PATHOGENESIS OF ATHEROSCLEROSIS AND ALPHA LIPOIC ACID AS A POTENTIAL THERAPEUTIC AGENT AGAINST ATHEROSCLEROSIS - A REVIEW

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Abstract

Studies have found the association between hypercholesterolemia with oxidative stress and atherogenesis. Atherosclerosis has become one of the leading causes of mortality among industrial countries due to abnormal cholesterol metabolism, inflammation of arterial wall and build-up of atherosclerotic plaque. This disease has been recently linked with alpha lipoic acid (ALA), a mitochondrial compound with antioxidative effects in water- and fat-soluble mediums, in both oxidized and reduced forms: lipoic acid (LA) and dihydrolipoic acid (DHLA), respectively. This article provides a comprehensive review of the development and progression of atherosclerosis and the roles and regulations of ALA as a potent antioxidant against atherosclerosis.

Keywords: *Atherosclerosis, Cholesterol, Oxidative Stress, Inflammation, Antioxidant, Alpha Lipoic Acid*

Introduction

Atherosclerosis has become one of the main factors that leads to the increment of mortality among industrialized societies (1). Most deaths have been contributed by the resulting arterial blockage causing deadly heart attacks despite other cardiovascular diseases and cancer (2). Cancer held the top rank of the mortality incidence until it was overtaken by atherosclerosis in the previous decades (3). While cancer can be described as an attack inside the body as triggered by mutations, atherosclerosis is a condition where the excess cholesterol from unhealthy diet and lifestyle accumulates and deposits in blood vessels (4).

This review focuses on discoveries which are crucial in the development and progression of atherosclerosis and the roles and regulations of alpha lipoic acid (ALA) as a potent antioxidant to counter the progression of this vascular disease.

Pathogenesis of Atherosclerosis

Cholesterol Homeostasis

Cholesterol is either produced through *de novo* biosynthesis in the body or transported through diet. This common lipid is a basic structural component of cell membrane, while approximately 20% of the body's total cholesterol resides in the human brain (5). It is also the major sterol component found in animal tissues that builds and maintains cell membrane, assists in the manufacturing of bile acids, serves as the precursor of steroid hormones synthesis and involves in the production of fat-soluble vitamins. In addition, cholesterol is responsible for cell signalling, nerve conduction and intracellular transport (6).

Cholesterol biosynthesis and homeostasis are crucial for cell growth and proliferation. Liver is the main organ responsible for maintaining cholesterol homeostasis.

Cholesterol level is regulated by three main factors: *De novo* biosynthesis mainly in the liver and intestines, intestinal absorption and bile excretion through liver cells. In the bloodstream, lipoproteins are responsible as cholesterol transport mediators (6). These lipoproteins are categorized into five main components: Chylomicron, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), high-density lipoprotein (HDL) and low-density lipoprotein (LDL). HDL is responsible in transporting cholesterol to the liver to be further metabolized and thus labelled as 'good cholesterol' (6). In contrast, LDL is responsible for transporting the cholesterol into peripheral tissues and labelled as 'bad cholesterol', rendering this lipoprotein as the key contributing factor for cardiovascular diseases (2, 7). As LDL is related to the development of atherosclerosis, Singh et al. (8) suggested that the impairment of cholesterol homeostasis may lead to the development of a variety of health disorders including atherosclerosis.

Regulation of HMG-CoA Reductase in Cholesterol Biosynthesis

In the cholesterol biosynthetic pathway, cholesterol is initially synthesized from acetyl-CoA which leads to formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA reductase is the crucial enzyme responsible for converting HMG-CoA into mevalonate in this rate-limiting step (8). In clinical practice, high cholesterol level can be pharmacologically regulated by inhibiting this enzyme (9). As a competitive inhibitor of HMG-CoA reductase, statins are a class of drug which is commonly used to treat hypercholesterolemia (6, 8, 10-12). The statins are categorised by Culver et al. (13) according to their respective potency to reduce the LDL level. Low potency category includes lovastatin and pravastatin whereas high potency category includes atorvastatin and simvastatin. Besides, lovastatin has been observed to inhibit the progress of cell proliferation by restricting G1 phase of cell cycle in rat fibroblast F111 cells (8).

