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Superoxide Generation Explains Common Features of Optic Neuropathies Associated with Cecocentral Scotomas

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Introduction

The pathophysiology of some of the more common optic neuropathies associated with cecocentral scotomas might be explained by a unifying hypothesis. This hypothesis is based on clinical features of these optic neuropathies, laboratory studies of the pathophysiology of how retinal ganglion cells die, some of which is unpublished, and biochemistry of reactive oxygen species generation within some of these disorders.

Optic neuropathies and cecocentral scotomas

Diseases of the optic nerve are associated with abnormalities of vision, primarily visual acuity, color vision, contrast sensitivity, and most relevant to this discussion, the visual field. The nature of the visual field defect usually reflects the location at which the disease affects the optic nerve. For example, glaucomatous optic neuropathy primarily affects retinal ganglion cell axons at the optic nerve head. A focal thinning of the optic disc will affect those axons which originated from retinal ganglion cells defined by the pattern of the retinal nerve fiber layer (1). Compression of the chiasm from a pituitary adenoma that affects primarily crossing fibers will generate unilateral or bilateral temporal visual field defects.

Cecocentral scotomas are distinctive in that there is visual field loss centrally, but there is involvement that includes the blind spot, i.e. a temporal predominance. This is different from pure central loss which only involves the blind spot as a result of the defect itself being large enough to subsume it. What is interesting about diseases associated with cecocentral scotomas is that they are relatively few in number, and share common clinical features. The main optic neuropathies with cecocentral scotomas are:

- 1) Leber hereditary optic neuropathy (LHON)
- 2) Nutritional optic neuropathy
- 3) Toxic optic neuropathy

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Conflict of Interest: None

4) Other hereditary optic neuropathies

My focus will be on the first three, creating a hypothetical framework for understanding their pathophysiology.

Clinical presentation of cecocentral scotomas

The optic neuropathies associated with cecocentral scotoma are painless and bilateral, and usually progressive. LHON is an exception in that the visual loss frequently starts unilaterally but eventually becomes bilateral in all cases. When only one eye is affected initially, the fellow eye follows within weeks to months although sometimes longer.

Optic disc edema is unusual in the optic neuropathies associated with cecocentral scotomas. In LHON, there may be a swollen optic nerve head with telangiectatic small vessels, but it is not true disc edema because it does not show evidence of vascular leakage on fluorescein angiography. Occasionally, acute toxic or nutritional optic neuropathies show elevation of the disc.

Cecocentral scotomas and pathophysiology

A cecocentral scotoma is a visual field defect that can be thought of as representing a biomarker of an underlying pathophysiological process. The retinotopic distribution of the cecocentral scotoma classically has been believed to reflect damage to the papillomacular bundle. However, the papillomacular bundle is not a single well-defined set of axons, but rather a concentration of axons that are primarily small in diameter and are within the area between the disk and the perifoveal macula (2). As pointed out by Plant and Perry (2) and others, the concept of the papillomacular bundle was a reverse induction from the clinical and histological findings associated with toxic optic neuropathy. It is tautologous that the papillomacular bundle is involved in similar diseases; i.e., those with cecocentral scotomas. For the sake of convenience and common usage, therefore, in the rest of this article, I will use the term papillomacular bundle to refer to the set of fibers arising from the foveal and parafoveal regions and approaching the temporal part of the optic disc, both directly and via an arcuate pathway.

The critical assumption underlying the framework being developed is that the common clinical presentation of optic neuropathies associated with cecocentral scotomas implies a common pathophysiological process. Specifically, my hypothesis is that these disorders have in common the generation of superoxide anion, or *superoxide* (O_2^-). Superoxide is an oxygen-containing free radical generated within cells in various processes. Relevant to this hypothesis, one of the main sources of superoxide is the reaction of molecular oxygen (O_2) with a free electron.

Superoxide is reactive, and among its reactions is that with nitric oxide (NO) to form peroxynitrite ($ONOO^-$). Peroxynitrite can react with other molecules, such as tyrosines, resulting in their nitration. Superoxide also reacts directly with macromolecules such as

proteins and nucleic acids. The scavenging of superoxide typically occurs via superoxide dismutases, of which there are three major types: intracellular (SOD-1 [Cu/Zn-SOD], mitochondrial [SOD-2 Mn-SOD], and extracellular (SOD-3).

