. In addition, ROS and, specifically, superoxide anions partici-

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pate in an important manner in the processes that are involved in the progression of atherosclerosis. In turn, oxidized LDL also are capable of enhancing generation of ROS *via* stimulation of NAD(P)H oxidase in endothelial cells and smooth muscle cells (6). Superoxide anions combine with nitric oxide to form peroxynitrite, thereby contributing to endothelial dysfunction and the subsequent alterations that are related to the loss of nitric oxide availability. Furthermore, enhanced superoxide formation results in either cell proliferation or apoptotic death of endothelial cells (7). Many data indicate that ROS contribute to the progression and complications of atherosclerosis by stimulating various intermediate and transcription factors that lead to the formation of adhesion molecules, cytokines, and metalloproteinases, which participate in the progression and complications of atherosclerosis (6).

Oxidative Stress in Uremia

Recent studies have shown that oxidative stress is highly present in patients with renal disease (1,3). It is known that LDL from uremic patients present an elevated susceptibility to oxidation, being an indication of accelerated atherosclerosis in these patients. Uremic oxidative stress is characterized from a biochemical point of view as a state of reactive aldehyde and oxidized thiol group accumulation, together with depletion of reduced thiol groups, which are particularly important as part of antioxidant defense. As a consequence of diminished renal catabolism and function, uremic oxidant mediators accumulate, favoring vascular cell dysfunction and progression of atherosclerosis. In addition to the mentioned oxidized thiol groups, homocysteine accumulates in uremic patients and may contribute to atherosclerotic disease (8). Epidemiologic studies have correlated hyperhomocysteinemia with atherosclerotic disease not only in the general population but also in hemodialysis patients. It should be mentioned that elevated inflammatory markers such as C-reactive protein and cytokines are highly prevalent in patients with ESRD (8). In fact, a linkage among increased oxidative stress, inflammation, and endothelial dysfunction in hemodialysis patients was described recently. Furthermore this synergistic linkage could contribute to increased cardiovascular risk in uremic patients (9).

Oxidative stress occurs when ROS exceed antioxidant defense, which is replenished continually by ingestion of nutrients. Malnutrition is relatively common in uremic patients and may contribute to increased oxidative stress (10). In fact, malnourished uremic patients present increased markers of oxidative stress than well-nourished uremic patients (9,10).

Anemia and Cardiovascular Disease

Anemia is a common and disabling feature of CKD. There is increasing evidence from epidemiologic studies of an association between anemia and cardiovascular mortality. The Atherosclerosis Risk In Communities (ARIC) study revealed that individuals with anemia had a worse prognosis than those with normal hemoglobin levels and demonstrated that anemia was associated independently with an increased risk for cardiovascular disease (11). Several studies showed that in patients with ESRD, low hematocrit levels were associated with a marked increased in cardiovascular morbidity and mortality (12). There are several reasons to explain the relationship between anemia and adverse cardiac outcomes. First, anemia is a marker of poor cardiac function. Second, it is a causative risk factor for cardiac ischemia, because coronary artery disease limits the ability to extract oxygen from hemoglobin. Third, the physiologic adaptive response to anemia is an increase in cardiac output. This initial compensatory benefit is limited, because a chronic adaptation to low hemoglobin levels may increase left ventricle growth in response to increased myocardial workload. In fact, several studies demonstrated the association between anemia and left ventricular hypertrophy in nonrenal patients and in patients who had CKD, were on dialysis, or received a renal transplant (13).

Correction of Anemia in Uremic Patients

Uremic patients are characterized by a predominant state of oxidative stress, and anemia seems to be a main cause for this redox imbalance (14). Regular supplements of intravenous iron and erythropoiesis-stimulating agents are standard therapies for treatment of anemia in patients with CKD. Consequently, correction of anemia in uremic patients, besides its primary beneficial effects, represents an effective approach to reduce oxidative stress and hence potential cardiovascular risk. There is increasing evidence that erythropoiesis-stimulating agents could protect from oxidative stress in patients who are on hemodialysis or peritoneal dialysis (1,3). Treatment with erythropoiesis-stimulating agents decreases patient morbidity and mortality, particularly as a result of cardiovascular disease in patients with ESRD (15). Furthermore, treatment of anemia with erythropoietin has been shown to produce regression of left ventricular hypertrophy in patients with CKD (16).

