



Minireview

Coenzyme Q₁₀ and idebenone in the therapy of respiratory chain diseases: rationale and comparative benefits

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Abstract

While there have been major advances in both the identification of the molecular basis and our understanding of mitochondrial pathology, the clinical management of patients with mitochondrial respiratory chain disease is still essentially supportive. Quinones are the only pharmacological agents that have proven some efficacy when, and only when, given to patients presenting with quite specific respiratory chain defects. In this article, after a short presentation of the coenzyme Q₁₀ molecule, its origin and distribution in human body, we summarize our present knowledge on its several physiological functions. We next discuss the rationale that justifies using different types of quinones in the therapy of mitochondrial disorders. We finally briefly review the available data obtained in the therapy of mitochondrial disorders by using quinones as either substitutive electron carriers or antioxidant compounds. © 2002 Elsevier Science (USA). All rights reserved.

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1. Coenzyme Q₁₀

Coenzyme Q₁₀ (CoQ₁₀; ubiquinone 50) content widely varies amongst tissues. CoQ₁₀ content of heart, kidney, liver (114, 66.5, 55 µg/g tissue, respectively) and intestine, colon, testis or lung (11.5, 10.7, 10.5, 7.9 µg/g tissue, respectively) differs by more than one order of magnitude in human [1]. Median values have been measured in skeletal muscle, pancreas, thyroid, spleen, and brain (40, 33, 24.7, 24.6, 13.4 µg/g tissue, respectively). A general trend to a decreased ubiquinone content with age has been reported, which appears to be, however, variable depending on the tissue studied [1]. Under most physiological conditions, the redox status of CoQ₁₀ rather than its absolute amount may be the crucial parameter to be looked at [2]. Theoretically, a decreased CoQ₁₀ content (or possibly an increased oxidation status) might reflect a reduced oxidative metabolism activity in tissues, resulting from a decreased need

for electron transfer and antioxidant capacities, especially with age. Alternatively, according to a more fashionable hypothesis, it could be one of the key events in a cascade leading to a decrease of oxidative and antioxidant capacity with age. In other words, a CoQ₁₀ decrease could be seen as a positive adjustment to decreased metabolic needs, or as a harmful progressive shortage with age. This latter hypothesis has led to a somewhat irrational and premature enthusiasm for using CoQ₁₀ as a miracle cure against the aging process, resulting in an indisputable effect on the health of the firms distributing it. However, real benefit of CoQ₁₀ supplementation for healthy individuals is still to be firmly established.

2. The role of ubiquinone in the mitochondria

CoQ₁₀ plays a pivotal role in the mitochondrial respiratory chain. It distributes the electrons between the various dehydrogenases and the cytochrome segments of the respiratory chain (Fig. 1A) [3]. It is in large excess

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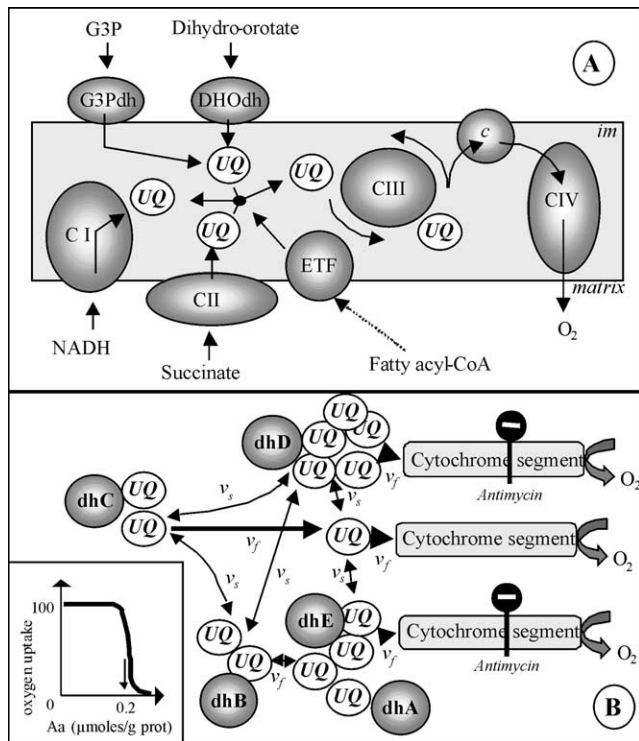


