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Review Article

Proatherogenic effects of 4-hydroxynonenal

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ABSTRACT

4-hydroxy-2-nonenal (HNE) is a α,β-unsaturated hydroxyalkenal generated by peroxidation of n-6 polyunsaturated fatty acid. This reactive carbonyl compound exhibits a huge number of biological properties that result mainly from the formation of HNE-adducts on free amino groups and thiol groups in proteins. In the vascular system, HNE adduct accumulation progressively leads to cellular dysfunction and tissue damages that are involved in the progression of atherosclerosis and related diseases. HNE contributes to the atherogenicity of oxidized LDL, by forming HNE-apoB adducts that deviate the LDL metabolism to the scavenger receptor pathway of macrophagic cells, and lead to the formation of foam cells. HNE activates transcription factors (Nrf2, NF-kappaB) that (dys)regulate various cellular responses ranging from hormetic and survival signaling at very low concentrations, to inflammatory and apoptotic effects at higher concentrations. Among a variety of cellular targets, HNE can modify signaling proteins involved in atherosclerotic plaque remodeling, particularly growth factor receptors (PDGFR, EGFR), cell cycle proteins, mitochondrial and endoplasmic reticulum components or extracellular matrix proteins, which progressively alters smooth muscle cell proliferation, angiogenesis and induces apoptosis. HNE adducts accumulate in the lipidic necrotic core of advanced atherosclerotic lesions, and may locally contribute to macrophage and smooth muscle cell apoptosis, which may induce plaque destabilization and rupture, thereby increasing the risk of athero-thrombotic events.

1. Introduction

Atherosclerosis and its cardiovascular complications are a major cause of morbidity and mortality and a worldwide economical problem. Atherosclerosis is a chronic metabolic and inflammatory disease of large and medium sized arteries, that silently and progressively evolves over decades and can be complicated by acute vascular events such as myocardial infarction or stroke [1,2]. Several risk factors contribute to atherogenesis, including inevitable systemic factors (aging, genetic susceptibility, gender) and classical risk factors (hypercholesterolemia, obesity, diabetes mellitus, hypertension), that can be modified through pharmacological approaches and lifestyle changes [2,3]. Among the

Abbreviations: ABCA1, ATP-binding cassette 1; AGEs, advanced glycation end products; AIF, apoptosis inducing factor; ALDH, aldehyde deshydrogenase; ANT, adenine nucleotide translocator; APAF1, apoptosis protease-activating factor-1; apoB, apolipoprotein B; ARE, antioxidant response element; Bcl2, B-cell lymphoma 2; BH4, tetrahydrobiopterin; Bid, BH3 interacting-domain death agonist; Bip/GRP78, binding immunoglobulin protein; BVH, bisvanillyl-hydralazone; CDK2, Cyclin-dependent kinase 2; CHOP, CCAAT-enhancer-binding protein homologous protein; COL1A1, collagen type alpha 1 chain; DISC, death-inducing signaling complex; DNPH, dinitrophenylhydrazine; ECM, extracellular matrix; EGF, epidermal growth factor; e-NOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ERK1/2, extracellular signal regulated kinase 1/2; Fas L, Fas ligand; GSH, glutathione; HDL, high density lipoprotein; 4-HHE, 4-hydroxy-2-hexenal; His, histidine; HMEC-1, human microvascular endothelial cells type 1; HNE, 4-hydroxy-2-nonenal; HO-1, heme oxygenase-1; Hsp70, heat-shock protein 70; HUVEC, human umbilical vein endothelial cells; IKK, IκBα kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; IRE1α, Inositol-requiring enzyme 1; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like ECH Associated Protein 1; KLH, keyhole limpet hemocyanin; LDL, low density lipoprotein; LDLR, LDL receptor; LOX-1, Lectin-like oxidized low-density lipoprotein receptor-1; 5-LO, 5-lipoxygenase; LPC, lysophasphatidyl choline; LPP, lipid peroxidation product; LPC, lysophosphatidyl choline; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinases; MDA, malondialdehyde; MMP, matrix metalloprotease; MPTP, membrane permeability transition pore; NAC, N-acetyl cysteine; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; NOX, NADPH oxidase; Nrf2, NF-E2-related factor 2 or Nuclear factor (erythroid-derived 2)-like 2; oxLDL, oxidized LDL; PARP, poly(ADP-ribose) polymerase; PDGF, platelet-derived growth factor; PDI, protein disulfide isomerase; PERK, protein kinase RNA-like ER kinase; PI3K/Akt, phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt); PPAR, peroxisome proliferator-activated receptor; Prx1, peroxyredoxin-1; PUFA, polyunsaturated fatty acid; RCC, reactive carbonyl compound; ROS, reactive oxygen species; S1P, sphingosine 1-phosphate; Shc, C-terminal Src homology; SK1, sphingosine kinase 1; SMC, smooth muscle cell; SR-A, class A scavenger receptor; TGFβ, transforming growth factor β; TXNIP, thioredoxin-interacting protein; TKR, tyrosine kinase receptor; TLR4, Toll-like receptor 4; TRAIL, TNF-related apoptosis-inducing ligand; Trx1, thioredoxin-1; UPR, unfolded protein response; UPS, ubiquitine-proteasome system; UV, ultra-violet radiations; VEGF, vascular endothelial growth factor

