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PII: S1359-6446(17)30471-3
DOI: https://doi.org/10.1016/j.drudis.2018.01.031
Reference: DRUDIS 2164

To appear in:

Received date: 28-9-2017
Revised date: 15-11-2017
Accepted date: 7-1-2018

Please cite this article as: Ruiz-Pesini, Eduardo, Emperador, Sonia, López-Gallardo, Ester, Hernandez-Ainsa, Carmen, Montoya, Julio, Increasing mtDNA levels as therapy for mitochondrial optic neuropathies.Drug Discovery Today https://doi.org/10.1016/j.drudis.2018.01.031

Increasing mtDNA levels as therapy for mitochondrial optic neuropathies

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Highlights

- Leber hereditary optic neuropathy (LHON) is a mitochondrial optic neuropathy (MON)
- Primary open angle glaucoma (POAG) can be a MON
- LHON and POAG risk factors include reduced mitochondrial DNA (mtDNA) quantity
- Increasing mtDNA levels is a potential MON therapeutic option

Leber hereditary optic neuropathy (LHON) is a rare, inherited mitochondrial disease. No treatment has shown a clear-cut benefit on a clinically meaningful end-point. Primary open-angle glaucoma is a frequent, acquired optic neuropathy. Lowering intraocular pressure reduces disease progression. However, current methods to decelerate this progression are recognized as being inadequate. Therefore, there is a clear need to look for new therapeutic approaches. The growing evidence indicates that primary open-angle glaucoma can also be a mitochondrial optic neuropathy (MON). Several risk elements are common for both diseases and all of them decrease mitochondrial (mt)DNA content. Based on these susceptibility factors and their molecular mechanism, we suggest herein pharmacological therapies targeted to increase mtDNA levels, oxidative phosphorylation capability, and mitochondrial energy production as treatments for MONs.

Keywords: LHON; POAG; RGCs; mitochondrial biogenesis; mtDNA; pharmacological therapy.
Teaser: Drugs that increase mitochondrial biogenesis are potential therapies for the mitochondrial optic neuropathies LHON and POAG.

Introduction

Optic neuropathies are an important cause of blindness worldwide. In many cases, a primary or secondary mitochondrial dysfunction, affecting retinal ganglion cell (RGC) survival, is the cause of these optic neuropathies. Unfortunately, there are no approved treatments for MONs. However, given the common risk factors for primary and secondary MONs, we propose herein pharmaceutical drugs that improve oxidative phosphorylation (OXPHOS) function and provide RGC protection in animal models.

Energy source in retinal ganglion cells

Retinal photoreceptor cells capture light information and, via bipolar and amacrine cells, conduct it to RGC dendrites and then down the optic nerve formed by RGC axons to the brain. Of the integrated sensory signals in the brain, 90% are of visual origin, which gives an indication of the work load of RGCs. RGC axons traverse the lamina cribrosa and their myelination is post laminar. The saltatory conduction in myelinated axons is more efficient, because depolarization and repolarization only occur at the nodes of Ranvier. By contrast, unmyelinated prelaminar and laminar RGC axons do not perform saltatory conduction and require a higher density of voltage-gated sodium channels and sodium/potassium ATP pumps. Therefore, the propagation of action potentials in these segments of the optic nerve demands greater energy consumption. Thus, these higher ATP demands explain why

physiologically increased numbers of mitochondria are found in RGC unmyelinated axons and why these cells are so vulnerable to OXPHOS impairments [1].

OXPHOS is a mitochondrial biochemical pathway that is more efficient than cytosolic glycolysis for ATP production, and is the main energy source for neurons. This system includes the electron transport chain (ETC) and ATP synthases. The ETC contains the respiratory complexes I (CI) to IV (CIV,), in which the reduction of NADH to oxygen electrons transfer is accompanied by protons pumped from the mitochondrial matrix to the intermembrane space, thus generating a proton electrochemical gradient. ATP is then synthesized when these protons come back to the matrix via ATP synthase. The first ETC complex, NADH:ubiquinone oxidoreductase, or CI, takes electrons from NADH produced after nutrient oxidation and transports them to the ETC. CI includes 44 different polypeptides, 37 encoded by genes on nuclear chromosomes and seven (p.MT-ND1-6 and p.MT-ND4L) by genes in mtDNA. These seven subunits are involved in proton pumping. Therefore, mutations in these proteins can affect OXPHOS function [2], which can then affect RGC survival; loss of RGCs can lead to blindness.

