Relevance of oxidative and carbonyl stress to long-term uremic complications

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Oxidative stress is a disturbance of balance between oxidants and antioxidant species. The existence of an increased oxidative stress in chronic renal failure is supported by evidence of increased lipid, carbohydrate, and protein oxidation products in plasma and cell membrane. Recent studies have implicated the oxidative stress in the nonenzymatic biochemistry leading to irreversible protein modifications. Reactive oxygen species may directly alter proteins with the eventual formation of oxidized amino acids. Alternatively, reactive carbonyl compounds formed by the oxidation of carbohydrates and lipids may indirectly lead to advanced glycation or lipoxidation of proteins. Chronic uremia is associated with increased modification of protein caused by reactive carbonyl compounds derived from both carbohydrates and lipids. Increased carbonyl modification of proteins subsequently results in the rise of plasma and tissue contents of advanced glycation end products and advanced lipoxidation end products, in which the deleterious biological effects have been revealed. This article focuses on the irreversible nonenzymatic modification of proteins, which might, at least in part, contribute to the development of complications associated with chronic renal failure and long-term dialysis, such as atherosclerosis and dialysis-related amyloidosis.

Oxidative stress is a disturbance of balance between oxidants and antioxidant species: The former (superoxide anion radical, hydroxy radical, lipid peroxide radical, etc.) predominates over the latter consisting of antioxidant enzymes [for example, superoxide dismutase, catalase, glutathione (GSH) peroxidase, GSH-S-transferase], thiols (for example, GSH, cysteine, albumin, and other protein thiols), urate, ubiquinol, tocopherol, ascorbate, carotenoids, etc.

The existence of an increased oxidative stress in chronic renal failure is supported by evidence of increased lipid, carbohydrate, and protein oxidation products in plasma and cell membrane, including increased serum ratios of oxidized to total ascorbate [1], oxidized to reduced GSH [2], and oxidized serum albumin (nonmercaptalbumin) to reduced albumin (mercaptalbumin) [3], increased serum levels of lipid peroxidation products [4, 5], glycoxidation products [6, 7], “advanced oxidation protein products” (AOPPs) [8], and protein carbonyls taken as markers of protein oxidation [9, 10]. Serum activity of GSH-dependent enzymes is lowered in hemodialysis patients [11, 12]. Dialyzable oxidants measured by electron spin resonance spectroscopy accumulate in uremic plasma [13].

The mechanism of uremia-associated oxidative stress is not completely understood. Activation of neutrophils and complement pathway during hemodialysis with an attendant enhanced reactive oxygen species production may contribute to oxidative stress [14]. However, the levels of oxidation products in plasma and cell membrane are elevated even in patients with renal failure prior to the initiation of dialysis therapy. Oxidative stress thus may result from uremia per se. Plausible explanations are impaired renal synthesis of antioxidant enzyme (for example, GSH peroxidase) [15], iron supplementation [16], and restriction of food intake, resulting in the depletion of selenium and GSH [17, 18].

Recent studies have implicated the oxidative stress in the nonenzymatic biochemistry leading to irreversible protein modifications. Reactive oxygen species may directly alter proteins with the eventual formation of oxidized amino acids, such as ortho-tyrosine and methionine sulfoxide [19]. Alternatively, reactive carbonyl compounds (RCOs) formed by the oxidation of carbohydrates and lipids may indirectly lead to advanced glycation or lipoxidation of proteins [20]. Chronic uremia is associated with increased modification of protein due to RCOs derived from both carbohydrates and lipids. Increased carbonyl modification of proteins subsequently results in the rise of plasma and tissue contents of advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs), whose deleterious biological effects have been revealed [20]. This article focuses on the irreversible nonenzymatic modification of proteins, which might, at least in part, contribute to the development of complications associated with chronic renal fail-
ure and long-term dialysis, such as atherosclerosis and dialysis-related amyloidosis.

