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Specific Cellular Responses to α-Tocopherol[1,2]

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ABSTRACT In the last 10 years precise cellular functions of α-tocopherol, some of which are independent of its antioxidant/radical-scavenging ability, have been revealed. Absorption of α-tocopherol from the gut is a selective process. Other tocopherols are not absorbed or are absorbed to a lesser extent. At the post-translational level, α-tocopherol inhibits protein kinase C and 5-lipoxygenase and activates protein phosphatase 2A and diacylglycerol kinase. Some genes [platelet glycoprotein IV/thrombospondin receptor/class B scavenger receptor (CD36), α-tocopherol transfer protein (α-TTP), α-tropomyosin, connective tissue growth factor and collagenase] are affected by α-tocopherol at the transcriptional level. α-Tocopherol also inhibits cell proliferation, platelet aggregation, monocyte adhesion and the oxygen burst in neutrophils. Other antioxidants, such as β-tocopherol and probucol, do not mimic these effects, suggesting a nonantioxidant, α-tocopherol–specific molecular mechanism. J. Nutr. 130: 1649–1652, 2000.

KEY WORDS: • vitamin E • α-tocopherol • antioxidant • cell proliferation • protein kinase C • gene expression

The term "Vitamin E" was introduced by Evans and Bishop (1) to describe a dietary factor in animal nutrition important for reproduction. It took >40 years (2) before vitamin E was associated with an antioxidant property and another 25 years to begin to consider the nonantioxidant properties of α-tocopherol (3,4). α-Tocopherol is the member of the vitamin E group of compounds (α- β- γ- and δ- tocopherols and tocotrienols) that possess extensive biological properties (5) and are found most prevalently in mammalian tissues (6).

Emulsified together with the fat-soluble components of the food, tocopherols passively reach the blood stream and eventually the liver. In the hepatocyte, the specific α-tocopherol transfer protein (α-TTP)[4] mediates the selective transfer of α-tocopherol into lipoproteins (7,8). A tocopherol-associated protein (TAP), capable of specific tocopherol binding was found recently to be present in a large number of tissues (9). TAP, by analogy with the homologous phosphatidylinositol-transfer protein (SEC14), may be a candidate responsible for the regulation of tissue α-tocopherol levels or of signal transduction–related reactions.

Antioxidant Capacity Can Coexist with Additional Functions of α-Tocopherol. A number of biomolecules, in addition to their antioxidant functions, may also have further properties. The estrogen, 2-hydroxyestrone, is 2.9-times more potent than α-tocopherol as an antioxidant (10,11), but this antioxidant activity is unrelated to its determination of secondary sexual characters. The principal function of all-*trans*-retinol in rhodopsin and vision is not related to its antioxidant properties (12). The sleep-wake regulation by melatonin in humans occurs through a receptor-mediated signaling function (13) and not through its free radical-scavenging properties (14).

All-*trans* retinoic acid binds to nuclear retinoid receptors, major regulators of gene expression (15), but it is also an antioxidant (16). Carotenoids (also a family of antioxidants) up-regulate connexin43 gene expression in fibroblasts and inhibit carcinogen-induced neoplastic transformation (17) via an antioxidant-independent mechanism.

The protective effect of α-tocopherol against LDL oxidation (18) and other free radical–induced damage is only one side of the coin. In fact, both a prooxidant effect (19) and an antityrosine nitrating action (20,21) of α-tocopherol have been described. Furthermore, the nonantioxidant action of α-tocopherol appears to be of particular relevance at a cellular level (3,4,22–26).

Molecular Mechanisms of α-Tocopherol Action at Cellular Level. In the sections, we discuss the actions of α-tocopherol at the cellular level, with a particular focus on the nonantioxidant properties of the molecule (**Table 1**).

