Short communication

Effect of Coenzyme Q_{10} supplementation on mitochondrial electron transport chain activity and mitochondrial oxidative stress in Coenzyme Q_{10} deficient human neuronal cells

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A B S T R A C T

Primary Coenzyme Q_{10} (CoQ_{10}) deficiency is an autosomal recessive disorder with a heterogeneous clinical presentation. Common presenting features include both muscle and neurological dysfunction. Muscle abnormalities can improve, both clinically and biochemically following CoQ_{10} supplementation, however neurological symptoms are only partially ameliorated. At present, the reasons for the refractory nature of the neurological dysfunction remain unknown. In order to investigate this at the biochemical level we evaluated the effect of CoQ_{10} treatment upon a previously established neuronal cell model of CoQ_{10} deficiency. This model was established by treatment of human SH-SY5Y neuronal cells with 1 mM para-aminobenzoic acid (PABA) which induced a 54% decrease in cellular CoQ_{10} status. CoQ_{10} treatment (2.5 μM) for 5 days significantly (p < 0.0005) decreased the level of mitochondrial superoxide in the CoQ_{10} deficient neurons. In addition, CoQ_{10} treatment (5 μM) restored mitochondrial membrane potential to 90% of the control level. However, CoQ_{10} treatment (10 μM) was only partially effective at restoring mitochondrial electron transport chain (ETC) enzyme activities. ETC complexes II/III activity was significantly (p < 0.05) increased to 82.5% of control levels. ETC complexes I and IV activities were restored to 71.1% and 77.7%, respectively of control levels.

In conclusion, the results of this study have indicated that although mitochondrial oxidative stress can be attenuated in CoQ_{10} deficient neurons following CoQ_{10} supplementation, ETC enzyme activities appear partially refractory to treatment. Accordingly, treatment with >10 μM CoQ_{10} may be required to restore ETC enzyme activities to control level. Accordingly, these results have important implication for the treatment of the neurological presentations of CoQ_{10} deficiency and indicate that high doses of CoQ_{10} may be required to elicit therapeutic efficacy.

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1. Introduction

Coenzyme Q_{10} (CoQ_{10}) functions as an electron carrier in the mitochondrial electron transport chain (ETC) as well as serving as a potent lipid soluble antioxidant (1). Primary CoQ_{10} deficiency is an autosomal recessive disorder with a heterogeneous clinical presentation. There are currently five distinct clinical phenotypes described: encephalomyopathy; severe infantile multi-systemic disease; nephropathy; cerebellar ataxia and isolated myopathy (Rahman et al., 2012). In most cases the family history suggests an autosomal recessive mode of inheritance. To date mutations in seven genes encoding components closely related with the CoQ_{10} biosynthetic pathway have been associated with primary human CoQ_{10} deficiency (Rahman et al., 2012; Emmanuele et al., 2012). Whilst the muscle symptoms associated with CoQ_{10} deficiency have been reported to improve in most cases upon CoQ_{10} supplementation, the neurological symptoms appear to be only partially ameliorated (Rahman et al., 2012). At present the reasons for the refractory nature of the neurological symptoms associated with CoQ_{10} deficiency to CoQ_{10} supplementation remain to be elucidated. However they may include; poor transfer of CoQ_{10} across...
the blood-brain barrier, irreversible structural and biochemical neuronal dysfunction or an inability of CoQ10 deficient neurones to utilise exogenous CoQ10. In order to investigate the effect of CoQ10 treatment at the biochemical level we have established a neuronal cell model of CoQ10 deficiency by treatment of neuronal SH-SYSY cells with para-aminobenzoic acid (PABA) (Duberley et al., 2013). PABA is a competitive inhibitor of the CoQ10 biosynthetic pathway enzyme, CoQ2 (Alam et al., 1975). 1 mM PABA treatment resulted in a 54% decrease in neuronal CoQ10 status which was accompanied by a global loss of ETC complex activities, a fourfold increase in mitochondrial oxidative stress and a potential reversal of ETC complex V activity (Duberley et al., 2013).

