Treatment Strategies for Leber Hereditary Optic Neuropathy

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Purpose of review

Leber hereditary optic neuropathy (LHON) is the most common primary mitochondrial DNA (mtDNA) disorder in the population and it carries a poor visual prognosis. In this article, we review the development of treatment strategies for LHON, the evidence base and the areas of unmet clinical need.

Recent findings

There is accumulating evidence that increasing mitochondrial biogenesis could be an effective strategy for protecting retinal ganglion cells (RGCs) in LHON. A number of clinical trials are currently investigating the efficacy of viral-based gene therapy for patients harbouring the m.11778G>A mtDNA mutation. For female LHON carriers of childbearing age, mitochondrial replacement therapy is being offered to prevent the maternal transmission of pathogenic mtDNA mutations.

Summary

Although disease-modifying treatment options remain limited, a better understanding of the underlying disease mechanisms in LHON is paving the way for complementary neuroprotective and gene therapeutic strategies for this mitochondrial optic nerve disorder.

Keywords: Gene therapy; idebenone; Leber hereditary optic neuropathy; mitochondrial disease; retinal ganglion cells.

Key Points

- Leber hereditary optic neuropathy (LHON) is characterized by the preferential loss of retinal ganglion cells (RGCs) within the papillomacular bundle resulting in a dense central scotoma.
- Agents that increase mitochondrial biogenesis have shown promise in mitigating the deleterious effects of the LHON mitochondrial DNA (mtDNA) mutations in the cell models studied.
- Gene therapy based on allotopic expression of wild-type *MTND4* is currently being investigated for patients carrying the m.11778G>A mtDNA mutation.
- Mitochondrial replacement therapy has been developed to prevent the maternal transmission of pathogenic mtDNA mutations.

Introduction

LHON (OMIM 535000) is the most common primary mitochondrial DNA (mtDNA) disorder with a prevalence of 1 in 31 000 – 50 000 in Northern European populations and an estimated incidence of 1 in 1,000,000 in the Japanese population [1–3]. Three point mutations within the mitochondrial genome, m.3460G>A (*MTND1*) m.11778G>A (*MTND4*), and m.14484T>C (*MTND6*), account for about 90% of all LHON cases. These mtDNA mutations disrupt critical complex I subunits of the mitochondrial respiratory chain, causing impaired cellular ATP synthesis and increased production of reactive oxygen species (ROS) [2,4]. The remaining 10% of LHON cases harbour rarer pathogenic mtDNA mutations, which individually have been reported in only a limited number of patients worldwide [4–6]. Furthermore, there is a subset of patients with a LHON-like presentation who are not found to harbour any clear pathogenic variants on whole mtDNA sequencing. Some of these cases could be caused by a combination of multiple mtDNA variants that synergistically trigger retinal ganglion cell (RGC) loss or deleterious variants in nuclear-encoded genes, such as *NDUFS2* [6–8▪▪].

Although the peak age of onset is between 15 and 35 years, patients with LHON have been reported from 2 to 87 years of age [5,9]. LHON typically presents with unilateral, painless, subacute, central visual loss with the fellow eye becoming involved within the following 6 months [10▪]. In about 25% of cases, there is simultaneous involvement of both optic nerves at first presentation [2,11]. An international consensus statement has defined 3 clinical stages in LHON based on the time from onset of visual loss, namely subacute (< 6months), dynamic (6 – 12 months) and chronic (> 12 months) [12▪▪]. In the subacute phase, there is

pseudopapilloedema due to swelling of the peripapillary retinal nerve fibre layer (RNFL), circumpapillary telangiectatic microangiopathy and vascular tortuosity. However, there is considerable heterogeneity in the appearance of the fundus and in about 20% of patients, the optic discs look normal, which can result in diagnostic delays [5]. As the disease progresses, there is rapid RGC axonal loss and pallor of the optic nerve head usually becomes evident within 6 weeks.