Low levels of α-tocopherol have been associated with increased risk for coronary artery disease and increased intake has been shown to be protective. Thus, scientific interest has gone beyond recognition of the role of oxidized LDL in atherogenesis, toward the understanding of the α-tocopherol response of crucial cells in the progress of atherosclerosis. Inhibition of smooth muscle cell proliferation, preservation of endothelial function, inhibition of monocyte-endothelial adhesion, inhibition of monocyte reactive oxygen species and cytokine release, and inhibition of platelet adhesion and aggregation are some examples of the cellular events that are regulated by α-tocopherol. These cellular complex events are associated with effects at a molecular level, both post-transcriptional and transcriptional.

Post-translational regulation of cellular reactions. In 1991, inhibition of protein kinase C activity by α-tocopherol was discovered to be at the basis of the inhibition of vascular smooth muscle cell proliferation by α-tocopherol (3,4,22–26).

A number of reports have subsequently confirmed the involvement of protein kinase C in the effect of α-tocopherol on different cell types, including monocytes, macrophages, neutrophils, fibroblasts and mesangial cells (27–34). α-Tocopherol but not β-tocopherol was found to inhibit thrombin-induced protein kinase C activation and endothelin secretion in endothelial cells (35). α-Tocopherol (but not β-tocopherol or Trolox) inhibits protein kinase C activity from monocytes, followed by inhibition of phosphorylation and translocation of

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[4] Abbreviations used: α-TTP, α-tocopherol transfer protein; CD36, platelet glycoprotein IV/thrombospondin receptor/class B scavenger receptor; PP_2A, protein phosphatase 2A; ROS, reactive oxygen species; SEC, secretory protein gene products; TAP, α-tocopherol associated protein.

TABLE 1

Effects of α-tocopherol and their supposed molecular mechanisms

Reaction	Proposed mechanism[1]	Reference
Inhibition of cell proliferation	NA	[24, 51]
Inhibition of platelet adhesion and aggregation	NA/ND/A	[30, 54–56]
Inhibition of monocyte-endothelial adhesion	NA/ND/A	[57–60]
Inhibition of ROS[2] in monocytes and neutrophils	A/NA	[27, 31, 36, 61–63]
Inhibition of protein kinase C	NA/A	[3, 4, 22–34]
Inhibition of α-tropomyosin expression	NA	[45]
Inhibition of liver collagen α1 (I) expression	ND	[46]
Inhibition of collagenase expression	NA	[47]
Modulation of α-TTP expression	ND	[48]
Inhibition of scavenger receptors class A (SR-A)	NA	[49]
Inhibition of scavenger receptors CD36	NA	[50]
Inhibition of ICAM-1 and VCAM-1 expression	ND	[60]

[1] A, antioxidant; NA, nonantioxidant; ND, not discussed
[2] ROS, reactive oxygen species; α-TTP, α-tocopherol transfer protein; CD36, platelet glycoprotein IV/thrombospondin receptor/class B scavenger receptor; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule.

the cytosolic factor p47(phox) and impaired assembly of the NADPH-oxidase and of superoxide production (36). α-Tocopherol has the important biological effect of inhibiting the release of the proinflammatory cytokine, interleukin-1β, via inhibition of the 5-lipoxygenase pathway (37).

Inhibition of protein kinase C by α-tocopherol in vascular smooth muscle cells is observed to occur at concentrations of α-tocopherol close to those measured in healthy adults (38). β-Tocopherol per se is ineffective but 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 (39). In vitro studies with recombinant protein kinase C have shown that inhibition by α-tocopherol is not caused by a tocopherol-protein interaction. α-Tocopherol does not inhibit protein kinase C expression as well. Inhibition of protein kinase C activity by α-tocopherol occurs at a cellular level by producing dephosphorylation of the enzyme, whereas β-tocopherol is much less potent (40). Dephosphorylation of protein kinase C occurs via the protein phosphatase 2A (PP_2A), which has been found to be activated by treatment with α-tocopherol in vitro (40,41).

