α-Tocopheryl hydroquinone is an efficient multifunctional inhibitor of radical-initiated oxidation of low density lipoprotein lipids

(vitamin E/tocopherol-mediated peroxidation/co-antioxidation/atherogenesis)

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ABSTRACT As the oxidation of low density lipoprotein (LDL) lipids may be a key event in atherogenesis, there is interest in antioxidants as potential anti-atherogenic compounds. Here we report that α-tocopheryl hydroquinone (α-TQH₂) strongly inhibited or completely prevented the (per)oxidation of ubiquinol-10 (CoQ₁₀H₂), α-tocopherol (α-TOH), and both surface and core lipids in LDL exposed to either aqueous or lipophilic peroxyl radicals, Cu²⁺, soybean lipoxygenase, or the transition metal-containing Ham’s F-10 medium in the absence or presence of human monocyte-derived macrophages. The antioxidant activity of α-TQH₂ was superior to that of several other lipophilic hydroquinones, including endogenous CoQ₁₀H₂, which is regarded as LDL’s first line of antioxidant defence. At least three independent activities contributed to the antioxidant action of α-TQH₂. First, α-TQH₂ readily associated with LDL and instantaneously reduced the lipoprotein’s ubiquinone-10 to CoQ₁₀H₂, thereby maintaining this antioxidant in its active form. Second, α-TQH₂ directly intercepted aqueous peroxyl radicals, as indicated by the increased rate of its consumption with increasing rates of radical production, independent of LDL’s content of CoQ₁₀H₂ and α-TOH. Third, α-TQH₂ rapidly quenched α-tocopheroxy radical in oxidizing LDL, as demonstrated directly by electron paramagnetic resonance spectroscopy. Similar antioxidant activities were also seen when α-TQH₂ was added to high-density lipoprotein or the protein-free Intralipid, indicating that the potent antioxidant activity of α-TQH₂ was neither lipoprotein specific nor dependent on proteins. These results suggest that α-TQH₂ is a candidate for a therapeutic lipid-soluble antioxidant. As α-tocopherolquinone is formed in vivo at sites of oxidative stress, including human atherosclerotic plaque, and biological systems exist that reduce the quinone to the hydroquinone, our results also suggest that α-TQH₂ could be a previously unrecognized natural antioxidant.

Recently, we proposed a novel molecular mechanism of radical-initiated lipid peroxidation in isolated LDL (5–7) and extended it to other isolated lipoproteins and to lipoproteins in human plasma (8–10). This model, referred to as tocopherol-mediated peroxidation (TMP), predicts that α-tocopherol (α-TOH, biologically the most active form of vitamin E) present in lipoproteins will aid the “entry” of radical oxidants into the particle by acting as a phase-transfer agent. Once inside, the radical will be present predominantly as α-tocopheryl radical (α-TO) that, under relatively mild oxidizing conditions, will initiate and propagate the formation of lipid hydroperoxides by acting as the lipid peroxidation chain-carrying species. This chain transfer activity of α-TOH is inhibited by either high rates of radical entry into the lipoprotein particle (resulting in radical-radical termination reactions) (6, 10) or the presence of suitable reductants capable of “exporting” the radical from the lipoprotein back into the aqueous compartment (11). Human blood plasma (12) and interstitial fluids (13) contain several such reductants, referred to as co-antioxidants (11). Of these, ubiquinol-10 (CoQ₁₀H₂) (14) and ascorbate (15) form the first line of non-proteinaceous antioxidant defence; in their presence, α-TOH efficiently protects the lipids in isolated LDL and plasma against in vitro oxidation (15–17).

