Vitamin E, Atherosclerosis and Thrombosis

Francesco Violi, Fausta Micheletta, Luigi Iuliano

Institute of Clinical Medicine I, University La Sapienza, Rome, Italy

Introduction

Vitamin E, a major lipid-soluble, chain-breaking antioxidant includes several tocopherols having the biological activity of RRR-alpha-tocopherol. Vitamin E circulates in the blood as free tocopherol bound to beta-lipoproteins and is present in cell membrane where it exerts a potent defence against lipid peroxidation (1). Blood concentration of vitamin E in humans ranges from 25 to 30 μM, depending on daily intake and body’s ability to absorb fat (1). In the last decade the scientific interest on biological activity of vitamin E increased because of a growing body of evidence linking this vitamin with atherosclerosis and its complications (2). Thus, the oxidative hypothesis of atherosclerosis suggests that LDL accumulates within vessel wall, in particular in the macrophages, as a consequence of its oxidative modification mediated by resident cells (3, 4). A reduced defence against LDL oxidation could favour this process and accelerate atherosclerotic progression. Accordingly, patients with coronary heart disease have lower plasma concentration of vitamin E than controls (2) and prospective studies demonstrated that a daily assumption of vitamin E reduces cardiovascular events (5). According to the oxidative hypothesis of atherosclerosis, this effect has been attributed to the inhibition of LDL oxidation. Alternative mechanism potentially implicated in the antiatherosclerotic activity of vitamin E includes its interference with the activity of platelet and monocyte, in which the intracellular redox status plays an important functional role (6, 7). As platelets and monocytes are both involved in the pathophysiologic process leading to atherosclerotic lesion, the interference of vitamin E with the biological function of these cells may represent another important tool to explore the antiatherosclerotic activity of vitamin E. This review will focus on the open issues related to the use of vitamin E in clinical studies and the potential usefulness in investigating platelet function and clotting activation in patients treated with vitamin E.

Lipid Peroxidation and Chain-breaking Antioxidants

Oxidation of lipids is one of the effects of free radicals-mediated attack to biological molecules. Polysaturated fatty acids are highly susceptible to peroxidation because α-methylenic hydrogens, which are positioned upon the carbon between two double bonds, have low bond-energy and can be abstracted by a free radical. Hydrogen abstraction is followed by molecular rearrangement and formation of a conjugated diene system in the molecule, which has a high absorption in the UV spectrum and is convenient to follow kinetically the peroxidation reaction, as commonly adopted for LDL oxidation (8). The following step is reaction with oxygen and formation of the peroxy radical (LOO°) intermediate. Peculiar of the lipid peroxidation is the chain reaction with recruitment of additional polysaturated fatty acids by intermediate radicals like carbon centered lipid radicals (L°), alkyl radicals (LO°), and peroxy radicals (LOO°), which perpetuate the reaction in presence of oxygen supply. The chain reaction can be interrupted if the radical-carrying lipid molecule is intercepted by an hydrogen donor having peculiar chemical-physical characteristics. By virtue of this action, these molecules are currently named chain-breaking antioxidants and the prototypical molecule in biological system is vitamin E, belonging to the class of phenolic antioxidants. The antioxidant activity of vitamin E is dependent solely on the phenolic ring with generation of a radical intermediate and a quinone. In fact, the antioxidant activity is not lost by changing the chromanol ring with a benzofuranol ring (IRFI005) or by changing the side chain (trolox) (9).

The peroxidation of fatty acids leads to fragmentation of the molecule that affects the physical and biological properties of the cell membrane and lipoproteins (10, 11). By-products of lipid peroxidation have been known to possess biological activity, such as cytotoxic activity (12), cause platelet aggregation (13), bronchoconstriction and vasoconstriction (14).