King's group (32) reported that prevention of glomerular dysfunction in diabetic rats can be achieved by treatment with α-tocopherol. Such a protection occurs through inhibition of protein kinase C. In this case, however, α-tocopherol would act on the diacylglycerol pathway by activating the enzyme diacylglycerol kinase, with consequent diminution of diacylglycerol and protein kinase C activation. In these studies, high glucose was responsible for the increased diacylglycerol synthesis counteracted, in the presence of α-tocopherol, by the activation of diacylglycerol kinase. The experiments of Azzi's group (42) were conducted at low glucose concentrations, and protein kinase C was found to be deactivated by α-tocopherol by means of its dephosphorylation. Other possible effects of α-tocopherol at a cellular level such as inhibition of lipoxygenase (37,43) have also been reported.

Transcriptional regulation of cellular reactions. Recently, the possibility of regulation of gene transcription by α-tocopherol has been analyzed (44). Upregulation (not mediated by protein kinase C) of α-tropomyosin expression by α-tocopherol and not by β-tocopherol (45) once more suggests a nonantioxidant mechanism. Long- and short-term α-tocopherol supplementation inhibits liver collagen α1(I) gene expression (46). In human skin fibroblasts, an age-dependent increase of collagenase expression can be reduced by α-tocopherol (47).

In rats, liver α-TTP and its mRNA are modulated by dietary vitamin E deficiency (48). Scavenger receptors, particularly important in the formation of atherosclerotic foam cells, are also modulated by α-tocopherol. Both class A (SR-A) activity in macrophages (49) and CD36 (the oxidized LDL scavenger receptor), in macrophage and smooth muscle cells, are down-regulated at the transcriptional level by α-tocopherol but not by β-tocopherol (50).

The following questions remain open. In some cases, differential effects of α-tocopherol and β-tocopherol have been found, pointing to a nonantioxidant mechanism at the basis of gene regulation (45,50). In other cases, however, only α-tocopherol has been tested, leaving the mechanism of α-tocopherol action unclarified. Furthermore, the involvement of protein kinase C has not been assessed in many cases, and it remains to be established whether the transcriptional regulation of certain genes by α-tocopherol is a direct or indirect consequence of protein kinase C inhibition by α-tocopherol.

Regulation of Integrated Cellular Functions by α-Tocopherol.

Effects of tocopherols on cell proliferation. α-Tocopherol, at concentrations of 50 μmol/L, inhibits rat A7r5 smooth muscle cell proliferation, whereas β-tocopherol is ineffective. When α-tocopherol and β-tocopherol are added together, no inhibition of cell growth is seen. Both compounds are transported equally in cells and they do not compete with each other for the uptake (51). The prevention of cell growth inhibition by α-tocopherol by β-tocopherol suggests a site-directed event to be at the basis of α-tocopherol inhibition rather than a general radical-scavenging reaction. The oxidized product of α-tocopherol, α-tocopherylquinone, is not effective, indicating that the effects of α-tocopherol are not related to its antioxidant properties (51). α-Tocopherol is responsible not only for the proliferation control of smooth muscle cells, but it exhibits similar functions in a number of different cell lines.

δ-Tocopherol, α-tocopherol and γ-tocopherol are (within experimental error) equally inhibitory (24). On the other hand, it appears that the inhibition by β-tocopherol is 10% of that exhibited by the others compounds. Tocotrienols, although possessing a greater antioxidant activity than tocopherols (52), inhibit cell proliferation to the same extent (24).

Janero's (53) series of 6-hydroxy-chroman-2-carbonitrile tocopherol derivatives have relative potencies in inhibiting cell proliferation that are not correlated significantly with their antioxidant properties (24). Probucol, a potent hydrophobic antioxidant, similar in its general properties to α-tocopherol, has been shown not to inhibit smooth muscle cell proliferation, but to prevent the inhibition by α-tocopherol, as is the case for β-tocopherol.

Inhibition of platelet adhesion and aggregation. It has been shown that α-tocopherol inhibits aggregation of human platelets by a protein kinase C–dependent mechanism both in

vitro and in vivo (30,54–56). Another study has indicated that both α- and γ-tocopherol decrease platelet aggregation and delay intra-arterial thrombus formation. The fact that γ-tocopherol was significantly more potent than α-tocopherol suggests that a simple antioxidant mechanism is not applicable to these effects. In fact, γ-tocopherol is much less potent than α-tocopherol as an antioxidant (55).