Besides causing oxidative damage, superoxide has an important role as a signaling molecule. Cells use small molecules such as nitric oxide to activate or suppress various processes within the cell. Superoxide has been recognized for many years to be such a molecule. For example, it is known to initiate mitosis in certain cells (3).

Over the last decade, our group demonstrated that superoxide plays a special role relevant to retinal ganglion cells (RGCs). Specifically, we showed that it signals the death of the cell body, or *soma*, when the axon is injured (4, 5). In other words, injury to the axon of the RGC caused an increase in superoxide within its soma. This finding was accomplished by imaging superoxide using specialized fluorescent probes in rat retinas after optic nerve transection. The increase in superoxide could be seen a few days after axonal injury, followed a day later by death of the RGC.

Using a variety of techniques, we established that the superoxide was not simply a result of the cell in the final throes of death, but, rather, a signal that both preceded and was necessary for death. We did this by showing that other drugs that decrease the levels of superoxide; (e.g., pegylated superoxide dismutase), both decreased the levels of superoxide and prevented the death of the RGC. Based on these studies, we concluded that superoxide was a signal for RGC death after axonal injury (5, 6).

Superoxide and cecocentral scotomas

How is superoxide relevant to optic neuropathies in which cecocentral scotomas occur? The next sections will discuss three specific optic neuropathies in turn; namely, LHON, the optic neuropathy associated with vitamin B₁₂ deficiency, and the toxic optic neuropathy caused by ethambutol. Each of these is associated with cecocentral scotomas, and for each of these, we have evidence that superoxide is generated at increased levels.

Leber hereditary optic neuropathy

In at least 95% of patients with LHON, there is a mutation at one of three sites within the mitochondrial DNA (mtDNA). The most common is the 11778 mutation, followed by the 3460 and 14484 mutations. There is a slow-to-rapid development of loss of vision in one or both eyes. If only one eye is involved initially, the second eye follows within weeks to months. The loss of vision typically occurs in the late teens or early 20s, but it can occur at any age. There is a strong male predominance, which is as yet unexplained, especially as the mtDNA is present in all cells (except mature erythrocytes) of both genders.

All of the three primary mutations that produce LHON are contained in mtDNA coding for components of the complex I of the mitochondrial electron transport chain. Complex I serves as a NADH:ubiquinone oxidoreductase. In other words, NADH, a product of the Krebs cycle, is oxidized at the same time that ubiquinone, a molecule that carries electrons between complexes, is reduced. Functionally, two electrons are transported from NADH to

ubiquinone at the same time that four protons (H^+) are transported across the inner membrane of the mitochondria into the mitochondrial intermembrane space.

This set of reactions has two results. First, electrons are transported to various loci in the electron transport chain. Second, protons are pumped across the inner membrane to form a voltage gradient. The voltage gradient, or electromotive potential, is the basis for another complex, complex V, to generate ATP in the process of transferring protons back across the inner membrane into the mitochondrial matrix.

For many years, it was assumed that the mechanism by which LHON mtDNA mutations in complex I caused visual loss was a deficiency of ATP; e.g., by decreased pumping of protons or some other mechanism. This made sense, given that ATP is essential for cellular function, and RGCs, with their very long axons relative to the size of their somas, would presumably have high ATP demands. However, there are some flaws in this assumption, related to the fact that RGCs are specifically affected in LHON whereas other cells are less commonly involved. We pointed out several years ago that diseases characterized by decreased ATP production from mutations in mitochondrial DNA usually are not associated with death of RGCs (7). Instead, they are associated with death of photoreceptors, as occurs in mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS; mtDNA position 3243) or neuropathy, ataxia, and retinitis pigmentosa (NARP; mtDNA position 8993). Frequently, there is abnormal extraocular muscle function, as well as other systemic involvement such as abnormal cardiac conduction deficits and pancreatic dysfunction. Tissues such as photoreceptors and extraocular muscles are highly energy-consuming, and any disruption of ATP production in MELAS or NARP usually will affect patients early in the course of their disease. Therefore, if there were a deficiency in ATP production in LHON, there should be effects on photoreceptors and not, or at least not only, on RGCs.

The second reason to suspect that ATP production is less relevant to LHON than previously believed is that studies of LHON cybrids demonstrate only a relatively small effect on ATP production. Cybrids are cells in which the mitochondria are from one cell source, usually having mutant mt DNA, whereas the rest of the cell is from another source, usually a wild-type dividing cell. This allows study of the specific effects of mt DNA mutations without affecting other aspects of cellular function. Surprisingly, the respiration deficits that would decrease ATP production are not particularly marked (8-12), although this is not always the case (13). Note that it currently is not possible to make cybrids from isolated RGCs because a dividing cell is necessary, and RGCs, like other neurons, do not divide.

