Role of oxidized low-density lipoprotein in the atherosclerosis of uremia

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Role of oxidized low-density lipoprotein in the atherosclerosis of uremia. Lipoprotein oxidation is involved in the genesis of atherosclerosis. In chronic renal failure (CRF), oxidative stress is enhanced because of an imbalance between pro-oxidant and antioxidant systems. Oxidative modifications of low-density lipoproteins (LDLs) occur not only at the level of lipid moiety, but also of protein moiety. We have shown that oxidation of LDL by hypochlorous acid (HOCl) in vitro, reflecting increased myeloperoxidase activity in vivo, leads to modifications of apolipoproteins such that the latter in turn are capable of triggering macrophage nicotinamide adenine dinucleotide phosphate-oxidase activation. These oxidative changes of LDL protein moiety, if shown to occur to a significant extent in uremic patients in vivo, may represent an important alternative pathway in the pathogenesis of atheromatous lesions.

It is well known that uremic patients have a high prevalence of atherosclerotic cardiovascular complications that greatly exceeds that of the general population [1]. This is true for patients with advanced chronic renal failure (CRF) who are not yet on dialysis and remain so after the initiation of maintenance hemodialysis [2–4]. Although the prevalence of cardiovascular-related deaths in the end-stage renal disease (ESRD) population is highest in the older population (~150 cardiovascular-related deaths per 1000 patient-years at risk), it is still substantial (40 cardiovascular deaths per 1000 patient-years at risk) in the group of 20 to 40 years of age [4].

Increasing attention has recently been directed to the role of inflammation [5], and the intervention of reactive oxygen species being generated by activated macrophages in the vessel wall has become increasingly recognized [6–8]. In particular, oxidative damage to low-density lipoproteins (LDL) and endothelial cells is postulated to be of prime importance in the development of fatty streaks, the early lesion in atherogenesis [9].

Oxidative modification of LDL in vitro requires the presence of high concentrations of free metal ion such as copper or iron, which are not easily available in vivo because of the presence of metal chelators [10]. In contrast, the involvement of chlorinated oxidants, which produce only minor changes of the LDL-lipid moiety, but strongly modifies its protein moiety [11], appears to be more likely in vivo. The presence of myeloperoxidase (MPO), which allows the formation of hypochlorous acid (HOCl) from hydrogen peroxide (H₂O₂) and chloride ion, has been demonstrated in human atheromatous lesions [12], together with markers of HOCl-induced protein damage [13].

Major efforts have been undertaken for several years to identify optimal indices of oxidative stress and to develop therapeutic strategies to counteract the deleterious effects of oxidants, the ultimate clinical goal being to reduce the excessively high incidence of cardiovascular complications of uremic patients.

INCREASED LIPID PEROXIDATION PRODUCTS IN HEALTHY SUBJECTS

A number of oxidative stress markers have been identified, including protein oxidation and lipid peroxidation products. The former are presented in detail in a companion article to this issue [14]. The latter include oxidation products of polyunsaturated fatty acids (PUFAs), which are located in phospholipids, glycolipids, triglycerides, and cholesteryl esters, which are extremely susceptible to oxidative degradation in living tissues. They further include lipid hydroperoxides such as hydroxyoctadecadienoic acid (HODE), which are products of enzymatic lipid peroxidation of linoleic acid; several reactive aldehydes comprising acrolein, 4-hydroxynonenal (4-HNE), and malondialdehyde (MDA), which are determined by the measurement of thiobarbituric acid reactive substances (TBARSs) in plasma; and a number of more recently shown phospholipid oxidation products. The latter can be subdivided into four classes: (1) phospholipids in which the unsaturated fatty acid contains
additional oxygen molecules, for example, isoprostanes and hydroxy fatty acids; (2) phospholipids in which the fatty acid is fragmented but remains attached to the glycerol backbone; (3) liberated free fatty acid fragments that occur as a result of fragmentation (for example, MDA) and free oxygenated fatty acids that are formed by hydrolysis of the phospholipids; and (4) lyso phosphatidylcholine, the hydrolytic product of the phospholipid backbone.

Isoprostanes are prostaglandin isomers that are produced nonenzymatically by free radical-catalyzed peroxidation of arachidonoyl lipids contained in cell membrane phospholipids [15] or circulating LDLs [16]. Isoprostanes are emerging as a new class of biologically active products of arachidonic acid metabolism of potential relevance to human vascular disease, causing significant renal vasoconstriction [15]. Enhanced urinary excretion of 8-iso-prostaglandin, namely 8-iso-PGF₂α, has been described in association with cardiac reperfusion injury and cardiovascular risk factors, including cigarette smoking, diabetes mellitus, and hypercholesterolemia [17]. 8-iso-isoprostanes are also found in atherosclerotic lesions [18, 19]. They can be measured by gas chromatography/mass spectrometry and specific immunoassays and appear to be most reliable markers of lipid peroxidation.

Free radical-induced oxidation of LDL generates two major reactive aldehydes, MDA and 4-HNE, which interact with lysine residues in apoB, the protein moiety of LDL. These protein adducts are found in oxidized LDL (oxLDL) and also in atherosclerotic lesions. Phospholipid endoperoxides such as levuglandin E₂ have also been shown to form protein adducts [20].

