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Review

Vitamin E and the Prevention of Atherosclerosis

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Abstract: Successful strategy for the prevention of coronary heart disease (CHD) in particular of atherosclerosis, require a detailed understanding of the underlying mechanism. It is now being recognised that dietary antioxidants, in particular vitamin E, will play an important role in designing future strategies. Although more and more beneficial effects of vitamin E on atherosclerosis are being described, the biochemical and cell biological mechanism underlying these benefits are not yet fully understood, preventing the use of vitamin E as therapeutic agent. Recent new findings have shed new light on the physiological role of vitamin E and suggest that it has a much broader array of biological activities than originally expected. In addition to its well described role as an antioxidant, it is becoming evident that vitamin E also can modulate the immune system, suppress local and chronic inflammation, reduce blood coagulation and thrombus formation, and enhance cell function and survival. This review summarises new findings from in vitro studies and discusses their potential relevance in human atherosclerosis.

Key words: Vitamin E, prevention, atherosclerosis, review, pathogenesis, cell, apoptosis, immunmodulation, hemostasis, macrophages

Introduction

The term "vitamin E" is often used to denote a mixture of biological active tocopherols. Tocopherols are lipophilic antioxidants found mainly in vegetables, oils, nuts and fish. Due to their radical scavenging properties tocopherols are potent inhibitors of lipid peroxidation, RRR-α-tocopherol being the most abundant and most active antioxidant. Radicals are produced in considerable quantities during normal metabolism and can be significantly increased by ischemia, inflammatory and degenerative process, ionising radiation, and other trauma. Vitamin E reacts with these noxious compounds and inactivates them. The reaction involves the abstraction of a hydrogen atom from the hydroxyl group (OH) of the chroman ring of vitamin E, resulting in the reduction of the radical to an unreactive product.

Free radical injury plays a key role in many chronic diseases including atherosclerosis, the main cause of death in adults in industrialised countries. Classic risk factors of

Abbreviations used: AggLDL, aggregated LDL; CHD, coronary heart disease; CMV, cytomegaly virus; DHT, delayed-type hypersensitivity skin test; FA; fatty acids; HAEC, human artery endothelial cells; HDL, high density lipoproteins; IL, interleukin; L•, lipid-centred radicals; LOO•, lipid peroxyl radical; LDL, low density lipoproteins; LFA-1, leukocyte associated antigen 1; MI, myocardial infarction; MM-LDL, minimal modified LDL; MUFA, monounsaturated fatty acids; NO, endothelium-derived relaxing factor; OH, hydroxyl group; OxLDL, oxidised LDL; PDGF, platelet-derived growth factor; PGI_2, prostaglandin I2, Prostacyclin; PP2A, protein phosphatase 2A; PTCA, percutaneous transluminal coronary angioplasty; PUFA, polyunsaturated fatty acids; SMC, smooth muscle cells; VLA-4, very late antigen 4; VLDL, very low density lipoproteins.

CHD such as hypertension, diabetes, hypercholesterinemia, adipositas and smoking do not sufficiently explain the pathogenesis of atherosclerosis. New risk factors have now been defined including suboptimal plasma levels of antioxidants, primarily vitamin E, C and β-carotene [1]. The prevention of CHD, and particularly of myocardial infarction (MI), is based on treating both asymptotic high-risk patients (primary prevention) and patients with established CHD (secondary prevention). It is now being recognised that dietary antioxidants, in particular vitamin E, could be part of a successful prevention strategy by the advantage of very low adverse effect at high intake doses. This review summarises in vitro effects of vitamin E and its potential role for the prevention of atherosclerosis.

