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Effects of tocopherols and 2,2′-carboxyethyl hydroxychromans on phorbol-ester-stimulated neutrophils

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Abstract

Tocopherol vitamers [e.g., alpha-, gamma- and delta-tocopherol (α-TOC, γ-TOC and δ-TOC, respectively)] and their water-soluble 2,2′-carboxyethyl hydroxychroman metabolites (e.g., α-, γ- and δ-CEHC) all possess antioxidant properties. As a consequence, and similarly to other natural antioxidants, vitamin E compounds may be useful in preventing inflammatory and oxidative-stress-mediated diseases. In this study, we investigated the concentration-dependent effect of tocopherols and water-soluble metabolites on a key event in oxidative stress, for example, the oxidative burst in neutrophils. It was found that not only α-TOC but also γ-TOC and δ-TOC as well as α-, γ- and δ-CEHC at physiological concentrations inhibit superoxide anion ($O_2^{\bullet-}$) production in phorbol-ester-stimulated neutrophils. This effect was mediated by the inhibition of the translocation and activation of protein kinase C (PKC) enzyme, which is the key event in the phorbol-ester signaling. Importantly, CEHCs were stronger inhibitors of PKC as compared with the vitamer precursors, and the gamma forms of both tocopherol and CEHC showed the highest inhibitory activities. Tocopherols, but not CEHCs, directly inhibit the fully activated nicotine–adenine–dinucleotide phosphate (NADPH) oxidase. However, none of the test compounds was able to directly scavenge $O_2^{\bullet-}$ when tested in a cell-free system. In conclusion, vitamin E compounds can control the neutrophil oxidative burst through the negative modulation of PKC-related signaling and NADPH oxidase activity. As an original finding, we observed that CEHC metabolites might contribute to regulate PKC activity in these cells. These results may have important implications in the anti-inflammatory and antioxidant role of vitamin E compounds.

Keywords: Vitamin E; Tocopherols; CEHC; Neutrophils; Protein kinase C; NADPH oxidase; Xanthine oxidase

1. Introduction

Vitamin E, since its discovery as one of the most important naturally occurring lipophilic antioxidants, was recognized to possess a number of biological functions that may justify its use in prevention and therapy of chronic-degenerative diseases associated with inflammation and aging, such as atherosclerosis, cardiovascular diseases or immune dysfunction [1]. This background has been supported by several observational and epidemiological studies, which almost exclusively investigated the alpha homologue of tocopherols (α-TOC) [1,2]. It is a chain-breaking antioxidant commonly reported to physiologically protect polyunsaturated lipids in cell membranes and lipoproteins from injury induced by reactive oxygen species (ROS).

However, vitamin E can also exert an important antioxidant role through the control of inflammatory cell activation such as neutrophils and monocytes, which are important sources of ROS such as superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide [3,4]. In these processes, the antioxidant activity of α-TOC is mainly due to the inhibition of protein kinase C (PKC) enzyme activity, which controls, among them, thyrosine phosphorylation-dependent signaling [5] and $O_2^{\bullet-}$ production [6] in inflammatory cells. However, during the past few years, increasing evidence has suggested that not only α-TOC but also gamma-tocopherol (γ-TOC) can exert significant antioxidant activity and other intriguing biological functions, which have increased the general interest of the scientific community on this hypomethylated form [7–9]. Thus, the common and long-lasting belief that it

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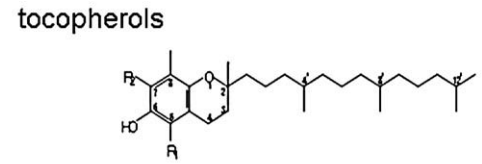

R1	R2	homologue
CH3	CH3	α
CH3	H	β
H	CH3	γ
H	H	δ

Fig. 1. Structure of tocopherols and 2,2′-carboxyethyl hydroxychromans.

could be of minor importance as compared with the alpha form because of its being less effective in some bioactivity tests and less abundant in some tissues has been overcome, and now, several lines of evidence suggest that it may play a role in preventing some oxidative-stress-mediated conditions [10–12]. γ-TOC was found to be a more potent anti-inflammatory agent as compared with α-TOC [11,12], and under some experimental circumstances, not only γ-TOC but also delta-tocopherol (δ-TOC) proved to be superior to the well-characterized α-TOC as a lipid antioxidant [13].