Fig. 1. The pivotal role of ubiquinone in the distribution of electrons in the mitochondrial respiratory chain. (A) Diversely distributed in the inner mitochondrial membrane, the various dehydrogenases compete to reduce the ubiquinone pool in the respiratory chain; (B) because of the specific location and redox properties of the various dehydrogenases, the rate of electron flow from a dehydrogenase to the cytochrome segments of the respiratory chain will be different for each dehydrogenase. This results in a variable ability of the dehydrogenases to reduce different domains of the ubiquinone pool which then behaves as kinetically compartmentalized (various electron exchange rates in the pool, from slow v_s to fast v_f). However, during the progressive inhibition of the cytochrome segments by an inhibitor such as antimycin (a specific complex III inhibitor), the pool function of ubiquinone ensures the redistribution of electrons to the residual noninhibited cytochrome segments. The quinone pool then behaves as kinetically homogenous, this accounting for the sigmoidal nature of respiratory chain inhibition by antimycin as schematized in the inset.

compared to any other component of the respiratory chain (RC) and forms a kinetically compartmentalized pool the redox status of which tightly regulates the activity of the dehydrogenases (Fig. 1B) [4]. The CoQ₁₀ pool function accounts for the non-linear dependence of the inhibition of electron flow through the RC on the concentration of antimycin, a specific inhibitor of RC complex III (Fig. 1B; inset) [5]. Finally, through the so-called Q cycle (see Fig. 6), the function of CoQ₁₀ allows protons to be extruded from the matrix to the intermembrane space along with electron flow through the RC complexes [6].

Besides its involvement in electron and proton exchanges in the RC, CoQ₁₀ can react with molecular oxygen to produce superoxides, depending on its redox

and protonation status (Fig. 2) [1]. Thus, the inhibition of electron flow through complex III by antimycin (see Fig. 1B) results in both the full reduction of *b*-type cytochromes (favored by the oxidation of cytochrome c_1) and the increased formation of unstable ubisemiquinone with ensuing superoxide production [7]. Both, the ATPase inhibition (oligomycin) and genetic defect (NARP mutation, a T8993G in the mitochondrial ATPase 6 gene) have also been shown to favor high membrane potential and quinone reduction, and a subsequent superoxide over-production [8]. Beside the pro-oxidant effect of ubisemiquinone, fully reduced CoQ₁₀ (ubiquinol) can scavenge a number of radical species, including superoxides, therefore acting as an antioxidant (Fig. 2). However, because CoQ₁₀ is highly hydrophobic, its natural radical targets are presumably hydrophobic radicals, such as liperoxide or tocopheryl radicals. Therefore, CoQ₁₀ can act as both a pro- and an antioxidant molecule [9]. Noticeably, in human cultured fibroblasts grown either in the presence of glucose or galactose, the lack (or strong decrease) of CoQ₁₀, reducing (but not nullifying) electron flow through the RC, does not increase oxidative stress (neither superoxide dismutase induction, nor increased liperoxidation) or susceptibility to oxidative stress, suggesting that at least in these cells (under the studied conditions) the antioxidant function of CoQ₁₀ is only of minor importance [10].

The partition of quinone molecules in the various compartments of a cell (membrane cores or surfaces, soluble phases) largely determines their efficiency as antioxidants *in vivo* (Fig. 3). Manipulating the hydrophobicity of quinones has resulted in the generation of numerous analogues that target various locations and has provided a choice of quinone analogues that can be selected in function of the exact location of radicals to be scavenged [11].

Finally, CoQ₁₀ has been shown or suggested to interact with several additional mitochondrial functions, including intermediate metabolism, biosynthesis pathways, and apoptosis either through the regulation of dehydrogenase activities, the generation of superoxides, or through direct interaction with other mitochondrial membrane components [12].

