Ubiquinol-10 ameliorates mitochondrial encephalopathy associated with CoQ deficiency

Laura García–Corzo, Marta Luna–Sánchez, Carolina Doerrier, Francisco Ortiz, Germaine Escames, Darío Acuña–Castroviejo, Luis C. López *

Departamento de Fisiología, Facultad de Medicina, Universidad de Granada, Granada, Spain
Instituto de Biotechnología, Centro de Investigación Biomédica, Parque Tecnológico de Ciencias de la Salud, Armilla, Granada, Spain

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ABSTRACT

Coenzyme Q10 (CoQ10) deficiency (MIM 607426) causes a mitochondrial syndrome with variability in the clinical presentations. Patients with CoQ10 deficiency show inconsistent responses to oral ubiquinone-10 supplementation, with the highest percentage of unsuccessful results in patients with neurological symptoms (encephalopathy, cerebellar ataxia or multisystemic disease). Failure in the ubiquinone-10 treatment may be the result of its poor absorption and bioavailability, which may be improved by using different pharmacological formulations. In a mouse model (Coq0/0) of mitochondrial encephalopathy due to CoQ deficiency, we have evaluated oral supplementation with water-soluble formulations of reduced (ubiquinol-10) and oxidized (ubiquinone-10) forms of CoQ10. Our results show that CoQ10 was increased in all tissues after supplementation with ubiquinone-10 or ubiquinol-10, with the tissue levels of CoQ10 with ubiquinone-10 being higher than with ubiquinol-10. Moreover, only ubiquinol-10 was able to increase the levels of CoQ10 in mitochondria from cerebrum of Coq0/0 mice. Consequently, ubiquinol-10 was more efficient than ubiquinone-10 in increasing the animal body weight and CoQ-dependent respiratory chain complex activities, and reducing the vacuolization, astrogliosis and oxidative damage in diemephalon, septum–striatum and, to a lesser extent, in brainstem. These results suggest that water-soluble formulations of ubiquinol-10 may improve the efficacy of CoQ10 therapy in primary and secondary CoQ10 deficiencies, other mitochondrial diseases and neurodegenerative diseases.

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1. Introduction

Coenzyme Q10 (CoQ) is a lipophilic molecule that is involved in the mitochondrial ATP synthesis because of its function as an electron between mitochondrial complexes I and II, as well as ETF-Q oxidoreductase, and mitochondrial complex III [1]. Moreover, CoQ10 functions as an antioxidant, which protects the cells both directly by preventing the oxidation of biomolecules and indirectly by regenerating other antioxidants such as vitamins C and E [1]. Due to these properties, oral supplementation with CoQ10 (in its stable oxidized form, ubiquinone-10) has been proposed for the treatment of diseases involving mitochondrial dysfunction and/or oxidative stress, i.e. primary mitochondrial disorders, Parkinson’s Disease, Alzheimer’s Disease, Amyotrophic Lateral Sclerosis, Huntington’s Disease or heart failure [2–6]. Moreover, oral ubiquinone-10 supplementation is the main choice in the treatment of primary (MIM 607426) and secondary CoQ10 deficiencies [7].

Despite the good expectation that ubiquinone-10 therapy has presented, the studies in different diseases, both at preclinical and clinical levels, have shown contradictory results. Specially, ubiquinone-10 seems to be less effective in improving the neurological symptoms and, in some cases, higher doses are needed to appreciate some clinical improvement [7]. The mild or completely lack of response to ubiquinone-10 therapy has been attributed to its low absorption and bioavailability that limit the increase of CoQ10 in cell mitochondria, where it is biologically active [8]. This limitation is even more important in the brain because the exogenous ubiquinone-10 must be able to cross the blood brain barrier. Thus, different strategies have been investigated to increase the absorption and bioavailability of the exogenous CoQ10. In this regard, water-soluble formulations of ubiquinone-10 seem to increase its bioavailability. Different studies have shown that concentration of CoQ10 in plasma after administration of water-soluble formulations of ubiquinone-10 is higher than that after supplementation with ubiquinone-10 administered as powder water-insoluble formulations [8]. Moreover, the plasma levels of CoQ10 are also higher.
2. Materials and methods

