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The role of electronegative low-density lipoprotein in cardiovascular diseases and its therapeutic implications

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A B S T R A C T

Cardiovascular disease (CVD) is a health problem of great concern to both the public and medical authorities. Low-density lipoprotein (LDL) has been reported to play an important role in both the development and progression of CVD, but studies are underway to determine how LDL exerts its effects. In recent years, it has been found that LDL has several subfractions, each of which affects endothelial function differently; L5, the most electronegative fraction, has been shown to be unique in that it induces an atherogenic response. This review examines the current knowledge concerning the relationships between L5 and CVD and highlights the role of L5 in the pathophysiology of CVD, especially with regards to atherosclerosis.

Key words: L5, Cardiovascular disease, Electronegative low-density lipoprotein.

Introduction

Cardiovascular diseases (CVDs), such as arrhythmias, myocardial infarction, and stroke, are the leading cause of death in industrialized nations. The underlying causes of CVD are endothelial dysfunction and atherosclerosis [1]. Risk factors for CVD include arterial hypertension, hyperlipidemia, hypercholesterolemia, obesity, diabetes mellitus (DM), and chronic

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smoking [1]. The most useful approach for treating and preventing CVD is managing the patient's low-density lipoprotein (LDL) cholesterol level [2].

Lipoproteins can be classified as high-density lipoproteins (HDL), LDLs, intermediate-density lipoproteins, very low-density lipoproteins (VLDL), or chylomicrons, according to their respective density ranges when separated by ultracentrifugation, which depends on the protein and lipid composition of the lipoprotein [3]. Each lipoprotein has specific functions, which are determined by the apolipoprotein and lipid content and by the organ in which it is synthesized. Circulating LDLs are a very heterogeneous group of particles that show differences in composition, electrical charge, and density [4]. Low-density lipoprotein becomes more electronegative after undergoing modifications, such as misfolding and lipid peroxidation [5,6].

In several studies, the subfractions of LDL have been separated, and the molecular characteristics of these subfractions have been evaluated. A preliminary assessment of the chemical composition of atheroma-derived LDL from human aortas was performed by Hoff et al. [7]. Electropositive LDL (LDL[+]) and electronegative LDL (LDL[−]) can be separated by using anion-exchange chromatography, as previously described by Avogaro et al. [8]. The chemical and functional characteristics of these LDL subfractions were later described by others [9]. Chen et al. [10] were the first to show that human LDL can be chromatographically separated into 5 subfractions (L1-L5) that are increasingly electronegative (Fig. 1). Because the protein distribution and many properties of L5 and L1 are largely comparable to those of LDL(−) and LDL(+), respectively, we will sometimes use these expressions interchangeably within this article, depending upon which pair was investigated in the cited article.

When compared to the other LDL subfractions, L5, the most electronegative subfraction, was found to be unique in that it induces dysfunction in cultured vascular endothelial cells (ECs) [10–12]. L5 has been shown to have proinflammatory and cytotoxic effects on ECs and, thus, is thought to be more atherogenic than other LDL subfractions [13]. In addition, some studies have recently shown a positive relationship between the progression of CVD and the level of L5 [14]. These findings could help clinicians better guide patients' lifestyle choices and select appropriate actions to prevent or intervene in CVD. In this review, we discuss the characteristics of L5 and examine the relationship between this most electronegative LDL subfraction and CVD.

Separation of LDL subfractions

Various methods have been used to separate LDL. Our laboratory uses anion-exchange fast protein liquid chromatography (FPLC) to divide whole LDL into 5 subfractions [12,15]. Briefly, venous blood is withdrawn from the subjects into tubes that contain EDTA. The blood is centrifuged at low speed for 15 min at 4 °C, and the plasma is collected. The LDL (d=1.019–1.063) can then be isolated by performing preparative sequential ultracentrifugation. For this technique, potassium bromide (KBr, MW: 118.99 g/mol) is used to adjust the background density of the plasma to 1.019 g/mL, and then the plasma is centrifuged at 186,000*g* (40,000 rpm, radius of rotor is 103.8 mm) for 24 h at 4 °C. The top fraction and underlying clear region are removed, and the rest of the plasma is pooled. Again, KBr is used to adjust the background density of the pooled plasma to 1.063 g/mL. Ultracentrifugation is performed at 186,000*g* for another 24 h at 4 °C. The supernatant, which contains the LDL, is collected from the top part of the tube. The LDL sample is dialyzed for 24 hours in a cold room (4 °C) in 20 mM Tris–HCl buffer (pH 8) containing 0.5 mM EDTA to remove the KBr. This dialysis step is repeated 2 more times, and then the samples are stored in a dark place (eg, in tubes covered by aluminum foil) at 4 °C.

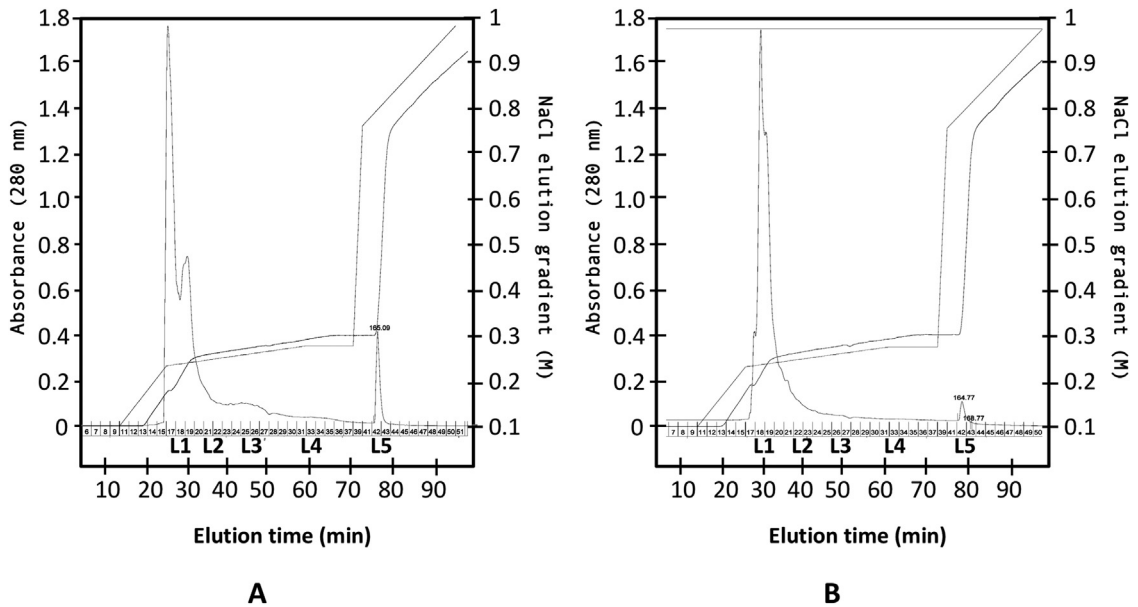

Fig. 1 – (A, B) Anion-exchange fast protein liquid chromatography elution profiles for plasma low-density lipoprotein isolated from diabetic patients (A) and control subjects (B). L1-L5, LDL subfractions; M, molar; NaCl, sodium chloride.