Commentary

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Therapeutic strategies for Leber's hereditary optic neuropathy: A current update

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Summary **Leber's hereditary optic neuropathy (LHON) is a rare mitochondrial retinopathy, caused by mutations in subunits of complex I of the respiratory chain, which leads to elevated levels of oxidative stress and an insufficient energy supply. This molecular pathology is thought to be responsible for the dysfunction and eventual apoptotic loss of retinal ganglion cells in the eye, which ultimately results in blindness. Many strategies, ranging from neuroprotectants, antioxidants, anti-apoptotic- and anti-inflammatory compounds have been tested with mixed results. Currently, the most promising compounds are short-chain quinones that have been shown to protect the vision of LHON patients during the early stages of the disease. This commentary gives a brief overview on the current status of tested therapeutics and also addresses future developments such as the use of gene therapy that hopefully will provide safe and efficient therapy options for all LHON patients.**

Keywords: Leber's Hereditary Optic Neuropathy, mitochondrial disease, quinones, therapy, clinical trial, antioxidants, neuroprotectants

1. Introduction

Leber's hereditary optic neuropathy (LHON) is a retinal neurodegenerative disorder, characterized by acute or subacute vision loss in one eye, generally followed by loss of visual acuity in the second eye within 2-4 months (*1,2*). Loss of vision in LHON patients is associated with dense central or centrocecal scotoma and impaired color vision. LHON predominantly affects young adult males of all ethnic groups, with a peak of onset in young adulthood. While in most cases vision loss is permanent, a minority of patients show spontaneous recovery of visual acuity by an unknown mechanism (*1,2*). LHON is regarded as one of the most prevalent mitochondrial diseases with an incidence between 1:30,000 and 1:50,000 (*2,3*). However, it can be assumed that LHON is still significantly underdiagnosed as optic atrophy of unknown origin. More than 100 years after the initial description of the disease, the first causative point mutation in the mitochondrial DNA (mtDNA) was identified (*4*) and at present more than 18 mtDNA alterations have been associated with LHON (*http://omim.org/entry/535000*). However, three so called primary mtDNA mutations account for about 95% of all LHON cases, 11778G>A (*ND4* subunit), 14484T>C (ND6 subunit), 3460G>A (*ND1* subunit), all of which lead to a dysfunction of complex I of the mitochondrial electron transport chain (*2*). As a result of this defect, decreased ATP synthesis and elevated levels of oxidative stress have been described (*5-7*), which are thought to impair the function and ultimately lead to apoptotic cell death of retinal ganglion cells (RGC) (*6-9*). Despite progress towards a better understanding of pathogenesis of LHON, currently no treatment options are available to patients and only a few years ago LOHN was regarded as untreatable. Furthermore, due to the spontaneous recovery potential seen in some LHON patients, any reports of treatment efficacy from small uncontrolled trials must be considered with extreme caution (Table 1). However, recent clinical data with redox-active electron carriers have demonstrated that protection and even recovery of vision is a realistic prospect in LHON.

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2. Neuroprotectants, anti-inflammatories and anti-apoptotic compounds

Given that the pathology of LHON seems to be largely

Table 1. Clinical results of different therapy approaches for the treatment of LHON

#	Reference /Sponsor*	Treatment	Dose	Type of study	Duration	No. of Patients	Primary Measure	Outcome	Clinical trial number	status
1	Newman *et al*. 2005 (*15*)	Brimonidine	0.15% 4 × daily	open-label, non-randomized prospective	2 years	9	Visual acuity	Brimonidine was unsuccessful in preventing second eye involvement. Trial closed prematurely because of low enrolment rate, and all patients failed the criteria for effectiveness during follow-up	n/a	finalized
2	Medical College of Wisconsin 2011	Near-infrared-light-emitting diode	50 mW/cm^2 2 × daily	non-randomized	12 months	4	Pre- and post-treatment electroretinogram	Unable to record primary measure as patients were unable to focus on target, no study results posted	NCT01389817	terminated
3	Buhmann *et al.* 2002 (*16*)	Mitoxantrone	12 mg/m^2 every 3-4 months	case study	48 months	1	Visual acuity	Recovery of visual function in patient with 11778G>A mutation 12 month after onset of vision loss	n/a	finalized
4	Huang *et al*. 2002 (*18*)	CoQ10	90-200 mg/day	case study	12 months	1	Visual acuity	Recovery of visual function in patient with 11778G>A mutation	n/a	finalized
5	Carelli *et al.* 2011 (*24*)	Idebenone	270-675 mg/day	non-randomized, retrospective	variable	103	Visual acuity	Increased recovery of vision in the treated group	n/a	finalized
6	Klopstock *et al*. 2011, 2013 (*23,25*)	Idebenone	900 mg/day	placebo-controlled, randomized, double-blind	6 months	85	Visual acuity	Protection against loss of visual acuity, improvement of visual acuity, treatment effect persisted even 30 months after termination of treatment	NCT00747487 NCT01421381	finalized
7	Sadun *et al*. 2012 (*28*)	EPI-743	300-1200 mg/day	non-randomized, open label	204-557 days	5	Anatomic and visual indices	4 out of 5 treated patients showed improvement of visual function	n/a	finalized
8	Mahidol University 2007	Curcumin	2 × 250 mg/day	placebo-controlled, randomized, double-blind	12 months	70	Visual acuity, visual field, electrophysiology	No study results released	NCT00528151	finalized
9	Quark Pharmaceuticals 2010	QPI-1007	variable doses	open-label, dose escalation, safety, tolerability study	12 months	48	Safety,dose-limiting toxicities, pharmacokinetics	No study results released	NCT01064505	finalized
10	Huazhong University of Science and Technology 2010	rAAV2-ND4, gene therapy	single injection	Safety and efficacy study	6 months	6	Visual acuity	Final data collection due by end of 2013	NCT01267422	recruiting

