



Could ornithine supplementation be beneficial to prevent the formation of pro-atherogenic carbamylated low-density lipoprotein (c-LDL) particles?



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ABSTRACT

Carbamylation (or carbamoylation) is a non-enzymatic post-translational modification process of lysine residues and protein N-termini, which occurs throughout the lifespan of both various plasma proteins and low-density lipoprotein (LDL) particles. Carbamylation results from the binding of isocyanates spontaneously derived from high levels of blood urea, environmental pollutants, nutritional sources and leads to the formation of potentially atherogenic carbamylated-LDL (c-LDL) particles. The carbamylation of LDL apolipoproteins is associated unfavorable downstream effects.

Ornithine is a non-proteinogenic amino acid, which plays a central role at the urea cycle function. The primary use of ornithine in supplements is to support athletic performance, liver function and wound recovery. Ornithine is structurally highly similar to lysine, and is only one carbon atom shorter in its side-chain. Therefore, we hypothesize that supplemented ornithine could compete with ϵ -amino groups of lysine residues found in apolipoproteins of native LDL particles in their binding to isocyanates and decrease c-LDL formation. This issue still remains unresolved in current literature and needs to be elucidated in experimental studies.

Introduction

Cardiovascular events are the leading cause of death in the developing and developed world [1]. Atherosclerosis plays a major role in the development of cardiovascular disease and Low-Density Lipoprotein (LDL) plays a major role in the etiology of atherosclerosis [2]. LDL particle carries cholesterol in systemic circulation, which is taken by endothelial cells via the LDL receptor [3]. Lowering LDL cholesterol is associated with decreased incidence of cardiovascular events [4] and the latest research indicate that LDL level seems to be never too low [5]. The current armamentarium of antihyperlipidemics is powerful but not perfect, mainly due to side effects and drug costs. For example, those who need proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, alirocumab & evolocumab, are now charged about 6000 \$/year. Therefore, there is still some room for new strategies to combat hyperlipidemia.

Nonenzymatic post-translational modifications of apolipoproteins in native LDL particles promote its molecular aging during its life-cycle in peripheral circulation [6–9]. In view of the close proximity of apolipoproteins and lipids within lipoprotein particles, the probability of adduct formation between reactive lipid peroxidation products and amino acid residues of the proteins is relatively high [10]. Accumulation of

oxidatively modified amino acid residues in LDL apolipoproteins is strongly associated with the alteration of LDL function such as reduced apoprotein exchange rate and receptor binding capacity [3,9]. Recently, a novel form of apolipoprotein modification, termed as carbamylation, has been described. Carbamylation of LDL refers to isocyanic acid driven non-enzymatic modification of apolipoprotein B in LDL. Emerging experimental and clinical data indicate that carbamylated LDL (c-LDL) plays a major role in the formation of atherosclerotic lesions [2,11]. However, the exact underlying pathophysiological mechanisms, particularly the role of c-LDL in endothelial dysfunction in the pathogenesis of atherosclerosis, remains to be elucidated.

In this manuscript, we focus on carbamylation process of apoB-100 found in LDL and its possible anti-carbamylation strategy with ornithine supplementation. The purpose of this manuscript is to hypothesize that ornithine (Fig. 1) supplementation may be used to competitively inhibit carbamylation of apolipoprotein B-100 in LDL particles by providing an alternative non-enzymatic carbamylation target, such as systemic free ornithine, to prevent c-LDL formation (Fig. 2) in peripheral circulation to slow down the development of c-LDL related atherosclerosis.

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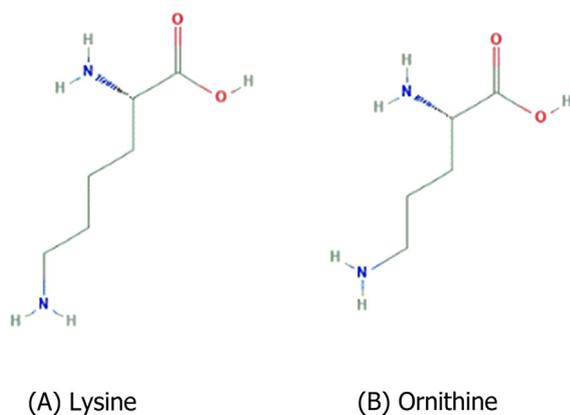
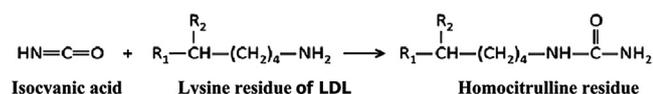


Fig. 1. Comparison of general chemical structures of lysine and ornithine (A) Lysine: IUPAC name: “(2*S*)-2,6-diaminohexanoic acid”; Topological polar surface Area: 89.3 Å² (B) Ornithine: IUPAC name: “(2*S*)-2,5-diaminopentanoic acid” Topological polar surface area: 89.3 Å²

(A) Carbamylation of protein bound lysine residue



(B) Carbamylation of systemic free ornithine

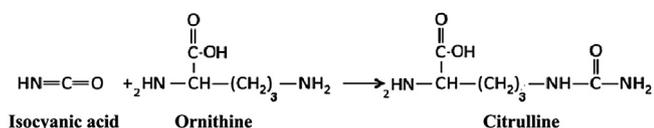


Fig. 2. Hypothesized competition between Lysine residue of LDL (A) and systemic free ornithine (B) for isocyanic acid in peripheral circulation.