2.1. Mice use and experimental treatment

Generation and characterization of Coq9<sup>+/−</sup> mice (C57BL/6 genetic background) were previously reported [9]. All experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the University of Granada (procedures CEEA 2009-254 and 2010-275) and were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS # 123) and the Spanish laws (32/2007 and R.D. 1201/2005). Mice were housed in the Animal Facility of the University of Granada under a specific pathogen free zone with lights on at 7:00 AM and off at 7:00 PM, and with unlimited access to water and rodent chow. Mice were sacrificed using CO<sub>2</sub> narcosis followed by cervical dislocation at 3 months of age.

The treatment consisted of administering ubiquinone-10 or ubiquinol-10 in the drinking water in a dose of 240 mg/kg bw/day. The treatment started at 1 month of age and the mice were sacrificed at 3 months of age. Ubiquinone-10 and ubiquinol-10 were provided by Kaneka Corporation (Japan) in a water-soluble formulation that contains dextrin, Arabic gum and ascorbic acid. A control group with vehicle at the same dose was also studied. The drinking water was changed twice a week.

2.2. Mitochondrial isolation

Cerebrum was homogenized in a glass-Teflon homogenizer in a proportion 1:5, w/v, in the homogenization medium A (0.32 M sucrose, 1 mM EDTA, 10 mM Tris–HCl [pH 7.4]) plus 0.2% fatty acid–free bovine serum albumin. Homogenate was centrifuged at 1000 g for 5 min at 4 °C to remove nuclei and debris. Mitochondria were collected from supernatants after centrifugation at 14,400 g for 2 min at 4 °C. The mitochondrial pellet was suspended in the corresponding buffer and an aliquot of each sample was used for protein determination [9].

2.3. Quantification of CoQ<sub>9</sub> and CoQ<sub>10</sub> levels in plasma, mice tissues and cerebral mitochondria

CoQ<sub>9</sub> and CoQ<sub>10</sub> from mice tissues were extracted by mixing tissue extracts with 1-propanol. After 2 min vortex, the solution was centrifuged at 11,300 g for 5 min. The resultant supernatant contained the lipid extract [9]. CoQ<sub>9</sub> and CoQ<sub>10</sub> from plasma and cerebral mitochondria were extracted in a hexane:ethanol mixture [10]. The lipid extract was injected in a HPLC system (Waters, Milford, USA) and the lipid components were separated by a reverse phase Symmetry C18 3.5 µm, 4.6 × 150 mm column (Waters, Spain), using a mobile phase consisting of methanol, ethanol, 2-propanol, acetic acid (500:500:15:15) and 50 mM sodium acetate at a flow rate of 0.9 mL/min. The electrochemical detector consisted of an ESA Coulochem III with the following setting: guard cell (upstream of the injector) at +900 mV, conditioning cell at −600 mV (downstream of the column), followed by the analytical cell at +350 mV [9]. CoQ<sub>9</sub> and CoQ<sub>10</sub> concentrations were estimated by comparison of the peak areas with those of standard solutions of known concentrations. The results were expressed in ng CoQ/mg prot.

2.4. CoQ-dependent respiratory chain activities

CoQ-dependent respiratory chain activities were measured in submitochondrial particles. To prepare submitochondrial particles, each mitochondrial pellet (100 µg prots) was suspended and sonicated in 100 µl of 0.1 M potassium phosphate buffer, pH 7.5. Complex I + II activity was measured at 30 °C in the presence of 0.5 mM potassium cyanide, 0.2 mM NADH and 0.1 mM cytochrome c, as the rotenone-sensitive reduction of cytochrome c at 550 nm [9,11]. The results were expressed in nmol reduced cyt c/min/mg prot. Complex II + III activity was measured at 30 °C in the presence of 0.5 mM KCN, 0.3 mM succinate and 0.01 mM rotenone. The reaction was initiated by addition of 0.1 mM cytochrome c and decrease in absorbance was monitored at 550 nm. The results were expressed in nmol reduced cyt c/min/mg prot [11].