Vitamin E and atherosclerosis

Pathogenesis of atherosclerosis

Atherosclerotic lesions can be classified as early, intermediate and late or complex lesions. It is now generally accepted that lesion formation is initiated as the result of either some form of insult to the lining of the artery wall or a general impairment of the endothelium [2, 3]. Endothelial dysfunction can rapidly lead to the development of a chronic inflammatory response, characterized by the transmigration and accumulation of peripheral blood monocytes and T lymphocytes in the vessel wall. Various factors expressed and released by vascular wall cells play an important role in the development and progression of atherosclerotic lesions. LDL accumulates in a concentration-dependent process in the subendothelial space and is oxidized by radicals or reactive oxygen species (ROS) formed during the inflammatory response [4]. Scavenger receptors present on monocyte-derived macrophages are responsible for the uptake of modified LDL, e.g. oxidised-LDL (OxLDL) and aggregated LDL (AggLDL). The unregulated uptake of modified LDL leads to the intracellular accumulation of lipids in macrophages resulting in their transformations into foam cells. The clustering of foam cells, i.e. the fatty streak, is the hallmark of the earliest microscopically detectable lesions. Interestingly, these lesions appear predominantly in branching regions of the arterial tree, areas subjected to high shear stress.

Fibrous plaques are palpably elevated areas of intimal thickening and represent the most characteristic lesions of advancing atherosclerosis. They consist of a central core of extracellular lipid and necrotic cell debris covered by a fibromuscular layer, or cap, containing a large amount of collagen and smooth muscle cells. The fibrous plaque contains monocyte-derived macrophages, smooth muscle cells and T-Lymphocytes, many of which are activated, as evidenced by HLA-DR expression [2].

Complex lesions are calcified fibrous plaques characterized by various degrees of necrosis, thrombosis and ulceration. With increasing necrosis and accumulation of gruel, the arterial wall progressively weakens. This can lead to the rupture of the fibrous cap resulting in aneurysms, haemorrhaging, arterial thrombus formation and ultimately the total occlusion of the vessel, i.e. the clinical manifestation of a heart attack.

Vitamin E and oxidation of low density lipoprotein

OxLDL plays a central role in all stages of atherogenesis [5]. This hypothesis is supported by numerous *in vitro* studies [6]. OxLDL has many potentially proatherogenic properties, including the ability to transform macrophages into foam cells [7]. A recent study showed, however, that OxLDL has to be aggregated to induce cholesteryl ester accumulation in human macrophages [8]. LDL oxidation may be induced by radicals, enzymes or various cells e.g. endothelial cells (EC), smooth muscle cells (SMC) and macrophages [4]. The abstraction of hydrogen from fatty acids (FA) such as polyunsaturated fatty acids (PUFA) in LDL particles results in the formation of lipid-centred radicals (L•) [9]. Not only FA but also apolipoprotein B-100 is modified during LDL oxidation [10]. L• lead to the formation of lipid peroxyl (LOO•) and alkoxyl radicals which in turn can initiate a radical chain reaction in neighbouring FA. In the absence of chain-breaking antioxidants, lipid peroxidation is terminated via a bimolecular process, e.g. L• + LOO•.

α-Tocopherol suppresses lipid peroxidation through two mechanisms: by trapping the initial oxidant or by reacting with the radical LOO• [9]. The resulting tocopheryl radical is much less reactive than L• and LOO• and can be reduced to α-tocopherol by ascorbic acid or other coantioxidants [9]. *In vitro* studies support the observation that α-tocopherol supplementation prolongs the lag phase during Cu^{2+}-mediated oxidation of LDL [9].

However, in the absence of coantioxidants, α-tocopherol acts as a prooxidant. The following mechanism for the action of vitamin E during LDL-oxidation has been proposed [9]: Radicals which encounter LDL particles react with and oxidise α-tocopherols on the particle surface and are transported outside the core of the LDL particles as tocopheryl radicals and may therefore react with lipophilic antioxidants. Hence, the balance between α-tocopherol, LDL and the level of coantioxidants is likely to determine, whether LDL will become oxidised or not. The proposed mechanism is supported by the finding that in the presence of physiological antioxidants such as vita-

min C, α-tocopherol clearly acts as an antioxidant [10]. This prooxidative property of vitamin E may explain why intervention studies with vitamin E alone in animal models of atherosclerosis showed no clear benefit and even apparently contradictory results [9]. In most studies, vitamin E showed no effect at all. In a few studies, a clear antioxidative and preventive effect was observed. Some studies even showed an increase in lesion formation after vitamin E treatment. Several clinical studies have demonstrated positive relationships between vitamin E intake and the prevention of atherosclerosis [11]. Future studies should therefore include both vitamin E and its coantioxidants in order to observe any protective natural effect by vitamin E.

