Mechanisms of Ageing and Development xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Oxidized LDL and NO synthesis—Biomarkers of endothelial dysfunction and ageing

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
Oxidized LDL
Nitric oxide
Ageing
Biomarker
Endothelial dysfunction

ABSTRACT

Oxidized LDL (oxLDL) and nitric oxide (NO) exert contradictory actions within the vascular endothelium microenvironment influencing key events in atherogenesis. OxLDL and NO are so far regarded as representative parameters of oxidative stress and endothelial dysfunction, new targets in prevention, diagnosis and therapy of cardiovascular diseases, and also as candidate biomarkers in evaluating the human biological age. The aim of this review is to explore recent literature on molecular mechanisms and pathophysiological relationships between LDL oxidation, NO synthesis and vascular endothelium function/dysfunction in ageing, focusing on the following aspects: (1) the impact of metabolic status on both LDL oxidation and NO synthesis in relation with oxidative stress, (2) the use of oxidized LDL and NO activity as biomarkers in human studies reporting on cardiovascular outcomes, and (3) evidences supporting the importance of oxidized LDL and NO activity as relevant biomarkers in vascular ageing and age-related diseases.

"longevity is a vascular question. A man is as old as his arteries"

William Osler, 1892

"It has been said that one is as old as one's arteries. In view of the supreme importance of endothelium in arterial function, I should like to modify. . . this statement by saying that

one is as old as one's endothelium."

Rudolf Altschul, 1954

1. Introduction

The vascular endothelium, with its broad spectrum of paracrine and autocrine functions, can be regarded as a multifunctional organ and "chief governor" of body homeostasis. Occupying a strategic location between the blood and tissues, the endothelial cells exist in a "high-risk position" and react progressively to aggressive factors, at first by modulation of the constitutive functions: permeability (*i.e.*, increased transcytosis of lipoproteins) and biosynthesis (*i.e.*, enhanced synthesis of the basement membrane and extracellular matrix) (Simionescu and Antohe, 2006; Sima et al., 2009). Even though the endothelial cells are resourceful cells that have the functional-structural attributes to adapt to the ever-changing surrounding milieu, to use innate mechanisms to confront and defend against insults, the ageing process induces a progressive failure of protective mechanisms, leading to vascular alterations (Dantas et al., 2012). It is becoming evident that ageing results in well-defined phenotypic changes and, as a consequence, a heightened susceptibility of the cardiovascular system to diseases, even in absence of traditional risk factors (*e.g.*, hypertension, hypercholesterolemia, diabetes, and smoking). Moreover, age-related alterations in cellular homeostatic mechanisms also impact the

Abbreviation: LDL, low-density lipoprotein; oxLDL, oxidized low-density lipoprotein; LOX-1, lectin-like oxidized LDL receptor-1; FFA, free fatty acids; ApoB-100, apolipoprotein B-100; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; ROS, reactive oxygen species; $O_2^{\bullet -}$, superoxide anion; $HO^{\bullet}$, hydroxyl radical; $LO^{\bullet}$, alkoxyl radical; $LOO^{\bullet}$, peroxyl radical; $ONOO^-$, peroxynitrite; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; NF-kB, nuclear factor κB; $p66^{Shc}$, 66-kDa isoform of Shc adaptor protein; BH_4, tetrahydrobiopterin; Dyslip, dyslipidemia; Hypergly, hyperglycemia; IR, insulin resistance.

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http://dx.doi.org/10.1016/j.mad.2015.03.003
0047-6374/

aged vasculature making it more liable to the damaging effects of the traditional pathophysiological conditions (Ungvari et al., 2010).

Endothelial dysfunction, a systemic pathological state defined as imbalance between vasodilating and vasoconstricting compounds produced by and acting on the endothelium, precedes development of atherosclerosis, leading to reduced vasodilation, pro-inflammatory and pro-thrombotic states (Deanfield et al., 2007).

In the last three decades, oxidation of low density lipoproteins (LDL) and nitric oxide (NO) synthesis have been discovered in parallel, studied extensively and considered as important mechanisms contributing to endothelial dysfunction, vascular ageing and disease. Oxidized LDL and NO exert contradictory actions within the vascular endothelium microenvironment influencing key events in endothelial dysfunction and atherogenesis such as: leukocyte adhesion, platelet aggregation and vascular smooth-muscle cell proliferation and migration. While oxidized LDL (oxLDL) – an oxidative stress biomarker has been identified as a non-traditional, pro-atherogenic emerging risk factor for coronary heart disease, NO is a free radical signal-transducing molecule that maintains the vasodilating tone, modulates in vitro lipid peroxidation reactions and alters pro-inflammatrory gene expression (Holvoet et al., 2008a,b; Borsa et al., 2012).

The LDL oxidation and NO activity are so far regarded as representative parameters of oxidative stress and endothelial dysfunction, new targets in prevention, diagnosis and therapy of cardiovascular diseases, and also as candidate biomarkers in evaluating the human biological age (Rodriguez-Manas et al., 2009; Verhoye and Langlois, 2009; Maiolino et al., 2013a,b; Zuliani et al., 2012; Paik et al., 2013; Burkle et al., 2015; Moreno-Villanueva et al., 2015a,b; Capri et al., 2015; Baur et al., 2015).

