OXIDATION LOW DENSITY LIPOPROTEIN AND ITS PATHOBILOGICAL CONSEQUENCE

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Abstract

Atherosclerosis was believed to be an inevitable consequence of aging. In the early eighties, oxidation of lipoproteins as a cause of atherosclerosis was proposed. It is now recognized that oxidized Low Density Lipoprotein (LDL) is not only a biomarker of atherosclerosis, but also a cause for it. LDL is a complex particle made up of cholesterol, cholesterol ester, phospholipids, triglycerides and one molecule of the protein Apo B. Since LDL and its components are susceptible to oxidation to varying extents, the actual species of oxidized LDL that is atherogenic in currently unidentified. Although oxidized LDL is detected in atherosclerotic plaques, the mechanism of in vivo oxidation has remained elusive. The only mechanism that has been deduced is from its in vitro oxidation, LDL undergoes distinct phase of oxidation in vitro and progresses through minimally modified LDL to completely oxidized LDL. The presence of unsaturated fatty acids in LDL make them more susceptibility to oxidation. With the surprising discovery of fatty streaks in the arteries of even newborn babies, it is evident that everyone is born with a possibility of developing

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Atherosclerosis is a word derived from the Greek languages; ‘arteria’ meaning gruel and ‘sclerosis’ meaning hardening. Atherosclerosis or hardening of gruel like substance in the arteries results in reducing the diameter of the arteries, and was believed to be an inevitable consequence of aging. In 1815, Hodgson published a monograph when he claimed that inflammation was the underlying cause of atherosclerosis (Hodgson 1815). In 1858, Virchow reported the presence of inflammatory cells in the atherosclerotic lesion and proposed that local injury to intima was the initiating stimulus for atherosclerosis (Meng 2005). However, the inflammation hypothesis was ignored for over a century in favor of the cholesterol theory which was put forth much later (Ravnskov 2002).

Several studies including Framingham study have shown that elevated serum cholesterol in an independent risk factor for heart diseases (Expert panel 2001, Heart Protection Study Collaborative Group 2002). However, for a given individual measurement of serum total cholesterol was useless in predicting the risk of heart diseases. Several studies showed that cholesterol did not correlate with degree of atherosclerosis (Oalmann et al. 1981, Sorlie et al. 1981, Stehbens 2001).

The discovery of lipoproteins in the blood carrying cholesterol and lipids gave a new lease to the cholesterol theory of heart diseases. High levels of low density lipoprotein (LDL) correlated with risk of cardiovascular diseases (CVD) better than high total cholesterol (Tomkins and Owens 2012). Hence LDL became the target of therapy for prevention of heart diseases (Steinberg et al. 1989). However, more than half of patients who had cardiac events were those whose LDL was in the optimal range.

In the early eighties the theory of oxidation as a cause of atherosclerosis was proposed (Parthasarathy 2010). According to this theory, LDL can undergo oxidation resulting in Oxidized LDL which is no longer recognized by the LDL-receptor, but is recognized by the scavenger receptor of macrophages.
It is now increasingly clear that oxidized LDL is not only a biomarker for atherosclerosis but also a cause for it. The ability of oxidized LDL to generate plaques has been investigated for over three decades. When LDL is oxidized in the intima, monocytes from the blood migrate to the intimal space to repair or remove the oxidized LDL. Repair mechanism involves monocyte/macrophage accumulation in an effort to clear cholesterol from the plaque. The macrophage does not have an LDL receptor and can only take up modified LDL. Modifications include oxidation, and glycation which can occur in the sub endothelial space. Once the macrophage takes up the LDL and becomes a foam cell it should be hypothetically be able to remove cholesterol to the reticulo-endothelial system for clearance but in the atherosclerotic process the macrophage gets trapped in the intima and is unable to escape due to the increase in size. When the LDL cannot be repaired it is removed by phagocytosis (Picard et al. 1992). These monocytes are unable to limit the level of oxidized LDL they engulf which results in an overload of lipids. The cells appear as “foam” filled and hence are called as foam cells. Foam cells are biologically active and signal the endothelium to recruit more monocytes. The smooth muscle cells also are recruited. Thus the process results in pushing up of the endothelial cell layer resulting in narrowing of arteries (Miller et al. 2003, Miller et al. 2005). Since LDL-oxidation can result in the initiation of this process, elevated LDL had a higher risk of elevated oxidized LDL. It is apparent that the LDL particle depends for its atherogenicity to a large extent on its ability to be modified. The modification that has drawn a lot of attention is oxidation. Essentially the release of free radicals is enhanced from conditions such as hyperglycaemia, ischaemia, and infection. The free radicals would be available to oxidise the LDL particle (Steinberg and Witzum, 2010). The major sites of oxidation on the LDL particle include Apo B and the polyunsaturated fatty acids but cholesterol and phospholipids can also be oxidised. There is one apo B molecule per particle and hence Apo B is considered a strong risk marker for atherosclerosis. The amount of oxidised phospholipid on LDL apo B100 appears to be a good marker of atherosclerotic progression (Ahmadi et al 2010).

