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The role of α -tocopherol in preventing disease

■ **Summary** A role of oxidative stress in atherosclerosis lies on experimental results carried out in vitro and in animal models. In humans, the supplementation with the antioxidant vitamin E has given in some cases supportive results and in others no effects. From in vitro studies, a large amount of data has shown that α -tocopherol (the major component of vitamin E) regulates key events in the cellular pathogenesis of atherosclerosis.

We first described the inhibition of protein kinase C (PKC) activity by α -tocopherol to be at the basis of the vascular smooth muscle cell growth inhibition by this compound. Subsequently, PKC was recognized to be the target of α -tocopherol in different cell types, including monocytes, macrophages, neutrophils, fibroblasts and mesangial cells. Inhibiting the activity of protein kinase C by α -tocopherol results in different events in different cell types: inhibition of platelet aggregation, of nitric oxide production in endothelial cells, of superoxide production in neutrophils and macrophages as well as impairment of smooth muscle cell proliferation. Adhesion molecule expression and inflammatory cell cytokine production are also influenced by α -toco-

pherol. Scavenger receptors, particularly important in the formation of atherosclerotic foam cells, are also modulated by α -tocopherol. The oxidized LDL scavenger receptors SR-A and CD36 are down regulated at the transcriptional level by α -tocopherol. The relevance of CD36 expression in the onset of atherosclerosis has been indicated by the protection against atherosclerosis by CD36 knockout mice. In conclusion, the effect of α -tocopherol against atherosclerosis is not due only to the prevention of LDL oxidation but also to the down regulation of the scavenger receptor CD36 and to the inhibition of PKC activity.

■ **Key words** α -tocopherol – atherosclerosis – vitamin E metabolism – CHD

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Atherosclerosis protection by vitamin E is seen in several animal studies

Hypercholesterolemia can lead to enhanced plasma oxidized LDL concentration and impaired endothelial function. Vitamin E can prevent some of these events by a number of mechanisms. In cholesterol-fed rabbits [1, 2], vitamin E fully prevented cholesterol-induced atherosclerotic lesions. In Watanabe rabbits, vitamin E added to the food inhibited LDL oxidation and caused a reduction of the atherosclerotic area [3]. In contrast, New Zealand White rabbits, fed a 1% cholesterol diet and 10.000 IU/kg α -tocopheryl acetate, showed

significantly more intima atherosclerotic proliferation [4].

In atherosclerosis-susceptible apolipoprotein E knockout mice, vitamin E deficiency, created by disruption of the α -tocopherol transfer protein gene, increased the severity of atherosclerotic lesions in the proximal aorta [5].

In a different study [6], male monkeys were given natural vitamin E during 3 years and their carotid arteries thickness was monitored by ultrasound analysis. Vitamin E was found to significantly inhibit the progression of the disease in both cases when the animals received the treatment at the beginning of the experiment, and when atherosclerosis had already started.

The effect of α -tocopherol in animals may not be only due to its antioxidant properties. Probucol, a powerful inhibitor of atherosclerosis in a number of animal models actively increased atherogenesis in LDLR-/- mice, even though it provided a very strong antioxidant protection of LDL [7]. Reduction of atherosclerosis by Probucol observed in some animal models is thus due to intracellular events which are absent in mice or to differences in the metabolism of probucol. A dissociation of atherogenesis from aortic accumulation of lipid hydro(pero)xides in Watanabe heritable hyperlipidemic rabbits has been also shown [8].

An extensive literature coverage of the subject is not appropriate at this time, but the cited studies show that antioxidants may be proatherogenic or antiatherogenic in different animal models and that LDL oxidation does not constantly correlate with atherogenic events.

Vitamin E protects humans against a number of disorders

Vitamin E deficiency is associated with a precise ailment: cerebellar ataxia. Mutations of the α -TTP gene lead to reduced α -tocopherol concentrations in plasma and tissues that ultimately lead to a severe syndrome named ataxia with vitamin E deficiency (AVED) [9]. Following a vitamin E therapy, some of the neurological symptoms of AVED may regress in some patients [10, 11]. Furthermore, vitamin E supplementation has shown beneficial effects for a number of disorders, in particular atherosclerosis, ischemic heart disease, and development of different types of cancer [12–14]. It appears evident that the biological role of vitamin E needs to be rediscussed, since its simple antioxidant function is not sufficient to explain all the effects shown by the molecule.

