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Commentary

Ubiquinone and tocopherol: Dissimilar siblings

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ABSTRACT

Research on antioxidants and their potential health benefits expanded over the last decades from basic science to the medical and nutritional fields. This included supplementation studies of both vitamin E compounds and the endogenous antioxidant ubiquinone, to prevent or alleviate cardiovascular diseases and their pathophysiological consequences. In many of these studies, only one antioxidant or one group of antioxidants was considered, disregarding the pharmacological and toxicological properties of their metabolites as well as possible cooperative and competitive effects on the overall physiological response. There are many – often indirect – effects, especially in gene regulation, observed on administration of both compound groups in cells, which have been assigned to these molecules without identifying the cellular targets. Therefore, this article focuses on direct chemical and biochemical effects of ubiquinone- and α-tocopherol-related compounds, which are evident from direct binding studies or direct interaction leading to chemical modification of the compounds. These groups include *para*-benzoquinones (ubiquinone and α-tocopheryl quinone) and chroma(e)nols (α-tocopherol and bicyclic ubiquinone derivatives). Their effects as antioxidants, co-antioxidants, and pro-oxidants as well as direct interactions with proteins are considered, pointing out similarities and dissimilarities of the two compound groups in a wider context. The review of the isolated findings about one or a few of these compounds in the literature, disregarding structurally related compounds, suggests that comprehensive structure/activity relationship studies including related compounds would promote the understanding of biological functions and pharmacological effects of ubiquinone- and α-tocopherol-related compounds.

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Abbreviations: α-CEHC, α-carboxyethyl hydroxychromane; α-CMBHC, carboxymethylbutyl hydroxychromane; cyt, cytochrome; CYP, cytochrome P450; DPPH•, diphenyl picryl hydrazyl; dUQ, decyl ubiquinone; LDL, low-density lipoprotein; LPO, lipid peroxidation; LPS, lipopolysaccharide; PBN, phenyl-*tert*-butyl nitrone; Q, quinone; ROS, reactive oxygen species; SMP, submitochondrial particles; SOD, superoxide dismutase; SPF, supernatant protein factor; TAP, α-Toc-associated protein; α-Toc, α-tocopherol (most active component of vitamin E); α-TQ, α-tocopheryl quinone (α-tocopherol quinone, vitamin E quinone); α-TTP, α-Toc transfer protein; UCa, ubichromanol; UCe, ubichromenol; UQ, ubiquinone; $UQ_{10}OH$, 3′-hydroxy ubiquinone (with 10 side chain isoprenyl units).

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1. Introduction

Antioxidative compounds of the ubiquinone (UQ)[1] and α-tocopherol (α-Toc) group are increasingly ingested as food supplements and often administered simultaneously [1]. The therapeutic targets for both compound groups are related to their antioxidant function and the requirement as cofactor in mammalian tissues. Therefore, the uptake of UQ and/or α-Toc to compensate endogenous deficiencies and to combat oxidative stress was shown to be beneficial [2–4]. According to the fundamental principles of drug actions at the molecular level (i) similar molecular structures can cause similar pharmacologic activities and (ii) the pharmacologic activity of a drug might not only be related to the parent compound administered, but also to its metabolites. Alternatively, for some drugs the administration of a pro-drug is more appropriate than to apply the active compound directly [5–7]. When these rules are applied to the antioxidants of the UQ- and α-Toc-group, several white spots of our current knowledge become visible. Due to their related structure, the question of drug interactions on the pharmacokinetic, but also pharmacodynamic level arises. This commentary attempts to draw attention to similarities and dissimilarities of α-Toc- and UQ-related compounds as well as to the often isolated scientific knowledge in this field.

2. Structural relationships, natural occurrence and basic functions

UQ (in its reduced form UQ-H_2) and α-Toc are well-known lipophilic antioxidants. They are regular constituents of human food and animal nutrition. Fig. 1 describes the structural similarities of UQ- and α-Toc-related compounds, which are of relevance in mammalian tissues. This scheme shows that also for UQ-related compounds a chromanol analog (ubichromanol, UCa) exists, which resembles α-Toc. Reactions 1/2 and 5/6 constitute the antioxidative defense mechanism of α-Toc and UCa_9, respectively, yielding the corresponding *para*-benzoquinones α-tocopheryl quinone (α-TQ) and hydroxylated ubiquinone ($UQ_{10}OH$) via their chromanoxyl radicals (α-$Toc^{\bullet}$, $UCa_9^{\bullet}$). Reverse reactions of *para*-benzoquinones to their corresponding chromanols are controversially discussed for mammalian tissues (reactions 3, 4, 7). The redox equilibrium of the *para*-benzoquinones with their corresponding semiquinone anions ($Q^{\bullet -}$) and hydroquinones (quinols, Q-H_2) is shown in reactions 8 and 9. The reversibility of these reactions qualifies them as redox shuttles (electron carriers), antioxidants, but also as pro-oxidants. The similarity of α-Toc, α-TQ, and UQ extends to their metabolism, yielding end products such as α-carboxyethyl hydroxychromane (α-CEHC), tocopheronic acid, and Q-acid I and II, respectively (Fig. 5). Some of these compounds will be considered in this article. α-Toc exhibits a rather uniform distribution in cellular membranes, which is proportional to the lipid content of the respective membranes [8]. In contrast, UQ is concentrated in certain organelle membranes [9,10], which partially require this natural *para*-benzoquinone as a cofactor for electron transfer enzymes. For α-Toc it has been shown that besides its antioxidant function it is involved in the regulation of genes related to lipid (including α-Toc itself) uptake and degradation, expression of extracellular proteins, inflammation, cell signaling and cell cycle [11].

Upon one-electron oxidation of α-Toc the corresponding chromanoxyl radical α-$Toc^{\bullet}$ is formed. Some of these radicals dismutate to α-TQ [12]. In this process, 50% of the α-$Toc^{\bullet}$ radicals lose the chance to be recycled back to α-Toc by co-antioxidants, such as ascorbate and UQ-H_2, so the primary antioxidant power of α-Toc is decreased. Recycling of compounds similar to α-TQ back to α-Toc can only be carried out by cyclases in plants [13]. This cyclization reaction, whether enzymatic or chemical, is generally facilitated by a double bond in the side chain of tocopherol precursors, which could be obtained from α-TQ by elimination of the side chain OH group as H_2O [14]. In mammals, there has been a single report documenting the reconversion of radioactively labeled α-TQ back to α-Toc [15], but no mechanism was presented. That this phenomenon is, however, not an isolated finding is supported by the repeated detection of the bicyclic ubiquinone derivative ubichromenol (UCe) in mammalian tissues [16–19]. Alternatively, α-TQ can be reduced to α-TQ-H_2 to regain some antioxidative power, but this process is slow compared to the recycling of α-$Toc^{\bullet}$, and the overall antioxidative efficacy of α-TQ-H_2 is smaller than that of the parent α-Toc [20].