Unfortunately, the statin treatment is accompanied with adverse side effects including myalgia, myopathy, myositis, gastro-intestinal discomfort, fatigue and insomnia (12). Likewise, the usage of statin contributes to an increase in the number of diabetes mellitus cases among postmenopausal women (13) and hepatoxicity which arises from elevated levels of liver enzymes, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (11).

Oxidative Stress and Other Factors Contributing to Atherosclerosis

Oxidative stress has been reported to be one of the causative criteria that links hypercholesterolemia with atherosclerosis (14). Previous studies conducted by Amom et al. (14) has found that initiation and progression of atherosclerosis are associated with formation of LDL. The LDL is oxidized by oxidants derived from macrophages, smooth muscle cells (SMCs) and vascular endothelial cells (15). This condition causes endothelial dysfunction and activates the endothelial cells to produce cytokines, growth factors and chemokines (16). The secretion of chemokines, such as CXCL8, CX3CL1, CCR5 and CCL2, attracts leukocytes into the intima layer and promotes leukocytes adhesion (17-20). The atherogenicity is enhanced as the presence of ox-LDL contributes to the alteration of LDL cell receptor uptake in various cells. The ox-LDL is favoured by scavenger receptors on monocytes, macrophages and SMCs. In this scenario, this lipoprotein is taken up by these receptors excessively and uncontrollably, which leads to accumulation of lipid and consequent development of lipid-rich foam cells (21-23).

Ox-LDL consists of complex products of oxidized lipids and negatively charged proteins which are assumed to be derived from the modification by aldehyde compounds. The net negative charges produced are crucial for the interaction with macrophages as it helps them to recognize the ox-LDL. Parthasarathy et al. (22) have demonstrated through *in vitro* study where the incubation of ox-LDL with macrophages has led to the accumulation of cholesteryl esters. Cholesteryl esters refers to the inactive form of transported cholesterol that are bound together with the lipoproteins in the bloodstream (9). In contrast, the incubation with native LDL has resulted in no accumulation of cholesteryl esters (22).

At the early stage of atherosclerosis, the lesion is worsened by the presence of oxidative stress which stimulates the inflammatory response by a plethora of pro-inflammatory cytokines, such as tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), interleukin-6 (IL-6), interferon-γ (INF-γ) and interferon-β (INF-β) (16, 24). Hence, this inflammatory response promotes the generation of hydroxy radicals, peroxides and superperoxides within the interior lining of the blood vessels (21).

Madamanchi and Runge (25) and Wang (26) have reported that atherosclerosis and other cardiovascular diseases are associated with excessive and abnormal mitochondrial oxidative stress and mitochondrial dysfunction. A sign of mitochondrial dysfunction is the decrease in the amount of ATP production pathologically caused by excessive cholesterol intake, hypercholesterolemia, oxidative stress, inflammatory response and lipotoxicity (25, 27). Under normal conditions, the antioxidant system in mitochondria helps to protect the organelle against mitochondrial reactive oxygen species (ROS) from disrupting their DNA, modifying the proteins and causing lipid peroxidation to occur. Despite being the main sources of ROS, excessive production of ROS can damage the mitochondria (28). When mitochondrial ROS is excessively generated, inflammation occurs which then causes atherosclerosis. The progression of atherosclerosis causes the antioxidant system to deteriorate as well. This finding indicates that mitochondrial fusion may be the novel therapeutic target for prevention against atherothrombosis and helps in stabilizing the plaque (26).