Vitamin E and cell death

Cell death, in particular apoptosis, may play an important role in atherosclerosis [12]. Apoptosis, also known as programmed cell death, is induced by a variety of different mechanisms including cell interactions with soluble mediators as well as by direct cell-cell interaction. Apoptotic features have been observed in cells of human atherosclerotic plaques. It is well known that OxLDL is cytotoxic for several types of artery wall cells, apparently by inducing both, apoptosis and necrosis. Preventing LDL oxidation is clearly one beneficial function of antioxidative vitamins resulting in increased cell viability. There are contradictory reports on the role of ascorbate and α-tocopherol in the prevention of apoptosis. On the one hand, both antioxidants prevent apoptosis in serum deprived HL60 cells [13]. Recent evidence suggests that reactive oxygen metabolites such as those found in OxLDL may affect the balance between apoptosis and cell proliferation, which may have a profound effect on the development of atherosclerotic lesions [14]. On the other hand, OxLDL-induced apoptosis in human macrophages is not prevented by either ascorbate or α-tocopherol [15]. Interestingly, transformation of human macrophages into foam cells results in loss of cellular vitamin E and a concurrent increase in the susceptibility to cell lysis by OxLDL [16]. Surprisingly, vitamin E supplementation did not improve cell viability. Clearly, more work is needed to clarify the role of vitamin E in vascular cell death.

Vitamin E and cell function

Endothelial cells

Endothelial cells regulate the exchange of nutrients and hormones between blood and tissues. Injury of endothelial cells, for example by OxLDL, mechanical stress, immunological injuries, homocysteine or toxins, leads to the dysfunction of these cells [2, 3]. Endothelium dysfunction contributes to the clinical manifestations of thrombosis, vascular spasm, as well as of atherosclerosis. Various pathways lead to these pathologies which involve one or several of the following: increased platelet aggregating factors and decreased anticoagulant factors, e.g. endothelium-derived relaxing factor (nitric oxide, NO), platelet-derived growth factor (PDGF), prostacyclin (PGI2) and endothelin.

Nitric oxide is important in the local control of vascular tone and the adhesion of platelet and monocytes to endothelium. OxLDL influences the effect of endothelium-derived nitric oxide, on the one hand, by inhibiting receptor-mediated endothelium-dependent arterial relaxation and, on the other hand, by degrading endothelium-derived nitric oxide directly [17]. NO, therefore, is sensitive to oxidative stress, and vitamin E may preserve NO bioactivity [18]. *In vitro* studies suggest that substances inhibiting nitric oxide synthase such as tocopherols may further be cytoprotective by inhibition lipid peroxidation [19]. In animal studies, arteries deficient in α-tocopherol showed a dose-dependent impairment of NO activity by OxLDL while α-tocopherol-rich vessels showed a marked increase in their resistance against OxLDL. How they may influence each other is at present unclear, but a radical mechanism is possible.

Similar studies on vitamin E and NO bioactivity in humans have not yet been reported. But factors such as dosage and duration of vitamin E supplementation are currently being discussed. It was also suggested that the beneficial effects of cholesterol lowering therapy on arterial relaxation may be enhanced in presence of vitamin E [18].

OxLDL also modulates the synthesis of other endothelial cell-derived mediators such as PDGF and chemotactic factors. OxLDL also regulates the expression of endothelium-derived adhesion molecules. Vitamin E appears to counteract the effects of OxLDL as vitamin E supplementation decreases PDGF synthesis *in vitro* [20] and protects human aortic endothelial cells (HAEC) from cytotoxic injury induced by OxLDL [21]. Furthermore, vitamin E reduces OxLDL uptake by endothelial cells, preserves the barrier function, increases the activity of cytostolic phospholipase A2 and cyclooxygenase, and augments PGI_2 release by EC [4]. These findings suggest that vitamin E acts both directly on EC as well as indirectly by modulation of OxLDL formation and activity.

