Introduction

Plasma lipoproteins are submicroscopic particles composed of lipid and proteins held together by noncovalent forces. Their general structure is that of a putative spheroidal microemulsion formed from an outer layer of phospholipids, unesterified cholesterol, and apolipoproteins, with a core of neutral lipids, predominantly cholesteryl ester and triacylglycerols. Although the microemulsion is the basic structural motif of lipoproteins, several different lipoprotein classes exist that differ in relative amount of lipids, in the protein/lipid ratio, and in the protein species present, resulting in differences in size, density, and electrophoretic mobility. Lipoproteins are generally classified by density into three major groups: very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Elevated levels of LDL-cholesterol and low concentrations of HDL-cholesterol are associated with increased risk for coronary artery disease (CAD).

Apolipoproteins

Apolipoproteins, the protein component of lipoproteins, are amphipathic in nature, in that they have both hydrophobic and hydrophilic regions, and can therefore interact with both, the lipids of the lipoprotein and the aqueous environment [1]. Because of the nature of these amphipathic regions apolipoproteins act as detergents, and have a major role in determining and stabilizing the size and structure of the lipoprotein particle.
Apolipoproteins can be grouped into two classes, the nonexchangeable apolipoproteins [apolipoprotein (apo) B-100 and apoB-48] and exchangeable apolipoproteins [apoA-I, apoA-II, apoA-VI, apoC-I, apoC-II, apoC-III, and apoE] [2]. The nonexchangeable apolipoproteins, i.e. apoB-48 and apoB-100, are highly insoluble in aqueous solutions and remain with the lipoprotein particle throughout their metabolism. On the other hand, the exchangeable apolipoproteins are soluble in aqueous solutions. It has been suggested that the levels of apoB, essentially the only protein component of atherogenic LDL, and apoA-I, the major protein component of antiatherogenic HDL, are better markers than LDL-cholesterol and HDL-cholesterol in the assessment of CAD risk.

The Role of High Density Lipoprotein (HDL) in Atherosclerosis

Epidemiological studies have firmly established an inverse correlation between plasma concentrations of HDL and their main apolipoprotein, apoA-I, and the risk of CAD [3-6]. The importance of low HDL-cholesterol level as a risk factor for incidence of CAD is underscored by the results of the Framingham Study, where 35% of CAD occurred in people with total cholesterol < 200 mg/dl [7]. Individuals who had a total cholesterol level of < 200 mg/dl, but had a HDL-cholesterol level of < 40 mg/dl, had the same high risk of CAD as individuals who had a total cholesterol level of 260 mg/dl [7]. Low levels of HDL-cholesterol increase CAD risk at every concentration of LDL-cholesterol [8]. Increase in total cholesterol level from 200 to 250 mg/dl doubles the risk of CAD whereas reducing HDL-cholesterol levels from 45 to 25 mg/dl also doubles the risk [9]. Therefore, for every 1.0 mg/dl increase in total cholesterol, the risk for CAD increases by 1% whereas for every 1.0 mg/dl decrease in HDL-cholesterol the risk of CAD increases by 2% to 3% [8,9]. However, a significant number of CAD events occur in subjects with normal LDL and HDL levels. Recent studies [10] suggest that the “quality” (i.e. anti-inflammatory property) of HDL may be more important than their “quantity” (i.e. their plasma levels).

The mechanisms whereby HDL protects against atherosclerosis have been the subject of intense investigation. Current knowledge suggests that HDL exerts its protective effect by a) inhibiting LDL oxidation [11,12], b) decreasing adhesion molecule expression resulting in diminished monocyte migration into the arterial wall [13], and c) via reverse cholesterol transport pathways by which excess cholesterol in the arterial wall is removed and is transported to the liver for elimination [14-20]. Thus, increasing the plasma concentrations of HDL and apoA-I and improving the “quality” of HDL are key steps in reducing atherosclerosis risk.

Current Approaches for Increasing the Levels of HDL and ApoA-I

The HDL-cholesterol concentration can be increased by drugs such as fibrates, statins, and niacin. Recently, the use of HDL and apoA-I as targets for the therapeutic intervention of atherosclerosis has gained momentum. Infusion or transgenic expression of human apoA-I results in increased HDL-cholesterol and protects against atherosclerosis in animal models [21-23]. In a more recent study, infusion of recombinant HDL consisting of apoA-I_{Milano} and lipid complexes has been shown to reduce atherosclerosis burden in humans [24]. Although the above approaches have contributed significantly to reducing atherosclerosis risk, the large amount of protein-lipid complex required for effectiveness limits their use as a therapeutic tool. ApoA-I has been hypothesized to exert its antiatherogenic property, in part, by its ability to increase
cholesterol efflux [25], activate the plasma enzyme lecithin:cholesterol acyltransferase (LCAT) [26], and scavenge lipid hydroperoxides from LDL [27]. These properties are associated with the lipid-poor form of apoA-I which is also referred to as the preβ HDL particle because of its mobility on agarose gels.