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various mechanisms involved in atherogenesis, the oxidative theory of atherosclerosis relies on the oxidation of low density lipoproteins (LDL) in the vascular wall and their implication in the formation of early atherosclerotic lesions [4–7].

Reactive oxygen species (ROS) and oxidants generated by activated endothelium are thought to initiate LDL oxidation, followed by auto-oxidation cycles that generate a huge variety of lipid peroxidation products (LPPs), exhibiting atherogenic, pro-inflammatory and pro-apoptotic properties [4,7–9]. Reactive carbonyl compounds (RCCs), including various aldehydes, are a family of highly reactive agents generated during polyunsaturated fatty acid (PUFA) peroxidation [10,11]. RCCs covalently bind to nucleophilic group of proteins, peptides, phospholipids and nucleic acids, thereby generating a "carbonyl stress" [10,12]. Among RCCs, 4-hydroxy-2-nonenal (HNE) is a highly reactive α,β-hydroxyalkenal, generated by the peroxidation of n-6 PUFA (arachidonic and linoleic acid), and one of the most extensively studied LPPs [13,14]. HNE is an electrophilic molecule that reacts non-enzymatically with histidine, cysteine, or lysine residues of proteins, leading to the formation of Schiff bases or stable Michael adducts with a hemiacetal structure [13,15,16]. Both Schiff bases and Michael adducts contribute to the cross-linking properties of HNE on proteins.

HNE exerts its atherogenic effects through several mechanisms, by targeting lipoproteins or cellular components. HNE generated during LDL oxidation is able to form HNE-apoB adducts, which are recognized by scavenger-receptors of macrophagic cells, thereby leading to foam cell formation. Moreover, HNE can be released during the degradation of oxLDL, or generated through oxidative stress and PUFA peroxidation in cell membranes [12,17,18].

The biological effects of HNE on vascular cells depend on its local concentration and on the expression of detoxifying systems, such as glutathione S-transferase, aldose reductase, and aldehyde dehydrogenase (ALDH), which rapidly neutralize and remove HNE from cells [19–21]. Physiological concentrations (0.1–1 μmol/L) of HNE induce hormetic responses, such as stimulation of adaptive responses and increase of cell resistance to oxidative attack and other stresses [16,22]. Moderate HNE concentrations, ranging from 1 to 10 μmol/L, trigger the accumulation of HNE-adducts and a variety of biological responses, such as inflammation and cell proliferation. Higher HNE concentrations, above 10–20 μmol/L, can induce cell dysfunction and apoptosis under condition of high oxidative stress [17,18,22,23]. However, important variations are observed in atherosclerotic lesions, from the lipid core to the periphery of the plaque, with very different local outcomes [11,17,18,22,23].

In this review, we present the mechanisms by which HNE and related aldehydes contribute to atherogenesis and may aggravate the outcomes of atherosclerotic lesions.

