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Review

Preventive effect of L-carnitine and its derivatives on endothelial dysfunction and platelet aggregation

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SUMMARY

Background and aim: Oxidative stress plays an important role in platelet activation and endothelial dysfunction. Exogenous and endogenous reactive oxygen species are associated with platelet activation and vascular dysfunction. Antioxidants have been shown to attenuate oxidative stress and consequently endothelial dysfunction by preventing inflammation, regulating vascular tone, and promoting anti-adhesive and antithrombotic properties. L-carnitine and its derivatives have been demonstrated to improve endothelial and platelet function against oxidative stress by several mechanisms, some of which cannot be found in other antioxidants. The role of L-carnitine and its derivatives in endothelial dysfunction and platelet activation will be reviewed here from the perspective of basic and clinical research.

Method: This study reviews *in vitro* and *in vivo* studies, clinical trials, and abstracts in the English language that have examined the protective effects of L-carnitine and its derivatives on endothelial dysfunction and platelet aggregation in pathological conditions. We searched experimental studies, clinical trials, and other review articles to obtain the materials.

Conclusions: Although *in vitro* physiological models, animal studies on vascular and platelet function, and some human studies on these systems are in favor of the preventive effects of L-carnitine and its derivatives on endothelial dysfunction and platelet aggregation, more clinical trials are needed to clarify the clinical importance of L-carnitine as a supportive option to maintain the normal homeostatic function of the vasculature and to prevent platelet activation.

1. Introduction

β-hydroxy-gamma-trimethyl aminobutyric acid (L-carnitine [LC]) is a vitamin-like and modified amino acid that plays an important role in supporting the body's metabolic activities. Carnitine homeostasis in mammals is maintained by a combination of biosynthesis, uptake from food sources, and highly efficient reabsorption of carnitine [1]. LC is a facilitator of the β oxidation of fatty acids, particularly in the heart and skeletal muscles. LC is an essential cofactor of carnitine palmitoyl transferase I, allowing the transport of long-chain fatty acids from the mitochondrial membrane and is involved in the regeneration of acetyl coenzyme A (acetyl-CoA) for the cycle of energy production in mitochondria, where β oxidation takes place [2]. LC prevents the accumulation of toxic compounds in mitochondria such as acyl groups and propionic acids as a result of a blockade in the normal metabolic pathway by trans esterification of CoA esters and then the removal of toxic compounds by the excretion of acyl-carnitine esters [1]. This reaction is freely reversible and is catalyzed by carnitine acetyl transferase in mitochondria, leading to an increase in free CoA. The accumulation of these toxic intermediates promotes insulin resistance in the heart and skeletal muscle as well as heart failure and ischemia [3]. In addition, there is evidence that LC is implicated in the protection of cardiac cells against ischemia, hypoxia, and oxidative stress via other mechanisms [4]. Individuals who are carnitine-deficient are, therefore, more prone to cardiovascular disease and diabetes.

Since the discovery of LC deficiency syndromes in the 1970s, the importance of LC has been increased as a medicine and a nutritional

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supplement. LC has been widely used for weight loss, enhancement of exercise performance, reduction of fatigue, and also treatment of carnitine deficiency [5]. In recent decades, studies have been done on other effects of LC in individuals who are carnitine-sufficient. There is growing evidence that high concentrations of LC provide beneficial effects in various diseases such as coronary artery disease, congestive heart failure, peripheral vascular diseases, type 2 diabetes, dyslipidemia, and hypertension [3]. Two recent meta-analyses have reported different results. Whereas the first one showed that LC was associated with a 27% reduction in all-cause mortality, a 65% reduction in ventricular arrhythmias, and a 40% reduction in anginal symptoms in patients experiencing acute myocardial infarction [4], the other one failed to demonstrate the benefits of LC use for the secondary prevention of cardiovascular disease – in contrast to older more positive studies [6].

In this article, we review the beneficial effects of LC on endothelial dysfunction and platelet aggregation, which are involved in the pathogenesis of diseases such as atherosclerosis, blood pressure, and coronary artery disease. Almost all the included studies are experimental because we focused on the pathogenesis and sought to clarify the role of carnitine.

2. Oxidative stress and platelet activation

Platelets are involved in pathophysiological processes such as arterial thrombosis. The activation of platelets is modulated by numerous factors and different cellular signaling pathways. Most of these factors and pathways are relatively well-characterized and are targets for antiplatelet therapy [7].