**ACCUMULATION OF RCOs AND AGEs/ALEs IN UREMIC (CARBONYL STRESS)**

Carbohydrates, lipids, and amino acids, abundantly present in human body, are the precursors for RCOs. Carbohydrates and ascorbate may generate RCOs, for example, glyoxal, methylglyoxal, arabinose, glycoaldehyde, 3-deoxyglucosone, and dehydroascorbate [21–25]. The RCOs react nonenzymatically with protein amino groups and form reversible Schiff-base adducts transformed subsequently into more stable, slowly reversible Amadori rearrangement products. Through a series of oxidative and nonoxidative reactions, they eventually form irreversibly AGEs, for example, carboxymethyllysine (CML), pentosidine, pyrraline, imidazolone, glyoxal-lysine dimmer (GOLD), and methylglyoxal-lysine dimmer (MOLD) [20].

Lipid peroxidation of polyunsaturated fatty acids such as arachidonate also generates RCOs [26, 27], for example, glyoxal, malondialdehyde, hydroxynonenal, and acrolein. These highly reactive RCOs combine with proteins and form ALEs, for example, malondialdehyde-lysine, hydroxynonenal, and acrolein-protein adduct, as well as AGEs, for example, CML, but not pentosidine [20]. Finally, RCOs are also generated during the myeloperoxidase-catalyzed metabolism of amino acids, l-serine, and l-threonine, for example, glyoxal, methylglyoxal, acrolein, and glycoaldehyde [28].

In chronic uremia, the levels of RCOs derived from a variety of sources are markedly increased in the plasma. Raised levels of glyoxal [29], methylglyoxal [29], 3-deoxyglucosone [30], dehydroascorbate [1], and malondialdehyde [5] have been demonstrated in the plasma of hemodialysis patients. Total RCOs have also been assessed by the reaction of 2,4-dinitrophenyldrazine with plasma: The yield of hydrazone formed by interaction with carbonyl groups is several times higher in uremic than in normal plasma [31].

The resulting levels of AGEs and ALEs are also raised in uremia. Pentosidine [6], determined by high-performance liquid chromatography (HPLC), and CML [5], determined by gas chromatography/mass spectrometry, are significantly higher in plasma and tissue proteins of hemodialysis patients than in normal or diabetic subjects. Other AGEs, such as GOLD and MOLD, also accumulate in uremic plasma [32]. The levels of malondialdehyde-lysine, taken as a surrogate marker for ALEs, are also raised in the plasma proteins of hemodialysis patients [5]. Uremia is thus characterized by the irreversible, nonenzymatic modification of proteins by RCOs derived from carbohydrates and lipids, that is, AGEs/ALEs.

Plasma levels of pentosidine [6] and CML [5] in hemodialysis patients are not correlated with the levels of fructoselysine, an Amadori product used as a marker of prevailing plasma glucose concentration. This is in contrast with the observation that in diabetic patients, tissue and serum levels of AGEs are correlated with fructoselysine [33, 34]. Similarly, the levels of malondialdehyde-lysine present in uremic serum are not correlated with triglycerides. These findings suggest that a factor(s) other than hyperglycemia and hyperlipidemia determines the rate of AGE and ALE formation in renal failure. Over 90% of the pentosidine and CML measured in uremic plasma was found in the albumin fraction [5, 6]. Their accumulation is thus not due to a decreased renal clearance of protein-linked AGEs or ALEs. It has therefore been hypothesized that the accumulation in uremia of AGEs/ALEs is the consequence of raised levels of RCOs derived from carbohydrates and lipids.

Pentosidine generation in plasma samples incubated under air for several weeks is markedly higher in uremic than normal plasma. This difference is still observed when plasma ultrafiltrates (5000 D filter cutoff) are incubated with normal human serum albumin. The pentosidine yield in the ultrafiltrate is significantly higher in predialysis than in postdialysis plasma samples. Inhibitors of carbonyl amine reaction with proteins, aminoguanidine and OPB-9195, significantly reduce the production of pentosidine in both control and uremic plasma [31]. These results support the concept that uremia is characterized by increased levels of low molecular levels of RCOs, precursors of pentosidine. Of interest, there is a clear correlation between pentosidine levels measured in plasma and that generated in vitro after incubation under air for several weeks [31]. If the in vitro pentosidine generation is determined by the initial RCO contents of plasma, this observation suggests that the plasma pentosidine level is an appropriate surrogate marker of the level of pentosidine precursor RCOs. The accumulation of various RCOs derived from carbohydrates and lipids in uremia and the subsequent carbonyl modification of protein are called “carbonyl stress” [20]. Under carbonyl stress, not only AGEs derived from carbohydrates but also ALEs derived from lipids accumulate in plasma and probably in tissue proteins as well.