Protection against human atherosclerosis has been observed in subjects taking high vitamin E quantities with the diet

A study of 16 European populations showed a strong inverse correlation between plasma concentrations of vitamin E and the risk of cardiovascular disease death [15].

In a case control study, the EURAMIC, α -tocopherol and β -carotene had no protective effect [16] although a large prospective cohort study (Nurses' Health Study) revealed that those who obtained vitamin E from supplements had a relative risk of nonfatal myocardial infarction or death from coronary disease of 0.54 [17].

Arterial imaging studies

The arterial wall thickness (IMT) can be measured non-invasively by ultrasound and consequently the extent of atherosclerosis at early, sub-clinical stages can be evaluated [18, 19]. In the EVA trial it was shown that higher red blood cell vitamin E was correlated with lower thickening of the arterial wall [20].

Also in the Kuopio Ischemic Heart Disease Study [21] a very significant inverse correlation between the progression of carotid artery narrowing and vitamin E plasma levels was found. The Antioxidant Supplementation in Atherosclerosis Prevention study (ASAP) has analyzed the effect of vitamin E and C on 3-year progression of carotid atherosclerosis [22]. Atherosclerotic progression, measured by IMT, was reduced by 74% in the male population receiving both vitamins. No effect on the arterial wall thickness has been found in the female group.

Using data from the Cholesterol Lowering Atherosclerosis Study (CLAS) [23], less carotid IMT progression was found for high supplementary vitamin E users when compared with low vitamin E users [24]. However, in the Study to Evaluate Carotid Ultrasound changes in patients treated with Ramipril and vitamin E (SECURE), showed no differences in atherosclerosis progression rates between patients on vitamin E and those on placebo, whereas treatment with ramipril showed a beneficial effect [25].

Although it appears from the majority of this type of studies that vitamin E protects against carotid thickening, more complex results are provided by the SECURE trial and by the ASAP trial.

Controlled intervention trials

The classical Alpha-Tocopherol beta Carotene (ATBC) trial, the Linxian China trial, the Cambridge Heart Antioxidant Study (CHAOS) in England, and Gruppo Italiano per lo Studio della Sopravvivenza nell' Infarto Miocardico (GISSI) in Italy have given contrasting results.

The CHAOS study, a secondary prevention trial that enrolled subjects with established heart disease [26], showed that vitamin E administration resulted in 77% reduction in the risk for nonfatal myocardial infarction. However, the results of the GISSI trial (11.324 patients who had survived a myocardial infarction) did not reach statistical significance for the group given vitamin E [27, 28]. A re-evaluation of the data [29–31] suggested more recently that cardiovascular mortality was significantly reduced by vitamin E in GISSI and the effect on overall survival showed a very favorable trend. The Linxian China Study tested four micronutrients on overall mortality and cancer mortality [32]. The subjects' small but

significant reductions in total and cancer mortality were observed in subjects receiving β -carotene, vitamin E, and selenium.

The ATBC trial tested the effects of vitamin E, β -carotene, and both micronutrients together in heavy smokers [33]. The subjects on vitamin E experienced 32 % lower risk of prostate cancer and a 41 % lower mortality from prostate cancer [34]. A statistically significant benefit was not found for either micronutrient on heart disease [35]. It should be noticed that, like the Linxian study, the ATBC trial used a much smaller dose of synthetic vitamin E than the CHAOS and the GISSI studies.

In the Heart Outcomes Prevention Evaluation study (HOPE) [36], primary outcomes, defined as myocardial infarction, stroke or cardiovascular disease death, did not differ after vitamin E administration.

In the Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease (SPACE) [37], a 46 % reduction was attained in the primary endpoint (myocardial infarction, ischemic stroke, peripheral vascular disease, and unstable angina).

The MRC/BHF Heart Protection Study Collaborative Group 2001 has carried out a randomized trial of cholesterol-lowering therapy and of antioxidant vitamins in 20,536 people at increased risk of coronary heart disease death. They have shown that statins can reduce the risk of heart attack or stroke by up to one third but vitamin C and/or vitamin E were without evident benefit. [Congress of the American Heart Association Scientific Sessions, 2001, November 11–14. Anaheim, California].