Superoxide is produced in LHON cybrids that are made in neuronal cell lines (14). For example, Wong and colleagues showed an approximate doubling of superoxide production in LHON 11778 or 3460 cybrids compared with control, non-mutant cybrids (14). They suggested that this effect was specific to differentiated neurons. However, they used a neuronal cell line that was different from RGCs and, thus, their findings would not explain the specific damage to RGCs in LHON.

How do LHON mutations result in an increase in superoxide? As mentioned previously, the function of complex I is as a NADH-ubiquinone oxidoreductase. If the transfer of electrons to ubiquinone is disrupted, then complex I will retain the extra electron, which then can react with free oxygen to form superoxide. In other words, the problem with the LHON mutations and their effects on complex I is less likely due to decreased ATP production, but instead due to an accumulation of superoxide from failure to transfer electrons down the electron transport chain to other complexes. This, in turn, results in those electrons reacting with molecular oxygen to form superoxide.

Why should an increase in superoxide in LHON lead to RGC death but not death of other cells? Mitochondria are present in all cells in the body except mature erythrocytes, and, therefore, superoxide should be increasing in those cells as well. The specificity to RGCs is therefore unexpected.

The answer is that superoxide is not only a free radical that can react with other molecules to cause oxidative damage, but it also is a signaling molecule. As discussed previously, superoxide is a signal for RGC bodies to die after the axon is injured. Our hypothesis (7) is that the increased superoxide “tricks” the soma of the RGC into thinking that axon injury has occurred, even though it has not. The RGC soma then initiates the process of apoptosis, misled by the aberrant superoxide signal, and eventually dies. Other cells in the body may not use superoxide as a signal for cell death, and, therefore, remain unaffected.

Partial support for this theory can be found in a study of mice in which superoxide dismutase-1 is knocked out, resulting in the levels of superoxide within the cell bodies being higher than normal (15). These animals have decreased numbers of RGCs, consistent with the premise (relevant to LHON) that increasing superoxide levels in cells other than RGCs nevertheless causes the RGCs to die, even when there is no axon injury and no direct injury to the RGCs.

In summary, LHON likely represents a disease in which superoxide is abnormally produced because of mutations in complex I of the mitochondrial electron transport chain. RGCs may be specifically sensitive to increased superoxide because it is a signaling molecule for cell death after axon injury. The increased superoxide therefore ultimately would result in specific loss of RGCs, with relative preservation of other cells within the nervous system and elsewhere in the body.

Vitamin B₁₂ deficiency optic neuropathy

Clinical background—“Nutritional amblyopia” is a term used for the optic neuropathy that arises from malnutrition. Examples include starved prisoners of war in Asia during World War II, severe malnutrition in alcoholics, those on inadequately supplemented vegan diets, patients with various gastrointestinal disorders or who have had intestinal surgery resulting in inadequate absorption of needed nutrients, and pernicious anemia in which, antibodies to intrinsic factor decrease absorption of vitamin B₁₂.

In virtually all cases, the causative factor is an abnormality of vitamin B₁₂, either from its absence in the diet or failure of absorption in the gastrointestinal tract. Occasionally,

abnormalities in metabolism or cell uptake of vitamin B₁₂ are causative. There are occasional cases of nutritional amblyopia from a deficiency of other nutrients, most commonly folate, and, rarely, vitamin B₆.

The clinical picture of vitamin B₁₂ deficiency-related optic neuropathy is slowly progressive bilateral visual loss, decreased color vision, cecentral scotomas, and the eventual appearance of temporal disc pallor, sometimes with excavation of the temporal disc. Interestingly, these disc features are similar to those seen in patients with LHON.

Vitamin B₁₂ has several roles within cells. Its absence causes megaloblastic anemia, subacute degeneration of the spinal cord, neuropsychiatric disorders, and, optic neuropathy. It is presumed that most of these abnormalities are due to its role as a cofactor for two enzymes, methionine synthase and methylmalonyl-CoA mutase, so that its deficiency will result in increased levels of methionine and methylmalonic acid, respectively. In some patients, vitamin B₁₂ levels in the blood may be in the “normal” range, but there are elevated serum levels of homocysteine and/or methylmalonic acid, implying that there is a functional vitamin B₁₂ deficiency despite the normal serum levels.