Inherited and acquired mitochondrial optic neuropathies

Leber hereditary optic neuropathy

LHON is a type of blindness that results from RGC dysfunction and loss. It is characterized by severe acute or subacute central vision loss in one eye followed soon thereafter by the other eye, and associated with dense central or centrocecal scotomas and impaired color vision. Pathological cupping of the optic disc is also a feature of longstanding LHON. The peak age of onset is during the second and third decades of life and its reported prevalence is <0.003 % [3]. CI specific activity and CI-driven ATP production rates are reduced in LHON lymphoblasts [4]. This disorder results mainly from pathologic mutations in mtDNA genes encoding CI subunits. Three of these mutations [m.3460G>A (*MT-ND1*), m.11778G>A (*MT-ND4*), and m.14484T>C (*MT-ND6*)] account for most cases of LHON, with a plethora of rare mutations accounting for the remaining cases [3]. However, most of these mutations are not severe and many carriers fail to express the phenotype [5]. Therefore, other factors must be involved in addition to the pathologic mutation. In fact, different mtDNA genetic backgrounds, such as haplogroup J, defined by particular combinations of genetic variants in this molecule, increase the risk for the disease [6]. It has been also found that males are especially affected and more than two-thirds of unaffected homoplasmic carriers are female [7]. Smoking increases LHON penetrance [8] and it has also been suggested that antiretroviral therapy triggers visual loss in individuals harboring LHON mutations [9]. Unfortunately, there are currently no Phase 1 clinical trial data supporting the use of any medication for the treatment of LHON [10].

Primary open-angle glaucoma

Primary open-angle glaucoma (POAG) represents approximately 85% of all glaucoma cases. Its global prevalence is 3.1 % for people aged 40 years or older. It is characterized by visual field defects, cupping of the optic disc, normal-appearing anterior chamber angle, and raised intraocular pressure (IOP). POAG is also the most-frequent cause of irreversible blindness and, similar to LHON, results from loss of RGC [11]. POAG is a multifactorial disorder [12], and likely represents a common phenotype resulting from several underlying pathophysiologies [4]. There is mounting evidence that, in some cases, POAG is a MON [4]. Similar to LHON, mitochondrial respiratory activity is decreased in POAG lymphoblasts [13]. These cells also exhibited lower CI specific activity, CI-driven respiration, and CI-driven ATP production rates. However, these CI functional defects in POAG were milder than in LHON [4,14]. It was also reported that, in glaucoma-resistant individuals, with raised IOP over many years and no optic neuropathy, lymphocyte ATP generation by ETC complexes was higher than that of control or susceptible individuals, with fast-progressing glaucoma and low IOP [15]. Although LHON mutations have been reported in patients with POAG [16,17], these or other mtDNA pathologic mutations are not a major cause of POAG. However, somatic mutations accumulate in mtDNA with increasing age [18], and age is an important risk factor for the disease, with POAG prevalence increasing with age [19]. Another significant risk factor for POAG is a raised IOP. Unilateral IOP elevation can be induced by episcleral vein cauterization (EVC) to establish a model of glaucoma, and an increased number of RGC mtDNA mutations and lower mtDNA levels were found in EVC eyes than in contralateral eyes. These changes were accompanied by significant reductions in CI activity [20]. Thus, all these results support mitochondrial dysfunction as another risk factor for some patients with POAG.

A bias of POAG maternal inheritance, compatible with mtDNA maternal inheritance, has also been reported [21–25]. Moreover, the number of mtDNA segregating sites, especially those in coding regions and also those nonsynonymous in CI genes, was higher in patients with POAG than in controls [26]. Similar to LHON, mtDNA haplogroups are also risk factors for glaucoma [27,28]. A higher POAG prevalence has been also reported in men than in women [19,29]. There also appears to be a potential association between POAG and heavy smoking [30]. Finally, a significant increase in the frequency of glaucoma was observed in patients treated with antiretroviral therapy [31].