3. The extra-mitochondrial functions of ubiquinone

In cells, ubiquinone distribution is far from being restricted to the mitochondria (about 1.4 $\mu\text{g}/\text{mg}$ protein; 1.86 in the inner membrane), and the highest ubiquinone concentration (2.62 $\mu\text{g}/\text{mg}$ protein) is, for example, found in the Golgi vesicles of rat liver cells [13]. In these cells, lysosomes, plasma membrane, peroxisomes, and microsomes all contain ubiquinone (1.86, 0.74, 0.29, 0.15 $\mu\text{g}/\text{mg}$ protein, respectively). The function of this

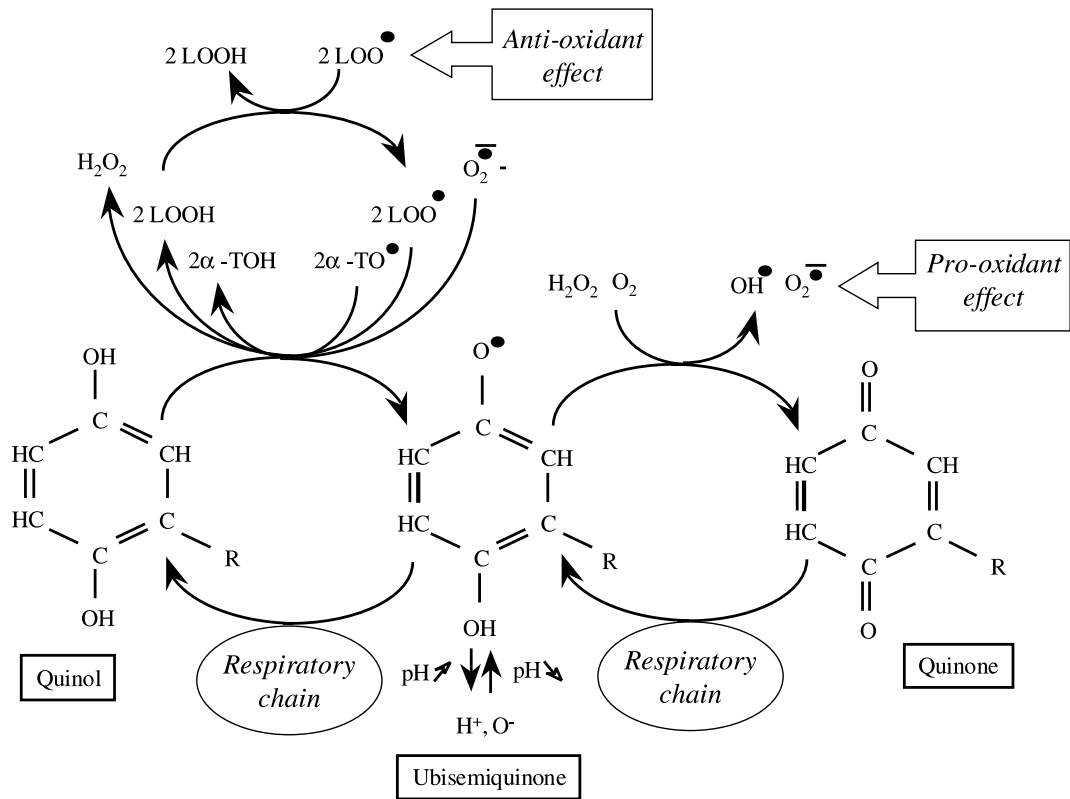


Fig. 2. The various anti- and pro-oxidant reactions possibly catalyzed by quinones. Pro-oxidant effect has been shown to be associated with the generation of the ubisemiquinone radical which readily reacts with oxygen and its derivatives to produce toxic radical oxygen species. The antioxidant function relies on the formation of the quinol form (fully reduced quinone) that can reduce oxygen, lipoperoxide and tocopheryl radicals, depending on their respective location in membranes. In the mitochondrial membrane, the redox status of the quinones is controlled by the activity of both the cytochrome segments and the several dehydrogenases that can bring about variable reduction level of the quinone pool depending on their location and redox properties.

