Bioorganic & Medicinal Chemistry Letters 21 (2011) 3693–3698

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

α-Tocotrienol quinone modulates oxidative stress response and the biochemistry of aging

William D. Shrader [a,*], Akiko Amagata [a], Adam Barnes [a], Gregory M. Enns [c], Andrew Hinman [a], Orion Jankowski [a], Viktoria Kheifets [a], Ryo Komatsuzaki [a], Edgar Lee [a], Paul Mollard [a], Katsuyuki Murase [a], Alfredo A. Sadun [d], Martin Thoolen [a], Kieron Wesson [a], Guy Miller [a,b]

[a] Edison Pharmaceuticals, Inc., 350 North Bernardo Avenue, Mountain View, CA 94043, USA
[b] Adjunct Clinical Instructor, Department of Anesthesiology, Critical Care Medicine, Stanford University, Stanford, CA 94305, USA
[c] Department of Pediatrics, Division of Medical Genetics, Lucile Packard Children's Hospital, Stanford, CA 94305-5208, USA
[d] Doheny Eye Institute, USC-Keck School of Medicine, 1450 San Pablo St., Los Angeles, CA 90089, USA

ARTICLE INFO

Article history:
Received 14 March 2011
Accepted 19 April 2011
Available online 24 April 2011

Keywords:
Redox
Antioxidants
Quinone
Aging
Digital Biology

ABSTRACT

We report that α-tocotrienol quinone (ATQ3) is a metabolite of α-tocotrienol, and that ATQ3 is a potent cellular protectant against oxidative stress and aging. ATQ3 is orally bioavailable, crosses the blood–brain barrier, and has demonstrated clinical response in inherited mitochondrial disease in open label studies. ATQ3 activity is dependent upon reversible 2e-redox-cycling. ATQ3 may represent a broader class of unappreciated dietary-derived phytomolecular redox motifs that digitally encode biochemical data using redox state as a means to sense and transfer information essential for cellular function.

The precise relationship between antioxidants, aging, and metabolic control remains a puzzling biological problem.[1–3] Many theories have been put forth to explain how antioxidants may be responsible for modulating the aging process.[4,5] Initial theories suggested antioxidants blocked free radicals, preventing oxidative injury, and preserving cellular structure and function.[6–8] While there have been numerous cellular and animal studies that have validated this concept,[9,10] few have been recapitulated in human studies of health and disease.[11]

Recent theories have attempted to link antioxidants contained in certain food sources with the genetic control of caloric expenditure and aging,[12–14] but initial promising animal studies[15–17] have not been reproduced in controlled clinical trials.[18] While a clear explanation of how antioxidants may work remains elusive, it is clear that individuals who eat diets high in naturally occurring antioxidants generally enjoy an improved quality of life with a lower incidence of diseases of aging: diabetes, obesity, and cardiovascular disease.[19–21]

To study how antioxidants may be beneficial, we have investigated diseases where both the mitochondria and oxidative stress have been implicated. While mitochondria have been implicated in a broad range of neurodegenerative and metabolic diseases we have specifically focused on a subset of unambiguous inherited mitochondrial diseases as models of oxidative stress and human aging. In these diseases quinone based antioxidants have registered positive preclinical and clinical responses.[22–29]

The most widely studied quinone in this class is Coenzyme Q_{10} (CoQ_{10}).[30–32] In contrast to single-electron donor antioxidants, CoQ_{10} undergoes reversible two-electron cycling.[33] There are numerous published and ongoing clinical trials demonstrating the clinical relevance of CoQ_{10},[34] but no systematic attempt has been made to identify why CoQ_{10} is active nor whether its marginal but clinically measurable activity can be improved upon.

CoQ_{10}'s mechanism of action is typically explained by an enhancement in mitochondrial electron transport function, neutralization of free radicals, or both.[35,36] Not generally considered is whether CoQ_{10}'s activity may be ascribed to other mechanisms or targets,[37,38] especially when administered at supra-physiologic doses where off-target effects are common.

Given the known role of quinones as biochemical redox signaling agents,[39] it may be that CoQ_{10} is a weak version of a more potent and unidentified quinone agonist. To test this hypothesis, we set out to identify and characterize dietary-derived quinones in search of potent quinone regulators of metabolic control and oxidative stress.

Herein we report that quinones derived from the hydrolysis of vitamin E—called vitamin E quinones[40]—are 1000–10,000 times

* Corresponding author.
E-mail address: bshrader@edisonpharma.com (W.D. Shrader).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.04.085

more potent than CoQ_{10} in a battery of human cell assays of oxidative stress. We explore the hypothesis that vitamin E quinones may be members of a new class of native and essential quinones critical to the control of metabolism and the aging process through redox signaling and information transfer and sensing mechanisms (Fig. 1).

To investigate α-tocotrienol quinone in detail, an efficient three-step synthesis was developed starting from an *Elaeis guineensis* extract enriched in γ- and δ-tocotrienol vitamin E isoforms. This is the first synthesis and characterization reported for the natural product, α-tocotrienol quinone (Scheme 1). Step one was an exhaustive aminomethylation of the γ- and δ-tocotrienol isoforms (**1**, **2**) contained within the *E. guineensis* extract.[41] This was accomplished by treating the crude *E. guineensis* extract with paraformaldehyde and 1-methylpiperazine to yield a mixture of mono- and diaminomethylated tocotrienol derivatives (**3**, **4**). During work-up, the aminomethylated derivatives were separated from the crude reaction mixture by protonation with formic acid, and extraction of the resulting ammonium formate salt into the acetonitrile layer of an organic/organic biphasic system with heptanes. Step two converted the crude aminomethyl species (**3** and **4**) to a single compound α-tocotrienol (**5**) by a reductive deamination with sodium cyanoborohydride in amyl alcohol at elevated temperatures.[42] Silica gel chromatography yielded high purity α-tocotrienol as a white waxy solid.[43]

