Fatty Acid Metabolism and Cell Proliferation. VII. Antioxidant Effects of Tocopherols and Their Quinones

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ABSTRACT

The antioxidant capacities of α- and γ-tocopherols (α-E and γ-E) and their quinones (α-EQ and γ-EQ) were determined in non-biological and biological systems. The non-biological system consisted of arachidonic acid [20:4 (n-6)], the oxidant cumene hydroperoxide, and a Fe³⁺ catalyst to facilitate malondialdehyde (MDA) formation from lipid peroxides. α-E and γ-E had similar antioxidant capacities in this system. α-EQ also functioned as an antioxidant, while γ-EQ exhibited a crossover effect by functioning as an antioxidant at low concentrations and a prooxidant at high concentrations. Biological lipid peroxidation in smooth muscle cells challenged with 20:4 (n-6) was measured both by MDA formation in confluent cultures and by cell growth in proliferating cultures. α-E, γ-E and α-EQ had similar antioxidant capacities, but γ-EQ was highly cytotoxic for cells in both confluent and proliferating cultures. Cellular retention of antioxidants was estimated indirectly from MDA formation when cells were loaded with an antioxidant (preincubation) and then incubated for varying periods of time in fresh media containing 20:4 (n-6). Cellular retention also was measured directly with tritiated α-E and tritiated α-EQ. These studies showed that cellular retention decreased in the sequence γ-EQ > α-E > α-EQ. Thus, cellular retention does not explain the enhanced antioxidant capacity of α-E compared to γ-E that has been reported for animal systems. The antioxidant capacity of α-E evidently is enhanced by its metabolism to a quinone which, unlike the quinone from γ-E, functions as a biological antioxidant.


INTRODUCTION

Previous studies in this series have focused on lipid peroxidation in cultured cells and its effect on cell proliferation. In the present investigation, tissue culture is used to compare the biological properties of naturally occurring tocopherols and their quinone metabolites.

The relative antioxidant capacities of α-tocopherol (α-E) and γ-tocopherol (γ-E) differ in biological and non-biological systems. Early studies generally concluded that γ-E was more effective than α-E in preventing non-biological lipid peroxidation (1,2). However, relative antioxidant effectiveness depended on the specific oxidizing system. For example, γ-E is much more effective than α-E in preventing peroxidation of lard at 100 C (1), yet Burton and Ingold (3) measured the rate constant for abstraction by peroxyl radical of the phenolic hydrogen and found that in this system α-E actually was slightly more reactive than γ-E. In contrast to non-biological systems, a number of early studies showed that α-E was far better than γ-E as a biological antioxidant (1,2). The α-E had much greater activity than γ-E in biological systems associated with a vitamin E deficiency such as fetal resorption, red cell hemolysis and muscular dystrophy (4). A recent study found that γ-E was only 37% as effective as α-E in reducing lipid peroxidation (pentane production) by iron-loaded rats (5).

Several investigators suggested that the difference in biopotency of α-E and γ-E was explained by differences in the tissue retention of the two compounds (2,6,7). Studies investigating α-E and γ-E found no significant differences in absorption, plasma transport or tissue uptake of these agents (8,9). Since the difference in biopotency of α-E and γ-E was found within minutes after intraperitoneal injection (10), Peake et al. (9) suggested that either intracellular binding or metabolism could be important in determining tocopherol activity.

The difference in biopotency of α-E and γ-E may be explained by differences in their conversion to quinones and the biological properties of these metabolites. Many studies show that α-E is metabolized to α-tocopherylquinone (α-EQ) (6-11). We found that α-E and α-EQ were both effective antioxidants in tissue culture and that high concentrations of α-EQ were less cytotoxic than α-E when they were added to cells in culture (14-19). Additionally, several early investigators reported that α-EQ, although less effective than α-E, prevented and cured the creatinuria, paralysis, weight loss and fetal resorption that are characteristic of the vitamin E deficiency state (20,21). The duration of the antioxidant effect is much shorter with α-EQ.
than with \( \alpha \)-E, suggesting a difference may occur in the cellular retention of these two agents (20). The metabolism of \( \gamma \)-E differs significantly from \( \alpha \)-E. The majority of \( \gamma \)-E is converted to metabolites such as the dimer, and little \( \gamma \)-E, unlike \( \alpha \)-E, is converted to \( \gamma \)-tocopherylquinone (\( \gamma \)-EQ) (8). Furthermore, \( \gamma \)-EQ has a different one electron redox potential than \( \alpha \)-EQ and little is known about the antioxidant capacity and other biological properties of \( \gamma \)-EQ that could be affected by its redox potential.