Smooth Muscle Cells

Migration of SMC from the media to the intima and SMC proliferation are central events in intimal thickening, the formation of fibrous plaques and in particular in restenosis after coronary artery balloon angioplasty [2, 3]. OxLDL induces the migration of SMC possibly by counteracting the inhibitory effect of endothelial cells on SMC

migration [22]. The synthesis of endothelin and the resulting SMC migration are inhibited by vitamin E [23]. These results are in agreement with the observed reduction of restenosis after percutaneus transluminal coronary angiography (PTCA) in patients which had received vitamin E supplements [17].

In vitro studies indicate that vitamin E also inhibits SMC proliferation, apparently by inhibiting protein kinase C and by activating protein phosphatase 2A (PP2A) [24]. This observation is supported by *in vitro* studies, which suggest that an oxidative stress-induced reduction of α-tocopherol in SMC leads to changes in gene expression and increased cell proliferation, apparently by a non-antioxidant mechanism [25]. Some forms of vitamin E such as α-tocopheryl quinone which have no antioxidant activity also block the activation of protein kinase C suggesting that redox processes are not involved. Other vitamin E analogous such as α-tocopherol inhibit SMC proliferation also by a nonantioxidant mechanism [26]. However, recent data by others support a more indirect mechanism for the effect of α-tocopherol on protein kinase C activation [18].

In a recent paper, vitamin E is reported to downregulate the expression of CD36, a receptor for OxLDL, in cultured aortic SMC [27]. However, CD36 expression in SMC has not been demonstrated in human atherosclerotic lesions putting into question the importance of this mechanism in human atherogenesis. More studies are required to further elucidate the role of vitamin E in SMC function, in particular with regard to human atherosclerosis.

Monocytes and macrophages

Macrophages are present in all stages of atherosclerosis. They play a key role not only as antigen-presenting cells for T-lymphocytes, but they also serve as scavengers of extracellular lipids, lipoproteins and noxious materials. Under the influence of chemoattractants, growth-regulatory molecules and cytokines, monocytes adhere to the endothelium and migrate into the subendothelial intima where they differentiate into mature macrophages [2, 3]. The uptake of modified lipoproteins by macrophages results in the massive accumulation of intracellular lipids, mainly cholesteryl esters. This transformation of lesional monocyte-derived macrophages into foam cells is the hallmark of the earliest detectable atherosclerotic lesions: the fatty streaks.

In macrophages, cellular levels of vitamin E appear to be regulated by the concentration of extracelluar vitamin E. The rate of vitamin E uptake in macrophages correlates with the extracellular α-tocopherol/cholesterol ratio. The transfer of α-tocopherol from LDL and very low density lipoproteins (VLDL) into macrophages – and vice versa – appears to occur mainly by diffusion while high density lipoproteins (HDL)-stimulated efflux of vitamin E appears to underlay a different mechanism [28]. A suboptimal supply of vitamin E to the vessel wall may favour an atherosclerotic macrophages phenotype and thereby indirectly enhance lesion formation and progression. This may further explain the strong inverse correlation between plasma vitamin E and clinical events related to atherosclerosis [29].

Il-1β is a proatherogenetic and proinflammatory cytokine which is a promoter of monocyte-endothelial cell adhesion *in vitro* [17]. The formation of interleukin 1β (IL-1β) by macrophages is inhibited by α-tocopherol. In patients supplemented daily with 1200 IU of α-tocopherol for 8 weeks, vitamin E not only inhibits adhesion of monocytes to the endothelium but also reduces the release of reactive oxygen species and other potentially pro-atherosclerotic factors [17]. *In vitro*, vitamin E dose-dependently blocks the expression of β_2-integrin-dependent cellular adhesion molecules in macrophages [18].

Mild oxidation of LDL results in the formation of minimally modified LDL (MM-LDL) which induces the expression of adhesion molecules on endothelial cells and monocytes. MM-LDL stimulates the release of monocyte chemotactic factors and macrophage stimulating factors from endothelial cells [17]. By preventing LDL oxidation, vitamin E may therefore reduce monocyte adhesion and subsequent transmigration in vessels resulting in decreased atherosclerosic lesions.