In this study we evaluated the effect of CoQ10 supplementation upon ETC function and mitochondrial oxidative stress in CoQ10 deficient neuronal cells.

2. Materials and methods

2.1. Materials

All reagents were of analytical grade and obtained from either Sigma–Aldrich Chemical (Poole, UK) or BDH Laboratory Supplies (Poole, UK).

2.2. Cell culture

The SH-SYSY neuroblastoma cell line was purchased from the European Collection of Cell Cultures (Health Protection Agency, Salisbury, UK). They are a thrice cloned cell line with a neuroblast-like morphology. The SK-N-SH cell line, from which they were originally cloned, has demonstrated various neurotransmitter activities: dopaminergic, acetylcholinergic and adenosinergic (Biedler et al., 1978).

SH-SYSY cells were treated with PABA as described by Duberley et al. (2013) over a 5 day incubation period. Following the 5 day treatment of the SH-SYSY cells with PABA (1 mM) the cells were then treated with CoQ10 (2.5, 5 or 10 µM) for 5 days with a further addition of 1 mM PABA. CoQ10 was solubilised in ethanol and to ensure optimal absorption was then incubated with media at 37 °C for 15 min prior to addition of the SH-SY-SY cells.

2.3. Quantification of CoQ10 status

The CoQ10 status of SH-SYSY cells was determined by reverse phase HPLC with UV detection at 275 nm according to the method of Duncan et al. (2005).

2.4. ETC enzyme activities

The activities of the ETC complex I (NADH:ubiquinone reductase; EC 1.6.5.3) II/III (succinate: cytochrome c reductase; EC 1.3.5.1 + EC 1.10.2.2) and IV (cytochrome c oxidase; EC 1.9.3. 1) were determined spectrophotometrically according to the methods described in Duberley et al. (2013). All ETC activities were expressed as a ratio to citrate synthase activity to account for mitochondrial enrichment (Selak et al., 2000).

2.5. Mitochondrial oxidative stress assessment

Mitochondrial superoxide levels were quantified using a mitochondrial targeted DHE derivative, Mitoxo™ (5 µM; Molecular Probes, Eugene, OR) as described by Duberley et al. (2013).

2.6. Mitochondrial membrane potential

Estimation of mitochondrial membrane potential (ΔΨm) performed using tetramethylrhodamine methyl ester (TMRM) in the SH-SYSY cells as described by Duberley et al. (2013).

2.7. Total protein determination

The protein status of the SH-SHSY cells was as determined according to the method of Lowry et al. (1951).

2.8. Statistical analysis

All results are expressed as mean ± standard error of the mean (SEM). One-way ANOVA was used for comparison of groups >2, with Bonferroni post hoc analysis. In all cases p < 0.05 was considered significant.

3. Results

CoQ10 treatment concentrations of 2.5, 5 and 10 µM were selected for investigation in this study as these are the approximate concentration ranges reached in the plasma of patients treated with oral CoQ10 supplementation (Bhagavan and Chopra, 2006; Miles, 2007).

The endogenous CoQ10 status of control cells (SH-SYSY cells untreated with PABA) increased significantly following a 5 day incubation with exogenous CoQ10. Increasing 9-fold (p < 0.005) following incubation with 2.5 µM CoQ10; 19-fold (p < 0.005) following incubation with 5 µM CoQ10 and 50-fold (p < 0.005) following incubation with 10 µM CoQ10 (Fig. 1). CoQ10 deficient neurons were also found to display a similar degree of exogenous CoQ10 uptake as the controls indicated by their increase in cellular CoQ10 status following CoQ10 treatment (Fig. 2).

Incubation of CoQ10 deficient neurons with exogenous CoQ10 (2.5, 5 and 10 µM) resulted in a progressive increase in ETC activities. ETC complex I, complexes II/III and complex IV activities increased from 38%, 46% and 31%, respectively of control levels to a maximum of 71%, 82% and 78% of control levels, respectively following treatment with 10 µM CoQ10 (Fig. 2).