The α-tocotrienol chroman (**5**) was converted to the α-tocotrienol quinone (ATQ3) by an oxidation with ceric (IV) ammonium nitrate in isopropyl acetate and water.[45] Analytically pure (*R*)-α-tocotrienol quinone was obtained as a yellow oil after silica gel chromatography, in an overall yield of 45% based on the starting γ- and δ-tocotrienol w/w content in the *E. guineensis* extract. To confirm the structure of (*R*)-α-tocotrienol quinone, a series of detailed ^{1}H, ^{13}C, HSQC, and HMBC NMR experiments were performed

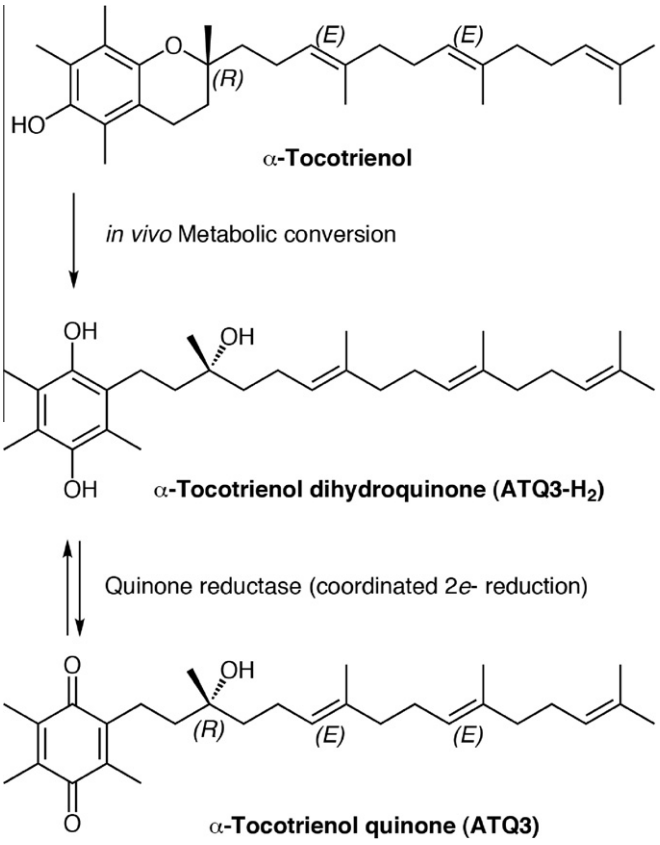


Figure 1. The conversion of α-tocotrienol to α-tocotrienol quinone.

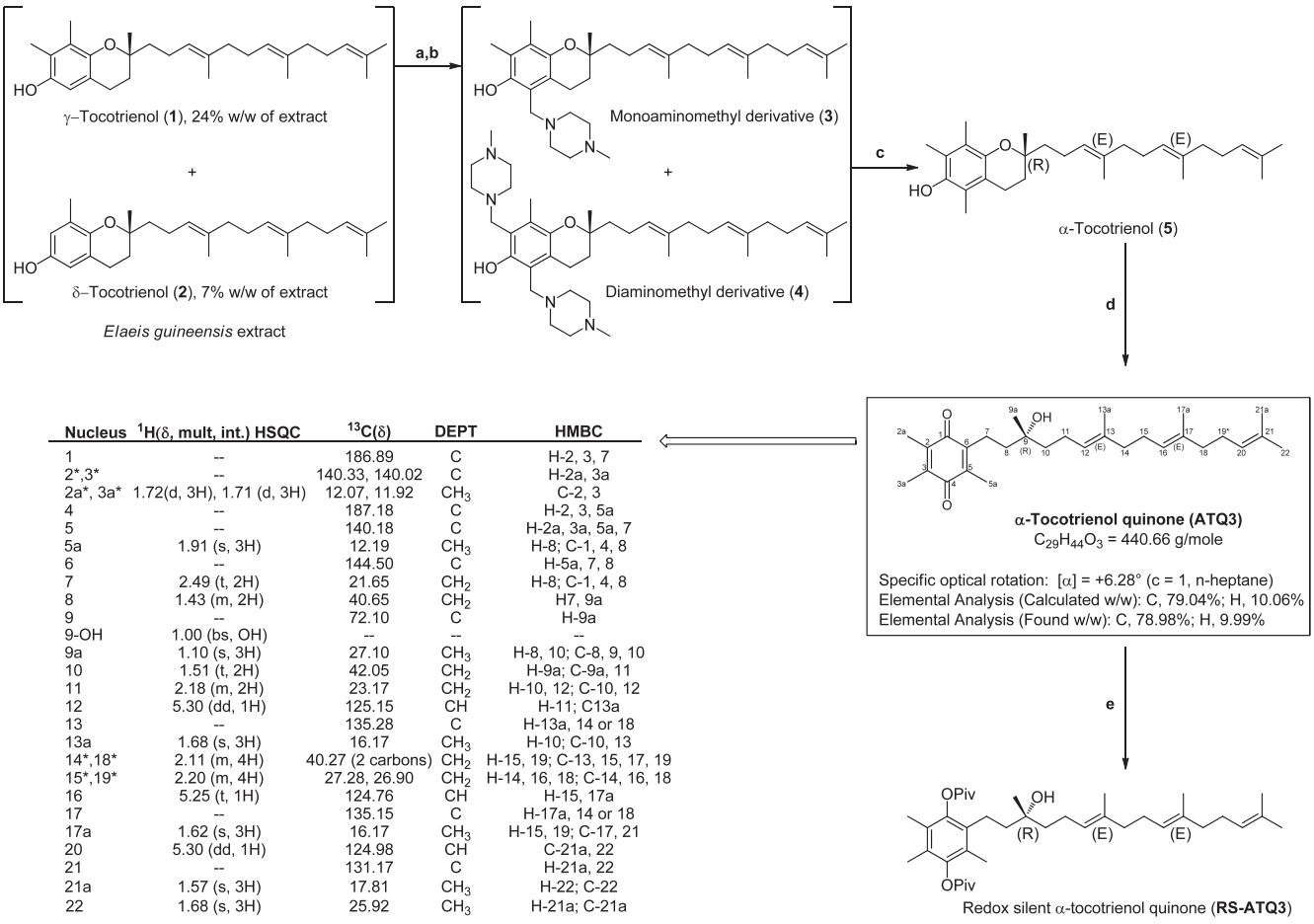
(Scheme 1 insert). To investigate the relationship of biological action with redox dependence, a redox silenced version, chemically locked in a single state was prepared by the hydride reduction of (*R*)-α-tocotrienol quinone to the dihydroquinone, with subsequent acylation and trapping as its bis-pivaloyl ester (RS-ATQ3).

