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Minireview

Non-antioxidant molecular functions of α-tocopherol (vitamin E)

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Abstract α-Tocopherol (the major vitamin E component) regulates key cellular events by mechanisms unrelated with its antioxidant function. Inhibition of protein kinase C (PKC) activity and vascular smooth muscle cell growth by α-tocopherol was first described by our group. Later, α-tocopherol was shown to inhibit PKC in various cell types with consequent inhibition of aggregation in platelets, of nitric oxide production in endothelial cells and of superoxide production in neutrophils and macrophages. α-Tocopherol diminishes adhesion molecule, collagenase and scavenger receptor (SR-A and CD36) expression and increases connective tissue growth factor expression.

Key words: α-Tocopherol; Non-antioxidant mechanism; Protein kinase C; Cell proliferation; Gene expression; Platelet aggregation; Oxygen burst

1. Introduction

Biochemistry textbooks describe vitamin E as a lipid soluble, chain breaking radical scavenger. This feature has been described in great detail but it appears not to include all of the properties of the different tocopherols comprised by the definition of vitamin E. Several tocopherols have special properties, which are unrelated to their antioxidant capacity. The present article is devoted to the description of those molecular aspects of the most potent vitamin E component, α-tocopherol, that cannot be accounted for by its antioxidant power.

2. α-Tocopherol protects against a number of disorders

Mutations of the α-tocopherol transfer protein (α-TTP) gene lead to a reduced α-tocopherol concentration in plasma and tissues and eventually to a lethal syndrome, ataxia with vitamin E deficiency (AVED) [1]. Vitamin E supplementation has protective effects against a number of disorders besides AVED, in particular atherosclerosis, ischemic heart disease and different tumors [2–4].

3. Selective uptake of α-tocopherol

The first example of a non-antioxidant reaction involving α-tocopherol is given by its uptake in the liver [5] where chylomicron remnants deliver it to the 32 kDa α-TTP. This protein has a higher affinity for α-tocopherol relative to the other tocopherols and tocotrienols. Some of the eight different side-chain isomers of racemic tocopherol are excluded from the plasma and secreted with the bile [6]. 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%; γ-tocopherol 9%; δ-tocopherol 2%; α-tocopherol acetate 2%; α-tocopherol quinone 2% [7]. The α-tocopherol transfer protein facilitates α-tocopherol incorporation into very low density lipoproteins that deliver it to peripheral cells [8].

4. Specific intracellular α-tocopherol binding proteins

α-TTP is mainly expressed in the liver, in some parts of the brain [9], in the retina [10], lymphocytes and in low amounts in fibroblasts [11], as well as in the labyrinthine trophoblast region of the placenta [12], where it may be responsible for regulating local α-tocopherol concentrations. Recently, a family of cellular tocopherol associated proteins (TAPs) has been identified [13]. TAP1 binds α-tocopherol better than the other tocopherols [14]. Present in all cells, TAPs may be specifically involved in intracellular tocopherol traffic, for example between membrane compartments and the plasma membrane, similar to the yeast secretory protein (sec14p). TAPs show GTPase activity and may modulate reactions like phospholipid/tocopherol signalling, phospholipid/tocopherol secretion or optimizing the tocopherol composition of membranes. TAP1 has recently been described as a cytosolic squalene transfer protein and enhances cholesterol biosynthesis by activating the enzyme squalene epoxidase [15], suggesting a possible involvement of TAPs in the transport of a number of hydrophobic compounds in competition with α-tocopherol (Fig. 1). Thus, these proteins may make α-tocopherol a suitable regulator in different cellular reactions. A further role for TAP1 has been suggested by Yamauchi's [14] group. Nuclear translocation of TAP1 and transcriptional activation have been suggested to occur under the influence of α-tocopherol.

Another tocopherol binding protein also preferentially interacts with α-tocopherol and may be responsible for intracellular distribution of α-tocopherol [16].