In addition to oxidative stress, atherosclerosis is caused by lipid retention, lipid oxidation by ROS and modification

of the lipids. These three conditions trigger chronic inflammation of the arterial wall, causing thrombosis and stenosis (29). Furthermore, this vascular disease can be intensified and provoked by several risk factors including hypertension, diabetes mellitus, obesity, cigarette smoking, genetic predisposition and family history of atherosclerosis (2).

Various types of leukocytes have been reported to be associated with the onset, progression and complication of atherosclerosis. However, the main leukocytes that are involved in this disease are monocytes and macrophages. The number of monocytes present in the bloodstream is increased as the disease complicates and worsens. The monocytes will then migrate into blood vessel wall, accumulate and differentiate into macrophages (24). The macrophages generate interleukin (IL)-1, TNF-α and monocytes chemotactic protein (MCP)-1, which then increase leukocytes adhesion and attract more leukocytes into the intima layer (30, 31).

The chemokines which activate those leukocytes trigger macrophages uptake of ox-LDL and transform the macrophages into foam cells. Interferon gamma (IFN-γ), TNF-like protein 1A (TL1A) and TNF-related weak inducer of apoptosis (TWEAK) are some of the cytokines involved in increasing the ox-LDL uptake by macrophages and regulating the foam cell formation (31). As the foam cells are formed, pro-inflammatory cytokines such as TNF-α and IFN-γ are secreted and the inflammatory response is multiplied (31-33).

The unbalanced level of cellular lipid and continued accumulation of ox-LDL lead to death of foam cells. The dead foam cells cause lipid to be deposited into necrotic core and SMCs to-migrate from the media to the intima layer of arterial wall. After the migration takes place, the SMCs start to proliferate, take up ox-LDL, produce collagen and secrete extracellular matrix (ECM) proteins. The secretion of ECM proteins aids in stabilizing the fibrous cap of atheromatous plaque (2, 31). However, the continuation of inflammatory response exhibited by pro-inflammatory cytokines, such as IFN-γ, TWEAK and interleukin-18 (IL-18), and the production of proteases such as matrix metalloproteinases (MMPs) and cathepsins by macrophages will degrade the stability of fibrous cap of the plaque (34).

The secretion of pro-inflammatory cytokines represses the synthesis of ECM proteins' stabilizing elements and collagen by SMCs and induces apoptotic effects of macrophages, foam cells and SMCs. This condition results in increased size of lipid-necrotic core and fibrous cap thinning (1, 31). Plaque rupture, necrosis and thrombosis may occur if the phagocytes are unable to clear the apoptotic macrophages efficiently (1) which ultimately leads to the clinical complications associated with this disease (33).

Also, macrophage-driven inflammation response has been reported to cause atherosclerosis. Upon accumulation of circulating monocytes in the atherogenic vessel wall, these monocytes differentiate into macrophages and lipid-rich foam cells. The macrophages particularly reduce overall plaque stability and promote thrombosis. These cells, therefore, become the key culprits associated with clinical complications due to atherosclerotic lesions (4).

Protective Effects of Alpha Lipoic Acid against Atherosclerosis

Alpha lipoic acid (1,2-dithiolane-3-pentanoic acid) (ALA), also known as thioctic acid, is commonly found in meats and vegetables (35) and is chemically identified in R- and S- enantiomers (36). This organosulfur compound has been proven to be a potent antioxidant (37). ALA exerts its antioxidative effect in both water- and fat-soluble mediums since it uniquely possesses hydrophobic and hydrophilic properties (38). The compound has an antioxidative effect in both oxidized and reduced forms: lipoic acid (LA) and dihydrolipoic acid (DHLA), respectively (39).

Initial stage of the atherosclerosis typically begins with endothelial dysfunction. The endothelium is pathologically activated due to excessive reactive oxygen species production. Such activation includes exposure of cell to adhesion molecules on the surface of epithelial cells including intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and platelet-endothelial cell adhesion molecule-1 (PECAM-1) (40). A study suggested the protective effect of ALA against endothelial dysfunction in induced rats (41) while another rat study demonstrated the inhibition of common glycoprotein expressed on endothelial cells called intercellular adhesion molecule-1 (ICAM-1) (42).