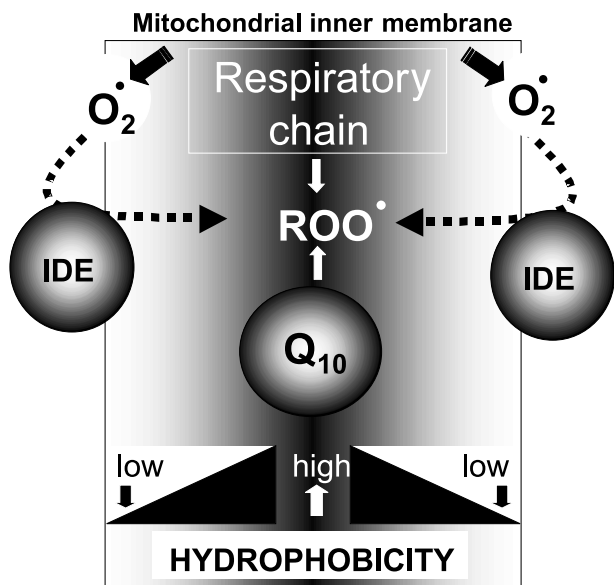


Fig. 3. Presumed spatial compartmentalization of the antioxidant effect of coenzyme Q₁₀ and idebenone in mitochondrial membranes. Essentially depending on their side-chain composition, the partition of reduced quinones in the different membrane domains (more or less hydrophobic) will determine the respective site of their antioxidant activity. IDE, idebenone.

extra-mitochondrial ubiquinone is still to be firmly established. Redox cycling of ubiquinone in association with specific redox chains in the Golgi and lysosomal membranes has been suggested to contribute to the unilateral proton distribution measurable in these organelles [14]. More generally, under its reduced form, it presumably exerts a broad antioxidant action in all cell membranes [1].

4. The extra-cellular action of ubiquinone

Besides its intra-cellular action, ubiquinone can also afford antioxidant protection to any component possibly facing an oxidative insult in the body fluids. In particular, quinones can act in blood by preventing (i) oxidation of low density lipoproteins (LDL), (ii) free radical damage caused by neutrophils in inflammatory diseases, and (iii) oxidative insult by endothelial cells caused by ischemic-reperfusion [1]. There is indeed convincing evidence that CoQ₁₀, which is poorly taken up by cells, is readily increased in blood after oral administration in both rat and human [1]. Through exog-

enous interactions with cell surfaces, circulating quinones have been suggested to affect the activity of the plasma membrane oxidoreductase (PMOR) activity which catalyses the reduction by cytosolic NADH of a variety of extra-cellular components, including oxygen to superoxide [15]. It may well be therefore that part of the beneficial effect presumably resulting from CoQ₁₀ administration is indeed related to its effect in the circulation.

5. CoQ₁₀ biosynthesis

The biosynthesis of CoQ₁₀ results from the condensation of a parahydroxybenzoate ring, arising from phenylalanine or tyrosine, with a 10-isoprenoid chain (Fig. 4). This polyprenoid side chain derives from mevalonate, which is also the precursor for cholesterol biosynthesis [16]. Prenylated parahydrobenzoate subsequently undergoes a series of modifications that includes hydroxylation, methylation, O-methylation, and decarboxylation reactions. The subcellular location of the several steps involved in this process is largely unknown in human. Few of the involved genes have been characterized in human, and none have been shown to

be responsible for inherited ubiquinone deficiency so far.

6. CoQs

CoQ₁₀ is a highly hydrophobic molecule, and one of the few antioxidants that human can synthesize and readily regenerate to its reduced active form after utilization. CoQ₁₀ is the predominant type in human, while mostly CoQ₉ is found in rat *Rattus norvegicus* [17] or the sunflower plant, *Helianthus tuberosus* [18]. Either shorter (CoQ₆ in *Saccharomyces cerevisiae*) or longer (CoQ₁₁ in the plant, *Capsicum* sp.) ubiquinone types can be found in various living organisms [16,18]. Length of the side-chain has been considered to be a potential factor interfering with life extent of different species, but this hypothesis has not been experimentally supported [19].

Besides natural CoQs, a bouquet of derivatives has been produced with the aim to improve the bioavailability, to target specific cell compartments, hoping to preserve or even improve the putative beneficial effect of CoQ₁₀ ([11] and references therein). So far, however, the only available pharmacological preparations are CoQ₁₀ itself and idebenone, a short chain analogue, with reduced hydrophobicity.

