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Evaluating the therapeutic potential of idebenone and related quinone analogues in Leber hereditary optic neuropathy

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ABSTRACT

Leber hereditary optic neuropathy (LHON) is an important cause of mitochondrial blindness among young adults. In this study, we investigated the potential of four quinone analogues (CoQ_1, CoQ_{10}, decylubiquinone and idebenone) in compensating for the deleterious effect of the m.11778G>A mitochondrial DNA mutation. The LHON fibroblast cell lines tested exhibited reduced cell growth, impaired mitochondrial bioenergetics and elevated levels of reactive oxygen species (ROS). Idebenone increased ATP production and reduced ROS levels, but the effect was partial and cell-specific. The remaining quinone analogues had variable effects and a negative impact on certain mitochondrial parameters was observed in some cell lines.

1. Introduction

Leber hereditary optic neuropathy (LHON) is a primary mitochondrial DNA (mtDNA) disorder characterised by bilateral sequential or simultaneous visual loss in young adults (Man et al., 2002). Affected patients develop a dense central scotoma and visual acuity deteriorates rapidly to 20/200 or worse. Three point mutations within the mitochondrial genome (m.3460G>A, m.11778G>A and m.14484T>C) account for about 90% of all cases, with the m.11778G>A mutation being by far the most prevalent (60–80%) cause of LHON worldwide (Newman and Biousse, 2004; Yu-Wai-Man et al., 2011). An abiding mystery of this disorder is why these mtDNA mutations have such a marked pathological predilection for retinal ganglion cells (RGCs) and their projecting axons within the optic nerve (Carelli et al., 2004). Spontaneous visual recovery has been reported in up to 25% of patients affected with LHON and the milder m.14484T>C mutation carries a relatively better visual prognosis (Newman and Biousse, 2004; Yu-Wai-Man et al., 2011). However, visual recovery is invariably incomplete and the majority of affected LHON carriers will remain severely visually impaired and classified as legally blind. The peak age of onset is in the second and third decades of life and the sudden onset of mostly irreversible visual loss in otherwise healthy young individuals has major socioeconomic consequences (Kirkman et al., 2009a). Treatment options for LHON remain limited and management is largely supportive, which is a frustrating situation for patients and their families, and the clinicians overseeing their care.

LHON has an estimated prevalence of 1 in 30,000 and there are considerable challenges, both practical and financial, in conducting adequately powered randomised controlled trials, which remain the gold standard for establishing the therapeutic efficacy and safety of a proposed intervention (Gorman et al., 2015; Man et al., 2003). Varying combinations of high-dose vitamins and supplements with putative mitochondrial antioxidant properties have been given to patients with LHON in the hope of improving the visual prognosis, but none of these drug cocktails are properly evidence-based (Pfeffer et al., 2013;

Abbreviations: ATP, adenosine triphosphate; CCCP, carbonylcyanide-3 chlorophenylhydrazone; CoQ_1, coenzyme Q_1; CoQ_{10}, coenzyme Q_{10}; DCF, 2′,7′-dichlorodihydrofluorescein diacetate; DQ, decylubiquinone; GAL, restrictive, galactose medium; GLU, permissive, high-glucose medium; Idb, idebenone; LHON, Leber hereditary optic neuropathy; MB, methylene blue; MRS, magnetic resonance spectroscopy; mtDNA, mitochondrial DNA; MTG, MitoTracker Green; OCR, oxygen consumption rate; OXPHOS, oxidative phosphorylation; PARP, poly (ADP-ribose) polymerase; PBS, phosphate buffered saline; RCR, respiratory control ratio; RFU, relative fluorescent unit; RGC, retinal ganglion cell; ROS, reactive oxygen species; SEM, standard error of mean; TMRE, tetramethylrhodamine ethyl ester.

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Yu-Wai-Man et al., 2014). A promising class of compounds for LHON is the ubiquinone family of molecules, in particular idebenone. Most of the mtDNA mutations that are known to cause LHON affect complex I subunits and as a result, there is impaired transfer of high-energy electrons to complex III, which is an essential step in mitochondrial oxidative phosphorylation (OXPHOS) (Carelli et al., 2004). The structural and functional defect of complex I triggers a downstream cascade of events, which ultimately compromises RGCs and precipitate progressive optic nerve neurodegeneration. There is ongoing debate whether the bioenergetic deficit in LHON directly commits RGC to cell death or whether it is driven primarily by increased reactive oxygen species, or possibly both having a synergistic deleterious impact (Carelli et al., 2004; Levin, 2015; Yu-Wai-Man et al., 2011). Irrespective of this unresolved point of contention, an attractive dual strategy would be to improve electron transfer along the mitochondrial respiratory chain to maximise OXPHOS and ATP production, whilst minimising in parallel ROS levels.