2.5. Blue native gel electrophoresis and immunoblotting for the evaluation of mitochondrial supercomplex pattern

Blue native gel electrophoresis (BNGE) was performed on mitochondrial fraction from cerebrum. The mitochondrial pellets were suspended in 140 µl in the homogenization medium A. An aliquot of each sample was used for protein determination. The remaining samples were then centrifuged at 17,000 g for 3 min at 4 °C. Mitochondrial pellets were suspended in an appropriate volume of buffer B (1 M 6-amiho-hexanoic acid, 50 mM Bis-Tris–HCl [pH 7.0]) to be at 10 mg/ml, and the membrane proteins were solubilized by the addition of digitonin (4 g/l) and incubated for 5 min in ice. After 30 min centrifugation at 13,000 g, the supernatant was collected, and 3 µl of 5% Brilliant Blue G dye prepared in 1 M 6-amiho-hexanoic acid was added [9]. Mitochondrial proteins (100 µg) were then applied and ran on a 3%–13% gradient native gel using electrophoresis system mini-PROTEAN Tetra Cell (Bio-rad). Western blot was performed using a mini Trans-blot Cell onto PVDF membranes and probes with specific antibodies against complex I, anti-NUDF9 (Abcam, ab14713), complex II, anti-ubiquinol-cytochrome c reductase Core Protein I (Abcam, ab110252) and VDAC1 (Abcam, ab14734) [9,12].

2.6. Mitochondrial complex I in-gel catalytic activity assay

Mitochondrial membrane proteins (100 µg) were applied and ran on a 3%–13% first-dimension gradient BNGE gel as described elsewhere [13]. The assay buffer contained 10 mg of NTB and 0.14 mM NADH added to 10 ml of 100 mM Tris/HCl, pH 7.4. After about 30 min the reaction was stopped using 5 mM Tris/HCl, pH 7.4 and scanned for densitometric quantitation.

2.7. Histology and Immunohistochemistry

Mice tissues were formalin-fixed and paraffin-embedded. Multiple sections (4 µm thickness) were deparaffinized with xylene and stained with hematoxylin and eosin (H&E). Immunohistochemistry was carried out in the same sections, using the following primary antibodies: anti-gliarial fibrillary acidic protein (GFAP) (Millipore, MAB380), anti-Neuronal Class III (β-tubulin (TUJ1)) (Covance, MMS-435P) and anti-8-hydroxy-2′-deoxyguanosine (8-OHdG) (QED Bioscience, 12501). Dako Animal Research Kit for mouse primary antibodies (Dako Diagnóstico S.A., Spain) was used for the qualitative identification of antigens by light microscopy. Sections were examined at 40–400 magnifications with an OLYMPUS CX41 microscope, and the images were scanned under equal light conditions with the CELL A computer program [9].

2.8. Statistical analysis

All statistical analyses were performed using the GraphPad scientific software. Data are expressed as the mean ± SD of seven–ten experiments per group. A one-way ANOVA with a Tukey post hoc test was used to
Table 1
Concentration of CoQ9 and CoQ10 in plasma of 3 months old mice after two months of treatment.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Plasma CoQ9 (μM)</th>
<th>Plasma CoQ10 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coq9+/+</td>
<td>0.27 ± 0.03</td>
<td>UND</td>
</tr>
<tr>
<td>Coq9XX + V</td>
<td>0.09 ± 0.01 **</td>
<td>UND</td>
</tr>
<tr>
<td>Coq9XX + Q10</td>
<td>0.07 ± 0.01 ***</td>
<td>1.36 ± 0.67</td>
</tr>
<tr>
<td>Coq9XX + Q3H2</td>
<td>0.05 ± 0.02 **</td>
<td>2.06 ± 0.65</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD of seven animals per group. V = vehicle; Q10 = ubiquinone-10; Q3H2 = ubiquinol-10. UND = undetectable.