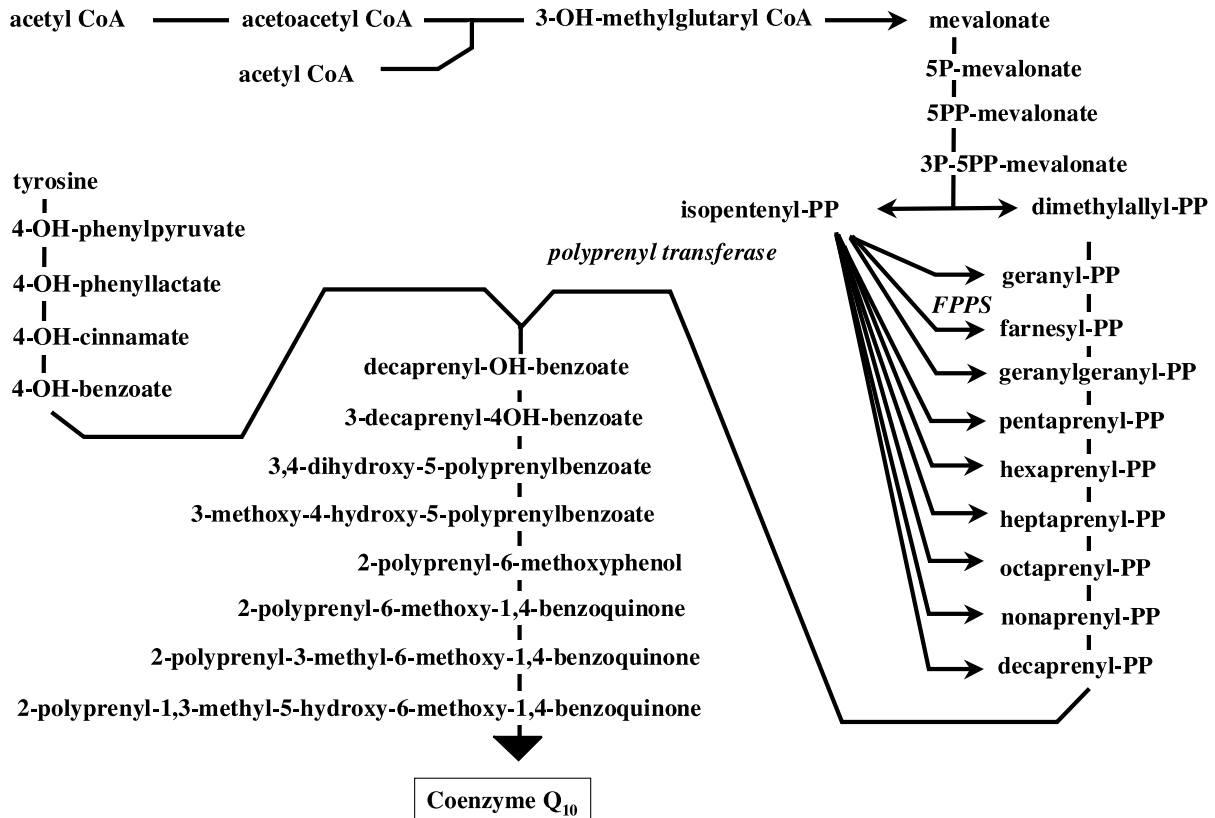


Fig. 4. A simplified view of coenzyme Q₁₀ biosynthesis. The precise intracellular location of part of the steps (cytosolic versus mitochondrial) remains to be established in human. Notice that mevalonate is mostly used as a cholesterol precursor. Finally, only part of the genes encoding the enzymes specifically involved in this biosynthesis is known in human. Enzymes are shown in italics. FPPS: farnesyl pyrophosphate synthetase.

7. Idebenone

Idebenone (6-(10-hydroxydecyl)2,3-dimethoxy-5-methyl-1,4-benzoquinone; Fig. 5) is a synthetic compound, initially patented by Takeda Chemical Industries, Osaka, Japan, which various properties were extensively reviewed by Nagy in 1990 [20]. The drug was introduced to the Japanese market as early as 1986. Initially its main indication was age-dependent impairments of brain functions. Its redox properties are those of a quinone analogue, with possible pro- and antioxidant activities, and ability to interact with other redox carriers including CoQ₁₀ itself in the mitochondrial respiratory chain. In rodents, intraperitoneal LD₅₀ was estimated to be about 800 mg/kg body weight while it would exceed 10 g/kg for subcutaneous and oral administration [21]. Reversible subacute toxicity in rodents was observed for 2,5 g/kg/d [22], while 500 mg/kg/d provoked diarrhea in beagle dogs [24]. Chronic toxicity study in the beagle dog resulted in similar figures with first signs appearing at 500 mg/kg body weight doses per os [23]. Idebenone was found to be neither immunogenic in rodents nor mutagenic in bacteria or mice. In human, idebenone is absorbed rapidly and circulating concentration increases in a dose-dependent manner (2 μM 2 h, after 100 mg oral supplementation), but rapidly tends to lower by excretion [24].