It is not known how and where LDL becomes oxidized during atherogenesis. However, oxidation most likely takes place in the subendothelial space where, at least at the late stages of the disease, the levels of oxidized lipids are approximately 10³-fold higher (17) than in plasma of severely diseased subjects (18). Despite such high levels of oxidized lipids, human atherosclerotic plaque contains large amounts of ascorbate and α-TOH when expressed per protein and oxidizable lipid, respectively (17). This could suggest that lipid peroxidation in the intima proceeds via TMP, perhaps within microenvironments from which aqueous co-antioxidants such as ascorbate are excluded. In such a case, lipid-soluble co-antioxidants that associate with LDL could conceivably be of

Abbreviations: AAPH, 2,2'-azobis(2-aminopropane) dihydrochloride; AMVN, 2,2'-azobis(2, 4-dimethylvaleronitrile); apoB, apolipoprotein B-100; CE-O(O)H, cholesteryl ester hydroperoxides plus cholesteryl ester hydroxides; CoQ₁₀, ubiquinone-10; CoQ₁₀H₂, ubiqui-

The oxidation of low density lipoprotein (LDL) is regarded as one of the early and key events in atherogenesis (1). As a result of the breakdown of oxidized lipids, LDL’s apolipoprotein B-100 (apoB) may become modified, and this can result in the uncontrolled cellular uptake of the lipoprotein, leading to the formation of lipid-laden “foam” cells (1, 2). Oxidized lipid component(s) may also be responsible for recognition of modified LDL, e.g., by the thrombospondin CD 36 receptor (3). In addition, oxidized LDL has many additional proatherogenic activities, so that inhibition of LDL lipid (per)oxidation might be beneficial and retard atherogenesis (4).
greater importance than aqueous co-antioxidants in the inhibition of TAME, and possibly atherogenesis.

Previous in vitro screening of a large number of natural and synthetic compounds for co-antioxidant activity (19) indicated high efficacy for hydroquinones. We now report on a group of lipophilic hydroquinones as powerful inhibitors of LDL lipid peroxidation. Among them, α-tocopheryl hydroquinone (α-TQH) was found to be most potent, capable of efficiently reducing α-TQ as well as directly scavenging aqueous radicals and reducing ubiquinone-10 (CoQ10) to CoQ10H2 in LDL, thereby also maintaining this co-antioxidant in the active form.

MATERIALS AND METHODS

Native LDL and high density lipoprotein (HDL) were isolated from fresh plasma by 2-h density ultracentrifugation (20). Where indicated, LDL was enriched with (21) or depleted of (10) α-TOH in vitro, or by isolation from plasma obtained from a vitamin E-deficient (FIVE) patient (22; 23) either during vitamin E supplements for 5 consecutive days (α-TOH-depleted) or after 2 or 5 days of vitamin E supplementation [partially or fully α-TOH-replenished samples, respectively (10)]. Before use, the lipoproteins were passed over two consecutive PD-10 columns (Pharmacia) or, for those prepared from the FIVE patient’s plasma, dialyzed against 50 mM of phosphate-buffered saline (PBS; pH 7.4). For peroxynitrite (ONOO−) oxidations, LDL was prepared in 200 mM of phosphate buffer (pH 7.2). All buffers were used with Chelex-100 (Bio-Rad) to remove contaminating transition metals.

LDL and HDL at the concentration indicated were oxidized under an atmosphere of air using 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) (Polysciences), soybean 15-lipoxygenase (SLO; type V, Sigma), CuSO4, ONOO− (prepared according to ref. 24), or hypochlorite (“OCI”, added as NaOCl) at the concentration indicated or by incubation in Ham’s F-10 medium in the presence and absence of human monocyte-derived macrophages (MDM), prepared as described (25). Hydroquinones were prepared freshly in ethanol and added as 10 µM final concentration [ethanol <1% (vol/vol)], with the appropriate volume of ethanol added to the control samples. The hydroquinones used were tert-butylnhydroquinone, 2,5-di-tert-butylhydroquinone (2,5-DTBHQ), and 3,5-di-tert-butylhydroquinone (3,5-DTBHQ) (Aldrich), α-TQH and 1,4-dihydroxy-2-(3-hydroxy-3-methylbutyl)-3,5,6-trimethyl-1,4-benzene (α-PQH2). α-TQH2 and α-PQH2 were prepared immediately before use by sodium dithionite reduction of α-tocopherol quinone (RRR-α-TQ) (Kodak or Acros) and 2-(3-hydroxy-3-methylbutyl)-3,5,6-trimethyl-1,4-benzooquinone (provided by C. Suarna, Heart Research Institute, Sydney, Australia), respectively (ε285 nm = 4075 M−1 cm−1).