The treatment of severe vitamin B₁₂ deficiency-related optic neuropathy is usually large amounts of vitamin B₁₂ delivered through a parenteral route, followed by continued supplementation at a lower dose. The commercially available form of vitamin B₁₂ in tablets is cyanocobalamin. Many believe that hydroxocobalamin is preferable because of its longer half-life, that the cyanide group on cyanocobalamin could be toxic to RGCs, or that hydroxycobalamin can detoxify free cyanide. Interestingly, the World Health Organization lists hydroxocobalamin and not cyanocobalamin in its list of essential drugs (16).

Vitamin B₁₂ as a superoxide scavenger—As part of a collaboration with Professor Zeev Gross of the Technion in Haifa, Israel, we assessed novel chemical entities to serve as superoxide scavengers for decreasing RGC death after axon injury. We tested metallocorroles, molecules that Gross and his laboratory had invented for redox chemistry. We tested their role as potential neuroprotective compounds and showed that some metallocorroles served as excellent superoxide scavengers in RGCs (17). They also were effective in decreasing RGC death after optic nerve transection in adult rats (18).

We noticed that the chemical structures of metallocorroles and vitamin B₁₂ were very similar, with both having a corrin ring. Based on this similarity, we hypothesized that vitamin B₁₂ could serve as a superoxide scavenger. A literature search found an article from the laboratory of Nicola E. Brasch at Kent State University showing that, cobalamin is a superoxide scavenger with catalytic activity approaching that of superoxide dismutase (19). We therefore embarked on a series of experiments to examine the ability of vitamin B₁₂, in the form of cyanocobalamin, to scavenge superoxide in RGCs and rescue them from cell death. While our results have been submitted for publication, the main findings are:

1. In the test tube, vitamin B₁₂ is an excellent scavenger of superoxide produced by reacting xanthine with xanthine oxidase.

2. Superoxide scavenges RGCs in neuronal-like cells (we used the RGC-5 photoreceptor-derived cell line that, despite its name, is **not** an RGC).
3. Intravitreal vitamin B₁₂ scavenges superoxide produced in RGCs after axon transection.
4. Intravitreal superoxide decreases RGC death after optic nerve transection.

Implications for understanding vitamin B₁₂ optic neuropathy—We hypothesize that vitamin B₁₂ has, in addition to its enzymatic cofactor roles with respect to methionine synthase and methylmalonyl-CoA mutase, a role as an endogenous superoxide scavenger in RGCs. Its deficiency would be specifically deleterious to RGCs because, as noted in the previous section on LHON, RGCs use superoxide as an intracellular signaling molecule for inducing soma apoptosis after axon injury. Given that superoxide is continuously being generated within cells as part of the mitochondrial electron transport chain, if superoxide is a signaling molecule, an RGC would require strict control of intracellular concentrations. Otherwise, the deregulated superoxide might induce aberrant cell death. The usual superoxide scavengers within cells, such as the various superoxide dismutases, theoretically should be sufficient, as they are for other cells. Given that RGCs do not divide and therefore cannot be replenished if they die, it is not surprising that they would develop a system for regulating superoxide levels that is more stringent than that of other cells.

We have suggested a role for vitamin B₁₂ as an additional superoxide scavenger. This is hypothetical, and further work will be needed to prove that this is indeed a mechanism relevant to RGCs. It is very difficult to induce optic neuropathy from vitamin B₁₂ deficiency in rats, and even more than 12 months of such deficiency does not cause an obvious loss of RGCs. Thus, it is possible that vitamin B₁₂ does not play the same role in rats as it does in humans. This is akin to other differences between rats and humans with respect to optic neuropathies; e.g., rats given methanol develop primarily a photoreceptor toxicity not an RGC toxicity. Another possibility is that rats are able to upregulate other superoxide scavenging molecules within cells in response to vitamin B₁₂ deficiency. Overall, this is an area of active research.

In summary, these data are consistent with the hypothesis that vitamin B₁₂ deficiency, the known cause of nutritional optic neuropathy, is due to an abnormality in superoxide scavenging, and that elevated levels of superoxide within RGCs would be responsible for the progressive bilateral visual loss associated with cecocentral scotomas that occurs from RGC death in this disease.