α-TQ formally arises from the two-electron oxidation of α-Toc. However, an effective conversion takes place only if the recycling of the chromanoxyl radicals (α-$Toc^{\bullet}$) is slowed down due to low ascorbate concentrations or a low UQ-H_2/UQ ratio in mitochondrial membranes (for example, state 3 respiration). Even when lipid peroxidation in mitochondrial membranes is artificially initiated, only about 10–15% of α-Toc is converted to α-TQ, although 80–90% of α-Toc is lost [21]. The remaining portion is most likely converted to tocopheryl quinone epoxides and lipid-linked products [22], which have not been toxicologically studied yet. Under physiological conditions in rat liver mitochondria oxidative processes result in 1–5% α-TQ of the total *para*-benzoquinone pool (mostly UQ) [23]. It has been shown that α-TQ can be reduced to α-tocopheryl hydroquinone (α-TQ-H_2) by several electron transfer enzymes (vide infra) [23], which can in turn act as an antioxidant [24]. Furthermore, it has been demonstrated that α-TQ can influence UQ functions, according to its structural similarity. On the other hand, there are also structural differences between UQ and α-TQ, which cause the non-equivalence of the two *para*-benzoquinones in the cell: polyisoprenyl versus phytyl side chains (i.e. unsaturated versus saturated side chains), the hydroxylated side chain of α-TQ and the ring substitution (methoxy versus methyl). Thus, a different distribution of polar and apolar groups in the two molecules might cause different interaction with quinone-binding proteins, especially redox enzymes. In addition, a decreased redox potential of α-TQ (E_{m7} α-TQ/α-TQ-H_2 = +50 mV as inferred from duroquinone [25]), caused by a different quinone ring substitution in comparison to UQ

[1] For ubiquinones (UQ_n), hydroxylated ubiquinones (UQ_nOH), ubichromenols (UCe_n), and ubichromanols (UCa_n) the index *n* indicates the number of isoprenic units in the side chain. Reduced quinones were depicted by the suffix –H_2. The abbreviations UQ, UCe, and UCa without index refer to these compounds in general.

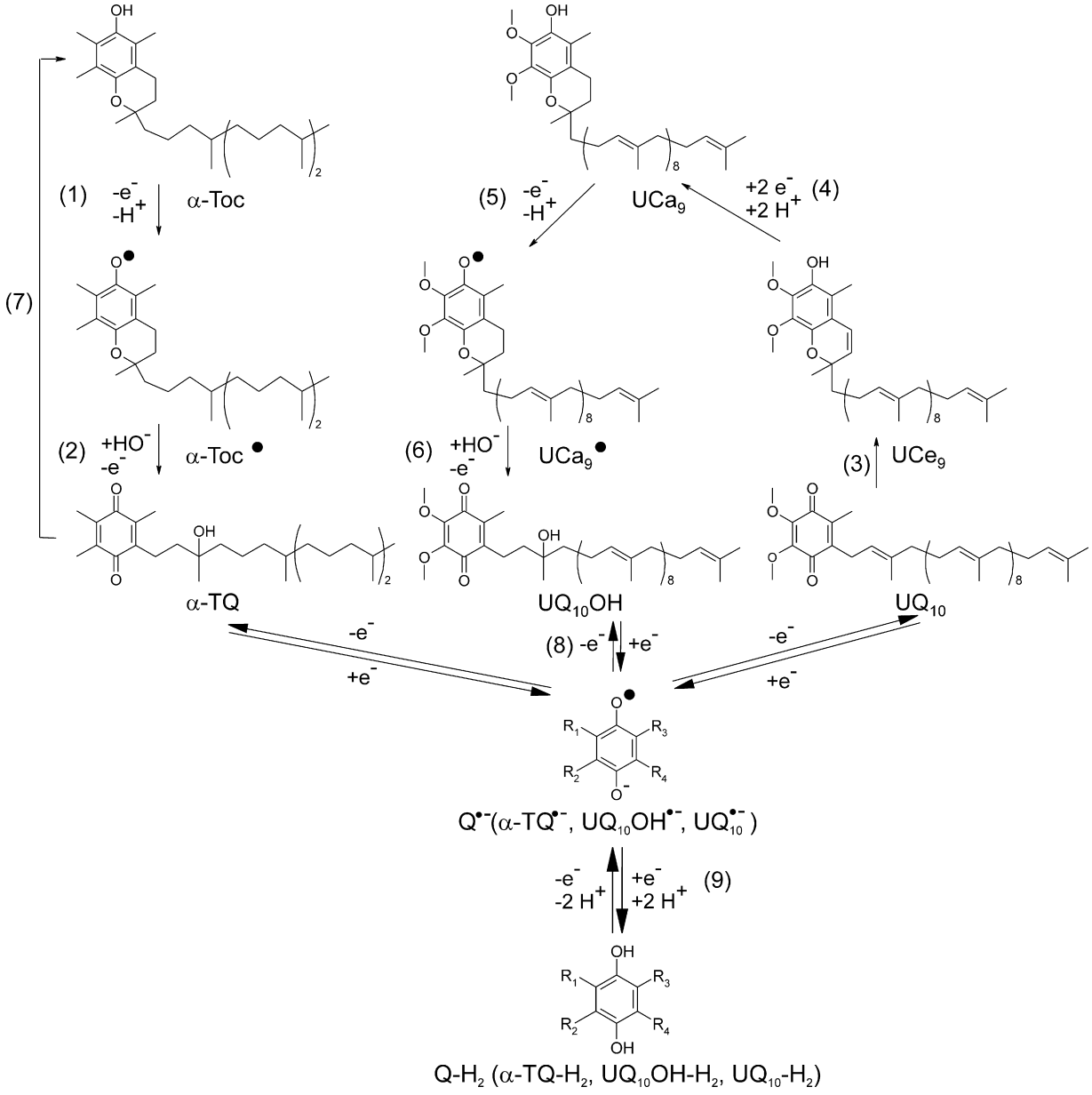

Fig. 1 – Scheme of chromane and *para*-benzoquinone compounds derived from α-tocopherol and ubiquinone-10. The compounds shown are: α-tocopherol (α-Toc), α-tocopheroxyl radical (α-Toc•), α-tocopheryl quinone (α-TQ), ubiquinone-10 (UQ_{10}), ubichromenol-9 (UCe_9), ubichromanol-9 (UCa_9), ubichromanoxyl radical (UCa_9•), side-chain hydroxylated ubiquinone-10 ($UQ_{10}OH$). The species $Q^{\bullet -}$ and Q-H_2 are common representations for the equilibrium of the quinones α-TQ (R_1, R_2, R_3 = CH_3; R_4 = phytyl), UQ_{10} (R_1, R_2 = OCH_3, R_3 = CH_3, R_4 = isoprenyl), $UQ_{10}OH$ (R_1, R_2 = OCH_3, R_3 = CH_3, R_4 = hydroxylated isoprenyl) with their corresponding semiquinone anions and hydroquinones (quinols), respectively.

(E_{m7} UQ/UQ-H_2 = +90 mV [26]) may be responsible for different properties as enzymatic substrates.

In order to analyze these structure/activity relationships in the group of bioquinones and related chromanols the question arises whether bicyclic derivatives of UQ (similar to α-Toc) exist in biological systems. In addition, alternative routes of ubiquinone delivery to tissues are of pharmacological interest for the treatment of pathophysiologic UQ deficiencies [27]. During the search for intermediates of oxidative phosphorylation in the 1960s, bicyclic ubiquinone derivatives have been detected for the first time and partially described [28]. However, the research interest declined as soon as the theory of chemiosmotic coupling [29] became accepted and the major function of UQ in the mitochondrial electron transfer was assigned. Formally, the compounds ubichromenol-9 (UCe_9) and ubichromanol-9 (UCa_9) can be considered as bicyclic isomers of UQ_{10} and UQ_{10}-H_2, respectively.