The pharmacokinetic parameters, oral bioavailability, and tissue distribution of ATQ3 were obtained in multiple species (Table 1). From these studies, ATQ3 was found to have good oral bioavailability. To support these studies, a LC/MS/MS bioanalytical method was developed in mice, rats, dogs and humans to detect ATQ3 in its oxidized state. The reduced dihydroquinone of ATQ3 present in fresh plasma or tissue samples was allowed to fully oxidize ex vivo in air to the quinone before quantification. The pharmacokinetic studies revealed that the presence of food increased the oral absorption and absolute bioavailability of ATQ3 in the rat from <5% to 89%. In the dog, the absolute bioavailability was 17% under fasting conditions and the C_{max} and AUC_{∞} increased by 16% and 28%, respectively, when administered with food. The elimination of ATQ3 in both rat and dog, demonstrated a similar biphasic decline in plasma levels with a terminal half-life of approximately 3.5 h (po) to 9.6 h (iv) for rat and approximately 7.5 h for both routes of administration for dog. The clearance was moderate in the rat (716–2086 ml/h/kg) and low in the dog (574 ml/h/kg). In human adults, a single po dose of 200 mg yielded peak plasma concentrations at 2 h with a C_{max} average of 167 ng/ml.

The high volume of distribution (V_{dss}) observed in the initial pharmacokinetic studies prompted further experiments to determine the tissue distribution of this particular metabolite of vitamin E (ATQ3). A series of distribution studies revealed that ATQ3 readily distributed into the brain, retina and heart. In mice, ATQ3 was dosed IP at 25 mg/kg and monitored at each time point ($n = 3$ per time point, $t = 0.5$, 4, 12 and 24 h) for ATQ3 levels in brain, heart, and plasma. Peak brain concentrations were observed at $t = 12$ h, with a brain-serum ratio of 1.83 (539 ng/g: 300 ng/ml). The heart tissue–serum ratio was 0.71 at $t = 0.5$ h, with a heart concentration of 848 ng/g and a plasma level of 1188 ng/ml. Distribution of ATQ3 into ocular tissues was measured at the conclusion of a 7-day dog po repeat dose study (po, QD, 300 mg/kg/day). Levels of ATQ3 in the retina were 11,200 and 755 ng/g in the cornea, with the associated peak plasma concentrations of approximately 10,000 ng/ml. ATQ3 concentrations were variable in vitreous humor ranging from below quantifiable levels to 682 ng/mL with no ATQ3 detected in the aqueous humor. This is in contrast to what has been reported for vitamin E members with the phytyl tail, such as α-tocopherol, which do not enter the eye or cross the blood–brain barrier.[46–49]

The conversion of the parent α-tocotrienol to ATQ3 metabolite was monitored in vivo by the simultaneous detection of both the parent chroman and the quinone metabolite using a modified LC/MS/MS method. In this study, α-tocotrienol was dosed either iv (5 mg/kg) or po (25 mg/kg) in rat with parent chroman and metabolite (ATQ3) monitored at each time point ($t = 0.1$–24 h). In both the iv and po study arms, the ATQ3 quinone metabolite concentration followed the parent α-tocotrienol chroman concentration at a level approximately 10-fold lower: 7–11% of parent concentration for iv, and 5–9% for po. This study demonstrated that the level of the ATQ3 metabolite was directly correlated to the level of its parent chroman—α-tocotrienol. The conversion of the parent α-tocotrienol to α-tocotrienol quinone could also be achieved in vitro by incubation of α-tocotrienol (20 μM) with human and mouse liver microsomes (25 μg). These conditions yielded a 15% overall conversion to ATQ3 relative to the initial levels of α-tocotrienol, and were independent of the presence or absence of NAD(P)H.

ATQ3 was not mutagenic when evaluated in the in vitro bacterial reverse mutation (Ames) assay and was negative in the CHO micronucleus test. A toxicological evaluation of ATQ3