Both the secretion of proteolytic enzymes and the activation of lipooxygenase in macrophages is inhibited by tocopherols [29]. Hence vitamin E appears to block proinflammatory activities of macrophages.

Changes in membrane structure induced by oxidative damage causes changes in the membrane cytoarchitecture which are likely to alter cell signalling so it was shown that the receptor response to CD8 monoclonal antibody is significantly decreased by the addition of tocopherol [30]. Vitamin E improves the chemotactic response of macrophages and their phagocytic function *in vitro* [31]. Interestingly, elevated cellular levels of α-tocopherol in mouse peritoneal macrophages and in human monocytes do not affect their ability to oxidise LDL *in vitro* [32]. This suggests that either cell-mediated oxidation of LDL under the conditions of this study is not dependent on cell-derived radical species or that cellular α-tocopherol is unable to inhibit their formation.

A study in rabbit peritoneal macrophages suggested that vitamin E downregulates scavenger receptor activity in macrophages [44]. In contrast to these findings and to previous studies performed in mouse macrophages and cell lines, a recent study in human cells showed that vitamin E has no effect on the transformation of macrophages

into foam cells [8]. The reason for these apparent contradiction is not clear, but species difference may certainly play an important role. During this formation of human foam cells the cellular level of α-tocopherol drops dramatically resulting in an increased susceptibility of foam cell to succumb to the membrane lytic properties of OxLDL [16]. Interestingly, vitamin E supplementation did not prevent the increased susceptibility to cell lysis. If this form of cell death is mediated by OxLDL-derived oxidants, as suggested by several groups, these results put into question the effectiveness of vitamin E as a cellular antioxidant, at least in the absence of physiological levels of coantioxidants, e.g. vitamin C.

Leukocytes

Leukocytes adhesion to endothelial cells is mediated by the expression of integrin molecules on the surface of leukocytes such as leukocyte function associated antigen (LFA-1), very late antigen (VLA-4) and αMβ-2 integrin or MAC-1 [33]. Oral supplementation of vitamin E decreases their expression level on leukocytes and reduces the adhesion of neutrophils to endothelial cells [34] suggesting an antiinflammatory effect. However, vitamin E enhances the proliferation, chemotaxis and bacterial killing power of polymorphonuclear leukocytes in the inflammation process [35]. With regard to atherosclerosis, the latter effect of tocopherol on leukocytes may be of particular importance when infections of the vasculature (see below) accompany the progression of lesions.

Vitamin E and its immunmodulatory effect

The presence of peripheral blood monocytes and T lymphocytes in the arterial wall are a sign of an active immune response characteristic for the chronic and local inflammatory responses to endothelial injury and dysfunction [2, 3]. Since chlamydia and cytomegaly virus (CMV) were first implicated as possible contributors to heart disease, other microbes have joined them as suspects suggesting that infections may at least contribute to the inflammatory compound of atherosclerosis.

Vitamin E appears to act as an immunosuppressant due to its ability to suppress both the humoral and cellular immune responses. [29]. Tocopherols decrease the release of arachidonic acid from membrane phospholipids, resulting in a decreased production of PGE_2. PGE_2 in turn suppresses antibody formation and delayed-type hypersensitivity skin test (DHT) lymphocyte proliferation. It is well-known that PGE_2 has an inhibitory effect on IL-2 production in activated T-cell. However, in a rat model for ageing, vitamin E supplementation increases IL-2 production [36]. PGE_2 appears to be decreased dose-dependably by vitamin E in rats [37]. However, the effective dose of vitamin E required for this effect was 4-fold to 6-fold higher than the amount of α-tocopherol taken up with normal diets putting into questions the immunosuppressive properties of vitamin E *in vivo* [38].