In human, the safety of idebenone (60–300 mg) was established on a long-term period by treating elderly patients for two years without remarkable side effects [25]. However, idebenone, as other quinone analogues, is a redox active compound with distinctive kinetics properties towards other redox carriers, including components of the respiratory chain complexes. In vitro,

it is able to favorably compete with natural CoQ₁₀ to mediate electron transfer in isolated mitochondria and to divert a variable part of the electrons at the expense of electron flow to oxygen, particularly from complex I [26]. Being thus readily reduced, idebenone revealed then as a powerful antioxidant at the surface of the mitochondrial inner membrane.

8. The rationale for the use of quinones in the therapy of mitochondrial diseases

Based on our present understanding of the role of quinones in the mitochondria, one should distinguish attempts aiming at either (i) restoring electron flow in the respiratory chain, (ii) providing electrons to the chain, or (iii) increasing mitochondrial anti-oxidant defenses.

8.1. Restoring electron flow

Restoring electron flow requires that CoQ₁₀, or its selected analogue, (i) reaches the targeted tissue, the cell and ultimately the mitochondria, (ii) get inserted in the mitochondrial inner membrane, (iii) and finally restores the electron flow by “filling a gap” (replacing a missing component) or “making a bridge” (a component is defective) between electron carriers. However so far, except in the case of CoQ₁₀ deficiency, neither CoQ₁₀, nor any of its analogues, has been demonstrated in vitro or in vivo to be able to actually restore electron flow in case of RC deficiency. In particular, providing exogenous CoQ₁₀, or any of its short-chain analogues, cannot restore in vitro electron flow through a defective complex