For the stability assay, α-TQH (10 µM final concentration) was added to native LDL, α-TOH-depleted LDL, fresh HDL, or Intralipid [20% (wt/vol); Pharmacia] diluted 10 times in PBS. The samples were then incubated at 37°C and aliquots were withdrawn at various time points. The extracts were then analyzed for α-TQH2 and α-TQ and, where applicable, for CoQ10H2 and CoQ10 as described below. A total LDL lipid extract was prepared by extracting the lipoprotein into chloroform (26).

EPR experiments were performed using a Bruker (Billerica, MA) ESP 300 spectrometer fitted with an X-band cavity and, at 20 mW, frequency 9.4 GHz and averaging the output from five successive accumulations with sweep time of 20.5 s. For generation of α-TQ′ (27), LDL (7.2 µM in apoB) was incubated with SLO (1 mg/ml) at 37°C for 5 min before EPR spectra were recorded in the presence of ethanol (control) or 10 µM of α-TQH2.

Aliquots (50–200 µl) of the lipoprotein samples were extracted with methanol/hexane and analyzed for α-TOH, CoQ10H2, and CoQ10H2, unoxidized lipids (cholesterol and cholesteryl esters), hydroperoxides of cholesteryl esters, and phosphatidyl choline (PC-OOH) by reversed-phase HPLC with electrochemical, ultraviolet, and chemiluminescence detection, respectively, as described (20), except that UV234 nm absorbance rather than chemiluminescence detection was used for cholesteryl ester hydroperoxides. The UV absorbance-based method detects both the cholesteryl ester hydroperoxides and hydroxides [referred to as CE-O(O)H]. Standards of cholesteryllinoleate hydroperoxides [used for CE-O(O)H] and PC-OOH were prepared as described in (20). The concentration of the various analytes was determined by area comparison with authentic standards, using free cholesterol as an internal standard for CE-O(O)H, α-TOH, CoQ10H2, and α-TOH as internal standard for α-TQH2 and α-TQ, measured as described in ref. 28. The quinones and hydroquinones were measured immediately upon generation of appropriate samples to avoid adventitious autoxidation upon storage. Determination of triglyceride hydroperoxides in oxidizing Intralipid was performed as described previously (8). Protein was determined by the bichinchoninic acid assay using bovine serum albumin as standard. The concentrations of LDL and HDL were calculated from the cholesterol determination, assuming a protein molecular mass of 500 and 35 kDa for LDL and HDL, respectively.

RESULTS AND DISCUSSION

Previous studies have shown that in freshly isolated LDL 60–80% of endogenous coenzyme Q is present as CoQ10H2 (29) and that upon exposure to aqueous or lipophilic radical oxidants, CoQ10H2 is converted to CoQ10 before the concomitant consumption of α-TOH and accumulation of CE-O(O)H and PC-OOH (see refs. 30 and 31). Fig. 1 confirms these results for a constant flux of aqueous peroxyl radicals (ROO·) produced from AAPH and, for the first time, shows that the initial levels of CoQ10H2 increased upon addition of either 2,5-DTBHQ (Fig. 1B) or α-TOH (Fig. 1C) to native LDL before oxidation. Addition of α-PQH2 or 3,5-DTBHQ, but not tert-butylhydroquinone, butylated hydroxytoluene (10 µM in ethanol), superoxide dismutase (1000 units/ml), or catalase (1000 units/ml) also increased the initial level of LDL’s CoQ10H2 (data not shown). The presence of 2,5-DTBHQ (Fig. 1B) or 3,5-DTBHQ (data not shown) reduced the rate of ROO·-induced CoQ10H2 oxidation, whereas α-TQH2 (Fig. 1C) and α-PQH2 (data not shown) completely prevented the onset of CoQ10H2 oxidation for up to 1 h, during which time α-TQH2 was oxidized stoichiometrically to α-TQ. Importantly, the presence of any of the five hydroquinones delayed the consumption of α-TOH and accumulation of CE-O(O)H, with the order of efficacy being α-TQH2 > α-PQH2 > 2,5-DTBHQ > 3,5-DTBHQ > tert-butylhydroquinone (partially shown in Fig. 1).