Carbamylation of LDL (c-LDL) and atherosclerosis

Protein carbamylation refers to nonenzymatic process which consists of the irreversible binding of electrophilic isocyanates to nucleophilic ϵ -amino groups of the amino acid residues in lysine-rich proteins [12–14]. Involvement of the role of c-LDL in atherosclerosis has only recently been highlighted [2,11 15,16]. Carbamylated proteins are derived from high concentrations of blood urea, transformation of nutritional thiocyanates by myeloperoxidase activity and various environmental pollutants [12,14]. All these aforementioned factors contribute to the formation of potentially atherogenic c-LDL particles [12].

Carbamylated LDL has strong atherogenic properties on endothelial cells by affecting the cell cycle, causing cellular injury and promoting monocyte adhesion through overexpression of intercellular adhesion molecule 1 and vascular cell adhesion molecule-1 [11,17]. It has been shown that, due to the formation of c-LDL, endothelial dysfunction occurs via lectin-like-oxidized LDL receptor-1 (LOX-1) activation and increased reactive oxygen species formation leading to endothelial nitric oxide synthase uncoupling [2]. It was also previously shown that c-LDL exerts its prothrombotic effects in vascular cells and platelets by activation of the LOX-1 receptor, leading to increased thrombus formation [11]. c-LDL induces the synthesis of LOX-1 receptor, which leads to cytotoxicity and accelerated monocyte adhesion to endothelial cells, which is also important in the formation of atherosclerosis [17]. Furthermore, increased LOX-1 expression via c-LDL binding also leads to p38-MAPK and NADPH-oxidase activation, reduces nitric oxide (NO) bioavailability, and impairs endothelial functions. It was also reported that c-LDL, but not native LDL, significantly reduces basal NO synthesis in human aortic endothelial cells [11]. In addition to chronic kidney

disease [18] and type 2 diabetes mellitus [19], because isocyanate sources are found in physiological conditions, a cumulative carbamylation of LDL is also expected during vascular aging.

Ornithine could compete with LDL and prevent the formation of carbamylated-LDL (c-LDL) formation

Ornithine is a non-proteinogenic amino acid found in meat, eggs, dairy. It plays a central role in the urea cycle. Currently, the main use of ornithine is to support athletic performance, liver function, and wound recovery. Ornithine is highly similar in chemical structure to lysine, only one carbon atom shorter in its side-chain. Comparison of the topological polar surface area of lysine and ornithine shows that they are very similar to each other (Fig. 1) [21,22]. The mature apo B-100 comprises a single polypeptide chain of 4536 amino acid residues, and there is one copy of the protein on an LDL particle. Chemical modification of functional groups in the apo B-100 molecule has shown that basic lysine and arginine residues are involved in binding to the LDL receptor. Apo B100 contains 33 analogues of Cardin-Weintraub arginine/lysine-based receptor ligand motifs and shares key lysine motifs [20]. Therefore, the similar three dimensional surface areas may result in LDL and ornithine having closely similar nucleophilicities in their binding to isocyanic acid (Figs. 1 and 2). Hence, ornithine supplementation may decrease the downstream effects of LDL on vasculature and/or it could prevent the transformation of LDL to c-LDL, which in theory would be expected to slow down atherosclerosis (Fig. 2).

Many interventions are used to prevent the undesired effects of oxidatively modified LDL-cholesterol, including life-style modifications and various antihyperlipidemic drugs, including the PCSK9 inhibitors which cost thousands of dollars per patient every year [23]. These interventions mainly depend on decreasing the systemic LDL levels by decreasing the synthesis of cholesterol, decreasing the absorption of various cholesterol metabolites in the gut and increasing the reuptake of LDL in the liver. In addition to these proven to be effective treatments, we hypothesize that ornithine supplementation could slow down the formation of c-LDL by competing with ϵ -amino groups of lysine residues found in apoB-100 of native LDL particles in binding isocyanic acid, and therefore decrease the formation of notorious c-LDL. With the identification and development of novel metabolic strategies, a new era for better and cheaper LDL management could be plausible. This issue still remains unresolved in current literature and needs to be investigated in experimental studies.

Conflict of interest

None

Funding

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mehy.2019.03.004>.

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