Vitamin E and Atherosclerosis

Atherosclerosis is a complex process involving entering, modification and accumulation of plasma lipoproteins, and recruitment and proliferation of cells in the artery wall. The process advances from a series of stages with the initial appearance of fatty streaks lesion, composed largely of foam cells which are lipid-englufed macrophages, which evolves towards the complex plaque consisting of a lipid core covered by a fibrous cap and areas rich in inflammatory cells (15). In the recent years a large body of evidence has been gathered in support of the hypothesis that free radical-mediated oxidative processes, in particular oxidation of LDL, and specific products arising therefrom play a key role in atherogenesis (3, 4, 11). Lipid peroxidation products, isoprostanes and oxidized epitopes of Apo B100, as a result of LDL oxidation, have been found in the atherosclerotic plaque (16-18), and antioxidants have been shown to inhibit atherosclerosis in animal models (19-21). This problem was specifically investigated in a study, that simulta-
neously analysed the relationship among oxidative stress, vitamin E supplementation and atherosclerosis in Apo E-deficient mice (22). This study showed an early increase in isoprostanes, indices of oxidant stress, in cholesterol-fed animals and its significant decrease after vitamin E supplementation, along with a significant decrease of atherosclerotic lesion in animals given vitamin E. We have recently studied the uptake of LDL by the atherosclerotic plaque of patients undergoing endarterectomy (23). Radiolabeled LDL intravenously injected have been localised in foam cells of the carotid specimen obtained from endarterectomy. The uptake of LDL was almost completely suppressed in patients treated for 4 weeks with vitamin E (900 mg/day).

Despite these findings, the clinical effects of vitamin E in human atherosclerosis, particularly in coronary heart disease, are still controversial. While observational studies showed that dietary intake of vitamin E is inversely related to coronary heart complications, supplementation studies gave conflicting results (24). The ATBC study analysed the clinical efficacy of 50 mg/day synthetic vitamin E in a population suffering from coronary heart disease, and showed no changes of cardiovascular events during the follow-up (25). The CHAOS study, which analysed the clinical efficacy of 400-800 IU/day natural vitamin E in a population affected by coronary heart disease, showed a significant reduction of cardiovascular events mainly dependent upon reduction of nonfatal myocardial infarction while cardiovascular death was increased but not significantly (26). In the GISSI-Prevenzione trial the effect of 300 mg/day of vitamin E was investigated in a population with previous myocardial infarction during a follow-up of 3.5 years (27). Differently from the CHAOS study, nonfatal myocardial infarction was unaffected by vitamin E while there was a nonsignificant trend towards a reduction of cardiovascular death (27). However, a secondary analysis disclosed a possible beneficial effect of vitamin E on all cardiovascular death (-20%) and sudden death (-35%). The HOPE study is a secondary intervention trial involving 9541 patients who were at high risk for cardiovascular events because they had cardiovascular disease or diabetes in addition to one other risk factor (28). Patients received 400 IU/day vitamin E. In the 4.5 years of follow-up vitamin E did not achieve statistical significance for cardiovascular outcomes.

The reason for these equivocal findings is obscure. There are several issues that must be taken into account when performing trials with vitamin E. First of all, the source of vitamin E must be carefully considered inasmuch as natural and synthetic vitamin E have different bioavailability. Based on study results, it has been concluded that bioavailability of synthetic vitamin E is approximately one-half of natural source of this vitamin (29). This important information is quite in contrast with the plasma levels obtained in clinical trials. Plasma concentration of vitamin E was 44.7 μM in the ATBC study (50 mg/day of synthetic vitamin E) and 51.1 μM in the CHAOS study (400 IU/day natural vitamin E). The MPV study, which assessed the influence of antioxidant treatment on restenosis rate in patients undergoing coronary angioplasty, further complicates this issue. A group of patients was in fact treated with 1400 IU/day vitamin E and, after 6 months of vitamin E supplementation, plasma levels were 60.8 μM (30). In contrast with pharmacokinetic studies, it is evident the lack of any relationship between daily dosage and plasma concentration of vitamin E. Unfortunately, the GISSI-Prevenzione study and the HOPE study did not provide any information on this specific point because plasma concentration of vitamin E after oral supplementation was not assessed.