UCe_9 can be synthesized easily by chemical isomerization catalyzed by basic solvents or photochemical treatment of ubiquinone [30,31]. In addition, UCe was detected in mammalian liver fractions (see Fig. 2A and B), kidney and intestine [28]. It is still unclear whether these levels of UCe arise from an

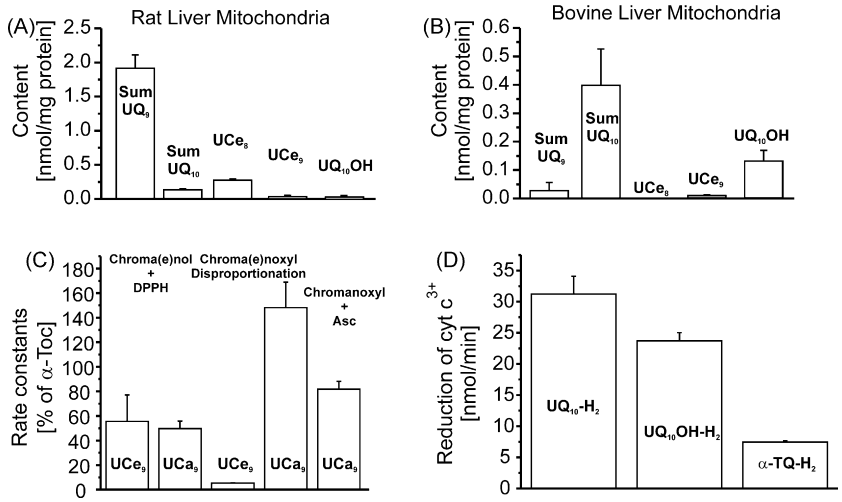

Fig. 2 – Antioxidant properties, activities as mitochondrial substrates and occurrence of bicyclic ubiquinone derivatives and related compounds. Occurrence of UQ derivatives in mitochondrial fractions from rat liver (A) (n = 2, data are means ± S.D.) and bovine liver (B) (n = 4, data are means ± S.D.). (C) Rate constants for the reaction of UCa_9/UCe_9 with the stable radical DPPH$^\bullet$, for the disproportionation reaction of the resulting $UCa_9^\bullet$/$UCe_9^\bullet$ radicals, and for the recycling of the $UCa_9^\bullet$ radicals by ascorbate (Asc) in comparison with the respective rates for the α-Toc derivatives ($UCe_9^\bullet$ recycling by ascorbate could not be measured due to low photochemical radical yield, experimental details see [39]) (n = 3, data are means ± S.E.). (D) Enzymatic reduction of cytochrome c^{3+} by isolated mitochondrial cytochrome bc_1 complex with reduced quinones as donors: UQ_{10}-H_2 (the native substrate), UQ_{10}OH-H_2 (UQ_{10}OH arises from UCa_9 oxidation), and α-TQ-H_2 (arises from α-Toc oxidation) (70 μM each, n = 7, data are means ± S.E.). For experimental details of (A), (B), and (D) see [35].

enzyme-catalyzed cyclization of UQ or food uptake. By isotopic labeling it was demonstrated that in rat liver UQ_9 was converted to UCe_8 (corresponding to reaction 3 in Fig. 1), but the reverse reaction barely occurred [19]. Even a link between UCe and α-Toc metabolism has been postulated [32]. In vitro, further chemical reduction of UCe_9 by metallic sodium leads to UCa_9, the α-Toc analog of UQ_{10}. So far, this compound was not detected in mammalian tissues. Early reports about the occurrence of bicyclic ubiquinone derivatives have been questioned by later findings that the chromatographic normal phase materials used in combination with saponification methods for the extraction of these lipophilic compounds may artificially form bicyclic derivatives from UQ [33,34]. However, using reversed phase materials for chromatographic separation and highly sensitive electrochemical detection the presence of UCe, but never UCa, in liver fractions was confirmed (Fig. 2A and B) [35]. In analogy to α-Toc, two-electron oxidation of UCa_9 leads to a *para*-benzoquinone with a hydroxylated side chain (UQ_{10}OH, Fig. 1), which is not identical to ubiquinone (UQ_{10}) [35]. Recently we showed that this modified ubiquinone (UQ_{10}OH) is present in liver mitochondrial fractions from bovines and rats (Fig. 2A and B). In both mitochondrial fractions the chain length of the predominant bicyclic UQ derivatives UCe_8 and UCe_9 was related to the predominant UQ species, i.e. UQ_9 (rat) and UQ_{10} (bovine), respectively. The reaction scheme in Fig. 1 demonstrates that a complete set of chromanols and corresponding *para*-benzoquinones with a hydroxylated side chain exists for α-Toc and UQ. Each group (α-Toc and UQ-related compounds) contains structurally related compounds, which partially coexist in mammalian cells. Therefore, it should be elucidated whether their cellular coexistence is indifferent, synergistic, or competitive in terms of pharmacological effects.

3. Separate effects as antioxidants

The general antioxidant property of α-Toc and reduced UQ (UQ-H_2) is the prevention of lipid peroxidation by scavenging of lipid peroxyl radicals [36,37]. The formation of the antioxidant-derived radical was widely studied for α-Toc [38].

A systematic comparison of α-Toc, UCe_9 and UCa_9 documented that in certain environments these compounds are radical scavenging antioxidants of similar efficiency [35]. The environment influences the reactivity of UCe_9/UCa_9 and α-Toc with the model radical (diphenyl picryl hydrazyl, DPPH$^\bullet$) in a different manner. In ethanol, the polarity of which approximates that of the polar head group region of phospholipid membranes, the reactivity of UCe_9/UCa_9 is ca. 50% with respect to α-Toc (Fig. 2C). In analogy to α-Toc$^\bullet$ the resulting chroma(e)noxyl radicals (UCa$^\bullet$, UCe$^\bullet$) can be recycled by other cellular reductants (co-antioxidants). Ascorbate and decyl ubiquinol as reductants rapidly recycle $UCa_9^\bullet$ (ca. 80% and at least 470%, respectively, in comparison with the recycling of α-Toc$^\bullet$) (Fig. 2C), whereas only rough limits for the recycling reaction could be determined for the recycling of $UCe_9^\bullet$ due to technical limitations. The disproportionation of the $UCa_9^\bullet$ radicals achieves rates faster than that of α-Toc$^\bullet$ radicals. In contrast, $UCe_9^\bullet$ radicals were very stable and decayed rather slowly (Fig. 2C) [39]. The reactivity of UQ-H_2 and α-TQ-H_2 with another model radical, galvinoxyl, is 2.5 and 4 times higher, respectively, than that of α-Toc in ethanol. A

similar ranking was also observed for the reaction of these hydroquinones with peroxyl radicals [20]. However, pro-oxidant activities of the resulting semiquinones have to be taken into account much more than for chromanoxyl radicals (vide infra). The disproportionation rate constant of the $UQ^{\bullet-}$ radical anion ($8 \times 10^4\ M^{-1}\ s^{-1}$) [40] is at least 10 times higher than that of α-Toc$^{\bullet}$.