Leukotrienes are a group of biological highly potent lipid mediators involved in intercellular communication, signal transduction and host defence mechanism. Excretion of leukotriene E_4, a metabolite of bioactive leukotriene, is inversely correlated to plasma α-tocopherol [39]. While many reports support an immunosuppressive role for α-tocopherols, some studies indicate α-tocopherols may also have immunostimulative properties. Some studies indicate that cellular immunity is improved through an increased T-cell differentiation by vitamin E while T-lymphocyte proliferation is enhanced [36]. Vitamin E increases the ratio of suppressor/killer T-lymphocytes which should results in a higher immunity. Conversely, vitamin E defiency can lead to both defective T-cell differentiation and incomplete maturation in thymus. On the other hand, α-tocopherol supplementation increases the number of positive antigen responses in the DHT [38]. *In vivo*, lymphocyte function may therefore be sensitive to any changes in vitamin E level [30]. How the immunomodulatory properties of vitamin E affect atherogenesis and lesion progression *in vivo* is not yet clear.

Vitamin E, hemostasis and plaque rupture

Further progress in the search for more effective and safe antithrombotic agents is coupled with an improved understanding of the factors involved in arterial and venous thrombosis. Arterial thrombosis is initiated by factors described by Virchow's triade. The continued growth of platelet mass depends on the presence of free thrombin. Thrombin is generated mainly after the activation of factor XII on the platelet surface by exposed collagen. Vitamin E decreases thrombin formation which results in reduced thrombus formation [45]. PGI_2 is a potent inhibitor of platelet aggregation and has vasodilatory activity. Many studies have shown that vitamin E supplementation causes a marked decline in prostacyclin levels. For example, prostacyclin produced by HAEC correlated positively with cell damage induced by OxLDL and negatively with vitamin E [40]. Thromboxane A_2, which is measured as thromboxane B_2, is a potent cellular regulatory agent with strong platelet-aggregating activity. Vitamin E supplementation decreases thromboxane A_2-levels *in vivo* supporting the role of vitamin E as an antithrombotic agent [41].

Following plaque fissure, the earliest event is the adhesion and aggregation of platelets leading to thrombus formation. Different studies using high doses of α-tocopherol (400 and 1200 IU) support an inhibitory effect of

tocopherol on platelet adhesion and aggregation induced by collagen and ATP [17]. *In vitro* vitamin E inhibits collagen-induced platelet activation by blunting hydrogen peroxide formation [42]. At normal physiological plasma levels, vitamin E does not appear to affect platelet function [18]. Some studies suggest the inhibition of protein kinase C by vitamin E may account for the inhibition of platelet aggregation in response to fibrinogen, which plays an important role in thrombus formation. Fibrinogen levels are decreased in the presence of vitamin E, thereby reducing the risk of thrombus formation [29].

Finally, vitamin E reduces the formation of PAF (platelet-activating-factor) in polymorphonuclear leukocytes, also resulting in reduced platelet aggregation and activation. During aggregation, platelets release NO. Loading of platelets with vitamin E increases NO production, which in turn results in decreased platelet aggregation [18]. Reduced platelet superoxide production during aggregation may be the reason for the vitamin E-induced increase in NO production [18]. *In vivo*, platelet NO production correlated with α-tocopherol plasma level. The strong antithrombotic effect of vitamin E is due to its concurrent action at multiple sites of the Virchow`s triad. Patients treated with levels of 50 mg vitamin E per day are therefore at a higher risk for haemorrhagic stroke [43].

Conclusion

While several clinical studies underscore the importance of including vitamin E in strategies directed at preventing atherosclerosis, the molecular mechanisms underlying the benefits of vitamin E are not yet fully understood. The results from the recent studies we reviewed herein support the hypothesis that the benefical effects seen with vitamin E in the clinic may not be explained by a single molecular mechanism. The overall antiatherogenic potential of vitamin E appears to be the result of a large number of sometimes minor effects, acting in concert to prevent harmful side-effects of physiologically essential oxidative reactions, to increase cell resistance to oxidative damage and reduce uncontrolled cell death, to optimize the immune response and enhance cell-cell communications, and finally, to keep thrombosis in check. Clearly, vitamin E is more than just a radical scavenger but more work is needed to elucidate its full range of potentials.

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