Intravenous iron supplements are incorporated rapidly into the transferrin and ferritin system for iron transport and storage. However, large doses of intravenous iron may exceed storage capacity leading to certain amounts of unbound iron in plasma. Ferric iron can be reduced to the ferrous form, which, *via* the Fenton reaction, can produce the hydroxyl radical, one of the most potent ROS. This has raised a justifiable concern that intravenous iron may exacerbate oxidative stress and, hence, endothelial dysfunction, inflammation, and progression of cardiovascular disease (17,18).

Evaluation of redox status in erythrocytes has been used as a reliable method to evaluate oxidative stress and antioxidant defense in patients with CKD (19,20). We aimed to investigate the effects of intravenous iron treatment followed by subcutaneous α-darbepoetin treatment on erythrocyte redox status in nine nondialysis patients with CKD (Table 1). As expected, hematocrit and hemoglobin increased after intravenous iron and α-darbepoetin treatments. However, none of the treatments modified plasma concentrations of iron, ferritin, or transferrin or transferrin saturation. The results support the oxidant effect of intravenous iron because

Table 1. Effect of iv iron saccharate and α-darbepoetin treatments on erythrocyte redox status[a]

Erythrocyte Concentration (μmol/g Hb)	Baseline	Intravenous Iron	α-Darbepoetin	P
MDA	1.8 ± 0.1	3.2 ± 0.6[b]	2.2 ± 0.1	<0.05
GSSG	0.9 ± 0.1	1.5 ± 0.2	1.1 ± 0.2	NS
GSH	4.8 ± 0.8	3.3 ± 0.73	8.8 ± 1.8[c]	<0.01
GSH/GSSG	4.9 (25.5)	2.07 (2.18)	9.96 (41.1)	<0.05[d]
GPx	28.8 ± 2.3	41.6 ± 9.5[e]	17.5 ± 4.5	<0.05
GRed	2.6 ± 0.3	5.2 ± 1.1	3.6 ± 0.4	NS

[a]Data are means ± SD or median (interquartile range) as appropriate. GSH, reduced glutathione; GPx, glutathione peroxidase; GRed, glutathione reductase; GSSG, oxidized glutathione; Hb, hemoglobin; MDA, malondialdehyde.
[b]$P < 0.05$ baseline *versus* intravenous iron.
[c]$P < 0.05$ intravenous iron *versus* α-darbepoetin.
[d]Kruskal-Wallis test was performed because values were not followed a normal distribution.
[e]$P < 0.05$ baseline *versus* intravenous iron.

iron treatment was associated with the elevation of malondialdehyde levels, an index of lipid peroxidation. Erythrocyte GSH/GSSG ratio decreased after iron treatment. This was due to a net decrease of GSH without changes in GSSG. Administration of α-darbepoetin returned malondialdehyde levels to values comparable to those observed before iron treatment and markedly increased GSH/GSSG ratio. This was due to an important elevation of GSH by α-darbepoetin, without changes in GSSG. Similarly, The changes in GSH that were observed seem to be due to a marked increase of GPx activity after iron and a decrease after α-darbepoetin, together with moderate changes of GRed activity. These effects of the treatments on erythrocyte redox balance occurred without changes in serum creatinine, creatinine clearance, C-reactive protein, and homocysteine. This clinical study in a small number of patients with CKD shows that intravenous iron increases oxidative stress by diminishing erythrocyte antioxidant defense. Administration of α-darbepoetin rebalanced GSH/GSSG system, further supporting the clinical benefits of anemia correction with erythropoiesis-stimulating agents on oxidative stress in patients who have CKD and receive intravenous iron supplements.

Acknowledgments

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