Tocotrienols and Atherosclerosis

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Summary  The adherence of monocytes to the vascular endothelial cells is an important early event in atherogenesis. Monocyte adherence to endothelial cells is induced by oxidized low density lipoprotein (LDL) and mediated by multiple cell adhesion molecules including vascular cell-adhesion molecule 1 (VCAM-1). Enhanced endothelial expression of these molecules by oxidized LDL has been shown to be a critical step in foam cell formation and the development of atherosclerosis. Recent studies have demonstrated that vitamin E inhibited the expression of mRNA and protein for VCAM-1 by endothelial cells in response to stimulation with oxidized LDL or inflammatory cytokines. Compared to α-tocopherol, α-tocotrienol displayed a more profound inhibitory effect on adhesion molecule expression and monocytic cell adherence. Among the isomers of tocotrienol, δ-tocotrienol was a potent and effective agent for the reduction of cellular VCAM-1 expression and monocytic cell adherence. The inhibitory effects of vitamin E analogs on the adhesiveness of endothelial cells correlated with their intracellular concentrations. Anti-atherogenic effects of tocotrienols may be derived from their properties of cholesterol-lowering effect, protein kinase C inhibition, and modulation of cyclooxygenase cascade. Although recent human studies have raised some doubts on the efficacy of vitamin E in the prevention of progression of atherosclerotic lesions, the experimental studies using tocotrienol strongly support its positive effect on the reduction of risk of atherogenesis. Further studies will be necessary for clarifying the anti-atherogenic effect of tocotrienol and its bioavailability after oral administration.

Key Words: adhesion molecule, atherosclerosis, tocopherol, tocotrienol

Introduction

Increased adherence of monocytes to the endothelium constitutes one of early visible changes in atherosclerosis. Monocytes are initially attracted to lesion-prone sites by cell-adhesion molecules expressed on activated endothelial cells. The initial adhesion involves selectins, which mediate a rolling interaction, and is followed by firmer attachment by means of integrins. Adherent monocytes migrate into the subendothelial space under influence of chemoattractant molecules. Endothelium leukocyte adhesion molecule (ELAM), vascular cell-adhesion molecule 1 (VCAM-1) and intracellular cell-adhesion molecule 1 (ICAM-1) may be a candidate for the initial recruitment of macrophages, given their

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up-regulation in cultured endothelial cells in the presence of oxidized low density lipoprotein (ox-LDL) [1]. These proteins promote monocyte adhesion and subsequent migration into the intima where monocytes differentiate into macrophages. Recently, we found that α-tocopherol inhibited the expression of mRNA and protein for ICAM-1 and VCAM-1 by human umbilical vein endothelial cells (HUVEC) in response to stimulation with oxLDL or interleukin (IL)-1 [2]. In addition, we showed that α-tocopherol decreased the expression of CD11b/CD18 on human neutrophils and monocytes induced by oxLDL in both in vitro and ex vivo studies [2-4]. These data support that vitamin E supplementation may reduce the extent of the oxLDL-induced monocyte-endothelial interaction and, thus, be protective against atherosclerosis. This hypothesis is strongly supported by studies in murine models of atherosclerosis. In contrast, clinical trials using this vitamin have been giving a more confused picture than expected, with results ranging from a significant protective action to the absence of any effect. Recent Vitamin E Atherosclerosis Prevention Study (VEAPS) study have demonstrated that α-tocopherol supplementation (dl-α-tocopherol 400 IU/day) significantly raised plasma vitamin E levels, reduced circulating oxLDL, and reduced LDL oxidative susceptibility, however, the progression of the intima-media thickness of common carotid artery was not reduced by the treatment [5].

Recently the role of tocotrienols, other forms of vitamin E, has received renewed attention. It has been demonstrated that tocotrienol has a potent action for inhibition of lipid peroxidation [6], inhibition of monocyte adherence [7, 8], and neuroprotection [9] compared with tocopherol. This review focuses on recent advances that have revealed the mechanisms by which tocotrienol may exert its protection against atherosclerosis.