2. HNE and apoB modification in early atherogenesis

2.1. LDL oxidation and HNE generation

LDL oxidation in the intima, is a complex mechanism initiated by a local activation of endothelial cells that increases endothelium permeability, promotes the passage of LDLs in the intima, their retention on ECM (glycosaminoglycans and proteoglycans) and their oxidation by activated cells of the arterial wall [24,25]. The mechanism of LDL oxidation *in vivo* is probably not unique, and may result from several sources of ROS and oxidants, such NADPH oxidases, the mitochondrial electron transport chain, cellular lipoxygenases, myeloperoxidase, heme and iron and copper ions [7,26]. ROS production is increased by oxLDL uptake through scavenger receptors expressed by vascular cells such as the Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), which is present on endothelial cells and stimulates the expression and activation of NOX2 [27–29]. Transition metals such as copper or iron, or the presence of ceruleoplasmin, may enhance *in situ* the oxidation process, as evidenced by the presence in advanced lesions of LPPs issued from metal-catalyzed oxidation [7]. LDL oxidation can be induced *in vitro* by various systems generating free radicals, such as xanthine oxidase, organic free radicals, peroxides including PUFA peroxides, UV radiations (UV-C). Moreover, LDL oxidation occuring in the intima can be mimicked by incubating LDLs with cultured vascular cells or macrophages [7,30].

PUFAs in LDLs are a main target of the oxidative attack, which occurs in three phases during copper-induced LDL oxidation: 1/ the initial event is the consumption of endogenous antioxidants (tocopherols, carotens) present in LDL. The initiation of lipid peroxidation by free radicals consists in the attack on a double bond that generates highly reactive lipid peroxide radicals; 2/ amplification of oxidative attack when PUFA peroxide radical attacks another near PUFA, thereby initiating a novel cycle of peroxidation and 3/ termination of the process, when all the substrate has been comsumed. A huge variety of oxidized lipids are generated through LDL oxidation, in particular RCCs, such as dicarbonyl aldehydes (MDA), saturated (ethanal, propanal, hexanal), unsaturated aldehydes (acrolein) and highly reactive hydroxyalkenals. HNE, the most abundant α,β unsaturated hydroxyalkenal generated during LDL oxidation, is derived from n-6 PUFAs, while 4-hydroxyhexenal (HHE) is derived from n-3 PUFAs [12,13].

2.2. HNE-modification of apoB and proteins in foam cells and atherosclerotic lesions

2.2.1. ApoB modification and uptake by macrophages

LDL oxidation is a progressive process that forms a continuum of oxLDLs, ranging from minimally to mildly oxidized LDLs and extensively oxidized LDLs. Minimally oxLDLs and mildly oxLDLs are mainly altered in their lipid moiety, whereas extensively oxLDLs exhibit both lipid oxidation and apoB modification by aldehydes [7,31].

During increasing LDL oxidation, both structural (*e.g.* oxidized lipid content, apoB modification) and physicochemical (*e.g.* density, charge) properties of oxLDLs become progressively more severe. For instance, during the progressive oxidation of oxLDLs, apoB modification increases, thereby reducing the affinity for the receptors of vascular cells and macrophages. While native (non oxidized) LDLs are taken up through the LDL receptor pathway [32], minimally oxLDLs are taken up *via* the LDL receptor [33] and/or *via* LOX-1 that recognizes oxidized phospholipid epitopes [34]. Finally, extensively modified LDLs (acetylated LDLs, MDA-modified LDLs) and extensively oxLDLs are no longer recognized by the LDL receptor, but are avidly taken up by macrophagic cells through various scavenger receptors [35–38].

The uptake of mildly oxidized LDLs by macrophages also occurs through the scavenger receptor CD36, which is involved in oxLDL uptake and foam cell formation [39,40]. Extensively oxLDLs with apoB modified by aldehydes such as MDA and HNE, are taken up by the Class A scavenger receptor (SR-A) pathway in macrophages, and are involved in foam cell and fatty streak formation [40,41]. Pioneer reports from Goldstein and Brown have demonstrated that acetylated LDLs are more negatively charged than native LDLs, and cause the accumulation of cholesterol esters in macrophages, *i.e.* generate foam cells [32]. Interestingly, the exposure of J774A.1 macrophages to HNE stimulates the expression of CD36, possibly through 5-lipoxygenase (5-LO) activation by p38MAPK, which increases oxLDL uptake [42]. HNE also directly stimulates the expression of SR-A [43], thereby indicating that HNE contributes to foam cell formation and atherosclerosis progression.

LDL modification occuring during LDL oxidation is mimicked by incubating LDLs with malondialdehyde (MDA), which reacts specifically with the ε-aminogroups of lysine residues involved in the recognition of LDLs by the LDL receptor [44]. HNE is more effective than MDA to modify LDLs. It increases their negative charge, their relative electrophoretic mobility and the global molecular weight of apoB cysteine [45]. HNE-modified LDLs exhibit a decreased affinity for the apoB receptor [46], are taken up by macrophages and generate foam cells [47]. LDL modification by HNE has been extensively studied by