Mitochondrial Neuroprotection – Idebenone

Idebenone is a synthetic, short-chain analogue of ubiquinone, which is responsible for shuttling electrons from complexes I and II directly to complex III [13]. Idebenone has greater bioavailability compared with coenzyme Q10 (CoQ10) as it possesses a less lipophilic tail that allows it to penetrate the blood-brain barrier and mitochondrial membrane more readily [14]. The efficacy of idebenone was assessed in a randomized, double-blinded, placebo controlled study (RHODOS), which recruited 82 patients with LHON harboring one of the three common mtDNA mutations. Patients were randomised in a 2:1 ratio in favour of idebenone and a dose of 300mg three times a day was used over a treatment period of 24 weeks [15,16]. In addition to RHODOS, the visual benefit of idebenone was assessed as part of a retrospective review of 103 patients treated with idebenone at varying doses and treatment duration [17]. Both these studies support a visual benefit in a proportion of patients treated with idebenone and the likelihood of a positive response was increased with earlier initiation of treatment. This is in keeping with the hypothesis that in the acute stage of LHON, a proportion of RGCs are functionally suppressed, but have not yet committed irreversibly cell death, and this pool of RGCs can be rescued if treatment is provided within a critical time window. Idebenone has been approved by the

European Medicine Agency (EMA) to treat LHON and post-marketing studies are currently underway to collect additional safety and efficacy data [13].

Other Neuroprotective Agents

Two neuroprotective drugs, EPI-743 and MTP-131 (elamipretide), have been used for patients with mitochondrial disease [18,19]. In an open-labelled study, preliminary data of efficacy for EPI-743 was demonstrated in 5 patients with subacute LHON [20]. The safety and efficacy of a topical formulation of MTP-131 is currently being investigated in 12 patients with LHON with disease duration between 1 and 10 years (https://clinicaltrials.gov/ct2/show/NCT02693119, accessed 1st of November 2018).

Cyclosporine A blocks apoptosis by inhibiting mitochondrial permeability transition and its therapeutic potential in LHON has been evaluated in 5 patients with subacute, unilateral LHON, who had been diagnosed within 6 months of disease onset [21,22▪]. Cyclosporine A was not able to prevent second eye involvement at the treatment dose used (2.5 mg/kg/day) [22▪]. There is mounting evidence that mitochondrial dysfunction plays a key role in RGC loss in glaucoma [23,24▪▪]. Interestingly, supplementation with nicotinamide adenine dinucleotide (NAD) precursors, which are derivatives of vitamin B3, was able to prevent the development of glaucoma in aged mice by improving mitochondrial metabolism and blocking pro-apoptotic pathways [23,24▪▪]. Vitamin B3 and nicotinamide are therefore attractive neuroprotective molecules that could be pharmacologically modified to help promote neuronal survival in other optic neuropathies, including LHON.

Improving Mitochondrial Biogenesis

LHON is a complex disease and the phenotypic manifestation of the primary mtDNA mutation is influenced by secondary genetic factors, environmental triggers and hormonal influences [25,26]. The mitochondrial genome is a high copy number

genome and the factors that control a cell's overall copy number are thought to be relevant to both physiological and pathological states [27]. Interestingly, unaffected LHON mutation carriers and normal healthy controls have a higher mtDNA copy number compared with affected patients with LHON [25]. By extrapolation, agents that improve mitochondrial biogenesis could therefore prove beneficial by providing a compensatory cellular response in the context of impaired mitochondrial oxidative phosphorylation. LHON fibroblasts treated with oestrogen derivatives show increased mtDNA copy and importantly, there is reduced ROS levels and increased cell survival [28]. These observations could also explain the marked sex bias in LHON with the preponderance for male carriers losing vision being due, at least partly, to lower circulating levels of oestrogens [28–30▪▪]. Smoking is a major risk factor for visual loss in LHON and in keeping with this detrimental effect, LHON fibroblasts cultured in the presence of cigarette smoke condensates exhibit significantly decreased mtDNA copy number [31,32]. However, further experimental work is required to refine and test specific therapeutic agents that target mitochondrial biogenesis before moving into early phase clinical trials [33].

Near-Infrared Light Therapy

Photobiomodulation with near-infrared light-emitting diode (NIR-LED) arrays increases the production of cytochrome *c* oxidase in cultured primary neurons and it reverses the reduction in enzymatic activity caused by metabolic inhibitors [34]. Recent experimental data in axotomy models of neurodegeneration look promising, but the translational application of this therapeutic paradigm to optic neuropathies poses a number of technical and practical challenges that will need to be overcome[35]. A previous study failed to recruit a sufficient number of patients to explore whether near-infrared light therapy could influence the visual prognosis in

LHON (https://clinicaltrials.gov/ct2/show/NCT01389817, accessed 1st of November 2018).

