Physical and Chemical Properties of Plasma Lipoproteins

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Oxidized low density lipoprotein (LDL) is directly and indirectly involved in the development of atherosclerosis, and high density lipoprotein (HDL) plays a very important role in its defense. The physiological functions of LDL and HDL in atherosclerosis have been studied extensively, but the question of how physical and chemical properties regulate these physiological functions remains unanswered, and it is not known what degree of changes in the density, size and shape of LDL cause the loss of affinity to the LDL receptor and unlimited uptake to macrophages via scavenger receptors. Many epidemiological studies and in vitro experiments have shown antiatherogenic properties of HDL; however, attempts to prevent atherosclerosis by raising plasma HDL have not produced any successful results. This suggests the need to selectively raise a stronger antiatherogenic HDL subclass and verify experimental results by analyses of clinical data.

The present study was thus conducted to perform a systematic and detailed analysis of the sequential changes of the physical and chemical properties of LDL during oxidation in order to determine how those properties regulate the physiological functions, focusing on the changes in the buoyant density and particle size of LDL. An additional study focus was distribution of apolipoproteins among HDL particles, which would create HDL subclasses by their compositions and differentiates physiological significance of those particles in the cholesterol reverse transfer system, i.e., the particles containing apolipoprotein (apo)A-I but not containing apoA-II (Lipoprotein (Lp)AI) and the particles containing both of these apolipoproteins (LpAI:AII).

The sequential changes in physical and chemical properties of LDL during oxidative modification were investigated first, by the following methods. LDL isolated from fasting plasma was oxidized by incubation with human umbilical vein endothelial cells (HUVECs) (LDL 100 μg protein/ml in Ham's F10 medium, incubation at 37°C for 20 hr) or with copper ion (LDL 200 μg protein/ml and $CuSO_4$ 2.5 μM in phosphate buffered saline (PBS), incubation at 37°C for 0–20 hr). After oxidation, lipid and protein levels were measured, and each oxidized sample was subjected to analyses by electrophoresis and ultracentrifugation. A detailed analysis of chemical compositions

and an image analysis using transmission electron microscopy were carried out using samples incubated with copper.

The results revealed an increase in negative electric charge, the degradation of apoB, and a decrease in size accompanied by an increase in density as the oxidation process proceeded in both the HUVEC and copper ion oxidation methods.

The density (d) of oxidized LDL (OxLDL) induced by copper increased with the time of oxidization. The proportion of d>1.044 subclass was 63.8±8.1% after 2 hr of incubation with copper, demonstrating that denser LDL became more predominant with time. After 10 hr of incubation with copper, all LDL was present in the d>1.044 subclass. There were no significant changes in total cholesterol level or the total protein level throughout the incubation with copper. After 20 hr of incubation with copper, the triglyceride (TG) level decreased by 24%, and the lysophosphatidylcholine (LPC) level increased markedly and accounted for 44% of choline-phospholipid. The average diameter of OxLDL induced by copper showed the changes accompanied by the increase of deformed particles. The diameter decreased from 28.1 nm to 25.6 nm after 5 hr, but it increased to 27.2 nm at 20 hr of incubation with copper. The size and shape of the OxLDL tended to recover. These dynamic changes can be interpreted as a loss of fatty acyl groups from the core lipid components during oxidative degradation, along with an increase in LPC on the surface with a consequent remodeling and fusion of the particles.

Next, the data of CETP gene polymorphisms (1452G→A and D442G mutations), lipid levels, and apolipoprotein levels were reanalyzed using the fasting plasma collected in 1994–1995 from 314 Japanese individuals. The apolipoprotein ratio in LpAI:AII and the differences in physiological significance in reverse cholesterol transport and the metabolism of apoB-containing lipoprotein were examined.

The results clarified the following. (1) Regardless of the sex and CETP genotype of the subjects, any increase in HDL levels predominantly depended on the concentration of LpAI. (2) Regardless of sex, CETP genotype, and concentration of HDL cholesterol, human plasma contains a certain amount or more of LpAI:AII particles containing a fixed molar ratio of apoA-I to apoA-II, i.e., 2 to 1. (3) ApoA-I in LpAI was negatively correlated with all apoB-lipoprotein indicators (apoB, TG, nonHDL-C, and TG+HDL-C). In contrast, there was a smaller but positive correlation of apoA-I in LpAI:AII with all indicators of apoB-lipoprotein. (4) These correlations between HDL parameters and apoB were found independent of sex, but they were slightly influenced by CETP mutations with a reduced statistical significance.

The findings thus suggest that these two subclasses are functionally different for cholesterol transport and that LpAI is more antiatherogenic than LpAI:AII.

These findings contribute to a better understanding of the physiological roles of lipoproteins in atherogenesis, and they could lead to the development of new analytical methods and the design of strategies to lower the risk of atherosclerosis.