Leber Hereditary Optic Neuropathy—Light at the End of the Tunnel?

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Abstract: Leber hereditary optic neuropathy (LHON) is an important cause of mitochondrial blindness. The majority of patients harbor one of three mitochondrial DNA (mtDNA) point mutations, m.3460G>A, m.11778G>A, and m.14484T>C, which all affect complex I subunits of the mitochondrial respiratory chain. The loss of retinal ganglion cells in LHON is thought to arise from a combination of impaired mitochondrial oxidative phosphorylation resulting in decreased adenosine triphosphate (ATP) production and increased levels of reactive oxygen species. Treatment options for LHON remain limited, but major advances in mitochondrial neuroprotection, gene therapy, and the prevention of transmission of pathogenic mtDNA mutations will hopefully translate into tangible benefits for patients affected by this condition and their families.

Key Words: gene therapy, idebenone, Leber hereditary optic neuropathy, LHON, mitochondrial donation, neuroprotection, retinal ganglion cells

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Leber hereditary optic neuropathy (LHON) typically presents with acute or subacute painless central visual loss in young adults with a peak age of onset in the third decade of life.[1] It is a bilateral disease and the majority of patients present with visual loss in one eye followed by the fellow eye usually 3–6 months later.[2] In about 25% of cases, both eyes are affected simultaneously.[3] The prevalence of LHON has been estimated to be about 1 in 30,000 and it is the most common primary mitochondrial DNA (mtDNA) disease in the population.[4] About 90% of LHON carriers harbor one of three mtDNA point mutations (m.3460G>A, m.11778G>A, and m.14484T>C), with the m.11778G>A mutation being the most common cause of LHON worldwide accounting for 70–90% of all cases.[5–7] Leber hereditary optic neuropathy is characterized by marked incomplete penetrance and a male sex bias. Although a wide variability is observed between families, on average, the lifetime risk of visual loss for a male LHON carrier is about 50% compared with 10% for a female LHON carrier. Leber hereditary optic neuropathy results in severe central visual loss and the visual prognosis is poor with most patients remaining registered legally blind for the rest of their lives.[8] There are currently limited treated options for LHON, but a better understanding of the disease mechanisms that underpin retinal ganglion cell (RGC) loss in this disorder and transformative technological advances are opening new exciting avenues for therapeutic intervention.

MITOCHONDRIAL NEUROPROTECTION

Mitochondria produce adenosine triphosphate (ATP) through a complex series of biochemical reactions that involve the transfer of high-energy electrons along the mitochondrial respiratory chain. Reactive oxygen species (ROS) are an inevitable byproduct of mitochondrial oxidative phosphorylation and the levels are tightly regulated in physiological states to minimize oxidative damage to mitochondrial membranes and mtDNA. Unsurprisingly, mitochondrial dysfunction is associated with impaired ATP synthesis and increased ROS production, both of which contribute to the neurodegenerative consequences of pathogenic mtDNA mutations. A variety of mitochondrial cocktails consisting of supplements such as carnitine, creatine and L-arginine, and multivitamins have been used to counteract these deleterious consequences, but the evidence base for efficacy is weak.[3,9]

Ubiquinone is an essential carrier within the inner mitochondrial membrane that ensures the efficient transfer of electrons along the mitochondrial respiratory chain from complexes I and II to complex III.[10] Co-enzyme Q10 (CoQ10) and idebenone are two well-known ubiquinone analogs that are frequently prescribed to patients with mitochondrial disease. These two molecules have different molecular sizes and pharmacokinetics, and CoQ10, unlike idebenone, is not able to cross the blood-brain barrier.[11] Idebenone was therefore an obvious drug candidate for LHON and its safety and efficacy was investigated as part of a randomized controlled trial (RHODOS) that recruited 85 patients carrying the m.3460G>A, m.11778G>A, and m.14484T>C mtDNA mutations.[12] The patients were randomized in a 2:1 ratio to idebenone (300 mg three times per day) or placebo for a period of 24 weeks. Although RHODOS failed its primary outcome measure, a subgroup of treated patients was found to benefit and this observation was confirmed in a larger retrospective case series of 103 patients treated with variable doses of idebenone.[13] Based on the cumulative body of evidence, idebenone was