Of the most important intervention studies, CHAOS and SPACE are consistent with each other and a careful analysis of the GISSI study reveals that α -tocopherol supplementation resulted in significant effects. However the HOPE and the MRC/BHF Heart Protection Study Collaborative Group have given decisive negative outcomes. It is clear that the selection of the population, the amount of tocopherol, the ability of being absorbed, the genotypic and nutritional aspects of the population studied may be important in the understanding of the present discrepancies.

Selective uptake of vitamin E into the body

Vitamin E is taken up together with dietary lipids in the proximal part of the intestine. The tocopherols are re-assembled together with lipids and apolipoproteins into chylomicrons. Chylomicron lipolysis, facilitated by lipoprotein lipase, allows part of vitamin E to be distributed to tissues [38]. Chylomicron remnants deliver the other part of α -tocopherol to the liver, where, specifically recognized by the 32 kDa α -tocopherol transfer protein (α -TTP), is incorporated into VLDL, and then transported and delivered to peripheral cells

[39]. The plasma phospholipid transfer protein (PLTP) facilitates the exchange of tocopherol between LDL and HDL [40].

The scavenger receptor SR-B1 promotes the uptake of HDL tocopherol into type II pneumocytes [41], into cells constituting the blood brain barrier [42] and into the liver, where it is again specifically recognized by α -TTP, recycled and secreted in VLDL [43]. α -TTP gene mutation results in low serum and cell α -tocopherol. Thus, in the two factors needed for realizing an adequate level of α -tocopherol in the body are dietary availability and the expression of liver α -TTP. Relative affinities of tocopherol analogs for α -TTP, calculated from the degree of competition for the α form, are as follows: α -tocopherol, 100 %, β -tocopherol, 38 %; δ -tocopherol, 9 %; α -tocopherol, 2 %; α -tocopherol acetate, 2 %; α -tocopherol quinone, 2 % [44]. Some of the eight different side-chain isomers of racemic tocopherol are excluded from the plasma and secreted with the bile [45, 46].

Tocopherol binding proteins in tissues

α -TTP is expressed in the liver, in some parts of the brain [47], in the retina [48], lymphocytes and fibroblasts [49] as well as in the labyrinthine trophoblast region of the placenta. The latter may play an important role in supplying the vitamin to the fetus, and explains the fetal resorption occurring in rats fed a vitamin E deficient diet [50].

It is still unclear how many other α -tocopherol binding proteins exist, and which mechanism regulates α -tocopherol transfer and its concentration in peripheral cells. Recently, a novel tocopherol binding protein has been identified, the 46 kDa tocopherol associated protein (TAP) [51]. Ubiquitously expressed, TAP may be specifically involved in the intracellular transport of tocopherol, for example between membrane compartments and the plasma membrane, similar to the yeast secretory protein (sec14). Being provided with GTPase activity TAP may regulate functions, such as phospholipid/tocopherol signalling, phospholipid/tocopherol secretion or adjusting the tocopherol composition of membranes.

A 15 kDa tocopherol binding protein (TBP), which preferentially binds α -tocopherol, may be responsible for intracellular distribution of α -tocopherol [52, 53].

Molecular properties of α -tocopherol

■ Antioxidant and non-antioxidant functions

It is common believe that phenolic compounds like vitamin E exert only a protective role against free radical damage and that vitamin E is the major hydrophobic

chain-breaking antioxidant that prevents the propagation of free radical reactions in membranes and lipoproteins.

The antioxidant properties of vitamin E are well known [54] especially in connection with the prevention of LDL oxidation [55] although the correlation between LDL oxidation and atherosclerosis is not always evident [56, 57]. Alternative studies have suggested that α -tocopherol protection against LDL oxidation may be secondary to the inhibition of protein kinase C (PKC). This enzyme is responsible for triggering the release of reactive oxygen species with consequent lipid oxidation [58, 59].