Nucleus	^{1}H(δ, mult, int.) HSQC	^{13}C(δ)	DEPT	HMBC
1	--	186.89	C	H-2, 3, 7
2*,3*	--	140.33, 140.02	C	H-2a, 3a
2a*, 3a*	1.72(d, 3H), 1.71 (d, 3H)	12.07, 11.92	CH_3	C-2, 3
4	--	187.18	C	H-2, 3, 5a
5	--	140.18	C	H-2a, 3a, 5a, 7
5a	1.91 (s, 3H)	12.19	CH_3	H-8; C-1, 4, 8
6	--	144.50	C	H-5a, 7, 8
7	2.49 (t, 2H)	21.65	CH_2	H-8; C-1, 4, 8
8	1.43 (m, 2H)	40.65	CH_2	H7, 9a
9	--	72.10	C	H-9a
9-OH	1.00 (bs, OH)	--	--	--
9a	1.10 (s, 3H)	27.10	CH_3	H-8, 10; C-8, 9, 10
10	1.51 (t, 2H)	42.05	CH_2	H-9a; C-9a, 11
11	2.18 (m, 2H)	23.17	CH_2	H-10, 12; C-10, 12
12	5.30 (dd, 1H)	125.15	CH	H-11; C13a
13	--	135.28	C	H-13a, 14 or 18
13a	1.68 (s, 3H)	16.17	CH_3	H-10; C-10, 13
14*,18*	2.11 (m, 4H)	40.27 (2 carbons)	CH_2	H-15, 19; C-13, 15, 17, 19
15*,19*	2.20 (m, 4H)	27.28, 26.90	CH_2	H-14, 16, 18; C-14, 16, 18
16	5.25 (t, 1H)	124.76	CH	H-15, 17a
17	--	135.15	C	H-17a, 14 or 18
17a	1.62 (s, 3H)	16.17	CH_3	H-15, 19; C-17, 21
20	5.30 (dd, 1H)	124.98	CH	C-21a, 22
21	--	131.17	C	H-21a, 22
21a	1.57 (s, 3H)	17.81	CH_3	H-22; C-22
22	1.68 (s, 3H)	25.92	CH_3	H-21a; C-21a

Scheme 1. Synthesis of α-tocotrienol quinone. Reagents and conditions: (a) 1-methylpiperazine, paraformaldehyde, 120 °C, 18 h; (b) formic acid, liquid–liquid partition with acetonitrile and heptanes, aqueous base wash; (c) $NaBH_3CN$, amyl alcohol, 120 °C, 6 h, silica chromatography; (d) Ceric (IV) ammonium nitrate, isopropyl acetate, 0 °C, 0.5 h, silica chromatography; (e) $NaBH_4$, pivaloyl chloride, THF, silica chromatography. Proton and carbon NMR assignments of human metabolite, ATQ3 (all shift values in ppm, C_6D_6). *Assignment inconclusive.

Table 1
A summary of the in vivo plasma pharmacokinetics of ATQ3 in rat, dog, and mouse[44]

Parameter	Species								
Species	Rat	Rat	Rat	Rat	Dog	Dog	Mouse	Mouse	Human
Dose route	iv	po	iv	po	iv	po	ip	ip	po
Dose level (mg/kg)	2	25	2	10	2	10	25	25	2[b]
Compartment	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Brain	Plasma
C_{max} (ng/ml)	–	248	–	931 ± 83	–	442 ± 294	1188 ± 601	539 ± 232[a]	167 ± 110
t_{max} (h)	–	4.0	–	3.5 ± 0.2	–	2.8 ± 1.0	0.5	12	2
AUC_t (h ng/ml)	5882 ± 431	961	984 ± 117	4728 ± 349	3408 ± 589	2693 ± 1656	4835	7738[a]	–
AUC_∞ (h ng/ml)	5954 ± 411	–	1069 ± 134	4781 ± 351	3572 ± 587	2817 ± 1704	5862	10724[a]	–
$t_{1/2}$ (h)	3.5 ± 1.0	–	9.6 ± 0.7	3.5 ± 0.1	7.5 ± 0.7	7.6 ± 5.1	7.6	12.4	–
F (%)	–	4.1(fasted)	–	89(fed)	–	17 ± 6	–	–	–
Cl (ml/h/kg)	716 ± 50	–	2086 ± 359	–	574 ± 112	–	–	–	–
V_i (ml/kg)	117 ± 14	–	567 ± 299	–	95 ± 14	–	–	–	–
V_{ss} (ml/kg)	222 ± 81	–	11497 ± 1881	–	2535 ± 893	–	–	–	–
V_z (ml/kg)	3726 ± 1137	–	27392 ± 3014	–	6249 ± 829	–	–	–	–

[a] Tissue samples, units are ng/g and h ng/g.
[b] Average mg/kg dose for a 50 kg adult.

demonstrated a high therapeutic index between no-observable-adverse-effect level (NOAEL) and the levels required for pharmacologic activity. A 28-day repeat-dose toxicity study in both dogs and rats after oral (gavage) administration showed that the NOAEL of ATQ3 was 100 mg/kg. In both species and sexes at ⩾100 mg/kg/day there were dose-dependent prolongations of prothrombin time (PT) and activated partial thromboplastin time (APTT) relative to controls. The dose-limiting effect is an increase in coagulation times explained by weak vitamin K antagonism previously ascribed to this structural class of compounds.[50,51] All alterations in coagulation parameters had resolved by the end of the 4 week recovery period in both species and sexes.

To determine if ATQ3 had similar cellular pharmacology to other antioxidant quinones, CoQ_{10} and idebenone, we established

an assay using stressed primary human fibroblasts. Utilizing the inherent oxidative stress of fibroblasts from patients with mitochondrial disease, we further increased their oxidative burden by depleting cellular glutathione with an inhibitor of glutathione synthesis, L-buthionine-(*S,R*)-sulfoximine (BSO). As a result, while fibroblasts from healthy individuals retained full viability, patient fibroblast exhibited complete cell death within 48 hr of the BSO insult (~100 μM). As an example, we show the results obtained with primary fibroblasts of a Friedreich ataxia (FRDA) patient, a neurodegenerative disease resulting from a triplet expansion in the nuclear *frataxin* gene (Fig. 2a), as well fibroblasts from a patient with Leigh syndrome caused by a compound heterozygous mutation *SURF1* mutation (Fig. 2b). Cellular viability was measured with a redox independent dye Calcein-AM. The data presented herein shows that ATQ3 is a potent cellular oxidative-stress protectant with in vitro potency and efficacy of 10^2–10^4 times higher than either structurally-related quinones, such as CoQ_{10} and idebenone, or structurally dissimilar antioxidants such as polyphenolic stilbenes, represented by resveratrol. The importance of ATQ3 redox-cycling for compound efficacy was tested via a redox silent bis-pivoyl adduct of ATQ3, which showed no activity in any cell line tested.