Alpha Lipoic Acid in Mitochondria

In human, the liver plays a crucial role in synthesizing and metabolizing ALA. The organ was shown to be the site where ALA exerts its capability by reducing the side effects of a wide range of toxic agents (43). ALA is metabolized through mitochondrial β-oxidation and generated within mitochondria by lipoic acid synthase (44, 45). In addition, ALA serves as an essential component in mitochondria as it enhances the mitochondrial energy metabolic function (46). The molecule becomes a cofactor for multiple mitochondrial enzymes including alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase (47, 48). These two enzymes are involved in the central pathway for glucose oxidation and energy synthesis through the generation of adenosine triphosphate (ATP), namely the citric acid cycle (49).

Antioxidative Effect of Alpha Lipoic Acid

Not only is ALA crucial for the regulation of protein and carbohydrate metabolism, but it also has the ability to regenerate endogenous and exogenous antioxidants including vitamin C and E, chelate metal ions and increase the level of reduced glutathione (GSH) *in vitro* and *in vivo* (38, 50, 51). GSH is one of the core endogenous

antioxidants responsible for protecting cell components from ROS and reactive nitrogen species (RNS) (52). This antioxidant donates its proton to lipid membranes (53) and forms oxidized GSH, which is also known as glutathione disulfide (GSSG) (54). According to Zitka et al. (55), the ratio of GSH to GSSG (GSH/GSSG) is a major potential oxidative stress biomarker and a high ratio indicates that the cell components are protected from the reactive species (53).

Numerous studies have been conducted on ALA to investigate its antioxidant effect. The antioxidant properties of ALA are measured by determining the concentration level of malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARS), both of which are the end products of lipid peroxidation. It has been demonstrated that ALA is capable of quenching free radicals *in vitro* and *in vivo* (38) as the concentration level of MDA or TBARS is lower with the supplementation of ALA compared to the negative control (14). Moreover, ALA effectively prevents inflammation in the liver as it helps to lower the oxidative stress by reducing TBARS and hydrogen peroxide (H_2O_2) levels, similar to the mechanism of action in atherosclerosis (16).

Anti-inflammatory Effect of Alpha Lipoic Acid

Previous reports have indicated that ALA is able to protect against diseases correlated with the abnormality in the level of oxidative stress and metabolic reaction (56) while enhancing the function of vascular endothelium (57). ALA exerts anti-inflammatory effect (47) by reducing the atherosclerotic lesion growth and plaque formation (58).

ALA slows down the migration of T-lymphocytes, monocytes and macrophages towards the atherosclerotic lesions as proven by the count reduction of $CD3^+$ T-cells and $CD68^+$ cells within the plaque (56). $CD3^+$ cells are one of the pro-inflammatory T-cells involved in atherosclerosis (19) and $CD68^+$ cells are associated with monocytes and macrophages (34).

Vasodilatory Effect of Alpha Lipoic Acid

ALA is reported to have vasodilatory effect *in vivo* by improving acetylcholine-induced vasodilation (59) and down-regulating the expression of angiotensin-II receptor type 1 (AT_1 receptor) which subsequently reduces the vasoconstriction response to AT_1 (56). Acetylcholine refers to the chemical that affects directly on muscarinic receptors of vascular endothelium. As the muscarinic receptors are triggered by acetylcholine, nitric oxide is produced thereby promoting vasodilation (60, 61). On the contrary, the triggered AT_1 receptors lead to vasoconstriction of the vascular endothelium (56).