*Sponsor is listed for studies with no published results.

restricted to a degeneration of RGC cells, it appears obvious that any protection of RGC neurons against cell death should at least in theory alleviate the symptoms associated with LHON. Many compounds with RGC-neuroprotective properties such as memantine (*10*), valproic acid (*11*) and SIRT-1 activators (*12*) have been identified in other models and could also be tested in pre-clinical models of LHON and/or in clinical trials. Some promising pre-clinical results have been reported with compounds such as the antibiotic drug minocycline (*13*). However, unless their efficacy is confirmed in tightly controlled clinical trials, their potential for the treatment of LHON patients remains unclear. Many putatively protective compounds such as steroids, hydroxycobalamin and cyanide antagonists have been tested to treat or prevent the acute phase of vision loss, but without success (*14*). In one of the few clinical trials, the anti-apoptotic compound brimonidine, approved to treat ocular hypertension and open-angle glaucoma, was tested in an open-label, non-randomized prospective study (*15*). Although the LHON patients in this trial were in the early stages of the disease with discordant vision and had therefore the highest chance of recovery, brimonidine was unsuccessful in preventing second eye involvement. Curcumin, a compound that has also been associated with many protective activities, was tested in a placebo-controlled trial that started in 2007 (NCT00528151). However, the absence of published results and the lack of a follow up study at present suggest that probably no positive effects were obtained with curcumin. One interesting observation is derived from a patient suffering from the rare LHON-multiple sclerosis (MS)-like disease. Treatment of this single patient with the commonly used anti-MS drug mitoxantrone resulted in a time delayed visual recovery 12 months after acute onset of rapid sequential bilateral subtotal visual loss, which led the authors to suggest an immunological involvement in the pathology of this disorder (*16*). However, the structural similarity of mitoxantrone to naphthazarin could also suggest a direct neuroprotective activity (*17*). An entirely different approach is the use of gene silencing to down regulate the expression of apoptosis-inducing genes such as in the case of the experimental compound QPI-1007, which targets the expression of the pro-apoptotic enzyme caspase 2 (NCT01064505). Phase 1 safety and tolerability have been assessed in a dose escalation trial in healthy subjects and it remains to be seen how effective this approach will be when used in optic atrophy patients.

3. Antioxidants and Electron Carriers

Given the good evidence for elevated levels of oxidative stress in LHON, antioxidant treatment has been proposed or tested repeatedly. While pre-clinical models showed sufficient efficacy to take some compounds into the clinic, compounds that act as radical scavengers only, have so far failed to show convincing clinical evidence of usefulness. On the other hand, promising clinical results have recently been described with molecules that can reduce oxidative stress levels and simultaneously act as electron carriers to modulate mitochondrial electron flow. The most well-known member of this group is the endogenous coenzyme Q10 (CoQ10), which is utilized in many mitochondrial disorders. Although, a beneficial effect of CoQ10 in a single patient harboring the 11778G>A mutation was reported (*18*), results with CoQ10 in controlled trials in other mitochondrial indications have so far not been convincing (*19*), most likely due to its poor tissue bioavailability as a consequence of its very high lipophilicity. In contrast, idebenone, a short chain benzoquinone has shown some encouraging effects (*20*). Due to its balanced lipophilicity (logD = 3.9), it not only acts as a potent catalytic antioxidant but can shuttle between the cytoplasm and the mitochondria to transfer electrons into the mitochondrial electron transport chain under conditions where complex I is defective (*21,22*). This mechanism is dependent on the cellular levels and activity of NAD(P)H oxidoreductase (NQO1) and although there is no direct evidence that idebenone acts *via* an NQO1-dependent mechanism in LHON patients, biochemical data demonstrated a normalization of mitochondrial function associated, for example, with reduced lactate production (*21*). A number of earlier case studies and trials, which suggested that idebenone could have a therapeutic effect in LHON patients (*20*), provided the rationale for the first randomized, placebo-controlled study in LHON. This randomized placebo-controlled clinical trial (RHODOS, Rescue of Hereditary Optic Disease Outpatient Study; NCT01421381) included eighty five LHON patients carrying one of the three primary mtDNA mutations and were treated with 900 mg of idebenone per day for 24 weeks (*23*). Although patients receiving idebenone improved on average by six letters while subjects receiving placebo improved by three letters, this trial did not reach is pre-specified endpoint of "best recovery in visual acuity in either eye" measured by change in logMAR between baseline and week 24. However, when analyzing the change of all subjects' eyes separately to increase the power of the study (pre-specified secondary endpoint), the visual acuity of eyes of patients receiving idebenone significantly improved compared to those receiving placebo (p = 0.026). Furthermore, 28% of patients receiving idebenone and unable to read the eye-chart at baseline recovered sufficient visual acuity to read at least five letters on the eye chart compared to 0% of patients in the placebo group (*23*). The results from the RHODOS trial, together with similar data from a non-randomized, retrospective trial of idebenone in LHON that involved 103 patients (*24*) support a protective and