** p < 0.01 versus Coq9+/+.

compare the differences between groups. A P-value of 0.05 was considered to be statistically significant.

3. Results

3.1. CoQ10 levels in plasma and tissues of Coq9XX after 2 months of treatment

We previously reported that Coq9XX mice showed a significant decrease in both CoQ9 (the major form of ubiquinone in rodents) and CoQ10 levels at 3 months of age compared with the age-mated Coq9+/+ mice in all examined tissues (cerebrum, cerebellum, heart, kidney, hind legs skeletal muscle and liver) [9]. Two months of ubiquinone-10 or ubiquinol-10 therapies increased plasma levels of CoQ10 in Coq9XX mice, while levels of CoQ9 did not change in the same animals (Table 1). Treatment with vehicle did not produce any effects in the plasma CoQ10 levels. In tissues, a significant increase of CoQ10 after ubiquinone-10 treatment was only detected in liver and muscle. On the contrary, CoQ10 levels were significantly increased in the cerebrum, cerebellum, heart, kidney, liver and hind leg skeletal muscle of Coq9XX mice treated with ubiquinol-10 (Fig. 1). The increase of CoQ10 levels after ubiquinone-10 or ubiquinol-10 treatments was in parallel to a decrease in the CoQ9/CoQ10 ratio (Fig. S1), which indicates that these therapies did not affect the CoQ9 levels (Fig. S2). Compared to the vehicle group, the highest increase of CoQ10 after ubiquinone-10 or ubiquinol-10 treatments was found in liver and muscle, followed by heart, kidney, cerebrum and cerebellum. Only muscle and heart of Coq9XX mice treated with ubiquinol-10 reached similar CoQ9 levels than that of Coq9+/+ mice, while liver accumulated huge amounts of CoQ10 after ubiquinone-10 or ubiquinol-10 treatment (Fig. 1).

3.2. CoQ levels in cerebral mitochondria of Coq9XX after 2 months of treatment

Because Coq9XX mice develop mitochondrial encephalopathy [9], we evaluated the effects of the therapies on mitochondrial CoQ levels and mitochondrial respiratory chain function in cerebrum of the mutant mice. Mitochondrial CoQ10 levels were significantly increased only after ubiquinol-10 treatment in Coq9XX mice, while vehicle or ubiquinone-10 supplementation did not increase the mitochondrial CoQ10 levels (Fig. 2A). Considering the total mitochondrial CoQ pool (CoQ9 + CoQ10), ubiquinol-10 treatment increased mitochondrial CoQ levels (Fig. 2B) and decreased the CoQ9/CoQ10 ratio after ubiquinol-10 treatment (Fig. 2C).

3.3. CoQ-dependent mitochondrial respiratory chain activities and supercomplex pattern in cerebral mitochondria of Coq9XX after 2 months of treatment

Cerebral mitochondria of Coq9XX mice treated with vehicle showed a significant decrease of CI + III and CI + III activities compared to those of Coq9+/+ mice (Fig. 2D and E). Similarly to the Pdes2ΔεΔεΔε mice, the decrease in CI + III activity was higher than the decrease in CI + III activity [14]. The increase of CoQ9 levels in cerebral mitochondria of
Coq\textsuperscript{9/9}\textsuperscript{+} mice after ubiquinol-10 treatment induced a significant increase of CII + III activity (Fig. 2D) and the normalization of CII + III activity (Fig. 2E). On the contrary, vehicle or ubiquinol-10 treatments did not change the CoQ-dependent respiratory chain activities (Fig. 2D and E). The increase of the CoQ-dependent mitochondrial respiratory chain activities after ubiquinol-10 treatment was not due to an increase in the supercomplex I/III formation because the ratio supercomplex I/III/free complex III remained low in Coq\textsuperscript{9/9}\textsuperscript{+} mice after vehicle, ubiquinol-10 or ubiquinol-10 treatment compared to Coq\textsuperscript{9/9}\textsuperscript{+/+} mice (Fig. 3A, B and C). The ratio supercomplex I/III/free complex I (Fig. S3A), as well as complex I in gel activity, was similar in Coq\textsuperscript{9/9}\textsuperscript{+/+} mice and Coq\textsuperscript{9/9}\textsuperscript{+} mice (Fig. S3B), while the treatments did not produce any changes on these variables (Fig. S3).