In two ongoing FDA-approved, open-label human studies[52,53] in children and adults with inherited mitochondrial disease at doses between 100 and 400 mg three times daily, no significant elevation of clotting times or significant drug-related adverse events have been reported in approximately 6250 patient dosing days. Initial clinical data in subjects with inherited respiratory chain diseases of the mitochondria suggest ATQ3 has an acceptable therapeutic index and alters the natural course of disease. ATQ3 is being advanced into controlled clinical trials to verify these initial positive open-label clinical findings.

Because manifestations of inadequate levels of ATQ3 may be both subclinical and pernicious, ATQ3 deficiency syndromes may be hard to detect in otherwise healthy subjects. As children with inherited mitochondrial disease present with clinical characteristics similar to adult diseases of aging they may magnify the importance of ATQ3's role in metabolic function. Thus inherited mitochondrial disease may represent time-compressed 'models' of aging.

The observed biological activity in both laboratory and clinical settings raises the question of how to reconcile the relationship of ATQ3 with its purported precursor, α-tocotrienol, particularly in the broader context of redox signaling, metabolic control, and aging.

Preliminary studies suggest that ATQ3 may be derived from dietary sources of α-tocotrienol. Incubation of α-tocotrienol with either human or mouse microsomes resulted in substantial conversion of α-tocotrienol to ATQ3. While the precise mechanism of this transformation is under investigation, the generalized possibility that a vitamin E vitamer could be a 'pro-drug' (pro-vitamin) raises new questions about the biochemistry of vitamin E deficiency. We are actively investigating whether a vitamin E deficiency syndrome in otherwise healthy subjects might be explained in part by a vitamin E quinone deficiency and not a vitamin E (chroman) deficiency. Furthermore, various diseases of aging may be examples of the subclinical presentations of a vitamin E quinone deficiency syndrome.

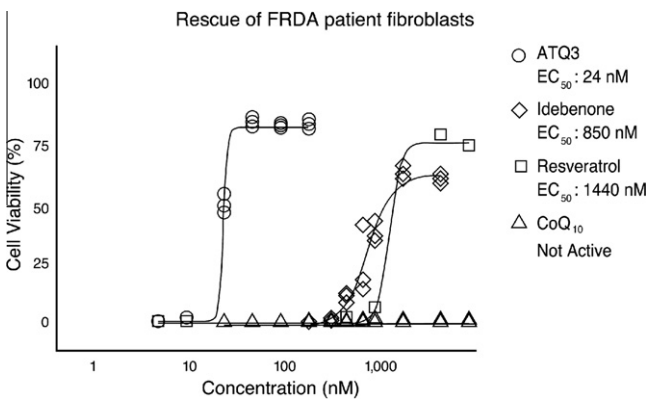


Figure 2a. Dose–response of ATQ3 versus other antioxidants in Friedreich ataxia (FRDA) primary human fibroblasts.

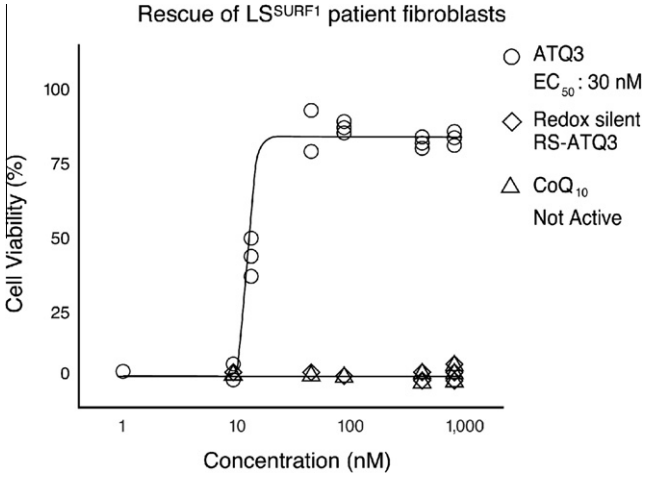


Figure 2b. Dose–response and redox dependence of ATQ3 for cellular activity in Leigh syndrome (*SURF1* mutation) primary human fibroblasts.

There is ample precedent for pro-vitamin biochemistry in, for example, vitamins A and D. But the fact that an active form of vitamin E may result from the conversion of a chroman to a quinone that is critical for metabolic control and aging has not been previously described.

The lack of cellular efficacy with the covalent modification of ATQ3 to its redox-silenced, bis-pivoyl adduct suggests that redox-cycling is critical to the mode of action of ATQ3. It may be that this structural modification also precludes target binding, though other covalent modifications have resulted in similar loss of activity, suggesting that the redox-cycling is the key factor. Additional experiments are underway to construct a periodic table of antioxidants correlating electronic (redox) properties with biological activity.

The observation that the biological activity of ATQ3 may have an obligate dependence on its redox properties may be reconciled through its involvement as a redox-signaling agent. Enzymes such as NAD(P)H:Quinone oxidoreductase 1 (NQO1, DT-diaphorase) that bind quinones have long been thought to detoxify such substrates.[54,55] But recent data now suggests these enzymes may be regulated by quinones. Further studies now also link oxidoreductases with oxidative stress response, cell death, and aging.[56–61] Compounds of the structural class of ATQ3 are also known NQO1 modulators.[62–64] We believe ATQ3 may exert its beneficial effects on metabolic control and aging through action at oxidoreductases—thus establishing the missing link between antioxidants and aging.