Given that LHON and POAG share several risk factors, here we provide a deep analysis of these factors that could provide clues to potential therapies.

New therapies for mitochondrial optic neuropathy

It has been observed that estradiol, the levels of which are higher in females, increases mtDNA content, oxygen consumption, and ATP levels in human cybrids harboring LHON mutations, which could explain the lower

prevalence of LHON in females [32]. By contrast, it was found that a significantly increased POAG risk was associated with later and earlier age of menarche and menopause, respectively, and the use of oral contraceptives for more than 5 years. Pregnancy is associated with decreased IOP during the third trimester. Therefore, increased estrogen states could confer neuroprotective effects and reduce POAG risk [33]. In fact, estradiol also increases mtDNA content, oxygen consumption, and ATP levels in human cybrids with no LHON mutations [32]. Interestingly, some studies report differences in peripheral blood mtDNA content between men and women [34] and, although it is not easy to carry out epidemiological studies on mtDNA copy number in human tissues distinct than blood, it was found that mtDNA levels in nontumorous liver tissue from male patients with hepatocellular carcinoma was significantly lower than that of female patients [35].

Other LHON and POAG risk factors, whether genetics, such as mtDNA haplogroup, or environmental, such as smoking or antiretroviral therapy, have also been associated with smaller amounts of mtDNA [36–38]. Thus, mtDNA levels were lower in haplogroup J than in H cybrids [36]. Smoking was significantly associated with the lowest blood mtDNA content in affected individuals. Moreover, cigarette smoke also condensate decreased fibroblast mtDNA copy number [37]. mtDNA depletion was observed in offspring from mothers with HIV-1 given zidovudine therapy [38]. These facts could explain why healthy LHON mutation carriers show higher blood mtDNA levels than do patients [7], and why glaucoma-resistant individuals also have a higher lymphocyte mtDNA content [15]. Although the quantity of RGC mtDNA from patients with MON is not known, the previous observations suggest a therapeutic strategy for MON that involves increasing mitochondrial biogenesis and, thus, mtDNA levels.

Mitochondrial biogenesis

We performed a PubMed bibliographic review (May 2017) using 'mitochondrial biogenesis' and 'mtDNA' as search terms and selected compounds that increase mtDNA copy number. Although environmental chemicals, such as benzene, hydrogen sulfide, methylmercury, perfluorooctanoate, or sulfur dioxide, increase mtDNA content, toxicities associated with them hamper their use as therapeutic agents. Other molecules associated with higher mtDNA levels are not so toxic (Table 1). A set of these drugs has been repeatedly associated with an increased mtDNA amount. For example, lipoic acid, lipoamide, thiazolidinediones, or polyphenols increase mtDNA copy number in varied cell types or tissues from mice, rats, and humans. This increase in mtDNA levels is frequently accompanied by elevation of mtDNA-encoded RNAs and proteins, number and activity of OXPHOS complexes, oxygen consumption, and amount of ATP.

Protective effects of retinal ganglion cells

Rotenone is a CI inhibitor that has been used to generate animal models of LHON [39]. Thus, following a daily intraperitoneal rotenone administration for 10 days, an increase in RGC apoptosis was observed. However, rosiglitazone treatment for 10 days after the administration of rotenone, significantly reduced the number of apoptotic RGCs [40]. Experimental induction of elevated IOP is frequently used to generate animal models of POAG [41]. It was reported that resveratrol significantly delayed RGC loss in a glaucoma model induced by injecting hyaluronic acid into the rat anterior chamber [42]. Given that RGC damage is a characteristic of both LHON and POAG, we conducted a literature search for animal models of RGC degeneration in which the compounds discussed above were tested. We performed a PubMed bibliographic review (May 2017) using the search terms 'retinal ganglion cells' and 'the particular drug' and selected for those compounds with an RGC neuroprotective effect shown in animal models. Many of the previously cited compounds had already been shown to have beneficial effects of RGC in different animal models (Table 2).