The aim of this review is to explore recent literature on molecular mechanisms and pathophysiological relationships between LDL oxidation, NO synthesis and vascular endothelium function/dysfunction in ageing, focusing on the following aspects: (1) the impact of metabolic status on both LDL oxidation and NO synthesis in relation with vascular oxidative stress, (2) the use of oxidized LDL and NO activity as representative biomarkers in human studies reporting on cardiovascular outcomes, and (3) evidences supporting the importance of oxidized LDL and NO activity as relevant biomarkers in vascular ageing and age-related diseases.

2. Relationships between NO synthesis and LDL oxidation in endothelial dysfunction

In the endothelial microenvironment, concurrently, a variety of substances that influence endothelial function have been recognized, but among them NO and oxLDL are the best characterized key players sharing significant antagonistic roles, and being involved in all phases of atherogenesis (Borsa et al., 2012).

Nitric oxide, a non-eicosanoid component of endothelial-derived relaxation factor (EDRF) is the most important vasodilating molecule being continuously synthesized by the endothelial constitutive isoform of nitric oxide synthase (eNOS and NOS III) under the action of different neurohumoral mediators such as acetylcholine and circulating hormones (catecholamines, vasopressin and aldosterone), plasma constituents (thrombin, sphingosine 1-phosphate), platelet products (serotonin, adenosine diphosphate), and autacoids (histamine, bradykinin and prostaglandin E4) (Michel and Vanhoutte, 2010).

In addition to maintenance of normal organ blood flow, endothelium derived NO has the following pleiotropic vasoprotective, cardioprotective and anti-atherogenic effects, summarized in numerous review articles (Michel and Vanhoutte, 2012; Ungvari et al., 2010; Jin and Loscalzo, 2010; Bermúdez et al., 2008): (1) prevents abnormal constriction (vasospasm) of the coronary arteries, which favors intraluminal clot formation and inhibits platelets aggregation and adhesion to endothelium surface (anti-thrombotic effect); (2) inhibits the release and action of the vasoconstrictor and mitogenic peptide endothelin-1, decreases endothelial permeability and reduces vessel tone, reducing lipoproteins flux into the vessel wall, vascular smooth muscle cell proliferation and migration (cell growth inhibition and anti-atherogenic effect) (Verma et al., 2003); (3) inhibits the NF-κB activation and determines a disruption of pro-inflammatory cytokine – induced signaling pathways; NO reduces the endothelial expression of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1(ELAM) (anti-inflammatory effect) (Rubio and Morales-Segura, 2004); (4) suppresses endothelial cell apoptosis and preserves the endothelial progenitor cell (EPC) function and regulation of tissue energy metabolism (anti-apoptotic, vasoprotective and cardioprotective effect) (Ungvari et al., 2010); (5) controls mitochondrial oxygen consumption and maintains cellular redox state. At physiological levels, NO is a highly reactive free radical that can also attenuate the metal/peroxide oxidative chemistry, as well as lipid peroxidation, and may limit oxidative injury to mammalian cells (antioxidative effect) (Wink et al., 2001; Müller et al., 2004).

Oxidative stress is one of the causative factors involved in ageing and pathogenesis of cardiovascular disease. While the chronological age is classified as major nonmodifiable risk factor for cardiovascular disease, the majority of modifiable atherosclerotic risk factors like hypertension, dyslipidemia, chronic hyperglycemia and cigarette smoking are real harmful stimuli that accelerate disease progression by augmenting the production of reactive oxygen species (ROS) (Nilsson, 2008).

The damaging effects of oxidative stress on cardiovascular system determine endothelial dysfunction through reduction in nitric oxide (NO) synthesis and bioavailability, inflammatory response, and lipid peroxidation. The endothelium is continuously exposed to various physiological molecules that may have a direct impact on nitric oxide actions (Chikani et al., 2004). Plasma lipoproteins, by virtue of their close interactions with endothelial cells in the vasculature and the susceptibility of their surface lipids to oxidative modification, are perfect biological "sensors" of oxidative stress in the arterial wall (Le, 2015). LDLs as main blood cholesterol carriers, containing relevant amount of polyunsaturated fatty acids (PUFAs) – major substrate for lipid peroxidation, are among various molecular targets the most affected by the oxidative stress associated with metabolic imbalance (hyperlipidemia, hyperglycemia, insulin resistance). Therefore, the oxidative modification hypothesis of atherosclerosis recognizes the crucial role of oxLDL as a byproduct of LDLs exposure to ROS (Steinberg and Witzum, 2010).

OxLDL promotes endothelial dysfunction and contributes to the atherosclerotic plaque formation, progression and destabilization, by several mechanisms described in numerous recent review articles (Maiolino et al., 2013a,b; Pirillo et al., 2013; Xu et al., 2012; Le, 2015): (1) chemotactic recruitment, activation, and proliferation of monocytes/macrophages in the arterial wall, through the induction of the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular-cell adhesion molecule-1 (VCAM-1), thus stimulating their binding to endothelial cells; (2) its identification and rapid uptake by macrophages, followed by foam cells formation; (3) stimulation of smooth muscle cells (SMCs) migration and proliferation in the tunica intima, following the increase of the expression of growth factors, such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF) by endothelial cells and macrophages. Subsequently, oxLDL stimulate collagen production by SMCs and increase secretion of matrix metalloproteinases 1 and 9 (MPP-1 and MPP-9) inducing SMCs apoptosis; (4) cytotoxicity exerted mainly on the endothelial cells, which

Please cite this article in press as: Gradinaru, D., et al., Oxidized LDL and NO synthesis—Biomarkers of endothelial dysfunction and ageing. Mech. Ageing Dev. (2015), http://dx.doi.org/10.1016/j.mad.2015.03.003