Lastly, we note that physical chemistry and material science investigators have exploited the binary nature of quinone redox states to synthesize solid-phase quinone computing chips to encode digital data.[65–69] We propose that dietary-derived quinones such as ATQ3 may represent nature's version of digital biology,[70–72] thereby explaining how select antioxidants may regulate metabolism and aging. We term compounds with these select

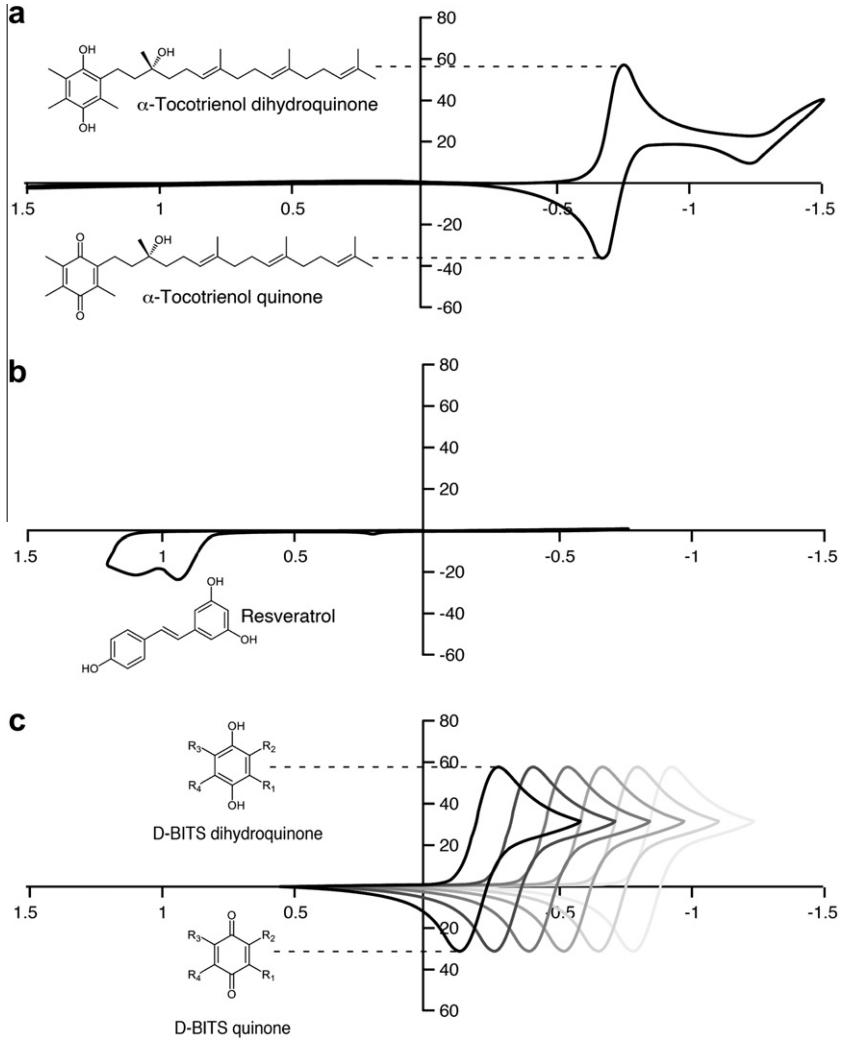


Figure 3. Cyclic voltammograms of ATQ3 (Fig. 3a), resveratrol (Fig. 3b), and generalized D-BITS (Fig. 3c). Cyclic voltammograms were obtained for ATQ3 and resveratrol using a BASi C3 electrochemical analyzer under standard reported conditions. Cyclic voltammograms were obtained using 2 mM analyte under the following conditions: anhyd acetonitrile with 0.1 M $(CH_3CH_2CH_2CH_2)_4N^+(BF_4)^-$ as the supporting electrolyte; working electrode Pt; reference electrode Ag/AgCl; sweep rate 100 mV/s. ATQ3 exhibits reversible oxidation reduction properties with a reduction potential of −650 mV, as evidenced by an equivalent current flow in oxidation and reduction phases (Fig. 3a). Resveratrol exhibits primarily irreversible oxidation with a single oxidation potential at 1050 mV (Fig. 3b). The generalized D-BITS case is presented that illustrates how nature could deploy structural changes in ring substitution patterns (here shown as a quinone) to alter redox properties and thereby discretely encrypt biological data (Fig. 3c).

functional properties D-BITS—Digital Biochemical Information Transfer and Sensing compounds.

Acknowledgments

We would like to thank Professor Sidney Hecht, Dr. Pat Confalone and Dr. Robert Ings, respectively, for their helpful discussions on redox biochemistry, synthetic methods and the pharmacokinetic data and analysis presented herein.