**MECHANISM OF CARBONYL STRESS: OXIDATIVE STRESS?**

The carbonyl stress of uremia is not completely understood. Two mechanisms should be considered. First, it may result from an increased generation of RCOs under oxidative stress. Under oxidative stress, the carbohydrates and lipids are modified by reactive free radicals. The autooxidation of carbohydrates yields glyoxal, arabinose, and glycoaldehyde and that of ascorbate, dehydroascorbate [22, 23, 25]. As described previously in this
article, lipid peroxidation and oxidation of t-serine and t-threonine yield glyoxal malondialdehyde, hydroxynonenal, acrolein, and glycoaldehyde [26–28].

The relationship between oxidative stress and carbonyl stress is further supported by the reports that serum levels of pentosidine are correlated with oxidative markers such as dehydroascorbate [1] and AOPP [8].

It should be pointed out, however, that RCOs derived from nonoxidative chemistry also rise simultaneously in uremic plasma without concomitant changes in glucose or lipid levels. 3-Deoxyglucosone and methylglyoxal are formed nonoxidatively by the decomposition of the Amadori product fructose-3-phosphate and of triose phosphates, respectively [18, 20]. Their plasma levels as well as that of their protein adducts are increased in the plasma of hemodialysis patients [29, 30, 32].

The second hypothesis for the uremic carbonyl stress rests on a decreased clearance or detoxification of RCOs. RCOs derived from both oxidative and nonoxidative chemistry of both carbohydrates and lipids have a rather low molecular weight so that their removal might rely on renal function in uremic and diabetic patients. [35, 36].

In addition to renal removal, a number of enzymatic pathways contribute to the detoxification of RCOs, for example, aldose reductases, aldehyde dehydrogenases, and the glyoxalase pathway [22]. A decrease in their efficiency can readily result in the simultaneous increase of a wide range of RCOs. Redox coenzymes, reduced GSH, and nicotinamide adenine dinucleotide phosphate [NAD(P)H] are particularly important elements for the activity of these pathways. RCOs, such as methylglyoxal and glyoxal react reversibly with thiol group of GSH to be subsequently detoxified by glyoxalase. NAD(P)H increases the activity of GSH reductase and replenishes GSH. Alteration of GSH homeostasis may impair the detoxification of RCOs and may increase the AGE/ALE genesis.

The GSH concentration in red blood cells and serum activity of GSH-dependent enzymes of uremic patients are indeed significantly lower than in normal subjects [2, 11, 12]. Increased RCOs levels might be the consequence of a decrease of thiol storage in uremia, for example, GSH, NADPH, and mercaptalbumin. This hypothesis is supported by our recent observation that the addition of GSH in both uremic and normal plasma lowers the generation of pentosidine after incubation in vitro (unpublished) and that GSH peroxidase activities correlate inversely with the pentosidine levels in the plasma of hemodialysis patients [12]. Similarly, several other groups have suggested that decreased GSH peroxidase activities increase the generation of hydrogen peroxide, which further accelerates the formation of AGEs/ALEs [37–39].

The decreased thiol concentration in uremia is as yet not fully understood. It might result from an increased consumption in order to detoxify an augmented load of reactive free radicals generated in uremia (oxidative stress). Alternatively, it might be derived from nonoxidative mechanism(s) as demonstrated in diabetes. Under these circumstances, the polyol pathway is activated by hyperglycemia and consumes NADPH for the reduction of glucose to sorbitol, a process catalyzed by aldose reductase [40]. NADPH is thus less available for GSH reductase so that GSH levels fall.