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Abbreviations: TAP, tocopherol associated protein; AVED, ataxia with vitamin E deficiency; α-TTP, α-tocopherol transfer protein; PKC, protein kinase C; PP_2A, protein phosphatase 2A

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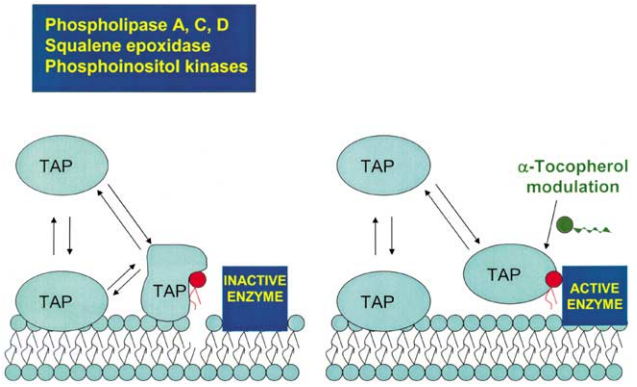

Fig. 1. Suggested mechanism of α-tocopherol modulated enzyme activation by phospholipid–TAP complexes.

5. Reactions of α-tocopherol at cellular level

Protein kinase C (PKC) inhibition is the cause of vascular smooth muscle cell proliferation inhibition caused by α-tocopherol [17,18]. This event takes place at concentrations of α-tocopherol considered optimal in human beings [19]. β-Tocopherol is per se ineffective but prevents the inhibition induced by α-tocopherol (Fig. 2). The radical scavenging properties of these two molecules are essentially equal [20]; thus the effect of α-tocopherol cannot be attributed to its antioxidant properties. This same phenomenon has been established in monocytes, macrophages, neutrophils, fibroblasts and mesangial cells [21–27]. In endothelial cells, thrombin-induced PKC activation and endothelin secretion are inhibited by α-tocopherol but not by β-tocopherol [28]. By inhibiting PKC in monocytes, the phosphorylation and translocation of the cytosolic factor p47 (phox) are also inhibited. Consequently, NADPH-oxidase assembly and superoxide production are impaired [29].

α-Tocopherol does not produce inhibition of recombinant PKC in vitro, thus excluding that the effect observed in cells is caused by tocopherol-protein interaction. α-Tocopherol does not inhibit PKC expression either. Inhibition of PKC activity by α-tocopherol is due, at cellular level, to dephosphorylation of the protein [30]. Protein phosphatase 2A (PP_2A) can be activated by α-tocopherol and causes dephosphorylation of PKC [30–32].

5.1. Transcriptional regulation by α-tocopherol

A non-antioxidant reaction induced by α-tocopherol causes up-regulation of α-tropomyosin expression by α-tocopherol; also in this case β-tocopherol is not effective [33]. Human skin fibroblasts exhibit an age-dependent increase of collagenase expression that can be diminished by α-tocopherol [34]. Dietary α-tocopherol modulates the expression of the liver α-TTP and of its mRNA [35]. Scavenger receptors are particularly important in the formation of atherosclerotic foam cells [36] and disruption of CD36 protects against atherosclerotic lesion. In smooth muscle cells and monocytes/macrophages, the oxidized LDL scavenger receptors SR-A and CD36 are transcriptionally down-regulated by α-tocopherol but not by β-tocopherol [37–39]. Recently (Villacorta et al., unpublished), the connective tissue growth factor transcription has been also found to be under the positive control of α-tocopherol.

5.2. Inhibition of monocyte-endothelial adhesion

Monocytes and neutrophils enriched with α-tocopherol decrease their adhesiveness both in vivo and in vitro [40,41] due to the down-regulation of adhesion molecule expression [42,43].

5.3. Inhibition of platelet adhesion and aggregation

α-Tocopherol inhibits aggregation of human platelets by a PKC-dependent mechanism, both in vitro and in vivo [23], and delays intra-arterial thrombus formation [44].