References and notes

1. Sohal, R. S.; Weindruch, R. *Science* **1996**, *273*, 59.
2. Finkel, T.; Holbrook, N. J. *Nature* **2000**, *408*, 239.
3. Garber, K. *Nat. Biotechnol.* **2008**, *26*, 371.
4. Beckman, K. B.; Ames, B. N. *Physiol. Rev.* **1998**, *78*, 547.
5. Sinclair, D. A. *Mech. Ageing Dev.* **2005**, *126*, 987.
6. Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 7915.
7. Cross, C. E.; Halliwell, B.; Borish, E. T.; Pryor, W. A.; Ames, B. N.; Saul, R. L.; McCord, J. M.; Harman, D. *Ann. Intern. Med.* **1987**, *107*, 526.
8. Shigenaga, M. K.; Hagen, T. M.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10771.
9. Behl, C.; Davis, J.; Cole, G. M.; Schubert, D. *Biochem. Biophys. Res. Commun.* **1992**, *186*, 944.
10. Forrest, V. J.; Kang, Y. H.; McClain, D. E.; Robinson, D. H.; Ramakrishnan, N. *Free Radical Biol. Med.* **1994**, *16*, 675.
11. Yusuf, S.; Dagenais, G.; Pogue, J.; Bosch, J.; Sleight, P. *N. Eng. J. Med.* **2000**, *342*, 154.
12. Lagouge, M.; Argmann, C.; Gerhart-Hines, Z.; Meziane, H.; Lerin, C.; Daussin, F.; Messadeq, N.; Milne, J.; Lambert, P.; Elliott, P.; Geny, B.; Laakso, M.; Puigserver, P.; Auwerx, J. *Cell* **2006**, *127*, 1109.
13. Heilbronn, L. K.; de Jonge, L.; Frisard, M. I.; DeLany, J. P.; Larson-Meyer, D. E.; Rood, J.; Nguyen, T.; Martin, C. K.; Volaufova, J.; Most, M. M.; Greenway, F. L.; Smith, S. R.; Deutsch, W. A.; Williamson, D. A.; Ravussin, E. *JAMA J. Am. Med. Assoc.* **2006**, *295*, 1539.
14. Criqui, M. H.; Ringel, B. L. *Lancet* **1994**, *344*, 1719.
15. Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P. J.; Poosala, S.; Becker, K. G.; Boss, O.; Gwinn, D.; Wang, M. Y.; Ramaswamy, S.; Fishbein, K. W.; Spencer, R. G.; Lakatta, E. G.; Le Couteur, D.; Shaw, R. J.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. *Nature* **2006**, *444*, 337.
16. Baur, J. A.; Sinclair, D. A. *Nat. Rev. Drug Discovery* **2006**, *5*, 493.
17. Shindler, K. S.; Ventura, E.; Dutt, M.; Elliott, P.; Fitzgerald, D. C.; Rostami, A. *J. Neuroophthalmol.* **2010**, *30*, 328.
18. Wade, N. In *The New York Times*: New York Edition; January 11, 2011.