Coenzyme Q_{10} (CoQ_{10}) is natural lipid-soluble quinone analogue and as a result of its intrinsic hydrophobic properties, it freely circulates within the mitochondrial inner membrane (Hargreaves, 2014). The molecule contains a redox active benzoquinone ring that is conjugated to an isoprenoid side chain consisting of 10 isoprenyl units, with the actual number of these units forming the basis of the biochemical nomenclature. In its reduced form, CoQ_{10} is the predominant electron carrier of the mitochondrial respiratory chain and it mediates the efficient shuttling of electrons from complexes I and II, and other flavoprotein dehydrogenases, to complex III. As a result, oral supplementation of CoQ_{10} has been used for a broad range of mitochondrial OXPHOS diseases, but with the exception of patients with primary CoQ_{10} deficiency, there is no convincing evidence of any clear benefit (Hargreaves, 2014). A major limitation of CoQ_{10} is its inability to cross the blood-brain barrier and to rectify this biophysical constraint, newer-generation quinone analogues have been developed to increase the bioavailability of the active moiety and potentially maximise its therapeutic potential. One such compound is idebenone (2,3-dimethoxy-5-methyl-6-(10-hydroxydecyl)-1,4-benzoquinone), which has a shorter side chain and is a hydrosoluble molecule compared with CoQ_{10} (Imada et al., 1989; Nagai et al., 1989). Idebenone has shown promise as a treatment modality for patients with visual loss secondary to LHON, but only a subgroup of patients with the m.11778G>A mutation seems to benefit, and there is still uncertainty about the magnitude of the visual benefit when compared with the natural history of the disease (Carelli et al., 2011; Klopstock et al., 2013; Klopstock et al., 2011; Newman, 2011).

In order to obtain pre-clinical data and support the case for early-phase clinical trials, we have optimised an *in vitro* functional test panel to investigate the effects of candidate drug molecules on key aspects of mitochondrial function with the use of patient-derived primary fibroblasts (Golubitzky et al., 2011; Soiferman et al., 2014). As reported previously for patients with nuclear-encoded complex I respiratory chain disorders, such an experimental approach can provide not only new insights into disease mechanisms, but it is also an attractive, cost-effective approach for targeted drug screening. In this study, we have made use of primary fibroblast cell lines carrying the m.11778G>A mutation to firstly explore the pathological consequences of this mtDNA mutation on mitochondrial function and cell survival, and secondly, to investigate whether supplementation with various quinone analogues could potentially rescue (or alternatively exacerbate) the observed disease phenotype.

2. Materials and methods

2.1. Patients and fibroblast cell lines

Primary fibroblast cell cultures (LHON A-D) were established from skin biopsies obtained from four unrelated white Caucasian affected male LHON carriers harbouring the m.11778A>G mutation at homoplasmic levels (Table 1). All four patients presented with a classical pattern of visual loss characterised by bilateral sequential optic neuropathy and rapid painless visual deterioration to counting fingers (CF) or worse. None of them were treated with CoQ_{10}, idebenone or other quinone analogues after a confirmed molecular diagnosis had been made and prior to a skin biopsy being taken. One patient (LHON-B) experienced a significant amount of visual recovery that was, however, limited to his right eye. Visual acuity in that eye improved from CF at the nadir to 20/30 about one year after first disease onset and in the absence of any specific treatment. All the skin biopsies were taken after the onset of visual loss with the patient's informed consent. This study had the relevant institutional approval and it complied with the Declaration of Helsinki.

Table 1
Clinical details of affected LHON carriers.

Patient	Onset (Yrs)[a]	Smoker[b]	Visual recovery[c]	Visual acuity[d]	
				RE	LE
LHON-A	21	No	No	HM	CF
LHON-B	18	No	Yes	20/30	CF
LHON-C	45	Yes	No	CF	CF
LHON-D	18	No	No	HM	HM

CF = counting fingers; HM = hand movements; LE = left eye; RE = right eye; Yrs = years.
[a] Age of onset of visual loss.
[b] Smoking status prior to disease onset.
[c] Spontaneous visual recovery with none of the patients having been treated with quinone analogues following disease onset.
[d] Best-corrected visual acuity at the last clinic visit, which was at a similar level at the nadir except for the right eye of patient LHON-B.

2.2. Materials

Aliquots of coenzyme Q_1 (CoQ_1), coenzyme Q_{10} (CoQ_{10}), decylubiquinone (DQ, Sigma-Aldrich, Rehovot, Israel), and idebenone (Idb, Santhera Pharmaceuticals, Liestal, Switzerland) were kept frozen as 10 mM stock solutions in DMSO. MTG (Molecular Probes, Eugene, Oregon, USA), TMRE and DCF (Biotium, Harvard, CA, USA) were diluted and stored according to the manufacturer's instructions. Unless otherwise stated, reagents were obtained from Sigma-Aldrich (Rehovot, Israel).

2.3. Tissue culture and experimental conditions

Fibroblasts were maintained in permissive DMEM (Biological Industries, Kibbutz Beit Haemek, Israel) medium containing 4.5 g glucose (GLU) per liter and supplemented with 10% fetal calf serum, 50 μg/ml uridine, and 110 μg/ml pyruvate at 37 °C under 5% CO_2 conditions. Cells were seeded in triplicates at a concentration of 4 × 103 cells/100 μl on four identical 96 well microtiter plates. The following day, the medium was removed and the wells were washed once with phosphate buffered saline (PBS) before the addition of fresh DMEM (GLU) medium or a restrictive glucose-free DMEM medium to prevent ATP production from glycolysis (Biological Industries, Kibbutz Beit Haemek, Israel) supplemented with 10% dialyzed fetal calf serum and 5 mM galactose (GAL). Quinone analogues at a final concentration of 1 μM were added to the growth medium as previously described (Golubitzky et al., 2011; Soiferman et al., 2014). Cell growth and mitochondrial function were assessed after a 72-hour incubation period.

2.4. Assessment of cell growth

Cell growth was assessed by measuring cellular content with a colorimetric method based on methylene blue (MB) staining of basophilic cellular components, which is independent of redox status (Pelletier et al., 1988). Cells in the microtiter wells were fixed with glutaraldehyde