Endothelial cells in the normal condition produce prostacyclin and nitric oxide (NO) or limit the accumulation of some substances that inhibit the adhesion and aggregation of platelets, subsequently maintaining the antithrombogenic properties of the vascular system [8]. The vascular system is vulnerable to oxidative damage caused by free radicals, the concentration of which is increased in the body through different ways. Moreover, free radicals appear to be the principal mediator of cellular dysfunction in various pathological conditions — including coronary heart disease, heart failure, and renal failure [9,10]. This condition occurs when oxidants cannot be sufficiently eliminated by an adaptive compensatory antioxidant [11]. Several conditions may produce reactive oxygen species (ROS) and reactive nitrogen species in the body; these conditions include strenuous physical exercise, inflammatory processes, cigarette smoking, reperfusion after ischemia, and many chemical agents [12]. A partial reduction in oxygen leads to the generation of active free-radical derivatives of molecular oxygen, which exert critical regulatory functions in the vasculature. Moreover, several studies have recently suggested that ROS as a newly defined modulator participate in the regulation of platelet activation. However, overproduction of exogenous or endogenous ROS is associated with platelet activation and formation of platelet-rich thrombi overlying the damaged endothelium and consequently atherothrombosis and many cardiovascular diseases [13,14]. Furthermore, ROS contribute to endothelial dysfunction by stimulating smooth muscle cell proliferation, lipoprotein oxidation (one of the initial steps of atherosclerotic plaque formation), and direct cellular damage at the myocardium [10,14,15]. ROS include superoxide anion (O_2^-) as a radical and central structure and other free radicals derived from O_2^- [13]. Superoxides react rapidly with the platelet- or endothelium-derived NO to form peroxynitrite ($ONOO^-$). $ONOO^-$ is a highly reactive oxidative species that, similar to O_2^-, has devastating effects on other large biomolecules [7,10,16]. $ONOO^-$ switches endothelial nitric oxide synthase (eNOS) as an anti-atherosclerotic NO^--producing enzyme to a superoxide-producing enzyme (uncoupled eNOS), which is involved in atherosclerotic and thrombotic processes. Superoxide dismutase react with O_2^- to form hydrogen peroxide (H_2O_2) [7,13,17]. Superoxides react with lipids and generate lipid alkoxyl and peroxyl radicals, which promote lipid radical chain propagation reactions. These radicals can react with NO and form lipid $ONOO^-$. Thus, NO bioavailability is decreased by oxidative stress. NO, as a potent inhibitor of platelet activation produced by NOS from endothelial cells and platelets but in pathological conditions, produces more $ONOO^-$ [7,13,17]. Cellular glutathione peroxidases can neutralize $ONOO^-$ and H_2O_2. Accordingly, these systems have a crucial role in the inhibition of pathological oxidative stress reactions [18].

Platelets possess enzymatic facilities linked to the plasma membrane; these facilities are referred to as the plasma membrane redox system, which is involved in different processes [19]. The plasma membrane redox system in platelets mainly encompasses NADH and NADPH oxidases. Free radicals or other stimuli such as thrombin and collagen activate phospholipase A2(PLA2) and phospholipase C, which hydrolyse phospholipids to form arachidonic acids and lyso-platelet activating factor (PAF) and indirectly ROS, may inactivate PAF-acetyl hydrolase – the enzyme that degrades PAF as a potent platelet aggregation agonist. Arachidonic acids can subsequently be converted into eicosanoids (prostaglandins, thromboxanes, and leukotrienes) by cyclooxygenases (COX) and lipoxygenases, whereas acetyl transferases generate PAF from lyso-PAF. Neutrophils and platelets are major sources of lipid mediators such as eicosanoids (prostaglandins, thromboxanes, and leukotrienes) and PAF – involved in the regulation of vascular tone, pathogenesis of thrombosis and tissue ischemia, amplification of local inflammatory response, and induction of the further recruitment and activation of leukocytes and platelets [8,20–24]. In addition, arachidonic acids activate protein kinase C, an enzyme that is known to stimulate NADH and NADPH oxidases. Stimulating agonists activate NAD(P)H oxidases, and NAD(P)H oxidases form superoxides outside the cell before superoxides are converted into H_2O_2. H_2O_2 acts extracellularly and stimulates other platelets and neutrophils as well as the endothelium, independent of the aggregation process in platelets. This process occurs during thrombus and clot formation and during inflammation and atherosclerosis. Also, NADPH oxidases produce O_2^- and H_2O_2 in cells, where they function as a second messenger, and lead to the activation of αIIb/β3-integrin. Furthermore, O_2^- or H_2O_2 increases the release of adenosine diphosphate (ADP), resulting in increased platelet recruitment. Levels of NAD(P)H/NAD(P)+ are maintained, while O_2^- and H_2O_2 are generated. The NADPH oxidase complex consists of cytosolic factors (p47phox and p67phox) and 2membrane-bound subunits (p22phox and gp91phox), catalyzing 1-electron transfer from NADPH to molecular oxygen and generating O_2^-. Well-known agonists such as thrombin, collagen, and immunological stimuli induce the phosphorylation of phosphatidylinositol 3-kinaseand protein kinase C. In this way, small GTPases (Rac1 or Rac2) and cytosolic factors (p47phox and p67phox) translocate to the plasma membrane. This translocation is needed for the catalytic activity and consequently aggregation. ROS accelerate this phenomenon by reducing the threshold for platelet activation to thrombin, collagen, ADP, or arachidonic acids, and may even induce spontaneous aggregation [7,13,14,19,20,25–29].

3. Oxidative stress and endothelial dysfunction

The vascular endothelium has an important role in keeping vascular homeostatic functions by preventing inflammation, regulating vascular tone, and promoting antiadhesive and antithrombotic properties. The initial step in atherogenesis that leads to endothelial dysfunction is imbalance between the production of vasoconstrictor substances such as endothelin-1 and vasodilators

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