To assess the ability of α-TQH2 to “incorporate” into LDL, the hydroquinone (final concentration 10 µM in ethanol) was added to the lipoprotein emulsion (1.3 µM in apoB) and placed on ice for ~0.5–1 min; the LDL was subsequently gel filtered (using cold PBS) through two sequential PD-10 columns, after which 96% and 86% of the α-TQH2 added was recovered with LDL. In addition, in a separate experiment, 10 µM of α-TQH2 was added to different LDL samples (1 ml each) prepared from three different subjects (concentration of LDL 1.2, 0.8, and 1.5 µM apoB, respectively), after which the individual LDL samples were re-isolated by 2-h density gradient ultracentrifugation (20). Recovery of total tocopherolquinoline expressed as the sum of α-TQ and α-TQH2 in such re-isolated LDL was 84 ± 4.1% (mean ± SD, n = 3) of the total
tocopherylquinone in LDL prior to centrifugation. Together, these results indicated that the majority of the added α-TQH₂ associated strongly with LDL.

Because substantial amounts of α-TQ are present in extracts of human atherosclerotic plaque (17), and cells can reduce α-TQ to α-TQH₂ (28, 32), we tested the ability of the hydroquinone to inhibit LDL lipid oxidation initiated by different oxidants. As can be seen from Table 1, α-TQH₂ was highly efficient in protecting LDL lipids against either AAPH, AMVN, SLO, Cu²⁺, or Ham’s F-10 medium in the presence and absence of MDM. Examination of the kinetics of lipid oxidation revealed that for each oxidant used, α-TQH₂ was consumed before CoQ₁₀ (as shown in Fig. 1 for AAPH), indicating that α-TQH₂ not only effectively suppressed lipid peroxidation but did so in preference to CoQ₁₀H₂, itself regarded as a first line of LDL’s antioxidant defence (14, 16).

All of the above agents have been demonstrated to be able to oxidize LDL’s lipids via TMP (10). We next tested the antioxidant efficacy of α-TQH₂ for LDL exposed to ONOO⁻ (≤500 mol per mol apoB) and −OCI (≤1100 mol per mol apoB), as these oxidants can react via nucelophilic attack. α-TQH₂ also protected LDL lipids from such oxidants, as judged by the decrease in both the accumulation of CE-O(O)H and the consumption of CoQ₁₀H₂ and α-TOH. These results indicate that α-TQH₂ is an outstanding antioxidant for LDL’s lipids. Preliminary experiments indicate, however, that α-TQH₂ is less able to inhibit the oxidation of apoB induced by −OCI and ONOO⁻, as assessed by its inability to inhibit the loss of the tryptophan fluorescence by these oxidants (data not shown). This may suggest that α-TQH₂ is not an efficient antioxidant for LDL’s protein, although this requires further examination. It is noteworthy that apoB is the major initial target for −OCI (33), for the group of oxidants that peroxidize LDL via TMP, the lipoprotein lipid components are initially the major targets.