The present article reports our knowledge about the role of oxidatively modified LDL in the cardiovascular complications of ESRD patients, including recent findings by our own group.

ROLE OF OXIDIZED LIPOPROTEINS IN ATHEROSCLEROSIS

Oxidation of lipids and lipoproteins such as LDL has long been recognized as a key event in the atherogenic process via the induction of vessel wall inflammation [5]. Oxidatively modified compounds enter the vascular subendothelial space and cause injury, especially in the setting of reduced antioxidant defense, to both endothelial and underlying smooth muscle cells [21, 22]. Whereas only a small fraction of circulating LDL displays the chemical and immunological characteristics of minimally modified LDL, heavily oxidized lipids, including oxLDL, oxidized sterols, isoprostanes (discussed later in this article), and products of the lipooxygenase pathway, are mainly found in atherosclerotic lesions [23].

The numerous mechanisms that lead to oxLDL formation in the population at large cannot be outlined here, but have been reviewed recently [24]. We would like to stress, however, that in addition to the predominantly reported and experimentally used oxidation of the lipid moiety of LDL, oxidation of the protein moiety also occurs, that is, of its major apolipoprotein (apo), namely apoB. This can occur through the generation of lipid hydroperoxides, followed by their breakdown and release of aldehydes and ketones, such as MDA and 4-HNE, which can modify lysine residues on apoB. Another pathway is via the activation of MPO, which amplifies the oxidative potential of H₂O₂ by generating highly reactive species such as HOCl and tyrosyl radical [25]. The latter initiate LDL lipid peroxidation and generate o,o'-dityrosine cross-links in proteins [25, 26]. Protein-bound dityrosine levels are markedly increased in human atherosclerotic lesions [27], suggesting that tyrosyl radical may play a role in LDL oxidation in vivo.

Enzymatically active MPOs as well as lipoproteins and other proteins oxidatively modified by the action of MPO, have been identified in human atherosclerotic vascular lesions. Moreover, oxLDLs isolated from atherosclerotic tissue in such patients are not enriched in lipid oxidation products characteristically induced by the action of free metal ions, pointing to the importance of amino acid-derived aldehydes generated by MPO in the formation of atherosclerosis. Thus, hypochlorite-modified proteins, including oxLDL, which might correspond to advanced oxidation protein products (AOPP), have been shown to be present in human atherosclerotic lesions [13].

In diabetes, auto-oxidation of carbohydrates, lipids, and ascorbate leads to advanced glycation end-products (AGEs) or glycation products. Both lipid peroxidation and glycoxidation favor the formation of protein carbonyls, which have been identified in the thickened intima of arterial walls and may play a role in atherogenesis [28].

OXIDIZED LIPOPROTEINS AND ATHEROSCLEROSIS IN UREMIC PATIENTS

In addition to qualitative lipoprotein changes in CRF patients by increased carbamylation and AGE transformation, evidence for excessive oxidation of lipoproteins includes the demonstration in recent reports of an increase in plasma lipid peroxidation markers such as MDA and conjugated diene concentrations in lipoprotein particles, peripheral blood cells, and adipose tissue [29, 30], although no such evidence was found in previous studies [31, 32], and the observation of an increase in plasma oxLDL levels [33] as well as in circulating reactive antibodies directed against them [34]. However, there has been no demonstration to date by immunohistochemistry or other methods that atherosclerotic lesions in CRF patients contain materials reactive with antibodies directed against oxLDL, as has been shown in the
general population. Although longitudinal studies in nonrenal patients have shown that the titers of circulating antibodies directed against oxLDL were an independent cardiovascular risk factor for atherosclerosis progression [35], this has not yet been demonstrated in uremic patients.

The study of the susceptibility of LDL to undergo oxidation in vitro in CRF patients has yielded conflicting results [32, 33, 36, 37]. This discrepancy is probably due to the fact that a number of factors influence the duration of the lag phase, which was used in these studies to measure the resistance of LDL to oxidation. Since it is difficult to reconstitute the complexity of all interactive factors in vitro, this may explain why in vitro conditions do not necessarily reflect the in vivo situation [38, 39].

Vitamin E appears to be an important agent in the protection against free radical-induced oxidative damage of LDL and biological membranes. Thus, long-term vitamin E administration has been reported to reduce F2-isoprostane levels significantly in aging kidney [40]. Interestingly, oral vitamin E supplementation or dialysis with vitamin E-modified membrane has recently been shown to protect chronic hemodialysis patients against oxidative stress [41, 42].

The reported inefficiency of vitamin E to protect LDL particles against oxidation in CRF patients despite a normal level may be due to an alteration of its function, probably resulting from vitamin C deficiency [57]. However, it has been reported that vitamin E was unable to prevent oxidative protein modification [43]. Vitamin E, to be protective against oxidation, requires ubiquinol (CoQ9H2) to regenerate its active, reduced form [44]. Extracellular CoQ9H2 is incorporated into and transported within lipoprotein particles, particularly LDL. Ubiquinol has been found to inhibit the initiation and propagation of lipid peroxidation synergistically with vitamin E. It is possible that the decrease in plasma CoQ9H2 levels in uremic patients is due to increased consumption of CoQ9H2 by free radicals. We previously have shown a decrease in plasma ubiquinol levels in CRF patients [45]. Since, in addition, uremic patients generally present lipoprotein disturbances with increased triglyceride-rich lipoprotein particles, this together with reduced lipid-soluble antioxidant levels probably represent an increased susceptibility to undergo oxidative modification.