Interestingly, the decreased thiol concentration alters the antioxidant systems and may mimic an oxidative stress. In the absence of oxidative stress, the decreased thiol concentration increases the ratios of oxidized to total ascorbate, of oxidized to reduced GSH, and of nonmercaptalbumin to mercaptalbumin as well as the levels of lipid peroxidation products and of glycoxidation products on protein in the serum. The serum activity of GSH-dependent enzymes also decreases. Thus, the lines of evidence taken to indicate a uremia-associated oxidative stress may be interpreted differently. The existence of an oxidative stress in diabetes has also been controversial. Recently, however, Wells-Knecht et al have demonstrated that age-adjusted levels of ortho-tyrosine and methionine sulfoxide, markers of oxidative protein damage, are not increased in the skin collagen of diabetic patients, and they postulated that there is no need to invoke an increase in oxidative stress in diabetes [41].

It therefore remains of particular interest to establish whether chronic renal failure is associated with oxidative stress. Measurements of ortho-tyrosine and methionine sulfoxide in tissue proteins from uremic patients should provide critical evidence in this regard. A precise answer would also indicate whether the decreased thiol concentration in uremia is due to an oxidative stress or to another nonoxidative mechanism. Whatever the answer to these questions, it remains clear that uremia is characterized by AGE and ALE modification of proteins due to the accumulation of carbohydrate and lipid-derived reactive carbonyl species.

IMPLICATIONS OF CARBONYL STRESS

Several lines of evidence have suggested links between carbonyl stress and the development of complications associated with chronic renal failure and long-term dialysis. Dialysis-related amyloidosis is a serious bone and joint destruction associated with chronic renal failure. It develops in two stages [42]. The preclinical stage is characterized by histologic evidence of β2-microglobulin deposits in the joints several years before the onset of clinical or radiological signs [43]. Amyloid fibrils precipitate in the absence of macrophages or bone destruction. The subsequent clinical stage is characterized by arthralgias, carpal tunnel syndrome, and radiologically visible bone cysts. Amyloid deposits are surrounded by macrophages and bone resorption. Recent immunohistochemi-
Table 1. Biological effects of AGEs/ALEs and RCOs

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<td>Intracellular signaling [61, 64, 65]</td>
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Characteristics of AGEs/ALEs and RCOs

- **AGEs/ALEs**
  - Chemotaxis of monocytes [52, 53]
  - Inflammatory cytokine secretion from macrophages [52, 54]
  - Collagenase secretion from synovial cells [52]
  - Osteoclast-induced bone resorption [55]
  - Proliferation of vascular smooth muscle cells [56]
  - Aggregation of platelets [57]
  - Intracellular signaling [63]

- **RCOs**
  - Structural alteration of matrix proteins
  - Resistance to the action of calcitriol
  - VEGF production from endothelial and mesothelial cells [62]
  - Cell apoptosis [65, 66]
  - Intracellular signaling [61, 64, 65]

Biosynthesis of AGEs/ALEs and RCOs in Uremia

**Table 1** presents the biological effects of AGEs/ALEs and RCOs, which include chemotaxis of monocytes, inflammatory cytokine secretion from macrophages, collagenase secretion from synovial cells, osteoclast-induced bone resorption, proliferation of vascular smooth muscle cells, and cell apoptosis. These effects are mediated by AGEs/ALEs and RCOs, which are structurally altered matrix proteins and resistant to calcitriol action.

**CONCLUSIONS**

Despite several lines of evidence, the presence of an oxidative stress in renal failure remains to be conclusively established. Nevertheless, chronic renal failure is a state of carbonyl stress, and accumulation of RCOs from various sources may lead to AGE/ALE formation. This carbonyl stress may occur due to oxidative stress or from other nonoxidative causes, such as depletion of thiols. Whatever its cause, the carbonyl stress alters the structure and function of cellular and matrix proteins and may contribute to the development of uremia-associated complications.

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APPENDIX

Abbreviations used in this article are: AGE, advanced glycation end product; ALE, advanced lipoxidation end product; AOPP, advanced oxidation protein product; CML, carboxymethyllysine; EGFR, epidermal growth factor receptor; GOLD, glyoxal-L-lysine dimmer; GSH, glutathione; MAPK, mitogen activated protein kinase; MOLD, methyglyoxal-L-lysine dimmer; NAD(P)H, nicotinamide adenine dinucleotide (phosphate); RCO, reactive carbonyl compound; VEGF, vascular endothelial growth factor.

REFERENCES