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approved by the European Medicine Agency under exceptional circumstances in June 2015 for use in patients with visual loss from LHON.[14] A recently convened expert panel has recommended that idebenone should be started as soon as possible (at a dose of 300 mg three times per day) for patients with LHON with visual loss of less than one year. This regimen should be continued for at least one year to determine whether there is a treatment effect and if a clinically relevant response is observed, treatment should be continued for one year after the plateau phase has been reached.[15] The expert panel concluded that there was not enough evidence to recommend treatment for chronic LHON cases. Given that impaired mitochondrial bioenergetics and oxidative damage are major pathophysiological players in LHON, other molecules with neuroprotective properties are being considered, including EPI-743 and MTP-131.[16,17] These two drug compounds and other future ones will need to be rigorously assessed as part of properly powered trials with a sufficiently long period of treatment to determine whether they can improve the visual prognosis in LHON.[18]

MITOCHONDRIAL BIOGENESIS

Compared with the nuclear genome, mitochondria have a very high copy number genome, which can lead to an admixture of different mtDNA variants. Mitochondrial biogenesis refers to the tightly regulated process that controls the cell's mtDNA copy number under both physiological and pathological states, which can also be influenced by environmental factors. Leber hereditary optic neuropathy is emerging as a complex multifactorial disease and there is now convincing evidence linking environmental triggers, in particular smoking, with an increased risk of visual loss among at-risk carriers.[19] However, the prominent male bias seen in this mitochondrial disorder requires further explanations and hormonal factors have emerged as possible disease modifiers given the obvious differences between the sexes.[20] Estrogen derivatives have been found to reduce ROS levels and rescue the viability of LHON cell lines, and this improved survival has been ascribed to an upregulation of mitochondrial biogenesis.[21,22] This encouraging in vitro data is supported by the observation made in human blood samples with unaffected LHON carriers having a higher mtDNA copy number compared with those who have experienced visual loss from LHON, pointing towards a neuroprotective effect.[22] Furthermore, patient cell lines exposed to cigarette extracts show a decrease in mtDNA copy number, impaired mitochondrial oxidative phosphorylation, and ROS detoxification, providing a strong causal link between smoking and an increased risk of visual loss in LHON.[23]

GENE THERAPY

The eye is ideally suited for gene therapy and the development of safe and effective viral delivery vector systems have led to several development programs for inherited eye diseases.[24,25] Gene therapy for LHON has been hampered by the double membrane structure of mitochondria and the relatively impervious properties of the mitochondrial inner membrane.[26] To circumvent these physical barriers, an indirect approach, known as allotopic expression, has been developed whereby the replacement gene of interest is delivered to the nuclear genome with a modified adeno-associated virus (AAV) vector and the encoded protein has a mitochondrial targeting sequence, which allows for its import into the mitochondrial compartment.[27,28] Preclinical studies for the *MT-ND4* mutation (m.11778G>A) have shown rescue of the cellular phenotype and RGCs in murine models of LHON.[29,30] Based on these findings, a number of clinical trials have been initiated using AAV2 viral delivery systems and the preliminary results are encouraging, suggesting that allotopic expression could be an effective strategy to preserve RGCs and improve visual function.[31–34] It should be stressed that the visual benefit of this gene therapy approach for LHON still requires confirmation, including the best window of opportunity for intervention and whether the effect can be sustained after a single intravitreal injection of the viral vector. There are also some concerns about the level of RGC transfection with this mode of delivery and whether the imported ND4 subunit will integrate and form a stable complex I structure, which require further investigation.[35]