Another mechanism that could contribute to increased lipoprotein oxidation is the reduction in CRF patients of the activity of paraoxonase, a high-density lipoprotein-associated enzyme capable of hydrolyzing lipid peroxides [46].

The uptake of oxLDL by phagocytes may be greater in uremic patients than in healthy subjects because of an increased expression of scavenger receptors [47], for instance via peroxisome proliferator activator receptor (PPAR) [48], and this should lead to an increased formation of atheromatous lesions.

The demonstration of markedly elevated concentrations of HOCI-modified protein in the plasma of CRF patients [49, 50] and the presence of oxidatively modified lipoproteins in vascular structures [51] would indicate enhanced MPO activity in blood and various tissues of uremic patients, including the arterial wall. Thus such an increase really occurs has been recently demonstrated in the blood of chronic hemodialysis patients [52].

In a recent personal study, we have tested the hypothesis that oxidation of the LDL protein moiety might induce macrophage activation, as this has been described for oxidative changes of the LDL-lipid moiety [53]. The latter are classically produced by incubation with copper sulfate (CuSO4). We first incubated human LDL with either CuSO4 (Cu-oxLDL) or hypochlorous acid (HOCI-oxLDL) at various oxidant/protein ratios and for various periods of time. Thereafter, human THP-1 and U937-derived monocytes/macrophages in culture were exposed to either native LDL, Cu-oxLDL, or HOCI-oxLDL to examine their relative capacity to trigger macrophage nicotinamide adenine dinucleotide phosphate-oxidase activation as measured by lucigenin-amplified chemiluminescence. Compared with native LDL, which exerted no effect in this experimental model, HOCl-oxLDL elicited dose-dependent, potent chemiluminescence responses in both cell lines, under the condition that the cells maintained in culture were fully differentiated into macrophages by phorbol myristate acetate. In contrast, Cu-oxLDL only triggered a moderate chemiluminescence response in U937 cells and exerted little effect on THP-1 cells. Figure 1 shows these findings in a typical experiment. We then delipidated these oxidatively modified LDL to examine the relative importance of the lipid moiety in these responses. While delipidation did not affect the HOCI-oxLDL-induced chemiluminescence response, it abolished that induced by Cu-oxLDL. The fact that U937 cells exhibited greater chemiluminescence responses to both types of oxidatively modified LDL than THP-1 cells could be due, at least in part, to the higher expression of scavenger receptor CD36 by U937 than by THP-1 cells. These observations strongly support our hypothesis of a distinct role of the protein moiety in oxLDL-induced macrophage activation. However, the presence of oxidatively modified LDL protein moiety remains to be demonstrated in uremic patients.

In CRF patients, there is also an increased rate of LDL modification by advanced glycation end product (AGE), independent of the blood glucose level. Oxidative stress is almost certainly at the origin of enhanced AGE formation of proteins in nondiabetic uremic patients [49]. This process is due to oxidative modification of fructosylne (a compound containing a ketoamine link) and the formation of (carboxymethyl)lysine (CML)
and pentosidine. Elevated AGE levels lead, in turn, to the generation of reactive oxygen intermediates. AGE moieties are present on both the apoB and the lipid components of LDL in such patients. Experimental data suggest that circulating AGE peptides can impair plasma clearance kinetics of native LDL and may increase the susceptibility of LDL to oxidative changes. AGE-transformed proteins favor the development and progression of atheromatous lesions in diabetes via their endothelial receptor RAGE [54], and atherosclerotic lesions in diabetic, apoE-deficient mice can be suppressed by the administration of soluble RAGE [55]. A similar noxious effect on the vessel wall has been shown to occur in CRF patients with high AGE levels, even in the absence of diabetes [56].

Major questions that currently remain to be addressed are to what extent and by which precise mechanisms the oxidatively modified LDL contribute to the accelerated atherosclerosis of CRF patients, in addition to the numerous other mechanisms that have already been identified or that have been postulated [1]. The relative implication of altered proliferation and apoptosis of the vascular endothelium and smooth muscle due to direct noxious effects on the blood vessel wall, and that of the attraction and activation of inflammatory cells such as polymorphonuclear blood cells and monocytes/macrophages, in this greatly stimulated process remains to be screened in appropriate animal models and in uremic patients.

In conclusion, among the numerous mechanisms that contribute to the imbalance between pro-oxidant factors and antioxidant defense systems in CRF, we propose that oxidation of LDL protein moiety plays a potentially important part, favoring pro-oxidant forces. This would lead to a vicious circle in which increased generation of oxidized apolipoprotein would induce the latter to stimulate macrophage activity, which, in turn, would allow the generation of further oxidative stress.

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