The importance of adequate plasma concentration of vitamin E to obtain effective antiatherosclerotic effect has been clearly documented. In fact, it has been demonstrated that plasma levels of vitamin E are crucial to observe a beneficial effect on atherosclerotic lesion: in particular it was shown that the higher the plasma concentration of vitamin E the lower was the entity of atherosclerotic lesions (22). As the reduction of atherosclerotic plaque was due to the inhibition of oxidant stress it is important to establish the mechanism by which vitamin E, in virtue of its antioxidant property, exerts an antiatherosclerotic activity. Most pharmacokinetic studies focused on the effect of vitamin E on LDL oxidation but this method is unreliable for clinical purpose and likely accounts for the large variability of daily dosage of vitamin E necessary to inhibit LDL oxidation. Consistent with this suggestion is the lack of relationship between inhibition of LDL oxidation and reduction of atherosclerotic lesion elicited by this vitamin (31).

Measurement of F2-isoprostanes could represent a novel approach for pharmacodynamic studies with vitamin E. Thus, in experimental models there is a close inverse relationship between urinary excretion of F2-isoprostanes and plasma levels of vitamin E (22). F2-isoprostanes have also been found in human atherosclerotic plaque and could therefore represent a useful tool to assess the relationship between oxidative stress and atherosclerotic progression (16). It is interesting to note that isoprostanes have also biological activity inasmuch as they amplify the platelet response to common agonists and induce vasoconstriction (14). However, it is still to be established if isoprostanes are mere markers of oxidative stress or also implicated in the pathophysiology of human atherosclerosis.

On the basis of these observations, it must be underlined that neither observational nor interventional studies adequately investigated the bioavailability of vitamin E and its relationship with cellular and plasma antioxidant activity. In this context, it should be considered that bioavailability of vitamin E is strongly influenced by food intake, with very poor bioavailability when assumed before meals (32). For this reason we believe all the trials that do not contain adequate information on bioavailability and related antioxidant activity of vitamin E should be wisely interpreted.

**Vitamin E and Clotting System**

The influence of vitamin E on clotting system was suggested several decades ago after observing the tendency to thrombosis in vessels of vitamin E-deficient animals (33, 34). Activation of coagulation in the newborn has been correlated to the vitamin E status (35, 36). Jain et al. (36) found shorter recalcification time and lower vitamin E concentration in neonatal blood compared to maternal blood and significant correlation of clotting time with plasma vitamin E. In agreement with these data, in rats infused with endotoxin the consequent disseminated intravascular coagulation was more pronounced in animals fed on vitamin E-deficient diet (37).

The mechanism by which vitamin E affects the clotting system is not known. Experimental evidence suggests that vitamin E might modulate both transcriptional and/or post-transcriptional events of the coagulation system. At post-transcriptional level, vitamin E has been reported to modulate the assembly of prothrombin complex and thrombin generation (38). Influence of vitamin E at transcriptional level can be deduced by the well known effect of oxidant stress on the expression of several genes, such as c-fos and c-myc, translocation of nuclear factor κB, from cytoplasm into the nucleus, and prevention of these events by antioxidants (39-41). Several studies pointed out the influence of antioxidants on the expression of monocyte Tissue Factor (TF), a protein of the extrinsic coagulation pathway that converts factor X to Xa.

Crutchley and Que (7) demonstrated an increased monocyte expression of TF by exposing THP-1 cells to 5 to 10 μM Cu. Based on Fenton chemistry, this effect should be attributed to the formation of
oxygen-free radicals generated by copper ions. The relationship between oxidant species and monocyte expression of TF was also corroborated by using several types of antioxidants such as pyridoline dithiocarbamate, N-acetyl-cysteine, salicylates and BHT (41-43). Thus all these antioxidants inhibited monocyte expression of TF in vitro but it was unclear if they acted at transcriptional and/or post-transcriptional level.

Vitamin E has been also shown to inhibit monocyte expression of TF and thrombin generation at concentration as low as 50 μM (44). These data have been corroborated by clinical studies indicating that oral supplementation with vitamin E significantly reduces plasma levels of prothrombin fragment F1+2, a marker of thrombin generation in vivo, and monocyte expression of TF (45).

Taken together, these data indicate that vitamin E is able to interfere with the activation of clotting system by inhibiting monocyte expression of TF; this effect would be dependent upon its antioxidant activity inasmuch as inhibition of clotting system was coincident with reduction of oxidant stress.

