Chapter 1

Introduction

This background information provides our research question and rationale including the specific aims and predictation as following: oxidative modification of human low-density lipoproteins namely oxidized LDLs (oxLDLs) are believed to play a key role in the initiation and development of atherosclerosis (Gotto & Grundy, 1999). Strong evidences support are:

1). Monocytes/macrophages in culture take up oxLDL much more rapidly than they take up native LDL

2). oxLDL is present in plasma and in atherosclerotic lesions

3). oxLDL exhibits various of biological properties that would be proatherogenic factor

4). Severity of atherosclerosis in a number of animal models can be significantly ameliorated by treatment with a variety of antioxidant compounds

5). Gene targeting studies implicate a number of proteins believed to be involved in LDL oxidation and atherogenesis

In vivo, less amount of LDL is oxidized in the circulation; rather, LDL is thought to become oxidatively modified within the arterial wall, perhaps in the subendothelial space, where LDL particles are sequestered in a pro-oxidant environment. Our observation in coronary heart disease, hypertension and diabetes mellitus patients, their LDLs are susceptible to oxidize when challenged with $CuSO_4$ resulting short lag phase of conjugated diene formation (not published data). Furthermore, when we assessed their lipid peroxidation and protein oxidation, mildly degree oxidation has been shown. Recently, data from large prospective randomized clinical trials failed to demonstrate beneficial cardiovascular effects of antioxidants, which may have pro-oxidant properties with harmful and deterioused interaction. We have raised a question about “oxidative modification of LDL” theory and revisit the oxidative stress mechanisms that may be involved much more broadly in atherosclerosis.

oxLDLs influence cellular properties principally by attaching to specific receptors called “scavenger receptors” or SRs. Several oxLDL receptors have been identified so far including CD36, SR-A, SR-PSOX and lectin-like oxidized LDL (LOX-1). Among them, LOX-1 is characterized as the major receptor for oxLDL in endothelial cells of large arteries and veins, smooth muscle cells, fibroblasts, macrophages and platelets. The potential role of LOX-1 in atherosclerosis was proposed: (1) LOX-1 possesses a strong activity in binding, internalizing and proteolytically dedrading oxLDL (2) oxLDL upregulates LOX-1 expression leading to endothelial dysfunction/apoptosis (3) LOX-1 is dynamically upregulated by pro-atherogenic condition such as hypertension and shear stress etc and (4) LOX-1 is found in atheroma derived cells and is accumulated in human and animal atherosclerotic lesion *in vivo.*

Growing evidences indicate that upregulation of LOX-1 expression activated by oxLDL is mediated reactive oxygen species (ROS) especially superoxide anion and hydrogen peroxide (Cominacini, et al., 2000) and leads to vascular dysfunction (Li, Saldeen, Romeo, & Mehta, 2000). Moreover, downregulation of eNOS, enzymatic produced nitric oxide, is presented resulting endothelial dysfunction. Superoxide anion is also reacting with nitric oxide, a reactive nitrogen species, to form peroxynitrite. Both of reactive oxygen species (ROS) and reactive nitrogen species (RNS) led to oxidative modification of lipid, proteins and nucleic acids which then contributed to the pathophysiology process. However, this concept has changed in recent years with the recognition that these biomolecules are able to play a crucial role in signal transduction through specific modification of signaling proteins and called “redox signaling”. In fact, physiological level of ROS/RNS is certainly needed for cell function such as endothelial function to protect the vasculature. The apparent paradox in the roles of ROS/RNS as essential regulator of cellular functions and as toxic by products of metabolism may be, at least in part, related to difference in the species and concentration. For example, nitric oxide, which has both regulatory functions and cytotoxic effects depending on the enzymatic source and relative amount of nitric oxide generated.

Our considerable attention is which of species and regulatory redox signalings of ROS/RNS concerning three different degrees of LDL oxidation: mildly, moderately

and fully oxidation involved in the process of atherosclerosis. Meanwhile, we use the culture organ (vasculature) as the powerful model that composes of endothelium, smooth muscle cell, adventitial fibrous and extracellular matrix. The culture organ will provide interactive and co-operation function of those cells. Furthermore, we use iron chelators and antioxidant compound pretreatment in order to examine the ROS/RNS system as well. Our hypothesis focuses on in which species of ROS/RNS influence the vascular dysfunction through redox-sensitive signaling. Our specific aims are followed:

1). To determine the effect of oxLDL in various degree oxidation and doses on LOX-1 mRNA expression

2). To investigate ROS species involved in the response of LOX-1 expression activated by oxLDL in various degree oxidation and doses

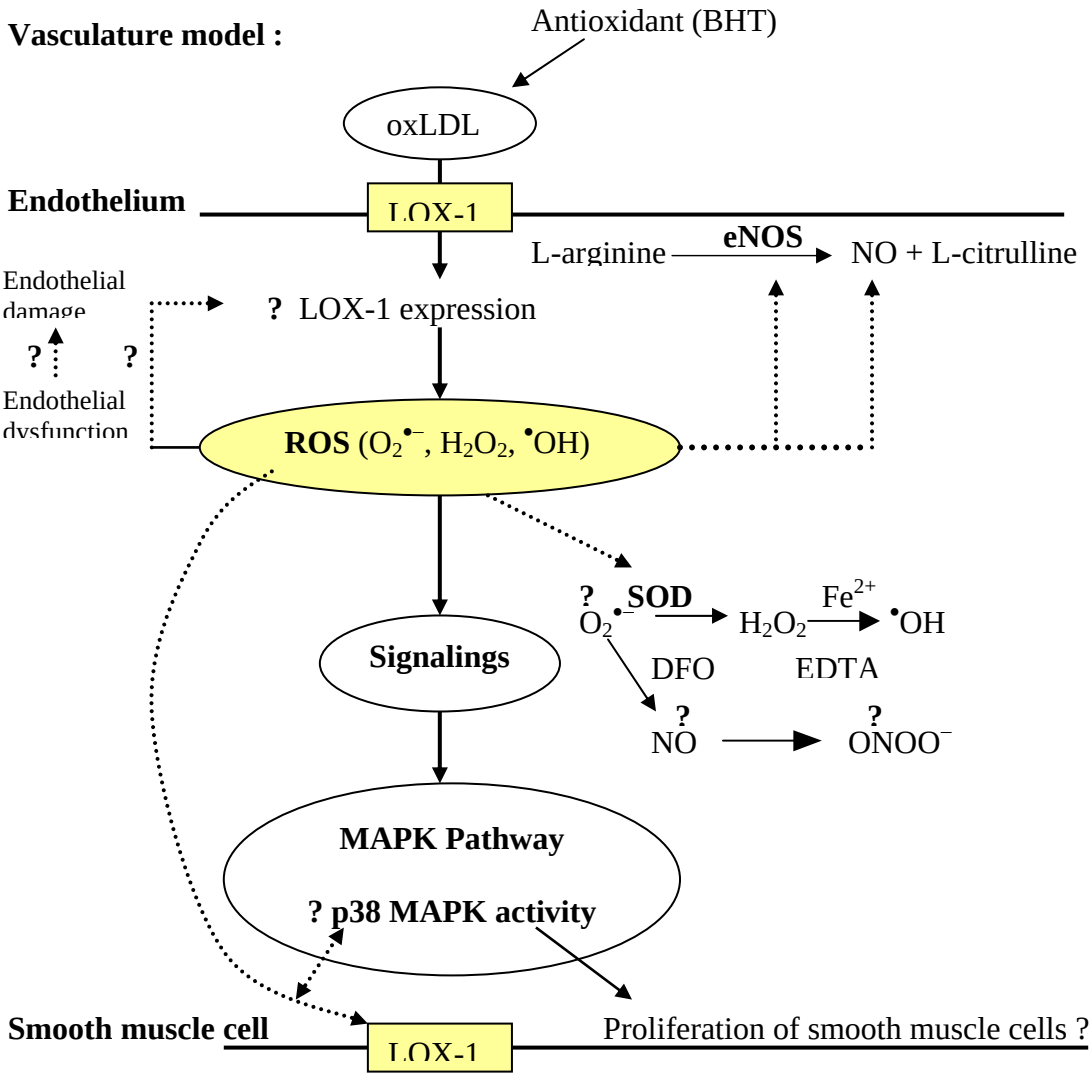
3). To determine the activity of superoxide dismutase (SOD) in order to estimate superoxide anion in the system.

4). To determine the eNOS mRNA expression and real-time nitric oxide generated for estimation of peroxynitrite formation and signaling

5). To study the role of ROS/RNS on redox-sentitive protein, P38 MAPK activity

6). Finally, to determine the structural changes of those perturbation

Scope of work in this study is present below.



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solid line indicates acceptable path