We next investigated the mechanism(s) by which α-TQH₂ exhibited such strong antioxidant activity for LDL’s lipids. Fig. 24 shows the rates of oxidation of α-TOH, CoQ₁₀H₂, and α-TQH₂ in LDL exposed to increasing rates (Rᵣ) of generation of ROO⁻. As predicted from the model of TMP and shown previously (6), the rate of α-TOH consumption was largely unaffected by changes in Rᵣ. Similarly unaffected was the rate of CoQ₁₀H₂ consumption (Fig. 2A), indicating that this antioxidant acted primarily as a co-antioxidant, i.e., reacting with α-TQ' rather than ROO⁻. In sharp contrast with α-TOH and CoQ₁₀H₂, the rate of α-TQH₂ consumption closely matched and increased with increasing Rᵣ, i.e., d(α-TQH₂)/dt = Rᵣ (Fig. 2A). Also, the rates of α-TQH₂ consumption remained largely unaltered when LDL (0.75 μM apoB) containing different concentrations of α-TOH was oxidized by 0.5 mM of AAPH. Thus, 0.81, 0.65, and 0.85 nmol α-TQH₂ liter⁻¹s⁻¹ were oxidized in AAPH-exposed, in vitro α-TOH-depleted, native and in vitro α-TOH-enriched LDL containing 0, 8.2, and 101.6 mol of α-TOH per mol apoB, respectively. In the same experiment, the corresponding rates of α-TOH oxidation were 0.26 and 1.2 nmol liter⁻¹s⁻¹ for the native and α-TOH-supplemented LDL, respectively. Thus, α-TQH₂ appeared to directly intercept at least some of the lipid peroxidation-inducing ROO⁻.

To rule out the possibility of an artefact with the above LDL samples whose α-TOH content was manipulated in vitro, we performed similar AAPH-induced oxidation experiments with lipoproteins containing different endogenous levels of α-TOH isolated from the plasma of a FIVE patient (see Materials and Methods and ref. 10). As was the case with the in vitro manipulated samples, the rates of oxidation of α-TQH₂ to α-TQ were the same despite the up to 10-fold different initial concentrations of α-TOH in the LDL samples from the FIVE patient (Fig. 2B). Similar rates of α-TQH₂ oxidation were also observed with the FIVE patient’s HDL samples that contained different amount of α-TOH (data not shown), demonstrating that α-TQH₂ can react with ROO⁻ independent of the levels of α-TOH and the apolipoprotein present. Under all conditions tested, α-TQH₂ was formed stoichiometrically from α-TOH. Also, the presence of α-TQH₂ completely prevented the ROO⁻-induced accumulation of CE-O(O)H and PC-OOH in the FIVE patient’s LDL and HDL for at least the first 3 hr of oxidation, whereas in the absence of α-TQH₂, the hydroperoxides accumulated at rates directly proportional to the levels of endogenous α-TOH (data not shown), in agreement with our recent observation (10). Furthermore, α-TQH₂ completely prevented the peroxidation of Intralipid (diluted 1:10 in PBS) incubated at 37°C with 2 mM of AAPH (data not shown). These results confirm that under these mild oxidizing conditions, α-TQH₂ acts as a prooxidant for lipid emulsions (6, 7), and demonstrate that α-TQH₂, effectively prevents this prooxidant activity, independent of the presence of protein(s).

We next examined a possible role of coenzyme Q in the antioxidant activity of α-TQH₂ For this, LDL enriched some
3-fold with CoQ_{10}H_{2} by dietary supplementation with CoQ_{10} (29) was exposed to AAPH in the presence and absence of α-TQH_{2}. Under all conditions α-TQH_{2} was consumed before and completely prevented the oxidation of CoQ_{10}H_{2} in agreement with the results shown in Fig. 1. More importantly, the rates of α-TQH_{2} oxidation were independent of the initial level of LDL’s CoQ_{10}H_{2} (data not shown), indicating that α-TQH_{2} acted independently of CoQ_{10}H_{2}.