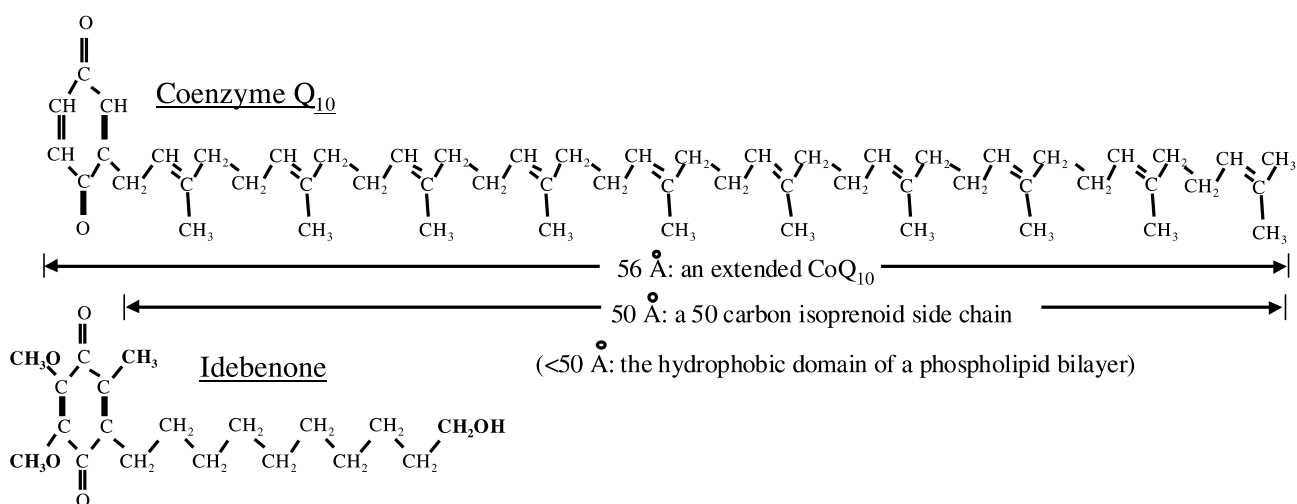


Fig. 5. Structural features of ubiquinone (coenzyme Q₁₀) and idebenone. Taking into account the extended dimension of CoQ₁₀, it appears that the molecule is too long to fit the hydrophobic domain of a phospholipid bilayer, except if folded. Notice the short side-chain of idebenone (10 carbons) as compared to CoQ₁₀ (50 carbons), the saturation of the idebenone side-chain, the presence of a terminal hydroxyl group, and of dimethoxy and methyl residues on the benzoquinone ring. All this concurs to make idebenone less hydrophobic than coenzyme Q₁₀, affecting its partition and diffusion in biological membranes.

Widespread CoQ₁₀ depletion has been identified in only one familial case due to a blockade in the biosynthesis of CoQ₁₀ resulting in the accumulation of the biosynthesis intermediate, prenylpyrophosphate [28]. Two affected children were efficiently treated by oral supplementation with CoQ₁₀. Presenting with major encephalomyopathy, the children, one being wheelchair bound before the treatment, are now (four years later) able to walk, run, and ride a bike for kilometers. The spectacular effect of CoQ₁₀ therapy in this case should prompt ones to make our best to detect primary defects of CoQ₁₀ before the occurrence of irreversible lesions. Functionally, CoQ₁₀ depletion can be best detected in human sample by studying RC enzyme activities that strongly depend on the CoQ₁₀ pool function, such as glycerol 3-phosphate or dihydroorotate cytochrome *c* reductase activity [28]. Succinate cytochrome *c* reductase appears much less dependent on the pool function, although being also reduced in the case of severe CoQ₁₀ depletion. Adding exogenous quinone analogues (i.e. idebenone, decylubiquinone, CoQ₂) during the enzyme assay restores normal activities. Noticeably, an artefactual loss of CoQ₁₀ pool-dependent activities can also result from an improper handling of frozen biological samples. Studying fresh material is therefore recommended as to convincingly establish CoQ₁₀ depletion, which should then be confirmed by direct quantification after extraction.

10. Friedreich ataxia and mitochondrial cardiomyopathy

Quinone administration also revealed a promising therapy to counteract the life-threatening cardiomyopathy that often develops in Friedreich ataxia (FRDA) [36]. FRDA is the most common hereditary ataxia originating from a GAA expansion in the first intron of the gene encoding frataxin [37], a mitochondrial protein most probably involved in iron–sulfur cluster synthesis or maintenance [38–40]. In human cultured skin fibroblasts, a decreased frataxin content hampers superoxide dismutase signaling [41]. Evidence for mitochondrial oxidative stress in patients suffering this condition are the loss of activities of mitochondrial iron–sulfur cluster-containing enzymes [38], perturbation of antioxidant reserves [42], and the increase of oxidized DNA species in patients' urine [43]. Because it is known (i) to have potent antioxidant properties, (ii) to cross the blood brain barrier, and (iii) to be readily taken up by cells even with a normal CoQ₁₀ content, idebenone (rather than CoQ₁₀) has been given to more than a 100 FRDA patients in our hospital. Noticeably, no sign of CoQ₁₀ depletion can be functionally evidence in FRDA patients by studying RC activity in various tissues. Thirty-eight of these patients received the drug for more than six months, and a significant decrease of heart hyper-

trophy (> 20%) was observed in about 50% of these patients [44,45]. No significant increase of heart hypertrophy was observed in any of the treated patients on this short period. Because the natural course of the disease irreversibly leads to a gradual increase of hypertrophy possibly resulting in heart dilatation, these results are quite promising for the treatment of this disease. Decreased of oxidized DNA species in FRDA patients' urine upon idebenone therapy has also been reported [43]. Whether idebenone will on a long term also improve the neurological condition remains to be studied. But the efficiency of the drug on the cardiomyopathy and the absence of side effects should also lead to consider giving the drug to presymptomatic patients. Because (i) cardiomyocyte hypertrophy is known to possibly result from superoxide production and (ii) Friedreich's ataxia is one of the few diseases for which such an overproduction is experimentally established, we believe that idebenone effect actually relies on its potent action as a superoxide scavenger *in vivo*. The efficiency of idebenone in counteracting mitochondrial cardiomyopathy in Friedreich's ataxia was an incentive to also evaluate its efficiency in mitochondrial cardiomyopathy of other origin possibly associated with quinone pool dysfunction, e.g., evidence of decreased cytochrome *c* reductase activities. Although anecdotal, the result from idebenone oral administration was an amazing improvement of the cardiac condition of one patient who was thereafter removed from the cardiac transplantation list [46].