Oxidized LDL Hypothesis

There is considerable evidence that inflammatory response induced by oxLDL contributes to the development of atherosclerosis [1]. OxLDL or their metabolites have been found in atherosclerotic lesions in both human and animal models [10], and contains several lipid-derived bioactive molecules

Fig. 1. Role of oxidized LDL in atherogenesis. Oxidized LDL (oxLDL) in blood stream stimulates the up-regulation of CD11b/CD18 adhesion molecule expression on monocyte (1). The entry of LDL into subendothelial space is followed by its oxidation by reactive oxygen species generated from smooth muscle cells and endothelial cells. OxLDL can induce endothelial dysfunction (2) and expression of cytokines (3), growth factors, and several cell surface adhesion molecules (4) that are capable of recruiting circulating monocytes and T lymphocytes into the intima. OxLDL activates the development of monocytes into macrophages where the uptake of oxLDL via the scavenger receptor is rapid (5, 6), and inhibits the migration of macrophages (8). OxLDL stimulates cell proliferation and transmigration of smooth muscle cells (7).
such as oxysterols (hydroxycholesterols and ketocholesterols), phospholipid peroxides, and fatty acid peroxides. OxLDL exhibits a wide variety of potentially atherogenic properties, including the stimulation of monocyte migration, the promotion of the adhesion of monocytes to cultured endothelial cells, and inhibition of endothelium-dependent vasodilatation (Fig. 1). OxLDL is avidly taken up by macrophages, resulting in foam cell formation [11]. Especially, oxLDLs have been shown to stimulate endothelial cells to express several proteins that contribute to the early stage of atherosclerosis, including monocyte chemotactic protein-1 (MCP-1), macrophage-colony-stimulating factor (M-CSF), VCAM-1, and ICAM-1 [7, 12].

It is well known that several cytokines, such as IL-1β and TNF-α, induce the surface expression of ICAM-1 and VCAM-1 on endothelial cells through the activation of redox-sensitive transcriptional factor, nuclear factor-κB. In our experiments, ICAM-1 and VCAM-1 expression on oxLDL- or oxysterol-stimulated HUVEC or human aortic endothelial cells (HAEC) was found to occur in the same fashion as after stimulation with IL-1β [2]. However, native LDL did not have any effect on the expression of endothelial adhesion molecules. These results suggest that oxLDL may induce almost the same signal transduction pathway for the synthesis of ICAM-1 and VCAM-1 as that stimulated by IL-1β, but the precise mechanism involved still needs to be investigated.

Apolipoprotein E-Deficient Mice and Atherosclerosis: Role of Adhesion Molecules

Recently, several useful models of atherosclerosis have been created by genetic alteration of lipid metabolism. The most widely used of these models involves a gene disruption of apolipoprotein E. Advantages of this model include the fact that the lesions develop at a much earlier age, exhibit more of the features observed in human and can be induced by both low and high fat diets. Similar to human and rabbit lesions, oxidized lipoprotein epitopes are found in lesions of apoE-deficient mice, and sera from these mice contain high titers of autoantibodies that recognize oxidized lipoproteins and can stain lesions in rabbits [13]. In apoE−/− mice, ICAM-1 is constitutively increased at lesion-prone site and VCAM-1 is also present at the same site as ICAM-1. Thus, adherence of monocytes may occur after an increase in one or more of adhesion molecules, which may act in concert with chemotactic molecules such as MCP-1, IL-8, and oxLDL. In these mice, it has been shown that additional knock out of the gene for vascular adhesion molecules, such as ICAM-1, platelet-endothelial cell adhesion molecule-1 (PECAM-1), and VCAM-1, results in significantly less atherosclerosis in the proximal aorta [14, 15]. These results indicate that inflammatory response including the expression of adhesion molecules plays a crucial role in the initial development of atherosclerosis.