Mitochondrial oxidative stress significantly (p < 0.0005) decreased in the CoQ10 deficient neurons following treatment with 2.5 and 5 µM CoQ10, falling below control levels (Fig. 2).

Following treatment with CoQ10, the mitochondrial membrane potential of the CoQ10 deficient neurons significantly decreased (p < 0.0005) from 120% to 65% of the control level with 2.5 µM CoQ10. Furthermore, treatment of the CoQ10 deficient neurons with CoQ10 improved the mitochondrial membrane potential to 65% of the control level with 2.5 µM CoQ10. Assessment of the mitochondrial membrane potential demonstrated a 9-fold increase in the ΔΨm of the CoQ10 treated SH-SYSY cells compared to those treated with PABA alone (2.5 µM).

![Fig. 1.](image_url) Cellular CoQ10 status of control SH-SYSY cells following a 5 day incubation with 0, 2.5, 5 and 10 µM exogenous CoQ10. Error bars represent standard error of the mean (SEM); statistical analysis was carried out using one-way ANOVA with Bonferroni post hoc analysis; levels of significance: **p < 0.005, ***p < 0.0005.
5 μM CoQ10 resulted in a significant (p < 0.005) 30% decrease into mitochondrial membrane potential to 90% of the control level (Fig 2).

4. Discussion

CoQ10 deficiency is a rare but often treatable disorder, however the neurological symptoms associated with the disorder are frequently refractory to treatment (Rahman et al., 2012; Emmanuele et al., 2012). In order to investigate this phenomena at the biochemical level in this study we have evaluated the effect of CoQ10 supplementation upon mitochondrial function and oxidative stress in CoQ10 deficient neurons.

The results of the present study have demonstrated a dramatic increase in cellular CoQ10 status following supplementation (2.5, 5 and 10 μM CoQ10) indicating that neurons are able to take up exogenous CoQ10 and that poor cellular uptake of this quinone may not be a contributory to the refractory nature of the neurological symptoms associated with CoQ10 deficiency. In the CoQ10 deficient neurons, cellular uptake of exogenous CoQ10 was accompanied by an increase in ETC complexes I, II/III and IV activities. However, CoQ10 treatment failed to restore ETC activities to control levels. This suggests the possibility that insufficient CoQ10 was reaching the inner mitochondrial membrane and therefore higher doses of CoQ10 maybe required to ameliorate the deficit in ETC activity. The inability of CoQ10 even at a concentration CoQ10 of 10 μM to fully restore ETC activity maybe the result of the reported low mitochondrial uptake of exogenous CoQ10, with only approximately 11% of this quinone being reported to reach the mitochondria (Bentinger et al., 2003). The preponderance of exogenous CoQ10 is distributed to other organelles including the plasma membrane, endoplasmic reticulum and the lysosomes (Bentinger et al., 2003). In this study we did not assess ETC complexes I–III (NADH: cytochrome reductase; EC 1.6.5.3 + 1.10.2.2) since it has been previously reported that complexes II and III activity maybe a more sensitive marker of a diminution of CoQ10 status (Montero et al., 2008). Although determination of complexes II and III activity assesses the integrated function of complexes II and III no assessment of the individual activities of these enzymes was undertaken in this study and further work is required to evaluate the effect of a CoQ10 deficiency upon their individual activities. The similar migration patterns and protein levels of ETC complexes I, III and V of the CoQ10 deficient neurons compared to controls following Blue Native Gel electrophoresis indicated that at least for these ETC complexes a CoQ10 deficiency had not resulted in a decreased stability or impaired assembly of these enzymes (Duberley et al., 2013). Therefore, in this study no assessment of the effect of CoQ10 supplementation was undertaken on the stability of the ETC complexes in the CoQ10 deficient neurons. However, further work will be required to assess the effect of a neuronal CoQ10 deficiency upon the stability of ETC complex II (succinate: ubiquinone reductase; EC: 1.3.5.1) and complex IV.