The formation of atherosclerotic plaque requires that the LDL be attached to the endothelial surface. Once the LDL particle has been attached to the

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endothelial surface further changes must occur before the cholesterol-rich particle becomes part of the atherosclerotic plaque. LDL aggregation seems very important in this process. The mechanism whereby LDL particles fuse and coalesce into lipid droplets and thereby increase LDL in the artery wall is complex (Oorni et al 2000). Jayaraman et al., (2011) demonstrated that free fatty acids enhance LDL coalescence into lipid droplets. They suggest that lipid droplet formation contributes to the pro-atherogenic effects of FFA on LDL. Most of the lipids found in atherosclerotic plaques are present in lipid droplets and LDL derived small lipid droplets are prominent in atherosclerotic lesions (Asplund et al 2011). Modifications such as oxidation, lipolysis and proteolysis are prerequisites for droplet fusion. Enzymes from the Phospolipase A2 (PLA2) family hydrolyse phosphatidyl choline. Lipoprotein associated PLA2 preferentially hydrolyses oxidised phosphotidyl cholines in LDL and may promote fusion of LDL particles and thus contribute to its enhanced atherogenicity (Guyton and Klemp 1994). Thus lipoprotein associated Phospholipase A2 has emerged as a causative agent of atherosclerosis and as a new therapeutic target.

**LDL-subtypes and LDL-oxidation:**

Based on its density LDL has been sub classified into at least three classes. The large LDL, medium LDL and small dense LDL have been separated and classified (Austin and Krause 1995, Kraus and Burke 1982). The size of the LDL particle is about the same as the space between two adjacent endothelial cells, which is about 26nm. Particles of diameter less than 20.5nm are called as small dense LDL (Koba et al. 2008). These particles can easily pass through the gaps between endothelial cells and enter the intimal space where they can get oxidized. (Phillips et al. 2005) Although there is no clinically relevant method to measure the amount of small dense LDL, it has been shown that individuals with high density lipoprotein (HDL-C) less than 35mg/dl and Triglycerides more than 250mg/dl have small dense LDL. LDL particle when it has moved out of the blood into the intima, it is out of reach of antioxidants present in the blood. Hence it is susceptible to oxidative modification. An atherosclerotic plaque is an ideal location for the oxidation of LDL.
LDL-oxidation:

The LDL particle contains about 1600 molecules of cholesterol ester and about 170 molecules of triglycerides, which form a hydrophobic core of the particle. This is surrounded by about 700 molecules of phospholipids consisting mainly of Phosphatidylcholine(PC) and a small amount of sphingomyelin and lyso PC, and about 600 molecules of free cholesterol. This particle contains one molecule of the protein Apo B with 4536 amino acid residues. The LDL particle has about 2700 fatty acid molecules esterified with various molecules of the particle (Jialal and Devaraj 1996). About half of these fatty acids are polyunsaturated. Since the composition of the poly unsaturated fatty acid(PUFA) in the LDL particle is variable and since the particle contains varying amounts of antioxidants like α-Tocopherol, LDL oxidation proceeds by multiple mechanisms, particularly when the oxidation is induced by different oxidants(Steinberg and Witztum 2010). Thus it is difficult to define what is actually oxidized LDL since there can be a variety of lipid and protein oxidation products as well as changes in the physical structure and properties (Chehin 2001). Which of these is actually atherogenic is impossible to identify currently.

Mechanism of in vitro oxidation:

In vitro oxidation of LDL has been a convenient model to understand the in vivo process. Several studies have used Cu^{++} or Fe^{++} to initiate LDL oxidation in vitro (Quehenberger 1988). The oxidation of LDL has been shown to proceed through well defined phases. Once the oxidation has been initiated the lipids of the LDL particle undergo oxidation by free radical catalyzed mechanism (Young and McEneny 2001). The conjugated double bond of polyunsaturated fatty acids of the lipid molecules in the primary target of the oxidant. The reactive methylene group between the two double bonds loses a proton. This is the key step in the initiation of lipid oxidation. The unstable radical then rearranges the double bond to give a conjugated diene. This conjugated dienes absorbs UV light maximally at 234nm and provides a convenient method for monitoring the progression of oxidation (Lynch and Frei 1993).

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In the presence of antioxidants, the oxidative effect of pro oxidants can be minimized or abolished. Thus the antioxidant present on LDL particle can prevent the lipid oxidation and there will be no 234nm absorbing conjugated dienes formed. This phase is called as the lag phase. The length of this phase is decided by the amount of antioxidant present on the LDL particle and the nature of the oxidant (Aviram and Fuhrman 1998).

Once the antioxidants are consumed, the lipids of LDL get rapidly oxidized with a large increase in the absorbance at 234nm. This is called as propagation phase. The propagation phase reaches a plateau where no more 234nm absorbing substances are formed.

If the oxidation is continued further, more and more oxidation products of lipids will be formed by cleaving the conjugated dienes. Thus if oxidation is monitored further, there will be a decline in the conjugated dienes and this phase is called the decline phase. In this phase, the lipid degradation products would react with the protein part of the LDL particle. The amino groups of proteins are highly susceptible to reaction with lipid oxidation products like aldehydes and ketones. At this stage the LDL particle is no longer recognized by the LDL-receptor and hence it has to be cleared by the scavenger cells through binding the scavenger receptor.

**Oxidation of LDL in vivo:**

There is adequate evidence to suggest that the process of LDL oxidation seen in vitro studies is also taking place in vivo. Several cell types produce powerful oxidants as anti microbial defense. Mitochondrial oxidation can also produce oxygen free radicals. When pro oxidants are more than the anti oxidants, they can cause LDL oxidation. Thus pro inflammatory stimuli can initiate oxidative steps resulting in LDL oxidation.

**Conclusion:**

Whatever the cause of initiation of LDL-oxidation, it can be a powerful initiating factor for atherosclerosis. Once initiated the atherosclerosis progresses
until the end point is reached namely the death of the individual. Surprisingly, even new born babies were found to have the fatty streaks in their arteries (Napoli et al 1997). This implies that everyone is born with a possibility of developing atherosclerosis. The life style one chooses decides whether this regresses or progress of full blown atherosclerosis.

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