3.4. Histopathological evaluation and oxidative damage in brain of Coq\textsuperscript{9/9}\textsuperscript{+} after 2 months of treatment

Coq\textsuperscript{9/9}\textsuperscript{+} mice show white matter vacuolization, severe reactive astroglisis, reduction in neuronal dendrites and increased DNA oxidation, which were especially evident in diencephalon and brainstem [9]. The treatment with vehicle did not produce any change in these histopathological biomarkers because Coq\textsuperscript{9/9}\textsuperscript{+} animals similarly showed white matter vacuolization (Fig. 4C and D) and proliferation of astrocytes (Fig. 4K and L) in diencephalon, as well as increased DNA oxidation in diencephalon and septum–striatum (Fig. 5C, D, K and L) compared to Coq\textsuperscript{9/9}\textsuperscript{+/+} mice (Fig. 4A, B, I and J; Fig. 5A, B, I and J). Treatment with ubiquinol-10 did not reduce the vacuolization (Fig. 4E and F) and astrogliosis (Fig. 4M and N) in diencephalon of Coq\textsuperscript{9/9}\textsuperscript{+} mice, while the immunoreactivity against 8-OHdG was slightly decreased in both diencephalon (Fig. 5E and F) and septum–striatum (Fig. 5M and N). On the contrary, treatment with ubiquinol-10 was able to reduce the vacuolization (Fig. 4G and H) and astrogliosis (Fig. 4O and P) in diencephalon, as well as the DNA oxidation in both diencephalon (Fig. 5G and H) and septum–striatum (Fig. 5O and P). In brainstem, however, both ubiquinol-10 and ubiquinol-10 treatments were able to reduce the astrogliosis and DNA oxidation, as well as increase neuronal immunoreactivity (Figs. S4 and S5). Nevertheless, the vacuolization still persisted with both treatments (Figs. S4 and S5). The histology
structure of kidneys, muscle and heart was similar in all experimental groups (Fig. S6).

3.5. Consequences of the treatments in the animal weight

Cog9<sup>XX</sup> mice show a reduction in the body weight between the age of 1 and 5 months [9]. Oral supplementation with vehicle or ubiquinone-10 did not produce any significant effect in body weight. On the contrary, ubiquinol-10 treatment significantly increased the body weight in both male (Fig. 6A) and female (Fig. 6B) Cog9<sup>XX</sup> mice after two months of treatment, compared to Cog9<sup>XX</sup> mice treated with vehicle (Movie S1).

4. Discussion

Therapy based on oral supplementation with ubiquinone-10 has shown contradictory results in the treatment of primary and secondary CoQ<sub>10</sub> deficiencies, mitochondrial diseases and other neurological diseases like Parkinson’s Disease, Alzheimer’s Disease, Amyotrophic Lateral Sclerosis or Huntington’s Disease [2–7]. These controversial results may be due to the poor absorption and bioavailability of ubiquinone-10 [7,10,15]. In this study, we demonstrate that a water-soluble formulation of ubiquinol-10, the reduced form of CoQ<sub>10</sub>, was more effective than that of ubiquinone-10 in increasing the levels of CoQ<sub>10</sub> in tissue homogenates and cerebral mitochondria, resulting in an increase of CoQ-dependent respiratory chain activities in the cerebrum of a CoQ deficient mouse model with mitochondrial encephalopathy (Cog9<sup>XX</sup> mice). As a consequence, ubiquinol-10 was more efficient than ubiquinone-10 in reducing the vacuolization, astrogliosis and oxidative damage in Cog9<sup>XX</sup> mice, thus increasing the animal body weight.