4. Cooperativity as antioxidants

α-Toc-related and UQ-related compounds coexist in different amounts in lipid membranes. The ratio of α-Toc to UQ varies in rat liver mitochondrial membranes from about 1:1 in the outer membrane to 1:10 in the inner membrane because of severalfold higher UQ concentrations and slightly lower α-Toc concentrations in the inner membrane [23]. In contrast, in microsomal fractions an excess of α-Toc over UQ (ratio about 1:0.3) was observed [23]. Although rate constants for the reaction of α-Toc ($33 \times 10^5\ M^{-1}\ s^{-1}$) and UQ-$H_2$ ($3.4 \times 10^5\ M^{-1}\ s^{-1}$) [41] with lipid peroxyl radicals in homogenous solution were in favor of α-Toc, for liposomal membranes their activities were rather similar [42] due to recycling of α-Toc$^{\bullet}$ by UQ-H_2 [37]. In contrast, the reaction of α-Toc ($5.12 \times 10^3\ M^{-1}\ s^{-1}$) and UQ_{10}-H_2 ($4.7 \times 10^3\ M^{-1}\ s^{-1}$) with aroxyl radicals in ethanol was about equally effective [43]. The importance of the cooperativity depends on the concentration ratio of α-Toc/UQ-H_2 and on the ability of membrane-bound enzymes to regenerate UQ-H_2. At high ratios, α-Toc acts as the preferred radical scavenging antioxidant and the resulting α-Toc$^{\bullet}$ is reduced by other reductants including ascorbate and UQ-H_2. In contrast, if UQ-H_2 dominates as in the inner mitochondrial membrane, it can become a primary radical scavenger [37]. In certain microenvironments the simultaneous presence of both antioxidants prevents the pro-oxidative activity of α-Toc [44,45]. Another link is related to the interaction of *para*-benzoquinones of both compound groups with enzymatic binding sites. The coexistence of α-TQ and UQ in mitochondrial membranes suggests competition between the two compounds at sites at which UQ undergoes cyclic reduction and oxidation.

Based on thermodynamic data of duroquinone (tetramethyl-*para*-benzoquinone), which is a simplified model of α-TQ, one would expect that the direct reduction of α-TQ by UQ-H_2 is not favored. Nevertheless, in mitochondrial membranes reduced α-TQ was detected [46]. Model experiments revealed that a direct interaction is only favored in a polar environment in which partial deprotonation of UQ-H_2 accelerates electron transfer [47]. This is in agreement with general thermodynamic findings that redox reactions are much faster between a charged and a neutral species than between two neutral reaction partners [25]. Due to the high pK_a values of quinols (pK_a UQ_{10}-H^-/UQ_{10}-H_2 = 11.3 [48]) these redox processes are expected to be rather slow at physiological pH values. In this context, a transhydrogenase activity of the mitochondrial electron transfer complexes transferring redox equivalents from UQ-H_2 to TQ has been suggested [47,49]. This activity (vide infra) has advantages and disadvantages for cellular functions. On the one hand it provides α-TQ-H_2, an additional antioxidant [24], but on the other hand redox-equivalents of UQ-H_2 are wasted. Furthermore, additional reduction of α-TQ by extra-mitochondrial enzymes is probably involved in α-TQ-H_2 formation (vide infra) [50].

5. Pro-oxidative side effects

There is no doubt that chromanols (α-Toc, UCa) and reduced *para*-benzoquinones (UQ-H_2, UQOH-H_2 and α-TQ-H_2) can act as radical scavenging antioxidants. However, the fate of the resulting metabolites also determines the overall benefit for the cellular defense against oxidative stress. In general, two different pathways have been considered in the past to be possible reasons for pro-oxidative reactions: (i) formation of harmful radical intermediates by the parent compound or its metabolites, and (ii) reduction of transition metal ions mediating oxidative stress.

(i) It has been assumed that recycling of α-Toc$^{\bullet}$ is important to prevent excessive loss of antioxidant equivalents [37] and to prevent pro-oxidant activities of this intermediate [44,51–53]. Due to the limited recycling ability within low-density lipoprotein (LDL) particles, only reduction of α-Toc$^{\bullet}$ by other (preferably aqueous) reductants is supposed to prevent α-Toc-mediated peroxidation. Although there is kinetic evidence for α-Toc$^{\bullet}$-mediated peroxidation and this effect is partially due to the specific situation in LDL particles (single or few α-Toc$^{\bullet}$ molecules per particle), no conclusive reaction mechanism for this phenomenon has been postulated [51,54,55]. So far direct hydrogen-abstraction from lipids by α-Toc$^{\bullet}$ [51] or by α-TocOO$^{\bullet}$ (a peroxyl radical of α-Toc$^{\bullet}$) [44] has been postulated. However, Mukai and co-workers [56] have shown that direct H-abstraction by tocopheroxyl analogs is in the range of $10^{-2}\ M^{-1}\ s^{-1}$ while the antioxidant reaction of α-Toc with fatty acid peroxyl radicals is 10^6–$10^7\ M^{-1}\ s^{-1}$ [57]. In this context, the possible existence of another pro-oxidative intermediate of α-Toc oxidation is of interest. Knapp and Tappel assumed that the α-Toc$^{\bullet}$ radical exists in an equilibrium with a 5a-C carbon-centered tocopheryl radical [58], which was held responsible for the formation of 5a-linked tocopherol dimers (dihydroxy dimers) as one of the side products of α-Toc oxidation (Fig. 3). These dihydroxy dimers were suggested to contribute to skin protection by α-Toc [59,60]. Unfortunately the mechanistic assumption involving carbon-centered tocopheryl radicals has been repeated by other authors [61,62] even decades after the first publication without critical evaluation. The existence of such a carbon-centered radical would be potentially dangerous in the cell due to its more aggressive nature in comparison with phenoxyl radicals, a fact which can be deduced from the pecking order of free radicals [63]. This reaction mechanism [58] was recently tested by isotope labeling in combination with spin trapping experiments [12]. The formation of 5a-C-centered radicals was studied with chromanol model compounds having the ^{12}C atom replaced by a ^{13}C atom in the 5a position of the chromane ring system. In addition, chromanoxyl formation was blocked by acetylation. After irradiation of these compounds with UV light in the presence of the spin trap compound phenyl *tert*-butyl nitrone (PBN), the additional isotopic hyperfine splitting only became visible if the 5a-carbon atom was activated by a photo-labile bromine substitution (Fig. 3). In contrast, the