Anti-atherogenic effect of food ingredients has been demonstrated using this model. Recent study shows that dietary consumption of red wine [16], soy protein [17], chitosan [18], pinus pinaster oil [19], pomegranate juice [20], ginger extract [21], or resveratrol [22] by apoE-deficient mice significantly attenuates the development of atherosclerotic lesions. These anti-atherosclerotic effects are, in part, associated with the inhibition of monocyte-endothelial interaction induced by oxLDL.

Tocotrienols

Tocopherols and tocotrienols share similar structure with the exception of their side chains. While
tocopherol has a saturated phytyl tail, tocotrienol possesses as unsaturated isoprenoid side chain (Fig. 2). Both tocopherols and tocotrienols have four isomers. Designated as α-, β-, γ-, and δ-, which differ by the number and position of methyl groups on chroman ring. Tocotrienols are found in high concentrations in palm oil and rice bran [23]. Other natural sources include coconut oil, cocoa butter, soybeans, barley, wheat germ, meat, and eggs [24]. Although no difference in radical scavenging activity between α-tocopherol and α-tocotrienol was found in hexane, the activity of α-tocotrienol in scavenging peroxyl radicals is 1.5-fold higher in liposomes compared with α-tocopherol [6]. In rat liver microsomes, the efficacy of α-tocotrienol to protect against Fe²⁺+NADPH-induced lipid peroxidation was 40 times higher than that of α-tocopherol. It is speculated that the side chain of tocotrienols altered the membrane distribution when compared with tocopherols [27]. Packer et al. [25] have summarized that greater antioxidant activity of α-tocotrienol results from the following: 1) more uniform distribution in membrane bilayer, 2) stronger disordering of membrane lipids, 3) more effective collision with radicals, 4) greater recycling activity of chromanoxyl radical, and 5) recycling activity correlated with inhibition of lipid peroxidation. Recently, Yoshida et al. [26] have compared the antioxidant properties for these 8 vitamin E homologues. They obtained the following results: 1) the corresponding tocopherols and tocotrienols exerted the same reactivity toward radicals and the same antioxidant activities against lipid peroxidation in solution and liposomal membranes; 2) tocopherols gave more significant physical effect than tocotrienols on the increase in rigidity at the membrane interior; and 3) tocotrienols were more readily transferred between the membrane and incorporated into the membrane than tocopherols. These data indicate that superior antioxidant activity of tocotrienols in membranes depends on the structure of the hydrophobic side chain, which is important for the mobility of the molecule, and that, in homogenous solutions, the reaction rate constant between vitamin E and the peroxyl radical depends mainly on the number of methyl groups on the chromanol nucleus [25].

Inhibition of Atherosclerosis in Murine Model by Tocotrienol

Black et al. [27] evaluated the effects of palm-vitamin E, α-tocopherol, and palm-carotenoids on apolipoprotein E+/- female mice, which develop atherosclerosis when fed diets high in triglyceride and cholesterol. With supplements of 0.5 g/100 g or 1.5 g/100 g palm-vitamin E, the size of the lesions was 92 or 98% smaller, respectively (Fig. 3). In contrast, the 0.5 g/100 g α-tocopherol and palm carotenoid supplements had no effect. These results indicate that this antiatherogenic effects of palm-vitamin E is probably due to the content of tocotrienols in the supplement. Qureshi et al. recently showed that the tocotrienol-rich fraction (TRF25) and didesmethyl tocotrienol (d-P25-T3 with no methyl groups on the chromanol ring) of rice bran inhibited atherosclerotic lesions in C57BL/6 apoE-deficient mice [28] and cholesterogenesis in hereditary hypercholesterolemic swine [29], and demonstrated a superior efficacy of tocotrienols compared with α-tocopherol. In their experiments, mice were fed the tested diet with or without 100 µg d-α-tocopherol, TRF25 or d-P25-T3/g. They have demonstrated that the novel tocotrienols have significant antiatherogenic effects.

