New insights into the effects of the protein moiety of oxidized LDL (oxLDL)

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New insights into the effects of the protein moiety of oxidized LDL (oxLDL). Oxidative stress has been implicated in the cardiovascular complications in chronic renal failure patients. Lipoprotein oxidation is involved in the genesis of atherosclerosis. Both the lipid and the protein moieties of low-density lipoproteins (LDL) are subject to oxidation. We have shown that oxidation of LDL by hypochlorous acid (HOCl) in vitro, reflecting increased myeloperoxidase (MPO) activity in vivo, leads mainly to modifications of apolipoproteins, such that the latter in turn induce high rates of apoptosis in a human monocyteic cell line via a caspase-dependent pathway. These in vitro oxidative changes of LDL protein moiety, if shown to occur to a significant extent in uremic patients in vivo, may represent an important pathway in the pathogenesis of atherosclerosis.

OXIDATION OF LOW-DENSITY LIPOPROTEINS (LDL) ON PROTEIN MOIETY: A NOVEL PATHWAY IN ATHEROGENESIS?

Lipoprotein oxidation is involved in the genesis of atherosclerosis. Several methods have been developed to promote LDL oxidation in vitro. However, it proved to be difficult to identify the mechanisms that are physiologically relevant in vivo. Both the lipid and the protein moieties of LDL are subject to oxidation, and both can be oxidized in vivo [1]. Oxidation of the LDL-lipid moiety is commonly thought to represent the initial step of oxidative LDL modification. Nevertheless, in vitro, this oxidative pathway requires the presence of high concentrations of free metal ions (e.g., copper or iron), and due to multiple redundant mechanisms for chelating redox-active free metal ions in vivo, the role of free transition metal ions has been questioned. Moreover, Heinecke’s group cannot find evidence of metal-induced lipoprotein damage at early stages of human atherosclerotic plaque formation [1]. Although lipid peroxidation could also lead to oxidative modification of apolipoprotein B100 (apo B100), the core protein of LDL, another pathway, namely the excessive generation of hypochlorous acid (HOCl), can transform the lipoprotein into a high-uptake form for macrophages without significant lipid (per)oxidation. Apo B100 is the main target for this oxidant [2]. A recent study indicated that HOCl also alters the physiologic properties of high-density lipoproteins (HDL) by generating a proatherogenic lipoprotein particle, particularly modified on the protein moiety [3]. The enzyme myeloperoxidase (MPO) catalyzes the production of HOCl from hydrogen peroxide (H₂O₂) and chloride in activated neutrophils and monocytes, which are present under inflammatory conditions. The importance of MPO as a potential in vivo oxidant is underlined by the presence of enzymatically-active MPO in human atherosclerotic lesions [4]. Moreover, it has been demonstrated that MPO is able to bind to LDL, enhancing site-directed oxidation of the lipoprotein [5]. Most importantly, specific HOCl-dependent oxidation markers were identified in the development of atherosclerotic lesions, strongly supporting the involvement of the MPO-H₂O₂-chloride system. Both mass spectrometry analysis [6] and immunohistochemical studies [7, 8] demonstrated that HOCl-generated protein oxidation products are present in human atherosclerotic lesions. The pathophysiologic relevance of protein oxidation products, such as plasma advanced oxidation protein products (AOPP), has already been documented in the context of coronary artery disease in nonuremic subjects [9].

PROTEIN OXIDATION PRODUCTS AND UREMIA

Oxidative stress has been implicated in the cardiovascular complications that affect chronic renal failure (CRF) patients [10]. We have previously shown that uremic patients, in addition to having several lipoprotein disturbances, present protein oxidation products, such as AOPP or carbonyls [11, 12]. An increase in plasma 3-chlorotyrosine level, another specific HOCl oxidation protein product, has been also demonstrated in chronic hemodialysis
patients [13]. The demonstration of markedly elevated concentrations of HOCl-modified protein in the plasma of CRF patients [12–15], and the presence of oxidatively modified lipoproteins in kidney vascular structures [16] would indicate enhanced MPO activity in blood and various tissues of uremic patients, including the arterial wall. In keeping with this, it has been shown that basal plasma MPO activity levels of patients on regular hemodialysis treatment were higher than those of healthy controls [17]. Of note, we recently found that plasma AOPP was associated with common carotid artery intima-media thickness (CCA-IMT), a process probably exacerbated by intravenous iron administration in patients undergoing epoetin therapy [18]. This observation supports the role of protein oxidation products in the early atherosclerosis of dialysis patients.

DIVERSE BIOLOGICAL EFFECTS OF HOCl-oxLDL

Growing evidence shows that HOCl-oxLDL exert biological functions distinct of those known for copper-oxLDL. Zabe et al [19] showed that HOCl-oxLDL has the potential for altering platelet function, with properties different from those of the copper-oxidized counterparts. Likewise, Woenckhaus et al [20] demonstrated that oxLDL generated by metal ion catalysis predominantly release monocyte chemotactic factors, such as MCP-1 (monocyte chemotactic protein-1), whereas HOCl-oxLDL induce the secretion and synthesis of the chemokine interleukin 8 in human monocytes. Moreover, we showed in our laboratory that HOCl-oxLDL were more potent than copper-oxLDL in inducing macrophage respiratory burst and retained this activating potential even after delipidation. This observation is in support of a crucial role of the protein moiety of oxLDL in the macrophage-mediated oxidative injury involved in atherogenesis [21].

Atherosclerosis can be considered as a chronic inflammatory disease associated with enhanced apoptotic cell death in vascular cells, including smooth muscle cells [22], endothelial cells [23], and monocyte-macrophages [22]. This type of apoptosis is partly induced by oxLDL. In these studies, the latter are classically produced by incubation with copper sulfate. In a recent study [24], we tested the hypothesis that oxidation of LDL-protein moiety might induce monocyte-macrophage apoptosis, as this has been described for oxidative changes of the LDL-lipid moiety [22]. As shown in Fig. 1, HOCl-oxLDL induce high rates of apoptosis in the human monocytic THP-1 cell line proportionally to both the degree of LDL oxidation (data not shown) and the concentration of LDL protein via a caspase-dependent pathway. The type of cell death occurring in atherosclerotic lesions may be of importance. Apoptosis is widely recognized as a “clean” death because apoptotic cells are rapidly engulfed without inducing an inflammatory response. However, this dogma was recently challenged by studies showing Fas-mediated activation of several pro-inflammatory genes during the apoptotic process [25]. In addition, with regard to atherosclerotic plaques, recent in vitro studies have suggested that the removal of apoptotic cells may be inefficient in such a complex tissue [26]. Moreover, apoptosis, through its procoagulant and proadhesive potentials, may play a critical role in both plaque and blood thrombogenicity, and may be an important step in the transition from stable to unstable atherosclerotic disease [27]. In keeping with this, an increased number of MPO-expressing macrophages is detected in eroded or ruptured plaques, causing acute coronary syndromes, but not in human fatty streaks, supporting a possible role of protein oxidation products in atheroma complications, in particular, acute coronary syndromes [28].

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