The non-antioxidant properties of tocopherol have been indicated by several experiments in which the four tocopherol analogues had effects that could not be correlated with their anti-oxidant capacity. Furthermore, the selective uptake and transport of α -tocopherol appears to represent the evolutionary selection of a molecule with unique functions not shared by other antioxidants.

■ Effects of α -tocopherol at cellular level

PKC inhibition was found to be at the basis of the vascular smooth muscle cell growth arrest induced by α -tocopherol [60–62]. It occurs at concentrations of α -tocopherol close to those measured in healthy adults [63]. β -Tocopherol, per se ineffective, prevents the inhibitory effect of α -tocopherol. The mechanism involved is not related to the radical scavenging properties of these two molecules, which are essentially equal [64]. This phenomenon has been confirmed in a number of different cell types, including monocytes, macrophages, neutrophils, fibroblasts and mesangial cells [65–72]. α -Tocopherol, but not β -tocopherol, inhibits thrombin-induced PKC activation and endothelin secretion in endothelial cells [73]. It inhibits also PKC dependent phosphorylation and translocation of the cytosolic factor p47(phox) in monocytes, with consequent impairment of the NADPH-oxidase assembly and of superoxide production [58].

In vitro studies have shown that inhibition of recombinant PKC by α -tocopherol is not caused by a tocopherol-protein interaction. In addition, α -tocopherol does not inhibit PKC expression. Inhibition of PKC activity by α -tocopherol occurs at the cellular level by producing dephosphorylation of the enzyme, whereby β -tocopherol is much less potent [74]. Dephosphorylation of PKC occurs via the protein phosphatase PP₂A, which has been found to be activated by the treatment with α -tocopherol [74–76].

■ Transcriptional regulation by α -tocopherol

Upregulation of α -tropomyosin expression by α -tocopherol, and not by β -tocopherol occurs via a non-antioxidant mechanism [77, 78]. In human skin fibroblasts the age-dependent increase of collagenase expression can be reduced by α -tocopherol [79]. The liver α -tocopherol transfer protein (α TTP) and its mRNA are modulated by dietary vitamin E [80]. Scavenger receptors are particularly important in the formation of atherosclerotic foam cells [81] and disruption of CD36 protects against atherosclerotic lesions. In smooth muscle cells and monocytes/macrophages, the oxidized LDL scavenger receptors SR-A and CD36 are down regulated at the transcriptional level by α -tocopherol but not by β -tocopherol [82–84].

■ Inhibition of monocyte-endothelial adhesion

α -Tocopherol enrichment of monocytes, as well as neutrophils, decreases adhesion to human endothelial cells both in vivo and in vitro [85, 86] and depends on the expression of adhesion molecules [87–89].

■ Inhibition of platelet adhesion and aggregation

α -Tocopherol inhibits aggregation of human platelets by a PKC-dependent mechanism both in vitro and in vivo [68, 90] and delays intra-arterial thrombus formation [91]. The studies reported above are consistent with the conclusions of Iuliano et al. [92] that circulating LDL accumulates in human atherosclerotic plaques and that such accumulation by macrophages is prevented by α -tocopherol in vivo. The protection by α -tocopherol may not be due only to the prevention of LDL oxidation, but also to the down regulation of the scavenger receptor CD36 and to the inhibition of PKC activity.

Despite a general agreement on the α -tocopherol inhibitory action at PKC level, the expression of several genes, such as CD36 [83], SR class A [93], collagenase [79], and ICAM-1 [88], appears to be regulated by α -tocopherol in a PKC independent way. Furthermore, a number of observations, such as PP2A [74] and diacylglycerol kinase [94] activation, 5-lipoxygenase [95] (Jialal et al. 2001) and cyclooxygenase (Wu et al. 2001) inhibition, still lack a mechanistic explanation.

Conclusions

From the study reported above three relevant conclusions can be derived. 1) It seems improbable that the effects of α -tocopherol, relevant to the protection against atherosclerosis, as described at a biomolecular and ani-

mal level, do not have a counterpart in the prevention of the human pathology. 2) The basis for the contradictory results obtained by similar clinical trials, carried out in different countries and by different research groups, is still obscure. 3) More adequate trial conditions on se-

lected populations are needed to see protective effects of α -tocopherol against human atherosclerosis.

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