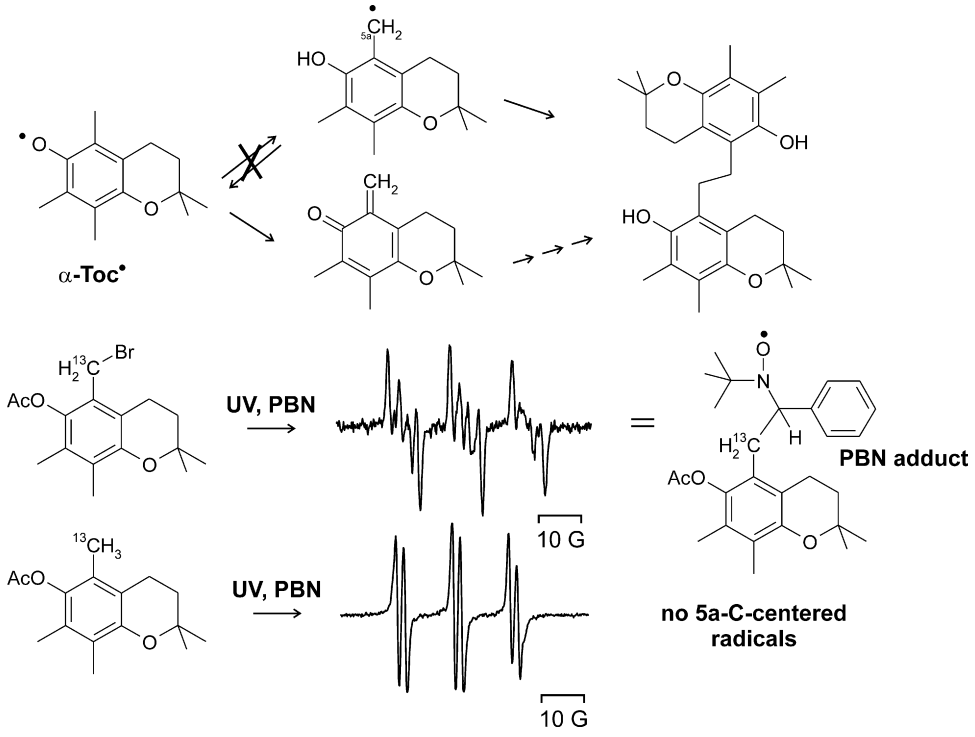

Fig. 3 – α-Tocopheroxyl radicals were postulated to be in equilibrium with 5a-C-centered radicals, which were thought to give rise to the formation of 5a-C-linked dimers and possibly to some pro-oxidative effects. However, spin-trapping experiments with 5a-^{13}C-labeled compounds and phenyl-*tert*-butyl nitrone (PBN) revealed that carbon-centered radicals could only be formed at this position if a labile group, such as Br, is introduced. For experimental details see [12].

non-brominated tocopherol analogues gave unspecific spin adducts without additional splitting (Fig. 3). These findings clearly disproved the mechanism supposed by Knapp and Tappel [58] and showed that chromanoxyl radicals did not equilibrate with 5a-C-centered α-Toc radicals. Consequently, this excludes the participation of such radicals in α-Toc-mediated peroxidation. Further studies on the reaction mechanism revealed the involvement of the *ortho*-quinone methide in many reactions following α-Toc$^{\bullet}$ formation [12]. This intermediate is widely neglected in biological research on this antioxidant. Therefore, the recycling of the α-Toc$^{\bullet}$ radical is probably aimed at increasing the efficiency of the antioxidant system by minimizing side reactions of the *ortho*-quinone methide.

It is evident from the basic studies of radical research that H-abstraction from chromanol and hydroquinone antioxidants leads to the formation of neutral primary antioxidant radicals under physiological conditions [64], the chromanoxyl and the neutral semiquinone radical, respectively. These primary chromanoxyl or semiquinone radicals have an extremely low affinity to undergo the reverse reaction by abstracting hydrogen atoms from other biomolecules. However, an equilibrium of these radicals with other species may counteract this positive view. Ubisemiquinones, although having no hydrogen abstraction capabilities, can significantly contribute to oxidative stress upon deprotonation, forming semiquinone anions ($UQ^{\bullet-}$). This has been demonstrated during UQ_{10}-H_2-inhibited lipid peroxidation (LPO) in liposomes. In these experiments, in which LPO was induced by decomposition of an azo-initiator, an UQ_{10}-H_2-dependent hydrogen peroxide release was observed [65]. Likewise ubisemiquinones of the cytochrome bc_1 complex, which are continuously formed as regular intermediates of mitochondrial respiration, are a major source of reactive oxygen species (ROS) in this cellular compartment [66]. This is because autoxidation of $UQ^{\bullet}$ preferably occurs via its deprotonated anion ($UQ^{\bullet-}$) [67], which, however, can only be formed in a (partially) polar environment. The quinone ring of UQ_{10} (and UQ_{10}-H_2) is partially located in the polar head group region of phospholipid bilayers [68]. Since the pK_a of $UQ^{\bullet}$ is 5.9 [67], $UQ^{\bullet-}$ formation can occur in polar head group regions of biomembranes at physiological pH values. The access of the semiquinone head group to the aqueous phase should depend on the length of the side chain below a certain threshold. For quinones with a short side chain this access increases the probability of autoxidation contributing to superoxide radical release. The increased application of short-chain homologues of UQ, such as idebenone and $MitoQ_{10}$, demonstrates the importance of this question. On the other hand the autoxidation of native ubisemiquinones in various membranes has been discussed as an important cellular function producing H_2O_2 via superoxide anion radicals ($O_2^{\bullet-}$) as an intracellular second messenger [69]. Furthermore, the autoxidizability of $UQ^{\bullet-}$ was used as an explanation for the prevention of α-Toc-mediated peroxidation in LDL particles by transferring the unpaired electron to oxygen in the aqueous phase in contrast to α-Toc$^{\bullet}$, which does not undergo autoxidation [45]. Among the compounds covered in this article, the α-Toc metabolite

α-TQ and its reduced form α-TQ-H_2 are of relevance for possible pro-oxidative activities. Analogous to UQ-H_2, α-TQ-H_2 is expected to form the semiquinone anion radical ($TQ^{\bullet -}$) in the course of its antioxidative reactions under physiological conditions, via its neutral precursor $TQ^{\bullet}$. Since the redox potential of the couple $Q/Q^{\bullet -}$ is more negative for α-$TQ^{\bullet -}$ ($E^0 = -250$ mV) than for $UQ^{\bullet -}$ ($E^0 = -110$ mV) [26], as inferred from hydrophilic analogues, α-$TQ^{\bullet -}$ should more easily undergo reaction with oxygen (O_2) forming $O_2^{\bullet -}$. On the other hand α-TQ-H_2 has a less positive redox potential (Q/Q-H_2) than UQ-H_2 (50 mV [25] versus 90 mV [26]). Therefore, α-TQ-H_2 should be more effective in reducing lipid peroxyl radicals than UQ-H_2.

(ii) Another pathway of pro-oxidative side effects is due to the fact that chemical antioxidants are reductants. Although they possess the beneficial feature of reducing lipid peroxyl radicals to their corresponding hydroperoxides, they can also reduce free metal ions (primarily Fe^{3+}, Cu^{2+}) to their pro-oxidative states (Fe^{2+}, Cu^{+}). This effect is best known for the hydrophilic antioxidant ascorbate, but also the reduction of Cu^{2+} by α-Toc and related compounds, leading to DNA damage, was described [70]. Overall, this effect is expected to increase with decreasing lipophilicity of the antioxidant. Therefore, under physiological conditions native α-Toc and UQ_{10} should contribute much less to pro-oxidative pathways than low molecular weight analogues.

6. Competition at protein binding sites

There are two major factors that modulate the effect of the quinones and chromanols considered in this article at protein binding sites: (i) structural differences of the quinone and chromanol head groups and/or of the lipophilic side chains; (ii) a different redox potential (in the case of electron transfer proteins). Accordingly, competition of α-Toc- and UQ-related quinoid compounds at protein binding sites can be expected, depending on the specificity/selectivity of such sites. In addition, competition for reducing equivalents can occur in case of redox proteins.

Following the principles of structure/activity relationships, competitive binding is expected for chromanols and *para*-benzoquinones within each group. Since UCa was not detected so far in mammalian tissues, it would only be of relevance if it was used as a drug. Coexistence of α-Toc and UCe was confirmed in liver tissues (Fig. 2A and B). Within the group of *para*-benzoquinones, α-TQ and UQ coexist in almost all mammalian cell types. In addition, UQ_{10}OH was detected in the liver [35]. Potential binding sites include those of redox enzymes, transporters, metabolic enzymes, and transcription factors.