Ubiquinone-10 in its pure form is a powder product that is insoluble in water and has partial solubility in lipids and organic solutions, and therefore it is poorly absorbed. The uptake of ubiquinone-10 is very low in brain because of the blood brain barrier. Moreover, the limitation of the exogenous ubiquinone-10 to reach mitochondria is one of the major problems for the CoQ<sub>10</sub> therapy because external ubiquinone-10 is distributed mainly in lysosomes and only a small amount, if any, is found in mitochondria [10,15]. To try to increase the absorption of ubiquinone-10, different ubiquinone-10 formulations have been manufactured and are currently available on the market. These formulations include powder-based compressed tablets, chewable tablets, powder-filled hard-shell capsules, softgels containing an oil suspension and water-soluble formulations in softgel or liquid forms [8]. The latter forms are based in the ability of dextrins to increase the solubility of poorly water-soluble compounds with no toxic effects [16]. Our study shows that water-soluble formulation of ubiquinone-10 in a dose of 240 mg/kg bw/day, which is equivalent to 30 mg/kg bw/day in humans [17,18] according to the body surface area [19], is able to increase CoQ<sub>10</sub> levels in plasma of CoQ<sub>10</sub> mice. The increase of plasma CoQ<sub>10</sub> concentration in Cog9<sup>XX</sup> mice was reflected in an increase of CoQ<sub>10</sub> levels in tissues of treated mice. The lowest increase of CoQ<sub>10</sub> in cerebrum and cerebellum may be due to the blood brain barrier. The highest increase of CoQ<sub>10</sub> in the liver may be explained by its mechanism of absorption in the gastrointestinal system. After gastric emptying, CoQ<sub>10</sub> is absorbed along with other lipids as chylomicron particles in the small intestine, and transported via lymph vessels to blood circulatory system and then taken up by the liver cells. In the liver, CoQ<sub>10</sub> is incorporated with lipoproteins and released into the blood, which is used as a transport vehicle to deliver CoQ<sub>10</sub> in other tissues [20,21]. In another study, using ubiquinone-10 in oil suspension (LiQsorb, Tishcon) in doses of 200 and 400 mg/kg bw/day for 3–4 months, the authors did not find any increase of CoQ<sub>10</sub> in kidneys of a mouse model of CoQ deficiency due to Pbs<sub>s</sub>2 mutation (Pbs<sub>s</sub>2<sup>lakd<sup>b</sup></sup>) [22]. Similarly, the water-soluble formulation of ubiquinone-10 used in our study did not show a significant increase in the levels of CoQ<sub>10</sub> in kidney, suggesting that the vehicle does not affect the absorption of ubiquinone-10 at tissue level.

Our study also shows that ubiquinol-10 has better absorption, bioavailability and tissue uptake than ubiquinone-10 [23,24]. Importantly, the increase of CoQ<sub>10</sub> after ubiquinol-10 treatment is not limited to tissue levels because CoQ<sub>10</sub> is also increased in mitochondria from the cerebrum of Cog9<sup>XX</sup> mice. As a consequence, ubiquinol-10 supplementation was able to increase the CoQ-dependent mitochondrial respiratory chain.
activities, while the oxidized form did not have this effect at mitochondrial level. However, Cl −/III activity in cerebrum of Cog7K−/− mice treated with ubiquinol-10 was still half of the activity in Cog9−/− mice. This result may be explained by the fact that ubiquinol-10 treatment was not able to normalize the supercomplex/free CLI ratio (Fig. 3A) [9], and this may be justified by two possibilities: a) the accumulation of 5-demethoxyubiquinone-9 in Cog9K−/− mice, which was not corrected by the treatments, could partially inhibit the transfer of electrons from Cl to CoQ, as it has been reported in Caenorhabditis elegans mutant clk-1 (analog to Cog7 in human and mouse) [25]; and b) the increase of total CoQ (CoQ9 + CoQ10) after ubiquinol-10 treatment did not reach the required total CoQ levels in the Q binding sites of Cl and CLI. Humans and mice have two CoQ forms, CoQ9 and CoQ10, which differ with each other in the length size of the polyphenyl tail. While the reason to synthesize two CoQ forms is not clear and some functions are attributed indistinctly to the two forms, an adequate CoQ9/CoQ10 ratio may be necessary for an optimal performance of mitochondrial bioenergetics, including a physiological proportion of CLI free and CLI bound to supercomplexes. In fact, each tissue has a particular value on CoQ9/CoQ10 ratio, with cerebrum and cerebellum being the tissues with lowest CoQ9/CoQ10 ratio in mouse (highest in human) [26]. This fact points out that CoQ9/CoQ10 ratio seems to be tightly regulated in a tissue specific way. In Cog9K−/− mice, a decrease in CoQ9/CoQ10 ratio was detected in cerebrum and cerebellum homogenates, as well as in isolated mitochondria from cerebrum (Figs. S1 and 2C), and the ratio was even lower after ubiquinol-10 treatment (Figs. S1 and 2C). Future therapeutic strategies focused in increasing the endogenous CoQ9 and CoQ10 biosynthesis could contribute to understand the importance of CoQ9 in mitochondrial bioenergetics.