Prevention and treatment of mitochondrial optic neuropathies

Significant changes in retinal structure occur during the conversion to LHON. Although a loss of RGC is documented in LHON, significant spontaneous recovery of visual acuity has been reported in 5–65% of cases, depending on the pathologic mutation [5]. This recovery commonly occurs during the first year, but is usually incomplete. Interestingly, significant vision recovery can occur without apparent retinal structure recovery [43]. During the earlier stages of glaucoma, RGCs have the capacity to recover function following periods of functional loss [44]. The mechanism for vision recovery is not understood but might be linked to the mitochondria of the RGCs that remain. Therefore, some of the compounds discussed above could be LHON therapies. By contrast, the administration of some of these drugs before the generation of RGC lesions could prevent posterior damage. For example, pretreatment with lipoic acid or ω-3 polyunsaturated fatty acids decreased RGC apoptosis in animal models (Table 2) [XX].

There are several scenarios in which these potential therapies might be important for MON. For example, they might be preventive treatments. POAG is not a common disease before 40 years of age. Thus, young maternal relatives of patients with POAG would be candidates for such therapy. Healthy carriers of LHON mutations would also be candidates. The peak incidence of LHON occurs during in the mid-20s [45]. Thus, younger individuals carrying the mutations are at risk and could benefit from these drugs. Moreover, in 75% of patients with LHON, the second eye is affected 2 or 3 months after the first. However, an interval greater than 12 months [46], even up to 18 years [47], occurs in some cases. In general, POAG is a bilateral disease, although the course of disease progression is often asymmetric, with one eye more adversely affected than the other eye [48]. Therefore, the sequential bilateral nature of MON can provide a window of opportunity for preventive treatment. Finally, some

patients with LHON spontaneously recover. It might be that supplying RGCs with these compounds could increase the chance of recovery. As previously discussed, different combinations of factors can result in POAG. Therefore, those POAG cases involving OXPHOS dysfunction would benefit from these therapies. The different therapeutic approaches to MON, including the drugs mentioned here, are reviewed elsewhere. [49].

Concluding remarks

Some of the drugs discussed above have shown beneficial effects in mouse models of a variety of pathologies, including diabetes, liver steatosis,; acute kidney injury;, Parkinson's disease, and X-linked adrenoleukodystrophy (see the supplemental references in the supplementary information online). Moreover, some of these compounds are already used in humans. Thus, increasing the amount of mtDNA and mitochondrial biogenesis should be now considered a potential therapeutic strategy for a range of diseases, including MON.

Acknowledgments

This work was supported by grants from the Instituto de Salud Carlos III (ISCIII) (PI14/00005, PI14/00070, PI17/00021, PI17/00166), Departamento de Ciencia, Tecnología y Universidad del Gobierno de Aragón (Grupos Consolidados B33), and the FEDER Funding Program from the European Union. The CIBERER is an initiative of the ISCIII.

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Table 1. Compounds that increase mitochondrial DNA copy number[a,b]