Recently, it was demonstrated that the water-soluble metabolites 2,2′-carboxyethyl hydroxychromans (α-CEHC, γ-CEHC and δ-CEHC), which are formed during the hepatic catabolism of tocopherols and tocotrienols, retain the scavenging function of peroxyl radicals and the inhibitory effect against peroxynitrite-mediated tyrosine nitration [14,15]. Moreover, CEHCs can inhibit not only the proliferation of some human cancer cell lines via down-regulation of cyclin-related signaling [16,17] but also inflammatory pathways [18].

However, the effect of γ-TOC and δ-TOC as well as their water-soluble CEHC metabolites on PKC activity and $O_2^{\bullet-}$ formation in neutrophils remains unexplored, leading to the hypothesis that other vitamin E compounds might contribute to the overall antioxidant-dependent protective role of α-TOC. Previously, we observed that γ-TOC can inhibit total PKC activity more effectively than α-TOC in C6 murine glioma cells [17]. To explain how just a small structural difference could produce such biological difference, recent studies carried out by our group on other antioxidants have confirmed that even a small change in the molecular structure of the test compounds can markedly affect their in vitro antioxidant properties, and the magnitude of this structure–activity relationship strongly depended on which kind of oxidative stress route or type of initiator is examined [15,19,20].

In this study, we investigated the effect of tocopherol vitamers (α-TOC, γ-TOC and δ-TOC) and their water-soluble metabolites (α-CEHC, γ-CEHC and δ-CEHC) on the oxidative burst response of phorbol–myristate–acetate (PMA)-stimulated human neutrophils. This was assessed by measuring $O_2^{\bullet-}$ formation, nicotine–adenine–dinucleotide phosphate (NADPH) oxidase activity and PKC activity and translocation. The antioxidant potency of the test compounds was also compared by assessing their ability to inhibit xanthine oxidase activity and to scavenge $O_2^{\bullet-}$ radicals.

2. Materials and methods

2.1. Chemicals

Cytochrome *c* (Type IV); xanthine; xanthine oxidase; Histopaque 1077; Hanks' Balanced Salt Solution (HBSS); α-, γ- and δ-tocopherol; dimethyl sulfoxide (DMSO); reduced NADPH; and PMA were from Sigma (St. Louis, MO, USA). CEHCs were a kind gift to Dr. Galli by Eisai Co., Japan. Stock and working solutions of tocopherols and their water-soluble metabolites were prepared in DMSO to achieve appropriate final concentrations in the reaction mixtures. All controls and test samples contained the same volume of DMSO (≤0.1%). The structure of tocopherols and water-soluble metabolites is presented on Fig. 1.

2.2. Neutrophil separation

Neutrophils were separated from fresh heparinized blood of 15 healthy volunteers after an overnight fasting according to the method of Boyum [21] using Histopaque 1077 gradient density centrifugation. None of the healthy volunteers was a smoker or was supplemented by vitamin E, but they had to have a sufficient dietary intake of vitamin E as assessed by a 72-h recall of diet (avarage α-TOC content in diet was 6–12 mg/day). Neutrophil purity over 95% and viability greater than 95% were ascertained by microscopic examination using Giemsa staining and trypan blue exclusion test, respectively. All volunteers gave their informed consent to participate in the study, which followed the guidelines of the Ethical Committee of the University of Debrecen.

2.3. Determination of tocopherol incorporation rate in neutrophils

Three milliliters of 1×10^6 cells/ml neutrophils was resuspended in HBSS (Sigma) containing 0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, 4.2 mM $NaHCO_3$ and 10 mM glucose. Cell suspension was incubated with individual tocopherols at 37°C in a humidified CO_2 incubator. Final compound concentrations in the cell media were set in order to simulate physiological condition in