Tocotrienols and Atherosclerosis

Tocotrienol d-P25-T3 can substantially reduce the growth of atherosclerotic plaques in all three of tested mouse genotypes (apoE+/+, apoE+/-, and apoE--/) and diet combinations that produce plaque. In order to confirm the superior anti-atherogenicity of tocotrienol compared to tocopherol, the bioavailability after its oral administration in rodents as well as human should be more clarified.

Inhibition of Cholesterol Synthesis by Tocotrienol

Although the exact mechanism of inhibition of atherosclerotic lesions by tocotrienols has not been elucidated, there is evidence suggesting that tocotrienols affect several distinct steps in the pathways leading to formation of complex atherosclerotic lesions (Table 1). In this review, we focused on following actions of tocotrienols: 1) inhibition of cholesterol synthesis and 2) regulation of inflammatory response of endothelial cells. Cell culture studies indicate that tocotrienols influence cholesterol synthesis by directly regulating the expression of 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMG-CoA reductase), principally through a post-transcriptional process involving accelerated degradation of the reductase protein [30]. This effect was ascribed to the side chain's unique ability to increase cellular farnesol, which in turn, has been shown to downregulate HMG-CoA reductase activity [23]. Among the four isomers, δ-tocotrienol is the most potent HMG-CoA reductase inhibitor [31]. On the other hand, α-tocopherol has been shown to actually increase the activity of HMG-CoA reductase. However, there are some conflicting results in the literature regarding the cholesterol-lowering effects of tocotrienols in vivo. Hypercholesterolemia pigs fed a tocotrienol-rich diet showed a 44 and 60% decrease in total serum cholesterol and LDL-cholesterol, respectively [32]. When rats were fed an atherogenic diet, both α-tocotrienol and α-tocopherol significantly lowered plasma lipid concentrations [33]. Interestingly, dietary α-tocopherol attenuated the cholesterol-lowering effect of α-tocotrienol in both humans and chickens [34, 35]. In a double-blind, placebo-controlled trial, no effect of a vitamin E supplement rich in tocotrienols on serum lipid, lipoproteins or platelet function in men with mildly elevated serum lipid concentrations was found [36]. In addition to hypocholesterolemic property, tocotrienol may reduce the plasma levels of apolipoprotein B (ApoB) and lipoprotein (Lp) (a), which have been recognized as an independent risk factor for the development of atherosclerosis. In recent studies, tocotrienol was shown to decrease the plasma ApoB and Lp (a) levels by 15 and 17%, respectively [34, 37]. Further studies will be necessary for clarifying the cholesterol-, ApoB-, and Lp (a)-lowering effect of tocotrienol. Especially, the cellular concentration of farnesol, a candidate of HMG-CoA inhibition, should be determined in the liver after tocotrienol administration.

Tocotrienols and Endothelial Function

Recently, it has been reported that α-tocotrienol is a potent and effective agent in the reduction of cellular adhesion molecule expression and monocytic cell adherence [7]. Pretreatment of HUVEC with various concentrations of α-tocotrienol (5–25 μM) for 20 h followed by tumor necrosis factor (TNF)-α stimulation demonstrated an inhibitory effect on VCAM-1 cell surface expression as well as monocytic cell adhesion to HUVEC. Compared to α-tocopherol and α-tocophenyl succinate, α-tocotrienol displayed a more profound inhibitory effect on adhesion molecule expression and monocytic cell adherence. This inhibitory action of α-tocotrienol on TNF-α-induced monocyte adhesion was shown to be nuclear factor (NF)-κB-dependent and was interestingly reversed with co-incubation with farnesol and geranylgeraniol, suggesting a role for prenylated proteins in the regulation of adhesion molecule expression. In addition, they found that δ-tocotrienol is a potent and effective agent for the reduction of cellular VCAM-1 and E-selectin expression and monocytic cell adherence compared to α-tocotrienol and γ-tocotrienol [38]. Recently, we compared the effects of α-tocopherol and four isoforms of tocotrienol on the adherence of monocytes to endothelial

Table 1. Potential mechanisms by which tocotrienols inhibit atherosclerosis.