Finally, in considering the effects of vitamin E on clotting system anticoagulant activity of vitamin E metabolites should be taken into account. Vitamin E quinone has been shown to possess potent anticoagulant activity even higher than vitamin K (46). The role of vitamin E quinone in vivo after vitamin E supplementation has never been investigated. A recent study reported that vitamin E given orally 800 to 1200 IU/day does not prolong INR values (47). However, increase in haemorrhagic stroke in patients taking vitamin E in the ATBC trial is between 400 and 1200 IU of vitamin E for 14 days inhibits platelet aggregation but the concentration used (about 1 mM) was much higher than that usually achieved in human circulation after oral supplementation. In contrast, more recent studies demonstrated that extracellular OFR generated by redox cycling of iron, and Heme-iron of Hb, and cleavage of hydrogen peroxide to OH° induce platelet activation (48-53).

The effect of vitamin E on agonist-induced platelet aggregation has been studied in the last two decades. Previous study (54) demonstrated that vitamin E is able to inhibit in vitro platelet aggregation but the concentration used (about 1 mM) was much higher than that usually achieved in human circulation after oral supplementation. In contrast, more recent studies demonstrated that vitamin E, in a range of concentration between 50 and 500 μM, inhibits ex vivo platelet aggregation induced by phospholipase, arachidonic acid and collagen. The mechanism by which vitamin E inhibits platelet function is controversial because it is still to be clearly defined if it depends or not on its antioxidant property. Using phospholipase or arachidonic acid as agonists vitamin E reduced platelet aggregation by inhibiting the activity of protein kinase C, but this effect was not related to its antioxidant property (55). Conversely, the inhibition of collagen-induced PA by vitamin E could be attributed to its antioxidant property inasmuch as it blunted the release of H2O2 induced by collagen itself (56) and, in turn, the arachidonic acid and inositol metabolism (57).

The effect of vitamin E on PA has also been investigated after oral supplementation to healthy volunteers and patients at risk for atherosclerosis. Freedman et al. (55) demonstrated that a daily dosage between 400 and 1200 IU of vitamin E for 14 days inhibits platelet aggregation elicited by phospholipase myristate and arachidonic acid by interfering with platelet protein kinase C activity. Calzada et al. (58) administered 300 mg/day vitamin E for 8 weeks and demonstrated an inhibitory effect on PA induced by ADP, collagen and arachidonic acid. Finally, Pigottelli et al. (56) showed that 600 mg/day vitamin E for four weeks inhibited collagen-induced PA by interfering with platelet H2O2 formation and Ca++ mobilization.

The effect of vitamin E on platelet function has also been investigated in two clinical models, hypercholesterolemia and diabetes mellitus that are associated with enhanced oxidative stress and platelet hyperactivity in vivo (59, 60). In these studies there was a significant correlation between urinary excretion of isoprostane F2-III, a marker of oxidative stress, and 11-dehydro-thromboxane B2, a marker of in vivo platelet activation, suggesting a relationship between oxidative stress and platelet activation. After a daily administration of 100 to 600 mg vitamin E for 8 weeks the Authors also demonstrated a significant reduction of both isoprostane F2-III and 11-dehydro-thromboxane B2, further corroborating the hypothesis that oxidative stress is implicated in platelet activation and that vitamin E supplementation can modulate it.

Conclusions

While experimental studies with vitamin E demonstrated that this vitamin is able to interfere with atherosclerotic lesion, human studies are still inconclusive. A major issue related with the administration of vitamin E in humans is the choice of an adequate daily dosage. Until now daily administration of vitamin E has not been based on appropriate pharmacodynamic studies mainly because inhibition of LDL oxidation was the principal method used to assess its biological activity. Measurement of isoprostanes represents a new interesting approach for dose-finding studies in clinical setting associated with atherosclerotic complications. Due to the relevance of oxygen-free radicals in the activation of platelets and monocytes and the interference of vitamin E with such activation, assessment of platelet and monocyte function in humans may represent another methodological approach for pharmacodynamic studies and for the assessment of antiatherosclerotic activity of this vitamin. Furthermore it remains to be established if the analysis of plasma levels of vitamin E is adequate or not to investigate its biological activity or if other measurement such as intracellular vitamin E concentration is more useful to explore its activity in humans.

References


Received February 24, 2000  Accepted after resubmission November 13, 2000