The studies summarized above suggest that differences, in vivo, between the antioxidant capacities of \( \alpha \)-E and \( \gamma \)-E may be related either to their retention in cells or to their conversion to quinones. These studies also suggest that differences, in vivo, between the antioxidant capacities of \( \alpha \)-E and \( \alpha \)-EQ may be related to their retention in cells. In this study, we examine the cellular retention of \( \alpha \)-E, \( \gamma \)-E and \( \alpha \)-EQ, and because little previous work has been reported, we investigate the role of \( \gamma \)-EQ as a biological antioxidant.

**MATERIALS AND METHODS**

**Materials**

Arachidonic acid [20:4 (n-6)] was purchased from NuChek Prep (Elysian, Minnesota), purified by elution from a Unisil| column with hexane/ether (9:1, v/v), and used only when thin layer chromatography showed that lipid peroxides were absent (22). \( \alpha \)-E, \( \gamma \)-E and \( \alpha \)-EQ were purchased from Eastman Organic Chemicals (Rochester, New York). D-\( \alpha \)-[\( ^3 \)H]-Tocopherol (24 Ci/m mole) was purchased from Amersham International (Arlington Heights, Illinois). The tritiated \( \alpha \)-E was analyzed by HPLC and found to be partially oxidized to a mixture of \( \alpha \)-E and \( \alpha \)-EQ. Labeled \( \alpha \)-EQ (elution time 3.9 min) and labeled \( \alpha \)-E (elution time 5.1 min) were separated by elution from an Altex Ultrasphere ODS (4.6 x 250 mm) column with 100% methanol at a flow-rate of 1.5 ml/min. \( \alpha \)-EQ was purified by elution from an Ultrasphere ODS (4.6 x 550 mm) column.

\( \gamma \)-EQ was synthesized from \( \gamma \)-E by FeCl\(_3\) oxidation (23), and purity was established by HPLC in two systems: System I, elution from a Nucleosil 5 C-18 (4.6 x 150 mm) column with methanol:water (95:5) at a flow-rate of 1.5 ml/min; System II, elution from a Nucleosil 50-5 (4.6 x 250 mm) column with isopropl ether: hexane (9:1) at a flow-rate of 2.0 ml/min. Purity was 98.3% in System I (elution time 5.21 min) and 98.8% in System II (elution time 6.78 min). No single impurity exceeded 0.5%.

**Non-Biological Lipid Peroxidation**

The model system which has been described previously (18) contained 1 mM 20:4 (n-6) in 0.1 M phosphate buffer (pH 7.4) 2.84 mM cumene hydroperoxide (CHP) and 41 \( \mu \)M Fe\(^{3+}\) in a final volume of 1.2 ml. Mixtures were incubated at room temperature for 10 min. Lipid peroxides were assayed by a TBA procedure (14,17,18). In this procedure, the fatty acid which imparted turbidity to the acidified (TBA) solution was extracted with chloroform:acetic acid (2:1, v/v) before the absorbance at 532 nm was measured. Absorbance data are reported as nmole of malondialdehyde (MDA).

**Tissue Culture**

Primary cultures of smooth muscle cells were established from the dissected medial layer of guinea pig aorta from prepubertal males (24). The medium for growing cells to confluence (Growth Medium), the medium in lipid peroxidaion studies with confluent cells (Experimental Medium) and Cloning Medium have been described (18). Media were supplemented with fetal bovine serum (Sterile Systems, Logan, Utah; Hyclone lots 100348 and 010439). Cells from random confluent cultures were detached with trypsin and counted (25). Cells were used at passage levels 4 to 6. 20:4 (n-6), tocopherols and tocopherylquinones were dissolved in 95% ethanol and diluted with Experimental Medium or Cloning Medium. Control culture were treated with medium containing the same amount of 95% ethanol.

**Biological Lipid Peroxidation**

The intracellular lipid peroxidation of cells in tissue culture (14,17,18) was measured with cells seeded at \( 1.3 \times 10^4 \) cells/cm\(^2\) in flasks containing 4 ml of Experimental Medium. The cells were grown to confluence before treatments were initiated. Lipid peroxides, which were found only in bound intracellular lipids (14,17,18), were measured at the end of the treatment period by a TBA procedure as previously described (14,17,18). Lipid peroxides are reported as nmole of MDA/culture.

**Cellular Retention of Tritiated \( \alpha \)-E and \( \alpha \)-EQ**

Confluent cultures, preincubated for 24 hr with 20 \( \mu \)M \( \alpha \)-E or 10 \( \mu \)M \( \alpha \)-EQ, incorporated similar amounts of these compounds in cells (4.3 and 3.5 nmole/culture, respectively). Cellular uptake was estimated from the \( \alpha \)-E or \( \alpha \)-EQ remaining in incubation media and media from one rinse. The labeled cells were incubated in fresh media for an additional 24 hr and total radioactivity released into the media was mea-