It is generally accepted that α-tocopherol exerts its inhibitory action on a number of cell reactions by interacting primarily at the level of PKC. However, the expression of several genes, such as CD36 [38], SR class A [45], collagenase [34], ICAM-1 [46] and some integrins [47], appears to be regulated by α-tocopherol in a PKC independent way. There are also data indicating that α-tocopherol activates PP_2A [30] and that it inhibits 5-lipoxygenase [48] and cyclooxygenase [49]. These phenomena are still not fully characterized.

6. Conclusions

The inhibition of a number of important cellular reactions by α-tocopherol can be traced back to its effect on PKC. In its turn, inhibition of PKC has been shown to be due to the activation of the protein phosphatase PP_2A. The inhibition of PKC in many, but not all cells, may be relevant in explaining the anti-atherosclerotic and anti-tumor effects of this vitamin in vivo. Furthermore, the expression of a number of genes has been found to be under the non-antioxidant control of α-tocopherol, suggesting a new level of action of α-tocopherol in protection against disease. This compound, uniquely important for the human body, cannot be considered merely a simple antioxidant. A special conserved protein for its uptake and a family of proteins for its cellular distribution and action point to the need of the organism to select and protect this compound against radical damage and immediate metabolic degradation rather than use it to shield other cellular compo-

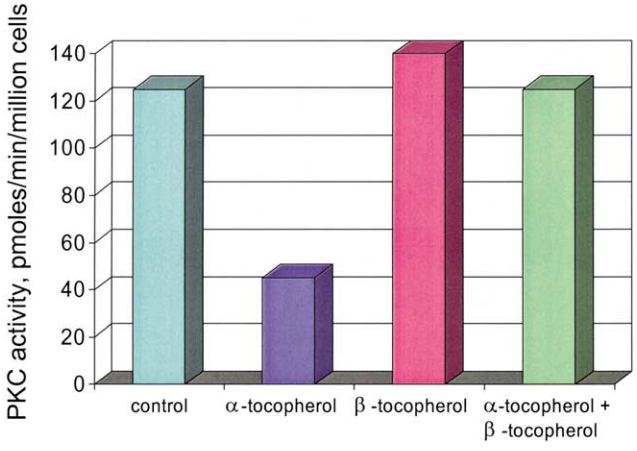

Fig. 2. PKC inhibition by α-tocopherol and not by β-tocopherol. Quiescent cells were re-stimulated to enter into the G_1-phase by adding 10% fetal calf serum in the presence or absence of α-tocopherol or β-tocopherol (50 μM). After 7 h cells were permeabilized and PKC was measured. Phorbol myristate acetate (100 nM) was added 60 min before assaying PKC activity. The basal kinase activity was subtracted in all samples and only the phorbol myristate acetate stimulated activity was considered [18].

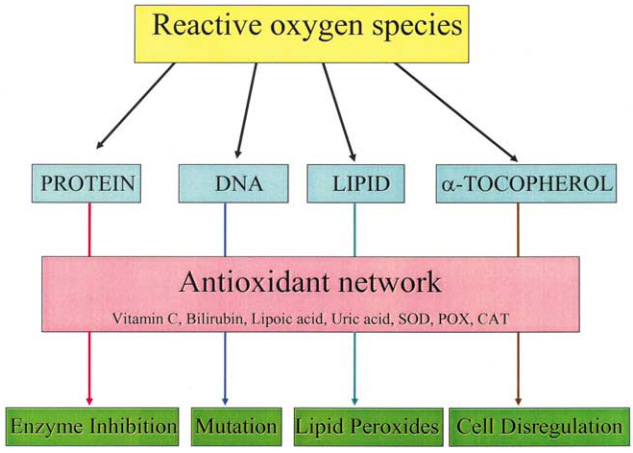

Fig. 3. Scheme of the network of cell antioxidants: they protect not only DNA, protein and lipid but also α-tocopherol against radical damage.

nents. Thus, the network of antioxidant systems of the organism should be considered not only valuable for the protection of DNA, lipid and protein against oxidative damage, but also for the protection of α-tocopherol (Fig. 3). Special non-antioxidant functions have been attributed more recently to γ-tocopherol [50], suggesting a general project of nature to utilize the compounds defined together with vitamin E in precise and unique non-antioxidant reactions.

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