**ApoA-I Mimetic Peptides as Therapeutic Tools for Reducing Atherosclerosis**

Human apoA-I is a 243 residue protein containing tandem repeats of eight 22-mer class A amphipathic helices [2]. The secondary structural motif responsible for apoA-I lipid association, the amphipathic α-helix, has been extensively studied [1,2,28]. The amphipathic α helix is a common secondary structural motif in biologically active peptides and proteins, including apolipoproteins [29,30], hormones [31], venoms [32], and in amphibian skin secretions [33]. Based on detailed analysis of their physico-chemical and structural properties, the amphipathic helices were grouped into seven distinct classes (A, H, L, G, K, C, and M,) [28,30]. In this classification, class A represented the lipid-associating amphipathic domains of apolipoproteins [28]. The most distinctive feature of class A is the unique clustering of positively-charged amino acid residues at the polar-nonpolar interface of the amphipathic helix and negatively-charged amino acid residues at the center of the polar face [28,34]. This unique structure is responsible for the higher lipid association properties of certain apolipoproteins [2]. In contrast, venom peptides are class L amphipathic helices and have a wide hydrophobic face with a high hydrophobic moment value and a polar face of only cationic amino acids. Helical wheel diagrams of class A (18A) and class L (18L) are shown in Figure 1A and 1B, respectively. The structural basis for the different membrane actions of these proteins lies in the distribution of charged residues on the polar face of the amphipathic helix [28]. Both class A and class L helices (whether as proteins or peptides) are membrane active, but have different properties. In this context, specifically-designed small, apoA-I mimetic peptides offer a powerful and more effective therapeutic tool to combat atherosclerosis.
Recent studies from our laboratory [35,36] have shown that synthetic amphipathic peptides (5F and 4F) which have many of the properties of apoA-I can also be atheroprotective. They have anti-inflammatory properties and inhibit the formation of atherosclerotic lesions in mice. These peptides could act directly by any (one or more) of the following means: (a) increase reverse cholesterol transport, (b) increase LCAT activation, (c) scavenge oxidized lipids and free radicals, and (d) induce apoA-I expression. Administration of class A peptides (5F and 4F) into atherosclerosis-sensitive mice has shown that these peptides inhibit atherosclerosis, but without changing plasma cholesterol levels [35,36]. However, administration of a class A peptide (4F) into mice infected with influenza A virus increased the levels of apoA-I and HDL and decreased the levels of LDL compared to control mice exposed to influenza A virus [37]. Administration of peptide 4F to apoE-null mice induced the formation of an apoA-I enriched cholesterol-containing particle which was of preβ HDL size and mobility and had higher paraoxanase activity [38]. As a result, lipid hydroperoxides in LDL are reduced, HDL becomes anti-inflammatory, and the preβ HDL-like particle stimulates cholesterol efflux and reverse cholesterol transport from macrophages. The peptide 4F, which also decreases arterial macrophage traffic [37], restores the balance between nitric oxide and superoxide levels [39], and dramatically improves vasodilation in hypercholesterolemia [39]. In general, it has a therapeutic effect and prevents atherosclerosis in atherosclerosis prone mice, has anti-inflammatory properties, and improves the ‘quality’ of HDL.

A Pro-linked dimer of a model class A amphipathic helical peptide 18A, 18A-Pro-18A, also referred to as 37pA (with a primary amino acid sequence of 18A=DWLKAFYDKVAEKLKEAF), has been shown to be more effective than human apoA-I in activating LCAT [40]. Lytic peptides, because of their large hydrophobic face, interact with membranes and self-associate to form pores [41]. On the other hand, class A peptides have been shown to be nontoxic even at high micromolar concentrations [36]. While class L peptides are cytolytic at high concentrations, class A peptides stabilize cell membranes and also inhibit class
L-mediated cell lysis [42]. This difference in their properties has been ascribed to their shape [42]. However, recent studies from our laboratory have shown that both the class A peptide, 37pA and the class L peptide 18L, enhance the synthesis and secretion of apoA-I in HepG2 cells, albeit at different effective concentrations [43]. We have shown that the secreted apoA-I is predominantly in the form of small HDL-like particles with preβ mobility [43]. Although this ability to improve the quality of HDL is seen in some of the amphipathic helixes, as mentioned above, it is not seen in all amphipathic helical peptides [44]. We have recently shown that the position of the aromatic residues on the non-polar face of Class A peptides plays an important role in determining their anti-atherosclerotic and anti-inflammatory properties [45].

Conclusion

Development of new and more efficient strategies to increase the level of HDL by enhancing apoA-I production and/or reducing apoA-I catabolism is both timely and of great clinical importance. In this context, apoA-I mimetic peptides have demonstrated promise. Understanding the mechanism of action and the structure-function relationship of these peptides is important in designing new iterations of these peptides with improved therapeutic potential in this emerging field of HDL targeted therapy.

References

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