6.1. Redox enzymes

The substitution pattern of the benzoquinone ring moieties of α-TQ, UQ_{10} and UQ_{10}OH is of major importance for binding to redox enzymes, a fact which has been shown with low molecular weight quinone derivatives and the natural quinone compounds [35,71]. In addition, the first isoprene units of the lipophilic side chain influence the binding to mitochondrial electron transport complexes [72,73]. Our own studies have shown that hydroxylation of the side chain of UQ_{10} (yielding UQ_{10}OH) is sufficient to significantly influence the binding properties of UQ [35]. Since UQ is a cofactor of several mitochondrial respiratory chain complexes, the interference of other bioquinones at these complexes is expected. In addition, extra-mitochondrial electron transfer could be disturbed. UQ has been shown to be involved in several electron transfer chains of mitochondria, the Golgi apparatus, lysosomes and plasma membranes [74].

6.1.1. *Mitochondrial electron transfer*

In the inner mitochondrial membrane α-TQ contributes about 1–5% to the total benzoquinone pool [23]. Although these are not extremely high concentrations, α-TQ was observed to act as weak competitive inhibitor of UQ-mediated electron transfer in submitochondrial particles and at the isolated complex III (ubiquinol:ferricytochrome *c* oxidoreductase, cytochrome bc_1 complex, EC 1.10.2.2, Figs. 2D and 4A). Even the hydroxylation of the side chain in reduced UQ_{10}OH leads to a decreased activity at this complex in comparison with reduced UQ (Fig. 2D). In addition, α-TQ decreased the release of superoxide radicals at the cytochrome bc_1 complex (Fig. 4B). Measuring electron transfer from respiratory complexes in submitochondrial particles to a short-chain α-TQ homologue, we found stronger binding to complex III than to complex I (NADH:ubiquinone oxidoreductase, EC 1.6.5.3) (K_M = 36 and 115 μM, respectively), whereas no reaction with complex II (succinate:ubiquinone oxidoreductase, EC 1.3.5.1) was detectable [23]. This demonstrates that competition of α-TQ with UQ at different UQ-binding sites occurs to a very different extent. Screening α-TQ, α-Toc, and UQ levels in tissues and organelles from animals with different pathophysiological conditions, we observed that the α-TQ concentration was decreased in liver mitochondria from rats subjected to endotoxic shock (Fig. 4C). Endotoxic shock causes the shock syndrome, resulting in multiple organ failure, which often leads to death. The exact mechanism of organ failure is not completely understood. There is a hypothesis that mitochondria are involved in this process [75,76]. Interestingly the decrease of α-TQ was accompanied by a faster mitochondrial state 3 respiration (Fig. 4C) and an increased mitochondrial production of superoxide radicals (Fig. 4D), in line with the oxidative loss of α-Toc and the inhibitory effect of α-TQ mentioned above, while the concentrations of cytochromes, total UQ, and α-Toc remained constant. This correlation suggests some regulatory function of α-TQ in the inner mitochondrial membrane, such as a feedback inhibition of mitochondrial respiration and/or ROS production under conditions of increased overall oxidative stress. In addition, the beneficial effect of α-TQ-H_2 (vide supra) should be considered. We found electron transfer from complex I and complex III to a short-chain α-TQ homologue (v_{max} = 23 and 6 nmol (mg protein min)$^{-1}$, respectively) [23], presumably resulting in the formation of the corresponding TQ-H_2 derivative. α-TQ-H_2 was indeed detected in vivo by several groups [77,78]. Other mitochondrial ubiquinone-binding redox enzymes are electron-transferring flavoprotein (ETF) dehydrogenase (EC 1.5.5.1) and 3-glycerophosphate:ubiquinone oxidoreductase (EC 1.1.99.5). ETF dehydrogenase shuttles electrons from reduced FAD generated by β-oxidation of fatty

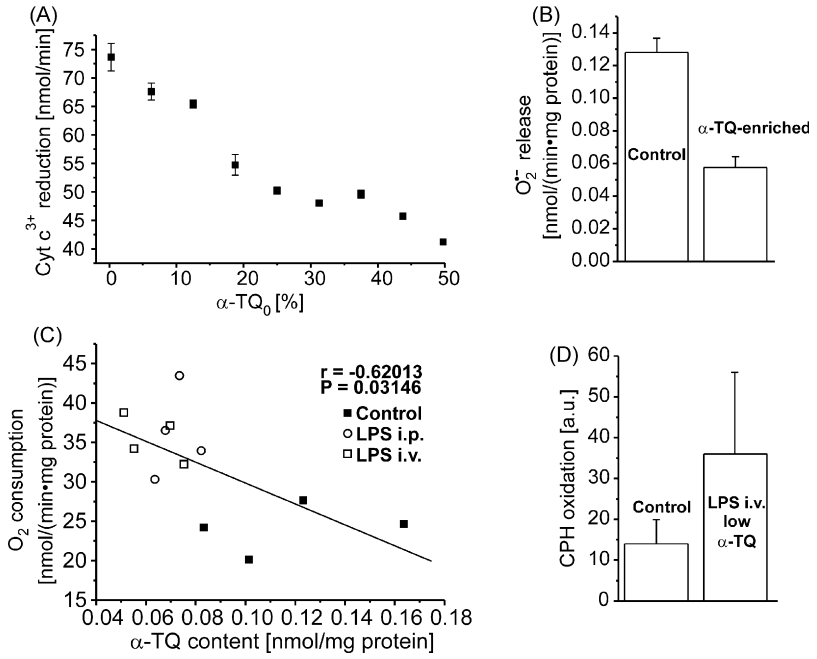

Fig. 4 – Modulation of electron transfer of respiratory enzymes by α-tocopheryl quinone (α-TQ). (A) Decrease of cytochrome c^{3+} reduction by the isolated cyt bc_1 complex in the presence of increasing concentrations of α-TQ_0 (a short-chain homologue of α-TQ; for experimental details see [47], $n = 2$, data are means ± S.E.). (B) Decrease of the release of superoxide radicals by submitochondrial particles (SMP) enriched with α-TQ versus control. Superoxide release rates were measured during succinate respiration using the SOD-sensitive reduction of acetylated cytochrome c^{3+} as detection system (for experimental details see [46], $n = 5$, data are means ± S.D.). (C) Correlation of the liver mitochondrial state 3 respiration rate in the presence of glutamate/malate (Merck, Germany; Sigma, USA) as substrates with the α-TQ content of liver mitochondria from lipopolysaccharide (LPS)-treated (LPS i.v., open squares; LPS i.p., open circles) versus control rats (solid squares) (unpublished, LPS *E. coli* 026:B6, Difco; USA). The LPS treatment of animals and isolation of mitochondria was described in [76]. The detection of α-TQ was carried out as described in [35] using 2 mg protein per sample and UQ_6 (Sigma, Germany) as internal standard. (D) Increased release of superoxide radicals was measured as oxidation of 1-hydroxy-3-carboxy-pyrrolidine (CPH, Alexis, Switzerland) by glutamate/malate respiring rat liver mitochondria from LPS-treated (i.v.) versus control animals ($n = 8$, data are means ± S.D.). For experimental details see [76].

acids in the mitochondrial matrix to the mitochondrial UQ pool. This enzyme, which is localized at the matrix face of the inner mitochondrial membrane, was shown to have slight preference for UQ homologues (UQ_4, $k_{cat} = 35.5\ s^{-1}$) over duroquinone (low molecular weight analogue of α-TQ, $k_{cat} = 18.1\ s^{-1}$) [79]. 3-Glycerophosphate:ubiquinone oxidoreductase, which is thought to deliver electrons from the outside of the inner mitochondrial membrane to UQ, has K_M values of 0.01–0.125 mM for UQ homologues, whereas data for α-TQ or analogues are not available [80].