Inhibition of monocyte-endothelial adhesion. α-Tocopherol enrichment of monocytes decreases agonist-induced, LDL-induced adhesion to human endothelial cells both in vivo and in vitro (57,58). Monocyte adhesion is paralleled by cell production of chemokines (59). Monocyte as well as neutrophil diminution of adhesion induced by α-tocopherol is dependent on the inhibition of expression of adhesion molecules (60). These events are relevant to the onset of inflammation as well as in the early stages of atherogenesis.

Inhibition of production of reactive oxygen species in monocytes and neutrophils. Inhibition of phorbol ester–induced generation of superoxide by α-tocopherol (31,61) has been attributed to protein kinase C inhibition. On the contrary, vitamin E inhibition of $O_2^{\bullet -}$ production in the promonocyte cell line THP-1 is due essentially to δ-tocopherol (62). α-Tocopherol also inhibits the respiratory burst in human monocytes via a mechanism involving protein kinase C inhibition (27), followed by attenuation of p47(phox) phosphorylation and membrane translocation (36,63). These studies provide strong evidence for an intracellular, antiatherogenic effect of α-tocopherol in monocytes.

Possible Links between In Vitro Cellular Events and α-Tocopherol–Related Disease. Clear links between cellular events and pathogenetic mechanisms cannot be established at the present time. However, some possible participation of the described cellular reactions in the onset of disease can be suggested.

Ataxia with vitamin E deficiency. This autosomal recessive neurodegenerative disease, whose clinical presentation is remarkably similar to that of Friedreich ataxia, is caused by mutations in the gene for α-TTP (7,64,65). Does protein kinase C activation, due to the very low α-tocopherol level, play a role in the onset of the disorders?

Atherosclerosis. Low levels of α-tocopherol have been associated with increased risk for coronary artery disease, and increased intake has been shown to be protective (66). This suggests a pathogenetic role for oxidized LDL and protection by α-tocopherol (67). The antiatherogenic effects of α-tocopherol may also be related to its action on crucial cells such as the inhibition of smooth muscle cell proliferation, preservation of endothelial function, inhibition of monocyte-endothelial adhesion, inhibition of monocyte reactive oxygen species and cytokine release, and inhibition of platelet adhesion and aggregation (29).

Cancer. Prostate and colorectal cancers seem to be affected by α-tocopherol (68,69). This may be related to the antiproliferative effects observed in vitro.

Diabetes. In vivo as well as in vitro evidence that vitamin E treatment can reverse protein kinase C activation, responsible for the glucose-induced vascular dysfunctions in diabetes, has been provided (70–72).

Inflammation. α-Tocopherol inhibits $O_2^{\bullet -}$ generation and cell adhesion (73) in monocytes and polymorphonuclear cells (31,36,61) via protein kinase C inhibition (27). These events are central to the inflammatory process.

Final Considerations. A number of cellular events controlled by α-tocopherol may be reconciled in large part by the existence of a common denominator, namely, the described protein kinase C regulation. Some alternative, but not contrasting interpretations exist concerning the molecular nature of α-tocopherol regulation of protein kinase C. Some other post-transcriptional effects, such as those related to the arachidonic acid cascade, are not yet understood in molecular terms. The possibility of a regulation of several genes by α-tocopherol is the most challenging experimental opportunity for future studies. Here, the existence of a common denominator (e.g., a receptor protein for α-tocopherol, an α-tocopherol sensitive transcription factor or an α-tocopherol sensitive promoter element) has been postulated. Recently, a new ubiquitous cytosolic α-tocopherol binding protein (TAP) has been discovered (9). Structurally, it belongs to a family of hydrophobic ligand-binding proteins including α-TTP, retinal binding protein, *cis*-retinal binding protein, SEC14, PTN 9 and rat secretory protein 45 (74). Future research will be directed to understanding whether this protein has, alone or in combination with others, α-tocopherol–specific receptor functions.

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