MITOCHONDRIAL REPLACEMENT THERAPY

As the mitochondrial genome shows strict maternal inheritance, preventing the transmission of pathogenic mtDNA mutations from mother to child has gained considerable attention over the past few years.[36] Mitochondrial replacement therapy (or mitochondrial donation) is a modified in vitro fertilization technique that involves the transfer of the parental nuclear material into a mitochondrial donor zygote carrying only wild-type mtDNA.[37,38] If the resulting embryo proceeds to a full-term pregnancy, the child's mitochondrial genome will be from the maternal donor and there have been some concerns about possible mismatch with the nuclear genome inherited from the biological parents.[39,40] Furthermore, as mitochondrial donation involves germline modification and any detrimental consequences might not become apparent until several years after birth, there is an ongoing ethical debate about the clinical application of this technique, more so for mitochondrial disorders such as LHON that are not life threatening.[41,42] The course of the argument was somewhat overtaken when it became public knowledge in September 2016 that a healthy child had been born in Mexico from mitochondrial donation.[43,44] The child's mother is heteroplasmic for the m.8993T>G mtDNA mutation in *MT-ATP6*, which in addition to the syndrome of neuropathy, ataxia, and retinitis pigmentosa (NARP) can also cause Leigh syndrome when present at high mutant levels. Although clinically asymptomatic, she suffered from multiple miscarriages and two of her children died at the age of 8 months and 6 years old from Leigh syndrome with the m.8993T>G mutation detected at levels exceeding 95%. The mutation was present a low level of heteroplasmy (2.4–9.2%) in the tissues obtained from the child and his development will be closely monitored until he reaches the age of 18 years old. A population-based study in the United Kingdom has estimated that about 150 female carriers of childbearing age would benefit from mitochondrial replacement therapy per year, with a significant proportion being mtDNA LHON carriers.[45] The law was modified in 2015 in the United Kingdom to allow for this technique to be used in strictly regulated clinics and as part of a long-term child follow-up program.[45] After a period of consultation, the Food and Drug Administration has concluded that further experimental and safety data are needed before mitochondrial replacement therapy can be considered as a reproductive option for women carrying disease-causing mtDNA mutations.[46]

LOOKING INTO THE FUTURE

A landmark discovery was the observation that somatic cells, such as fibroblasts, could be transformed into induced pluripotent stem cells (iPSCs) with a combination of virally delivered reprogramming factors.[47] Research into LHON and other mitochondrial optic neuropathies has been severely limited by our inability to study disease mechanisms and treatment paradigms directly in RGCs. The advent of this powerful and versatile technology has the potential to be transformative and a number of research groups have generated LHON iPSC lines to address these fundamental gaps in our knowledge base.[48,49] The differentiation of iPSCs into RGCs needs to be optimized further to generate a sufficient amount of cells for experimental manipulation and high-throughput drug screening, which will require close collaboration with industrial partners.[50] Although RGC replacement remains a challenging endeavor given the need for the transplanted cells to make the right retinotopic connections, it is possible that they could exert a neuroprotective influence, more so if genetically modified to overexpress RGC trophic factors that block the deleterious consequences of an underlying mtDNA mutation.[51]

CONCLUSIONS

Leber hereditary optic neuropathy is a devastating disease with major long-term consequences for patients and their families. Until recently, the management of this condition was limited to visual rehabilitation, genetic counseling, and the avoidance of potential environmental triggers. Research into LHON has now reached an important translational phase and there is considerable hope that a combination of approaches ranging from RGC neuroprotection to gene therapy and mitochondrial replacement therapy will help improve the visual prognosis for patients or achieve the breakthrough of preventing the maternal transmission of the causative mtDNA mutation. Although the road ahead remains challenging with the need for multidisciplinary collaborations and substantial research funding for clinical trials, there is certainly light at the end of the tunnel in our efforts to develop effective treatments for LHON.

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