Compound	Cell/tissue	Animal
Acetyl-L-carnitine	Brain, liver, muscle	Rat
All-trans retinoic acid	Hepatocytes, HepG2 cell line	Human
	Liver, 3T3-L1 cell line	Mouse
Benzene	Blood	Human
Caffeine	Myotubes	Rat
Catalpol	Muscle	Mouse
cGMP-PDEase inhibitors (cilostamide, trequinsin, sildenafil)	Kidney	Mouse
Conjugated linoleic acid	Myotubes	Mouse
DL-3-n-butylphthalide	H9c2 cell line	Rat
Fatty acids	Muscle	Rat
Forskolin	Adipocytes	Human
Hydrogen sulfide	Hepatocytes, smooth muscle cells	Mouse
Idebenone	Early neural progenitor cells	Human
Lipoamide, Lipoic acid	Adipocytes, ARPE-19 cell line	Human
	3T3-L1 cell line	Mouse
	Liver	Rat
Lithium chloride	Dami cell line	Human
Methylmercury	Immortalized neural progenitor cells	Human
Nicotinamide riboside	Fibroblasts	Human
	Muscle	Mouse
Perfluorooctanoate	Liver	Rat
Polyphenols (curcumin, epigallocatechin gallate, hydroxytyrosol, oligonol, quercetin, resveratrol, rutin)	Coronary arterial endothelial cells, fibroblasts, HepG2 cell line, muscle, umbilical vein endothelial cells	Human
	Aorta, brain, brown adipose tissue, kidney, liver, MN9D cell line, muscle, 3T3-L1 cell line	Mouse
	Brain, hepatocytes, kidney, muscle, PC12 cell line	Rat
PPARα agonist (pirinixic acid)	Adipocytes	Human
Pyrroloquinoline quinone	Early neural progenitor cells	Human;
	Hepa1-6 cell line	Mouse
S-nitrosoacetyl penicillamine	Brown adipoctyes	Mouse
Sphingosine 1-phosphate	HepG2 cell line	Human
Sulfur dioxide	Brain	Rat
Tetramethylpyrazine	HeLa cell line	Human
Thiazolidinediones (ciglitazone, pioglitazone, rosiglitazone)	Adipose tissue, NT2 cell line, SH-SY5Y cell line, umbilical vein endothelial cells	Human
	Adipose tissue, brain, C2C12 cell line, spinal cord, 3T3-L1 cell line	Mouse
Thyroxine	Liver	Rat
Valproic acid	Fibroblasts	Human
β-adrenoceptor agonists (formoterol, nebivolol, carvedilol)	Umbilical vein endothelial cells	Human
	Heart, kidney, 3T3-L1 cell line	Mouse
	Renal proximal tubule cells	Rabbit
Δ^9-tetrahydrocannabinol	SH-SY5Y cell line	Human
ω-3 PUFAs (DHA, EPA)	Brown adipocytes, C2C12 cell line, white adipocytes	Mouse
AICAR	HeLa cell line, umbilical vein endothelial cells	Human
5-HT 1F receptor agonist (LY344864)	Heart, kidney, liver	Mouse

[a]Abbreviations: AICAR, 5-aminoimidazole-4-carboxamide ribonucleoside; cGMP-PDEase inhibitors, cyclic guanosine monophosphate-selective phosphodiesterase inhibitors; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PPARα, peroxisome proliferator-activated receptor alpha; ω-3 PUFAs, omega-3 polyunsaturated fatty acids; 5-HT, 5-hydroxytryptamine.

[b]Grey background indicates toxic compounds. Bibliographic references for all these compounds can be found in the supplementary information online

Table 2. Compounds with retinal ganglion cell neuroprotective effects[a]

Compounds	**Model**	**Animal**
All-*trans* retinoic acid	ON axotomy	Frog
Caffeine	IOP (VC)	Rat
Forskolin	IOP (anterior chamber saline infusion)	Rat
Idebenone	Rotenone (intravitreal injection)	Mouse
Lipoic acid	ONC	Cat
	Diabetes, IOP (DBA/2J)	Mouse
	IOP (anterior chamber methylcellulose injection), ONC	Rat
Lithium chloride	ONC	Rat
Nicotinamide riboside	EAE	Mouse
Polyphenols (epigallocatechin gallate, resveratrol)	EAE, IOP (anterior chamber microbeads injection), ONC	Mouse
	IOP (intracameral hyaluronic acid injection)	Rat
Sphingosine 1-phosphate analog (fingolimod)	IOP (microbeads injection)	Rat
Tetramethylpyrazine	Intravitreal NMDA injection	Rat
Thiazolidinediones (pioglitazone, rosiglitazone)	ONC, Rotenone (intraperitoneal injection)	Rat
Valproic acid	NTG (GLAST KO)	Mouse
	ONC	Rat
Δ^9-tetrahydrocannabinol	IOP (VC)	Rat
ω-3 PUFAs (DHA, EPA)	ONC	Mouse
	IOP (VC), ONH lesion	Rat

[a]Abbreviations: DBA/2J, genetic model; DHA, docosahexaenoic acid; EAE, experimental autoimmune encephalomyelitis; EPA, eicosapentaenoic acid; GLAST KO, glutamato/aspartate transporter gene deletion genetic model; IOP, intraocular pressure; NMDA, *N*-methyl-D-Aspartate; NTG, normal tension glaucoma; ON, optic nerve; ONC, ON crush; ONH, optic nerve head; VC, veins cauterization; PUFAs, polyunsaturated fatty acids.

Bibliographic references for all these compounds can be found in the Supplementary Material