In cells with normal oxidative phosphorylation the mitochondrial membrane potential is maintained by the proton pumping activity of the ETC (Abramov et al., 2010). However, if the ETC is impaired as has occurred in the CoQ10 deficient neurons in the
present study then ATP synthase (complex V) may start to work in reverse, hydrolysing ATP and pumping protons across the inner mitochondrial membrane to maintain the membrane potential (Abramov et al., 2010). A reversal of complex V activity has been associated with an increase in mitochondrial membrane potential to above control levels in cells harbouring mitochondrial DNA mutations in ETC complex I (Abramov et al., 2010). Furthermore, in a previous study by Duberley et al. (2013), a rapid decrease in mitochondrial membrane potential following treatment of the CoQ10 deficient neurons with oligomycin (specific complex V inhibitor) was observed indicating that a reversal of complex V activity was maintaining the supra-control level of mitochondrial membrane potential in these cells. Following CoQ10 treatment of the CoQ10 deficient neurons the accompanying increase in ETC enzyme activities (Fig. 2) would enable the mitochondrial membrane potential to be entirely maintained by the proton pumping capacity of the ETC rather than the reversal of complex V activity. This transition would be accompanied by a decrease in membrane potential from the supra-control level resulting from reversal of complex activity to a level reflecting the proton pumping capacity of the ETC which in this study only reached 90% of the control level following treatment with 5 μM CoQ10.

Davey et al. (1998) reported that a deficiency in neuronal ETC complex I activity to 75% of control level may be sufficient to perturb oxidation phosphorylation. Therefore, the maximal restoration of ETC complex I activity to 71% of control levels following supplementation of the CoQ10 deficient neuronal cells with 10 μM CoQ10 (Fig. 2) may suggest that oxidative phosphorylation is still compromised within these cells. This is supported by the fact that mitochondrial membrane potential does not reach control levels following CoQ10 supplementation. However, quantification of cellular ATP status would be required to confirm/refute this hypothesis.

CoQ10 supplementation (2.5 and 5 μM) was effective at significantly reducing mitochondrial oxidative stress (p < 0.0005) to below control levels. In contrast, CoQ10 supplementation at these concentrations was unable to fully restore ETC activities to control levels. This disparity may result from the different concentrations of CoQ10 required in the inner mitochondrial membrane to facilitate both its ETC and antioxidant function, the latter possibly requiring a lower concentration of the quinone. Furthermore, CoQ10 present in the outer mitochondrial membrane may also contribute to the lowering of mitochondrial oxidative stress within this organelle (Bentingger et al., 2007). Although reactive oxygen species (ROS) have been frequently associated with cellular damage they have important roles in physiological signalling pathways (Thannickal and Fanburg, 2006). Therefore, a decrease in ROS levels to below control levels may be detrimental to cellular function and further work is required to investigate this phenomena. The persistence of the ETC deficiency in the absence of increased mitochondrial oxidative stress following CoQ10 supplementation may indicate that other factors in addition to oxidative damage to the protein subunits of the ETC complexes and the membrane phospholipids maybe contributing to the global loss of ETC activity associated with neuronal CoQ10 deficiency (Kowaltowski and Versaci, 1999; Duberley et al., 2013). CoQ10 is an essential cofactor for the inner mitochondrial enzyme, dihydroorotate dehydrogenase which is involved in de novo pyrimidine synthesis (López-Martin et al., 2007). Since defects in nucleotide metabolism have been associated with mitochondrial DNA depletion, impairment of dihydroorotate dehydrogenase activity as the result of a paucity in CoQ10 availability may be a contributory factor to the global loss of ETC activity (López-Martin et al., 2007; Duberley et al., 2013).

In conclusion, the results of the supplementation study have indicated that the plasma CoQ10 level of ≥10 μM may be required to elicit a significant improvement in neuronal ETC activity. This level of plasma CoQ10 may however be higher than that achievable with current oral CoQ10 formulations. Furthermore, at present it is uncertain whether plasma levels of CoQ10 following supplementation represents those of CSF, in view of the reported limited transfer of CoQ10 across the blood brain barrier.

Confict of interest

None.

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