Mitophagy Modulation

Mitophagy refers to the selective autophagocytosis of mitochondria and this tightly regulated process allows the elimination of damaged mitochondria that would otherwise compromise the cell's survival. The balance between mitochondrial biogenesis and degradation has been investigated in LHON cybrid models and the available data indicates that mitophagy is dysregulated with impaired mitochondrial function and cell survival [36,37▪]. Based on these findings, activation of mitophagy by pharmacological means is being explored as a potential therapeutic strategy for mitochondrial diseases.

Gene Therapy

The eye represents an ideal target organ for gene therapy given the relative ease of access for direct delivery of viral vectors [38]. A major challenge in the treatment of mitochondrial disease is the double-membrane nature of the mitochondrial compartment, which complicates the efficient import of exogenous proteins. To bypass this physical barrier, an alternative strategy is to express the replacement wild-type gene in the nuclear compartment and the resulting protein is designed to contain a mitochondrial targeting sequence, which directs for its importation into mitochondria [39]. Following the successful demonstration that allotopic expression of wild-type *MTND4* could rescue the phenotype both in cell and animal models of LHON, human clinical trials have been initiated using modified adeno-associated virus (AAV) vectors to deliver the gene construct [40–43▪▪]. These groundbreaking studies have shown the safety of this approach and the preliminary results obtained

are encouraging indicating the preservation of RGCs and a functional visual benefit in a proportion of treated eyes.

Mitochondrial Replacement Therapy

As the mitochondrial genome shows strict maternal inheritance, preventing the transmission of pathogenic mtDNA mutations from mother to child is a major area of research for a number of research groups worldwide. Two main *in vitro* fertilization (IVF) techniques have been developed, namely, pronuclear transfer and maternal spindle transfer, both of which require the contribution of a donor egg that carries only wild-type mtDNA [44]. The term “three-parent embryo” is frequently used in the lay press as the mitochondrial genome of the resulting embryo is derived from the donor egg whereas the nuclear genome is inherited from the biological parents. In 2015, mitochondrial replacement therapy was approved by both Houses of Parliament in the United Kingdom as a justifiable reproductive option with the caveat that it should only be carried out in a recognised centre of excellence and with long-term follow-up of the children born using this approach [45]. In contrast, the US Food and Drug Administration (FDA) has concluded that more experimental and safety data are needed before advocating mitochondrial replacement therapy as reproductive option for mitochondrial disease [33,45]. As the legal and ethical implications of mitochondrial donation was being openly debated, the media reported that maternal spindle transfer had resulted in the birth of a healthy baby boy who carried low levels of the m.8993T>G mtDNA mutation in *MTAP6* that had resulted in the premature death of two siblings from Leigh syndrome [46▪]. Mitochondrial replacement therapy remains controversial and both the regulatory framework and the consequences of modifying the germline need to be carefully considered [45].

Regenerative Medicine

The seminal discovery that somatic cells could be reprogrammed into pluripotent stem cells, so called induced pluripotent stem cells (iPSCs), has transformed the scope for regenerative medicine, in particular for progressive neurodegenerative diseases [47]. Although the generation of iPSCs carrying pathogenic mtDNA LHON mutations is now technically straightforward, their differentiation into RGCs and the generation of a homogenous population in sufficient quantity for experimental needs still remain challenging [48▪]. Other hurdles that will need to be overcome before considering the use of iPSC-derived RGCs in LHON and other optic neuropathies are the methods required to deliver the cells in a viable state to the right retinal location and providing the guiding signals needed to allow the axonal projections of the transplanted cells to make the correct retinotopic connections [49–51].

A summary of the clinical studies and treatment strategies for LHON is listed in Table 1.

Conclusions

LHON is a rapidly progressive, blinding disorder that typically affects young adults in their prime. The management of LHON remains largely supportive, but rapid advances in drug discovery, gene therapy and stem cell technology are providing researchers with new tools for rescuing RGCs in this disorder with the aim of stabilising vision and improving the visual outcome. Central to these endeavours is the need to ensure patient safety both in early phase experimental trials and as part of long-term follow-up studies.

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Conflicts of interest

PYWM holds a consultancy agreement with GenSight Biologics (Paris, France). NJ and JH none.

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