<table>
<thead>
<tr>
<th>Target</th>
<th>Biological function</th>
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<tbody>
<tr>
<td>Cholesterol</td>
<td>Inhibit its synthesis and reduce its plasma level</td>
</tr>
<tr>
<td>LDL</td>
<td>Inhibit its peroxidation</td>
</tr>
<tr>
<td>Lipoprotein (Lp)</td>
<td>Reduce plasma levels of apoLp B and Lp (a)</td>
</tr>
<tr>
<td>Endothelial cells</td>
<td>Decrease adhesion molecule expression</td>
</tr>
<tr>
<td></td>
<td>Inhibit chemokine production</td>
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<tr>
<td></td>
<td>Inhibit its adherence to endothelium</td>
</tr>
<tr>
<td>Platelet</td>
<td>Inhibit its adherence and aggregation</td>
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Vol. 34, No. 3, 2003
Fig. 4. Effects of tocotrienol on VCAM-1 expression induced by 7-ketocholesterol. Human aortic endothelial cells were grown and pretreated with α-, β-, γ-, and δ-tocotrienol (10 μM) for 24 h. Cells were washed and changed to media supplemented with 7-ketocholesterol (10 μM). ELISA was carried out to measure the surface expression of VCAM-1. Results represent mean±SEM. *p<0.01 vs. normal group and †p<0.05 and ‡p<0.01 vs. control group.

cells. In our study, HAEC confluent monolayers prepared in 48-well plates were stimulated with 7-ketocholesterol (10 μM) for 4 h. Subsequently, HAEC were washed with Hanks’ balanced salt solution, and U937 monocytic cells were added to each well and incubated at 37°C for 30 min. The plate was washed 3 times with Hanks’ balanced salt solution and fixed in 1% paraformaldehyde in phosphate buffered saline. The number of adherent cells was counted in twenty microscopic fields defined by an eyepiece and VCAM-1 expression was determined by enzyme-linked immunosorbent assay. To investigate the role of adhesion molecules in the adherence of monocyte to 7-ketocholesterol-exposed HAEC, monoclonal antibodies to adhesion molecules were used. Anti-VCAM-1 and anti-ICAM-1 monoclonal antibodies significantly reduced the monocyte adherence to HAEC, indicating that monocyte adherence to 7-ketocholesterol-stimulated HAEC is involved in VCAM-1 and/or ICAM-1-dependent pathway. In addition, α-tocopherol (10 μM) did not affect the number of adherent monocytes and VCAM-1 expression, in contrast, pretreatment of HAEC with either 10 μM α-tocotrienol, β-tocotrienol, γ-tocotrienol or δ-tocotrienol for 24 h prior to stimulation with 7-ketocholesterol all displayed significant inhibitory effects on monocyte cell adherence and VCAM-1 expression (Fig. 4). These results suggest that reduction of monocyte adhesion by tocotrienol is attributed to decreased adhesion molecule expression. Notably, δ-tocotrienol exerted a more profound inhibitory action on monocyte cell adherence when compared to α-tocotrienol and α-tocopherol.

Recently, Noguchi et al. [8] clearly gave reason for the differences among these tocotrienols in inhibitory effects on monocyte-endothelial interaction. They showed that α-tocotrienol accumulated in human umbilical endothelial cells to levels approximately 10-fold greater than that of α-tocopherol, and that the efficacy of tocotrienol for reduction of VCAM-1 expression and adhesion of monocytic cells to endothelial cells was also 10-fold higher than that of tocopherol. These results indicate that the inhibitory effects of vitamin E analogs on the adhesiveness of endothelial cells correlate with their intracellular concentrations. Although the exact molecular mechanism of inhibition of endothelial inflammatory response by tocotrienols has not been elucidated, recent reports suggest that tocotrienols affect the mRNA expression of several genes and activate/regulate the targeted proteins [9, 39, 40]. Recent advances in genomics and proteomics will prove the novel mechanism, probably beyond antioxidant, of inhibition of atherosclerosis by tocotrienols.

References


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