A striking feature of the treatment of LDL with α-TQH_{2} was the ability of the hydroquinone to rapidly reduce CoQ_{10} (see above). Fig. 3A shows the results of an experiment where in vivo CoQ_{10}H_{2}-enriched LDL (29) was first allowed to autoxidize until all coenzyme Q was present as CoQ_{10}, before the lipoprotein was placed on ice, 10 μM of α-TQH_{2} was added, and the lipoprotein was then incubated at 37°C. Addition of α-TQH_{2} to such LDL resulted in the instantaneous formation of CoQ_{10}H_{2}, whereas a slower reduction of CoQ_{10} was observed when either an organic extract of such LDL (redissolved in ethanol) or an ethanolic solution of authentic CoQ_{10} was used (Fig. 3A), similar to the situation when LDL-associated CoQ_{10} was exposed to HepG2 and red blood cells (35). Whether the very rapid reduction of CoQ_{10} by α-TQH_{2} plays a physiological role in the maintenance of extracellular coenzyme Q in its reduced, antioxidant active form appears unlikely because healthy human blood does not contain measurable α-TQH_{2}. However, the fact that several of the hydroquinones tested here are able to perform this reduction may suggest a role for a physiological hydroquinone, such as the reduced form of vitamin K, in the maintenance of circulating CoQ_{10}H_{2}, though this requires testing. What is clear is that the rapid reduction of CoQ_{10} by α-TQH_{2} was protein independent, as it was also observed when α-TQH_{2} was added to either in vivo CoQ_{10}-enriched) HDL or commercial Intralipid (data not shown).

The conversion of LDL’s CoQ_{10} to CoQ_{10}H_{2} was quantitative and required two molecules of α-TQH_{2} for the two electron reduction of each molecule of CoQ_{10} (Fig. 3B). This indicates that the reaction may be complex, perhaps involving semiquinone radicals. As superoxide dismutase did not affect CoQ_{10} reduction by α-TQH_{2} (data not shown), it appears that superoxide anion does not play a role here.

Similar to CoQ_{10}H_{2} (d[CoQ_{10}H_{2}]/dt = 97 ± 11 pmol liter⁻¹s⁻¹), α-TQH_{2} dissolved in ethanol at 10 μM and incubated at 37°C autoxidized linearly at a rate of 127 ± 5.6 pmol liter⁻¹s⁻¹. By contrast when added to LDL, HDL, or Intralipid incubated at 37°C, the levels of α-TQH_{2} remained unchanged for at least 5 hr, independent of the coenzyme Q and α-TQH_{2} concentrations present in the emulsions, suggesting that for presently unknown reason(s), lipid emulsions stabilize α-TQH_{2}.

Previous results suggested that α-TQH_{2} is also capable of directly reducing α-TO’ in alcohol/water mixtures (36) or micelles (19). We therefore tested whether α-TQH_{2} was also able to react with α-TO’ in LDL. For this, we incubated LDL

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<th>Table 1. α-TQH_{2} effectively inhibits LDL lipid peroxidation induced by different oxidants</th>
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*LDL (0.8–12 μM apoB) was supplemented with 10 μM α-TQH_{2} (+) or the appropriate volume of ethanol (−) immediately before oxidation. AAPH was used at 2 mM, AMVN at 1 mM, and SLO at 4 μg/ml. Cu^{2+} was used at either 16.7 (1) or 1.5 (2) molecules of Cu^{2+} per LDL particle. For cell-enhanced oxidation, LDL was diluted 5 times with sterile Ham’s F-10 medium and incubated in the absence (F-10) or presence (MDM) of ~10^6 MDM per well.

§Onset of oxidation refers to the first signs of significant decrease of CoQ_{10}H_{2} or α-TOH.