11. Oxidative stress, mitochondria, and quinones

A number of other RC deficiencies have been shown to induce an increased generation of mitochondrial superoxides [8,47]. For example, the ATPase deficiency caused by the NARP mutation has been shown to be associated with an increased superoxide production triggering the apoptotic cascade in cultured skin fibroblasts. *In vitro*, the use of a spin-trap was established to protect cell from the apoptotic death [8]. Unfortunately, because of the general blockade of the RC due to the ATPase defect, the effect of redox cycling antioxidants, which as quinones, require redox exchange with the RC, may be only limited.

Superoxide-triggered apoptosis might also participate to the quite specific (and not well-understood) optic nerve degeneration associated with Leber hereditary optic atrophy (LHON) caused by mutation in mtDNA. Indeed, it has been for long assumed that a high and specific energy demand of the optic nerve would account for such a specific involvement [48]. Obviously, signal transmission is an energy requiring process and is predictably sensitive to any significant energy restriction. However, neither decreased substrate oxidation nor re-

duced ATP production could be evidenced in cultured skin fibroblasts harboring the homoplasmic LHON mutation (more than 99% mutant mtDNA molecules with G11778A in the ND4 gene) (unpublished data). Besides, many other functions in many other organs presumably rely on energy for normal functioning as well. As a result, alternative explanation has been looked for, and one might hypothesize that a high sensitivity to radical oxygen species triggering cell death contributes to the selective optic nerve degeneration in LHON. Because of their known antioxidant properties, CoQ₁₀, and idebenone, sometimes in association with vitamin E, have been tested in patients with LHON. As early as 1992, idebenone has been claimed to show some efficiency in the therapy of LHON [49]. However, the question remains open of the usefulness of this drug in this condition, since despite several reports on the positive effect of quinones, no clinical trial has been organized so far.

In a number of situations, oxidative injuries resulting from mitochondrial dysfunction have been proposed to be key events. The consequences of ischemic/reperfusion either through or/and on the mitochondrial functions have been extensively studied and the oxidative stress is supposedly playing a major role in the course of this process [50]. Both CoQ₁₀ and its short-chain analogue, idebenone, have been reported to successfully counteract the effect of the reperfusion, presumably due to their antioxidant properties [51–53]. Similarly, although often lacking a strong experimental support, oxidative injuries to the central nervous system resulting from mitochondrial dysfunction have been frequently advocated to account for neurodegenerative diseases [54] and physiological aging as well [55]. Again, both CoQ₁₀ and idebenone have been recommended in all these conditions [56,57], opening a tremendous commercial market in developed countries. So far, no toxicity of these drugs has been shown, but their potential usefulness is still to be established. After an initial enthusiasm, the supposedly multi benefit resulting from CoQ₁₀ administration is still presumptive and long term, randomized, double-blind studies are required to demonstrate potential improvement.

12. Conclusion

Beside rare genetic defects affecting cell quinone biosynthesis, decreased CoQ₁₀ content, which might reduce electron flow through the RC and/or cell antioxidant defenses, has been taken for granted in association with a variety of age-related diseases or ageing itself. This has permitted the development of a considerable market for CoQ₁₀ in so-called developed countries. Actually, the amount of ubiquinone have been claimed, although seldom shown, to be of prime im-

portance for life span in different organisms, but CoQ₁₀ “inundation” does not seem to be as efficient in prolonging human life span. Any beneficial effect for the consumers remains even to be demonstrated, although it is taken daily by up to 10% of the elderly in some north European countries!

However, even if in a few particular cases, CoQ₁₀, or its analogues, represents one of the very few compounds which has shown an indisputable efficiency in the context of mitochondrial diseases, particularly Friedreich’s ataxia. This makes it essential to not miss the quinone-responsive cases when screening for RC deficiency.

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