In addition to its bioenergetics role, CoQ10 is one of the most important endogenous antioxidants in the cell [1]. CoQ10 deficiency is accompanied with an increased production of reactive oxygen species (ROS) and oxidative damage to biomolecules, which leads to an increased cell death in vitro [27,28] and in vivo [14]. Cog9K−/− mice also show an increase of 8-OHdG in diencephalon, septum–striatum and brainstem [9] (Figs. 5 and 4S). Both ubiquinone-10 and ubiquinol-10 reduced the immunostaining against 8-OHdG, but the reduction was higher with ubiquinol-10. This result may reflect the higher uptake of ubiquinol-10 compared to ubiquinone-10, its higher antioxidant capacity and/or an effect in reducing the leak of electrons through the mitochondrial respiratory chain.

Following the biochemical changes after the treatments, supplementation with ubiquinol-10 reduced the vacuolization and astrogliosis in diencephalon, septum–striatum and, to a lesser extent, in brainstem of Cog9K−/− mice. The lower efficiency of ubiquinol-10 treatment in reducing the histopathological changes in brainstem of Cog9K−/− mice compared to diencephalon and septum–striatum may be related to an early and irreversible damage in this area, which is particularly susceptible in Leigh
syndrome [29,30]. Notably, ubiquinol-10 was more powerful than ubiquinone-10 in reducing the histopathological changes in Coq9−/− mice, resulting in an increase in the body weight. These results are particularly important because patients with CoQ10 deficiency showed variable responses to ubiquinone-10 treatment and, in some cases, the treatment failed or did not show a clear response [31–40], which may be due to the reduced uptake of ubiquinone-10 [7]. Thus, our results suggest that ubiquinol-10 supplementation could improve the efficacy showed by ubiquinone-10 supplementation, which will be especially important in patients with encephalopathy or cerebellar ataxia associated to CoQ10 deficiency. In agreement with that, in a patient with CoQ10 deficiency, mental retardation, encephalopathy and dimorphic features due to a COQ4 mutation, ubiquinol-10 in a dose of 15 mg/kg bw/day had the same efficacy than ubiquinone-10 in a dose of 30 mg/kg bw/day [18].

5. Conclusions

Our results demonstrate that dextrin-based water-soluble formulations of ubiquinol-10 have better absorption and uptake at tissue and mitochondrial levels, which results in an increase of CoQ-dependent respiratory chain activities, reduction in vacuolization, astrogliosis and oxidative damage in different brain areas, and an increase of body weight in a CoQ deficient mouse model with mitochondrial encephalopathy. This data suggest that water-soluble formulations of ubiquinol-10 should be preferentially used for CoQ10 therapy. However, ubiquinol-10 supplementation did not completely rescue the encephalopathic phenotype of the Coq9−/− mouse model. Considering that mice and humans produce both CoQ9 and CoQ10, future therapeutic strategies focused in increasing both CoQ9 and CoQ10 levels could lead to obtain better results.

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Competing interest statement

None of the authors have competing interests to declare.

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