Furthermore, ALA also increases the level of mitochondrial aldehyde dehydrogenase-2 (ALDH2) activity *in vivo* and *in vitro* (62). As oxidative stress causes vulnerability and instability of the atherosclerotic plaques, ALDH2 helps to protect against the damage (63). ALDH2 is responsible for detoxification of reactive aldehydes such as 4-hydroxy-2-nonenal (4-HNE) that is formed during lipid peroxidation following oxidative stress (64). Activation of ALDH2 may lead to decreased amount of ROS production, thus preventing ROS-induced vasoconstriction (65). Li et al. (63) concluded that ALA aids in lowering the oxidative stress and enhances the ALDH2 activity.

Potential Cholesterol Lowering Effect of Alpha Lipoic Acid

As reviewed by Shay et al. (66), the diverse physiological actions of ALA include as an inducer of cellular signalling pathways, an insulin mimetic, a hypotriglyceridemic agent, a vasorelaxant/anti-hypertensive compound, a metal chelator, and an adjuvant for neuro-cognitive function.

Amom et al. (14) revealed the protective activity of ALA by reducing plasma total cholesterol and LDL levels while demonstrating anti-atherosclerotic properties in hyper-cholesterolemic-induced rabbits. Interestingly, Ying et al. (56) found that ALA did not significantly affect the level of triglycerides, VLDL, LDL and HDL.

Besides reducing plasma total cholesterol, triglycerides and LDL, ALA also reduces the cholesterol number of non-high-density lipoprotein (non-HDL), ox-LDL and lipoprotein (a) while increasing the levels of HDL and hepatic LDL receptor protein (58, 67, 68). Moreover, ALA is reported to prevent the accumulation and deposition of triglycerides by suppressing lipogenesis, increasing VLDL export, improving oxidation of hepatic fats (67) and decreasing lipid peroxidation (69).

Contrary to naturally occurring R enantiomers of ALA, the synthesized compound exists as a racemic mixture with equal composition of R and S enantiomers. Thus, the absorption and bioavailability of ALA have been studied following administration of the commercial racemic mixture. Some experimental studies have confirmed that R-ALA has greater biopotency in several metabolic pathways than its mirror structure (70). In humans, the therapeutic doses of ALA range from 200 to 1800 mg/day. This supplementation of exogenous ALA is clinically effective in the treatment of diabetes and the prevention of vascular disease, hypertension, and inflammation as the amount of ALA in plasma and human cells is inadequate to meet bodily needs (71).

Due to close association with increased oxidative stress and inflammatory pathways, ALA supplementation has been beneficial to prevent beta cell destruction, enhance glucose uptake, and provide antioxidant effects in slowing the development of complications related to diabetic neuropathy, retinopathy, and other vascular diseases (72, 73). The mechanism of dyslipidaemia regulation and anti-insulin resistance of ALA has been suggested to have a therapeutic role in ameliorating dyslipidaemia and insulin resistance caused by oxidative damage in obese patients with impaired glucose tolerance (74). However, the mechanism of action of ALA which leads to reduced plasma total cholesterol and LDL levels in human remains

to be elucidated. Likewise, the prooxidant properties of ALA supplementation should be further studied due to various direct or indirect reactions in human (75) and ALA-supplemented aged rats (76).

Conclusion

As the prevalence of atherosclerosis is increasing worldwide, many researchers have been studying the mechanism of atherosclerosis, so that effective therapies can be targeted. Many studies have proved that oxidative stress is one of the leading causes of arterial wall inflammation and atherosclerotic plaque build-up. By understanding and identifying the pathogenesis and causes of atherosclerosis, it is now clear that atherosclerosis is a chronic inflammatory disease caused by altered cholesterol metabolism that may lead to plaque build-up and thrombosis. Natural antioxidants are being widely studied as the potential therapeutic agent against atherosclerosis for alternative treatments and supplements among high-risk groups. Natural products are helpful in reducing the inflammatory and oxidative biomarkers on a cellular level. However, more large-scale clinical trials and large cohort meta-analysis should be conducted in order to allow these natural products to be fully developed as therapeutic agents.

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Conflict of Interests

The authors declared that they have no competing interests.

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