restorative activity of idebenone in patients, particularly those with recent disease onset. Interestingly, when the patients of the RHODOS trial were followed up 30 months after treatment had been terminated, it became clear that the protective idebenone effect still persisted (*25*).

A second quinone compound, which unlike idebenone belongs to the Vitamin E family of compounds is α-tocotrienol-quinone (EPI-743), was tested in small trials in several mitochondrial indications such as Leigh syndrome (*26,27*). At present, EPI-743 was evaluated only in a single open-label clinical trial involving five LHON patients with acute vision loss, where visual function improved in four out of five patients based on visual acuity or visual field (*28*). Similar to idebenone, EPI-743 is reduced by the enzyme NADPH quinone reductase (NQO1) however at a rate of less than 30% compared to idebenone (*29*). Due to its higher lipophilicity, it does not participate in the cytoplasmic-mitochondrial electron shuttling reported for idebenone (*29*). Based on the available literature, the most likely mode of action of this redox-active compound could be a strong antioxidative effect (*30*). Even though the clinical results with EPI-743 appear promising and raise hope in many LHON patients, they still have to be verified by tightly controlled studies with sufficient patient numbers.

It has to be noted here that a strict classification of compounds as antioxidants, neuroprotectants or electron carriers is realistically not possible, since many of the described molecules could potentially display several activities at once. This highlights the problem that even when compounds show activity in protecting visual acuity in LHON patients, we cannot necessarily deduce by what mechanism, which hinders the development of compounds with increased potency. Moreover, this uncertainty also makes claims of superiority of one compound over another based on pre-clinical data largely unfounded until a clear improved activity has been demonstrated in controlled, comparative clinical trials.

4. Gene therapy

In addition to pharmacological approaches, there is significant activity to directly correct the inherited genetic defect by expressing the functional mitochondrial protein in the retina (*31-33*). The application of gene-therapy in LHON is mainly based on the nuclear, allotopic expression of mtDNA-encoded genes, where the wild type version of the mutant mitochondrial complex subunit is delivered into the RGC *via* adeno-associated virus (AAV) (*31*). The underlying idea of this approach is that proteins that are normally expressed in the mitochondria can be produced in the cytoplasm and then imported into the mitochondria using specific mitochondrial targeting sequences. Once in the mitochondria, it is assumed that they can be correctly incorporated into the mitochondrial enzyme super-complexes to replace the defective subunits, thereby restoring normal electron flow and energy production. Although a significant critique of this approach has been voiced (*32,33*), successful results were reported using pre-clinical *in vitro* and *in vivo* models of LHON (*31,34*). Currently several clinical trials are in preparation or ongoing that universally aim to treat LHON patients by expressing the wild-type form of the *ND4* gene using AAV vectors (NCT01267422) (*35,36*). It has to be noted that due to its nature this approach will not be useful for all those patients that harbor mutations in different subunits than ND4. In this context it is therefore interesting to note that a second gene-therapy approach could be independent of the underlying mutation. This so far only pre-clinical work is based on the expression of a yeast-derived NADH-oxidase called Ndi. Encouragingly, the mitochondrial expression of this enzyme in a mouse model of LHON protected against RGC loss and preserved visual function (*37*). It will be exciting to see the first results of clinical gene-therapy trials in this field. However, these studies not only have to demonstrate sufficient efficacy with regards to a protection and restoration of visual acuity in LHON patients but also need to display a tolerable safety profile to become a viable treatment option either as independent treatment modalities or in combination with pharmacological strategies.

5. Conclusion

At present, effective and available treatment options for all LHON patients are still lacking. However, based on the recent encouraging results with quinone compounds in some patients in the early stages of the disease, LHON can no longer be seen as an untreatable disorder. Current evidence also suggests that the development of LHON-specific gene-therapy approaches could add to the therapeutic repertoire in the future. Over the next few years, a detailed understanding of the molecular pathology combined with the use of pharmacological therapies and potential genetic treatment options will therefore likely lead to significant therapeutic improvements for all LHON patients.

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