6.1.2. *Extra-mitochondrial electron transfer*

There are numerous UQ-mediated physiological electron transfer activities outside mitochondria. These include redox-driven proton translocation in non-mitochondrial membranes (Golgi vesicles [81], lysosomes [82]), and trans-membraneous electron transfer (plasma membranes [83]). In addition, there are enzymes, which reduce quinones besides other substrates and are involved in quinone detoxification (vide infra). Beyond this, quinone-mediated superoxide production as signaling process was suggested [69]. However, so far only a part of these activities was assigned to specific characterized enzymes. Microsomal NADPH:cyt P450 reductase (NADPH:hemoprotein reductase, EC 1.6.2.4) and NADH:cyt b_5 reductase (cyt b_5 reductase, EC 1.6.2.2) were found to contribute to the reduction of UQ and α-TQ [84-86]. Several other enzymes, which can reduce UQ at the expense of NADPH, have been described. One of them is a cytosolic NAD(P)H:(quinone acceptor) oxidoreductase (QR1, NQO1, DT-diaphorase, EC 1.6.5.2) [50]. The mechanism and significance of membrane-embedded UQ reduction by a soluble enzyme is not entirely clear. However, this enzyme (NQO1) was shown to reduce α-TQ to α-$TQ-H_2$ more rapidly (about 36–57 faster) than UQ_{10} [87], suggesting a competitive inhibition of UQ reduction under conditions of increased α-TQ formation, such as oxidative stress. This enzyme catalyzes a concerted two-electron transfer, contrary to the mitochondrial redox enzymes, and can generate the antioxidant α-$TQ-H_2$ without intermediate formation of the potentially harmful semiquinone (vide supra).

The homologous enzyme ribosyldihydronicotinamide dehydrogenase (NRH:quinone reductase 2, QR2, NQO2, EC 1.10.99.2) was shown to reduce UQ_0 (a hydrophilic UQ analogue) without preference for lipophilic natural UQ

derivatives [88]. In plasma membranes of hepatocytes and erythrocytes an UQ-dependent NADH:oxygen oxidoreductase activity was described [83]. Upon removal of UQ this activity was impaired, while addition of UQ and α-TQ to UQ-deficient membranes restored this activity to about the same extent [83]. Although UQ is the natural cofactor, also α-TQ can obviously exert this function. From human brain a non-specific NADPH:carbonyl reductase (EC 1.1.1.184) has been isolated, which reduced several endogenous and exogenous quinones. This enzyme reduced UQ_1 at a rate about 10 times higher than that for α-TQ and duroquinone [89]. Whether UQ_{10} is reduced by the NADPH:carbonyl reductase at the same rate as UQ_1 was not assessed in this study. Other flavoenzymes reported to be capable of reducing UQ are thioredoxin reductase (TrxR-1, EC 1.6.4.5, now EC 1.8.1.9), lipoamide dehydrogenase (LipDH, EC 1.8.1.4) and glutathione reductase (GR, EC 1.6.4.2, now EC 1.8.1.7) [90]. However, for these enzymes generally no information about an interaction with α-TQ is available.

6.1.3. *Blood coagulation*

Major key enzymes involved in blood coagulation are the vitamin K (naphthoquinone)-dependent carboxylase, which requires reduced vitamin K as substrate, and the warfarin-sensitive vitamin K epoxide reductase. A third enzymatic activity required for the initial reduction of vitamin K has not been completely elucidated yet [91]. There are some indications that both UQ and α-TQ can interfere with blood coagulation. Takahashi [92] demonstrated that α-Toc and UQ_{10} significantly extended the prothrombine time as marker for blood coagulation. Likewise, for α-TQ an inhibition of blood coagulation was reported [93], while another study claimed that other synthetic byproducts were responsible for this effect [94]. Later it was confirmed that for α-TQ this interaction was based on the inhibition of the vitamin K-dependent γ-glutamyl carboxylation of several blood clotting proteins [95]. The mechanism for both UQ and α-TQ could be similar due to their structural relationship. On the other hand, UQ_{10} supplementation was found to be responsible for the reduced anticoagulant effect of warfarin due to increasing its metabolism [96]. This might explain the paradoxical effect that the potential anticoagulant UQ can have a partially procoagulant effect in drug interactions with more potent anticoagulants, such as warfarin.

6.2. *Metabolic enzymes*

The lipophilic side chains of α-Toc and UQ compounds are obvious targets for the metabolic cytochrome P450 enzymes, in order to convert them to excretable metabolites. For α-Toc and UQ, there is a common metabolic pathway via ω-oxidation and subsequent β-oxidation of the side chain leading to similar carboxylated products.

The metabolism of α-Toc is split into a free radical pathway leading to α-TQ (Fig. 1) and an enzymatic pathway causing side chain oxidation of α-Toc and α-TQ (Fig. 5). The latter reactions are catalyzed in part by the microsomal cyt P450 system and especially isoforms CYP 4F2 [97] and CYP 3A [98] were suggested to be involved. This ends up in the formation of conjugated short-chain derivatives, α-tocopheronic acid derived from α-TQ [99] as well as α-CMBHC [100] and α-CEHC [101] derived from α-Toc (Fig. 5), bearing a carboxy group on the side chain. Whether α-tocopheronic acid can arise under physiological conditions from α-CEHC is still unclear [100], since conjugated chromanols are chemically protected from oxidation.

Metabolic degradation of UQ yields Q-acid I and Q-acid II (Fig. 5), which are excreted in conjugated (glucuronides [102], phosphates [103], sulfates [104]) and non-conjugated forms into the urine [102]. The similarity of these UQ metabolites to α-Toc metabolites suggests involvement of similar cyt P450 isoforms; an assumption which, however, has not yet been verified. In this case, the question arises whether α-Toc and UQ, upon uptake as nutritional supplements are competing for these metabolic enzymes giving rise to pharmacokinetic interactions.

6.3. *Transport and regulatory proteins*

Due to the structural similarity of α-Toc, UCe, and UCa as well as the related *para*-benzoquinones, other possibilities of competition could be the binding to transport proteins and regulatory proteins. A few proteins of this category specific or selective for α-Toc, α-TQ and UQ, have been identified. These include the α-Toc transfer protein (α-TTP) and the tocopherol-associated protein (TAP); the latter turned out to be a transcription factor [105]. In the mammalian liver α-TTP was found to be responsible for the specific retention of the α-congener after uptake of vitamin E members with the diet [106]. Stocker et al. [107] demonstrated that the discrimination between α-Toc and γ-/δ-Toc is mostly based on the interaction of aromatic methyl substituents of the chromanol head with hydrophobic amino acid residues. Therefore, the absence of some interactions in the γ- and δ-congeners, which are characterized by a smaller degree of ring substitution, explains their weaker binding to α-TTP. In addition, the interaction of the α-TTP with the methyl groups of the phytyl side chain is the prerequisite for the preference of the natural *RRR*-α-Toc over other stereoisomers. In contrast, the oxidation product α-TQ has only 2% of the binding affinity of α-Toc to α-TTP [106]. Due to this highly specific binding of α-Toc, an interaction of α-TTP with UQ seems unlikely (methoxy instead of methyl groups and a longer unsaturated side chain). This is also partially valid for UCa and UCe, although their head groups are more similar to α-Toc than to UQ. Another vitamin E binding glycoprotein (afamin) in human plasma, extravascular and cerebrospinal fluid has recently been described [108]. The binding of α-Toc to and the inhibition of phospholipase A2 [109] provide an important rationale for the link between antioxidative defense and directly anti-inflammatory mechanisms via prevention of eicosanoid production. Although binding of α-Toc was demonstrated for these proteins, no data on the selectivity for the α-congener over the other congeners and other α-Toc-/UQ-related compounds are available.