19. Anlasik, T.; Sies, H.; Griffiths, H. R.; Mecocci, P.; Stahl, W.; Polidori, M. C. *Br. J. Nutr.* **2005**, *94*, 639.
20. Trichopoulou, A.; Costacou, T.; Bamia, C.; Trichopoulos, D. *N. Eng. J. Med.* **2003**, *348*, 2599.
21. Mecocci, P.; Polidori, M. C.; Troiano, L.; Cherubini, A.; Cecchetti, R.; Pini, G.; Straatman, M.; Monti, D.; Stahl, W.; Sies, H.; Franceschi, C.; Senin, U. *Free Radical Biol. Med.* **2000**, *28*, 1243.
22. Rustin, P.; von Kleist-Retzow, J. C.; Chantrel-Groussard, K.; Sidi, D.; Munnich, A.; Rotig, A. *Lancet* **1999**, *354*, 477.
23. Ihara, Y.; Namba, R.; Kuroda, S.; Sato, T.; Shirabe, T. *J. Neurol. Sci.* **1989**, *90*, 263.
24. Saretzki, G.; Murphy, M. P.; von Zglinicki, T. *Aging Cell* **2003**, *2*, 141.
25. Rotig, A.; Appelkvist, E. L.; Geromel, V.; Chretien, D.; Kadhom, N.; Edery, P.; Lebideau, M.; Dallner, G.; Munnich, A.; Ernster, L.; Rustin, P. *Lancet* **2000**, *356*, 391.
26. Jonassen, T.; Larsen, P. L.; Clarke, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 421.
27. Chowanadisai, W.; Bauerly, K. A.; Tchaparian, E.; Wong, A.; Cortopassi, G. A.; Rucker, R. B. *J. Biol. Chem.* **2010**, *285*, 142.
28. Rucker, R.; Chowanadisai, W.; Nakano, M. *Altern. Med. Rev*. **2009**, *14*, 268.
29. Tchaparian, E.; Marshal, L.; Cutler, G.; Bauerly, K.; Chowanadisai, W.; Satre, M.; Harris, C.; Rucker, R. B. *Biochem. J.* **2010**, *429*, 515.
30. Shults, C. W.; Oakes, D.; Kieburtz, K.; Beal, M. F.; Haas, R.; Plumb, S.; Juncos, B. L.; Nutt, J.; Shoulson, I.; Carter, J.; Kompoliti, K.; Perlmutter, J. S.; Reich, S.; Stern, M.; Watts, R. L.; Kurlan, R.; Molho, E.; Harrison, M.; Lew, M. *Arch. Neurol.* **2002**, *59*, 1541.
31. Koroshetz, W. J.; Jenkins, B. G.; Rosen, B. R.; Beal, M. F. *Ann. Neurol.* **1997**, *41*, 160.
32. Matthews, R. T.; Yang, L. C.; Browne, S.; Baik, M.; Beal, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 8892.
33. Lagendijk, J.; Ubbink, J. B.; Vermaak, J. H. *J. Lipid Res.* **1996**, *37*, 67.
34. There are >50 Coenzyme Q clinical trials on register at the FDA—listed on www.clinicaltrials.gov.
35. Shults, C. W.; Haas, R. H.; Passov, D.; Beal, M. F. *Ann. Neurol.* **1997**, *42*, 261.
36. Ernster, L.; Dallner, G. *Biochim. Biophys. Acta: Mol. Basis Dis.* **1995**, *1271*, 195.
37. Beyer, R. E.; SeguraAguilar, J.; DiBernardo, S.; Cavazzoni, M.; Fato, R.; Fiorentini, D.; Galli, M. C.; Setti, M.; Landi, L.; Lenaz, G. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 2528.
38. Navas, P.; Villalba, J. M.; de Cabo, R. *Mitochondrion* **2007**, *7*, S34.
39. Ivleva, N. B.; Gao, T. Y.; LiWang, A. C.; Golden, S. S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 17468.
40. Cornwell, D. G.; Ma, J. Y. In *Vitamin E: Vitamins and Hormones Advances in Research and Applications*; Elsevier Academic Press Inc.: San Diego, 2007; Vol. 76, p 99.
41. Netscher, T.; Mazzini, F.; Jestin, R. *Eur. J. Org. Chem.* **2007**, 1176.
42. Netscher, T. In *Vitamin E: Vitamins and Hormones Advances in Research and Applications*; Elsevier Academic Press Inc.: San Diego, 2007; Vol. 76, p 155.
43. Ohnmacht, S.; West, R.; Sirnionescu, R.; Atkinson, J. *Magn. Reson. Chem.* **2008**, *46*, 287.
44. All pharmacokinetic parameters were determined using WinNonLin Professional, Version 5.3; Pharsight Corporation, Mountain View, CA.
45. He, L.; Galland, S.; Dufour, C.; Chen, G. R.; Dangles, O.; Fenet, B.; Praly, J. P. *Eur. J. Org. Chem.* **2008**, 1869.
46. Pappert, E. J.; Tangney, C. C.; Goetz, C. G.; Ling, Z. D.; Lipton, J. W.; Stebbins, G. T.; Carvey, P. M. *Neurology* **1996**, *47*, 1037.
47. Gilgun-Sherki, Y.; Melamed, E.; Offen, D. *Neuropharmacology* **2001**, *40*, 959.
48. Tanito, M.; Itoh, N.; Yoshida, Y.; Hayakawa, M.; Ohira, A.; Niki, E. *Lipids* **2004**, *39*, 469.
49. Tanito, M.; Yoshida, Y.; Kaidzu, S.; Chen, Z. H.; Cynshi, O.; Jishage, K.; Niki, E.; Ohira, A. *Invest. Ophthalmol. Vis. Sci.* **2007**, *48*, 396.
50. Dowd, P.; Zheng, Z. B. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8171.
51. Bettger, W. J.; Olson, R. E. *Federation Proceedings* **1982**, *41*, 344.
52. Alfredo A. Sadun, MD, PhD Doheny Eye Institute, USC-Keck School of Medicine, Principle Investigator, FDA IND #107,772, 'Emergency administration of EPI-743 to a single patient with Leber's hereditary optic neuropathy (LHON)'.
53. Gregory M. Enns, MB, ChB, FAAP Stanford University, Lucile Packard Children's Hospital Principle Investigator, FDA IND #107,401 'Emergency use protocol for EPI-743 in acutely ill patients with inherited mitochondrial respiratory chain disease within 90 days of end-of-life care'.
54. Ross, D.; Siegel, D.; Beall, H.; Prakash, A. S.; Mulcahy, R. T.; Gibson, N. W. *Cancer Metastasis Rev.* **1993**, *12*, 83.
55. Ross, D.; Siegel, D. In *Quinones and Quinone Enzymes, Pt B*; Academic Press Inc.: San Diego, 2004; Vol. 382, p 115.
56. Sollner, S.; Macheroux, P. *FEBS J.* **2009**, *276*, 4313.
57. Asher, G.; Bercovich, Z.; Tsvetkov, P.; Shaul, Y.; Kahana, C. *Mol. Cell* **2005**, *17*, 645.
58. Gong, X.; Kole, L.; Iskander, K.; Jaiswal, A. K. *Cancer Res.* **2007**, *67*, 5380.
59. Buryanovskyy, L.; Fu, Y.; Boyd, M.; Ma, Y. L.; Hsieh, T. C.; Wu, J. M.; Zhang, Z. T. *Biochemistry* **2004**, *43*, 11417.
60. Gaikwad, A.; Long, D. J.; Stringer, J. L.; Jaiswal, A. K. *J. Biol. Chem.* **2001**, *276*, 22559.
61. Merker, M. P.; Audi, S. H.; Bongard, R. D.; Lindemer, B. J.; Krenz, G. S. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **2006**, *290*, L607.
62. Faig, M.; Bianchet, M. A.; Talalay, P.; Chen, S.; Winski, S.; Ross, D.; Amzel, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3177.
63. Li, R. B.; Bianchet, M. A.; Talalay, P.; Amzel, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8846.
64. Siegel, D.; Bolton, E. M.; Burr, J. A.; Liebler, D. C.; Ross, D. *Mol. Pharmacol.* **1997**, *52*, 300.
65. Bandyopadhyay, A.; Miki, K.; Wakayama, Y. *Appl. Phys. Lett.* **2006**, *89*, 3.
66. Bandyopadhyay, A.; Acharya, S. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 3668.
67. Bandyopadhyay, A.; Sahu, S.; Fujita, D. *Appl. Phys. Lett.* **2009**, *95*, 3.
68. Bandyopadhyay, A.; Pati, R.; Sahu, S.; Peper, F.; Fujita, D. *Nat. Phys.* **2010**, *6*, 369.
69. Katz, E.; Willner, I. *Electrochem. Commun.* **2006**, *8*, 879.
70. Katz, E.; Privman, V. *Chem. Soc. Rev.* **2010**, *39*, 1835.
71. Privman, V.; Halamek, J.; Arugula, M. A.; Melnikov, D.; Bocharova, V.; Katz, E. *J. Phys. Chem. B* **2010**, *114*, 14103.
72. Szacilowski, K. *Chem. Rev.* **2008**, *108*, 3481.