$†The extent of lipid hydroperoxide accumulation was determined at a time where 20% of the initial α-TOH was depleted in the control, α-TQH_{2}-free LDL samples. The times required for this varied for the different oxidants and were approximately: AAPH, 140 min; AMVN, 315 min; SLO, 315 min; Cu^{2+} (2), 180 min; F-10, 300 min; and MDM, 210 min.

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$§For Cu^{2+} under condition (1), 25 μM of α-TQH_{2} was added and the level of CE-O(O)H accumulation was compared after complete α-TOH consumption and at a time of maximum peroxidation in the control LDL (~120 min), under these very strong oxidizing conditions only little lipid hydroperoxides accumulate as long as the vitamin is present (for example, see ref. 10).

$Five minutes represents the earliest time point measured, where there was already detectable CoQ_{10}H_{2} consumption.

The results shown represent means ± SD from three separate experiments from two to three different donors. ND, not determined.
In summary, the results presented show evidence for three distinct antioxidant activities of α-TQH$_2$, i.e., direct radical scavenging and reduction of both α-TQ and CoQ$_{10}$. Various compounds have been shown to both directly scavenge radicals and reduce α-TQ (see ref. 19). What makes α-TQH$_2$ exceptional is the apparent preference with which it becomes oxidized before CoQ$_{10}$H$_2$ (which thus far has been regarded the first line of lipophilic antioxidant defense), and the efficacy with which it prevents, rather than attenuates, lipid peroxidation in LDL and HDL exposed to different types of radical oxidants. Consistent with this, the one-electron reduction potential of α-TQH$_2$ is expected to be lower than that of CoQ$_{10}$H$_2$ (and ascorbate) (for example, see ref. 37), and the rate constant for the reaction of α-TQH$_2$ with α-TQ is greater than that for CoQ$_{10}$H$_2$ though slightly lower than that for ascorbate (36).

In humans, who do not synthesize α-TQ, the quinone is most likely formed by oxidation of α-TOH. Under normal conditions, significant concentrations of α-TQ or α-TQH$_2$ are not present in human tissues and fluids. By contrast, α-TQ has been detected in biological systems exposed to oxidative stress, such as plasma obtained during ischemia induced by cross-clamping (38) or in advanced atherosclerotic plaques, where α-TQH$_2$ is present as α-TQH$_2$ (17). This raises the possibility that α-TQH$_2$ could be formed in vivo under conditions where α-TOH oxidation occurs and a suitable reducing system for α-TQ exists (28). If so, α-TQH$_2$ may represent a previously unrecognized, highly effective natural antioxidant that could contribute to the protection of intimal lipids from oxidation. Also, in this case a potential contribution of α-TQH$_2$ formation may need to be considered when evaluating the in vivo antioxidant activities of vitamin E, particularly under conditions of severe oxidative stress.

Because of its outstanding antioxidant activity in the in vitro systems used here, a potential protective function of α-TQH$_2$ as a therapeutic antioxidant deserves consideration. Relevant to this, hydroquinones are potential carcinogens and cytotoxic agents, although these deleterious activities, at least in the case of tocopherol hydroquinones, are lowest for the α-form, probably due to its inability to give rise to Michael adducts (39). Also, tocopheryl quinones inhibit vitamin K-dependent carboxylase activity (40), and this could result in an anticoagulating action, although to our knowledge this action has not
be demonstrated in vivo. On the other hand, there is early literature demonstrating a beneficial effect of administration of relatively large doses of α-TQ or α-TQH₂ in the prevention of muscular dystrophy in rabbits and rats without apparent side effects. α-TQ and α-TQH₂ possess vitamin E activity (for example, see ref. 42), and this may be due to their conversion to α-TOH (43). Oral supplementation of humans with α-TQ results in low micromolar plasma levels of both α-TQ and α-TQH₂ (28, 45). Together, these results suggest that α-TQ and α-TQH₂ are non-toxic and that humans not only take up the quinone but also reduce it to the hydroquinone, and therefore warrant testing of α-TQ/α-TQH₂ as a potential therapeutic antioxidant.

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