α-TQ was found to specifically bind to a supernatant protein factor (SPF, a SEC-14 family member of secretory proteins), which is also involved in the cholesterol synthesis pathway [110]. SPF shows a clear preference for α-TQ over α-Toc; information about UQ binding is not available so far.

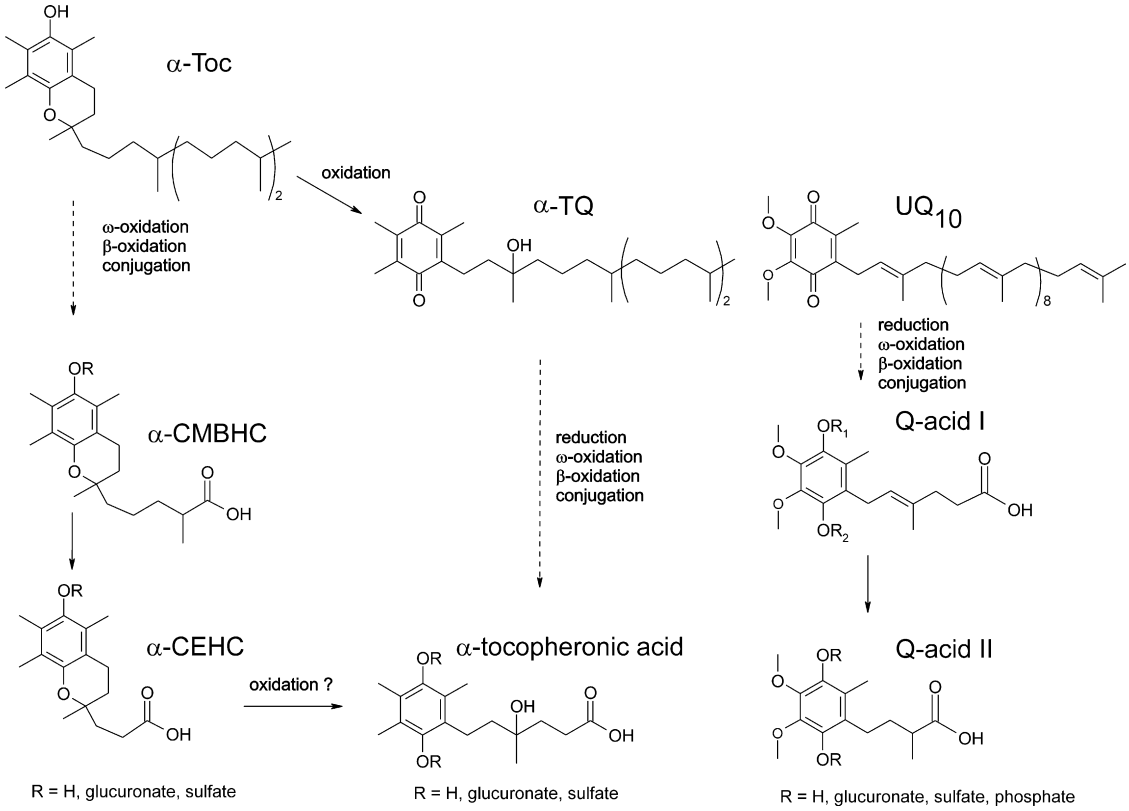

Fig. 5 – Metabolism of α-Toc and UQ-related compounds via subsequent ω- and β-oxidation. Typical end products of α-Toc metabolism, which are excreted in the urine, are conjugates of carboxymethylbutyl hydroxychromane (α-CMBHC) [115] and α-carboxyethyl hydroxychromane (α-CEHC) [101]. Conjugates of tocopheronic acid found in the urine are derived from α-TQ side chain oxidation [99]. In analogy to α-Toc, UQ is metabolized to 2,3-dimethoxy-5-methyl-6-(5′-carboxy-3′-methylpent-3′-enyl)-1,4-benzohydroquinone (Q-acid I) and 2,3-dimethoxy-5-methyl-6-(3′-carboxy-3′-methylpropyl)-1,4-benzohydroquinone (Q-acid II) [102].

Further studies have shown that SPF is identical to tocopherol-associated protein (TAP). Surprisingly, the majority of the literature focuses on α-Toc actions mediated via SPF/TAP, although α-TQ binds much more strongly according to Stocker [110]. SPF/TAP, besides stimulating cholesterol synthesis, was shown to be translocated into the nucleus in a α-Toc dependent fashion, resulting in the regulation of several genes [105]. Unfortunately, the activity of α-TQ and other compounds in this respect were not addressed so far. Furthermore, α-TQ binds to glutathione-S-transferase (GST) in liver cytosol, thereby inhibiting its function [111]. However, the major purpose of this binding seems to be the transport of α-TQ to other catabolic enzymes.

Several UQ-binding proteins (QBP) in rat liver cytosol have been described in the past [112], which however did not only bind UQ_{10} but also α-Toc and other lipophilic compounds. Obviously, these proteins are partially related to less specific lipid transporters and cytosolic quinone reductases (vide supra). Some effects of α-Toc on gene regulation of metabolic enzymes (especially CYP3A) are obviously mediated by binding to the nuclear pregnane X receptor (PXR) [113]. UQ was found to activate the expression of these enzymes to a lesser extent via PXR [114], supporting the idea that α-Toc-related and UQ-related compounds share a common metabolic route and possibly also common regulatory pathways. Whether UCa and UCe influence this pathway is not clear yet.

7. Conclusions

The study of structure/activity relationships is a domain of the development of new synthetic drugs. However, since there are naturally occurring or synthetic antioxidants with structural relationships, such as α-Toc- and UQ-related compounds (α-Toc, α-TQ, UCa, UCe, UQ, and UQOH), the studies in this field could profit from a systematic consideration of their properties. This overview shows that in spite of research for more than half a century there are still considerable gaps in the understanding of direct interactions of these compounds with physiological partners and in some cases also in the field of their mutual interaction. While the separate activities of α-Toc and its sibling antioxidant UQ in scavenging lipid radicals is well understood, their interaction in certain environments, such as low-density lipoprotein particles, is more difficult to interpret. Even more complex appears the interaction of α-Toc- and UQ-related compounds with redox enzymes and other protein binding sites. Although α-Toc- and UQ-related compounds share certain pathways, suggesting

also interference at other sites, many studies focused on a few or even a single compound of this group. Nevertheless, there are many indications that effects of these compound groups in the cell beyond their antioxidant activity are not independent. The data presented here show that cellular effects of α-Toc and UQ are interactive and sometimes synergistic. The latter is based not only on properties of α-Toc and UQ as antioxidants, but also on their interaction with proteins. The examples discussed above demonstrate that there are several pathways modulated by α-Toc and UQ, which would deserve a comprehensive consideration for all the chromanols and *para*-benzoquinones of the α-Toc/UQ families.

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