Ethambutol optic neuropathy

Ethambutol is commonly used to treat tuberculosis and other mycobacterial infections, especially multiple drug-resistant (MDR) tuberculosis. Ethambutol is the most common cause of toxic optic neuropathy (20, 21), and is characterized by progressive bilateral loss of visual acuity, acquired dyschromatopsia, cecocentral scotomas, and optic disc pallor. In some cases, there is a significant temporal preponderance of the visual field defect that sometimes respects the vertical meridian, suggesting that the damage is occurring primarily

at or near the optic chiasm. This is different from the classic cecocentral scotoma associated with presumed involvement of the papillomacular bundle.

The potential for ethambutol to produce an optic neuropathy limits in patients who have visual symptoms, underlining the need to understand this optic neuropathy and find mitigating strategies. Major problems are that it is difficult to predict who will develop the optic neuropathy, how it can be detected before RGCs are irreversibly damaged, and, once present, how to treat the visual loss other than by stopping the medication. In many cases of ethambutol optic neuropathy, there is reversibility when the visual loss is detected early. However, the literature is replete with cases demonstrating failure to completely or even partially reverse the loss of vision. Two studies demonstrated that 40-50-% of patients with ethambutol-related optic neuropathy had no visual improvement after ethambutol was discontinued, an unacceptably high failure rate for the only known potentially effective treatment (22, 23).

A priori there is no specific reason to suspect that ethambutol toxicity is associated with superoxide induction. However, in preliminary experiments that we have performed in rats, we found increased levels of superoxide after intravitreal injection of ethambutol, using the oxidation of hydroethidine as a marker for superoxide induction.

***In vitro* detection of superoxide induced by ethambutol by fluorescent microscopy**—We purified rat RGCs by sequential immunopanning and plating on poly-D-lysine/laminin-coated plates to provide a substrate for adherence and neurite outgrowth. The cells then were exposed to 3 mM ethambutol for 1 hr, with hydroethidine (1 mM) added in the last 30 min to detect superoxide generation. Hydroethidine reacts with superoxide to form 2-oxy-ethidium, which has specific excitation and emission characteristics. The cells then were imaged by fluorescent microscopy or with a fluorescent plate reader.

The addition of ethambutol resulted in a robust fluorescence signal, compared with vehicle control. To prove that ethambutol led to superoxide generation and not generation of some other reactive oxygen species that might also oxidize hydroethidine, some wells were incubated simultaneously with pegylated superoxide dismutase (500 U/ml), which enters cells and scavenges only superoxide and not other reactive oxygen species. This eliminated the ethambutol-induced fluorescence, implying that the fluorescence reflected an increase in superoxide alone.

The interpretation of these experiments is that ethambutol induces superoxide in cultured ratRGCs.

***In vivo* detection of superoxide induced by ethambutol using *in vivo* confocal imaging**—One eye of adult Long-Evans rats under anesthesia received an intravitreal injection of ethambutol (final concentrations 0, 0.3, 1, or 3 mM), along with hydroethidine (final concentration 100 μ M) to detect superoxide. The other eye was not injected and acted as a control rats were imaged 24 hr later with a Heidelberg HRA-2 confocal scanning laser ophthalmoscope using the 488 nm laser for excitation and a broad-spectrum 500-600 nm filter for emission detection. Eyes injected with 3 mM ethambutol demonstrated small but

definite production of superoxide in cells within the RGC layer that were not fluorescent prior to ethambutol injection, whereas equivocal induction was seen in eyes injected with the 0.3 or 1 mM concentrations of ethambutol. No fluorescence evidence of superoxide induction was seen in control eyes.

These data suggest that ethambutol can induce superoxide in RGCs and presumably lead to their death. Note that these experiments used an acute exposure to high concentrations of the drug. Chronic exposure experiments need to be performed to allow translational validation in patients. Furthermore, chronic exposure experiments to very high doses of ethambutol in rats demonstrated chiasmal lesions (24), and whether or not this chiasmal predilection is related to superoxide production is unclear. Finally, the mechanism for ethambutol causing an increase in superoxide is currently unknown but could be related to zinc chelation effects of ethambutol and the interaction of zinc with complex I (25).

Issues related to the superoxide hypothesis for cecocentral scotomas

Why the papillomacular bundle?

Even if superoxide is a common pathophysiological factor for LHON, vitamin B₁₂ deficiency optic neuropathy, and ethambutol optic neuropathy, this does not explain why a cecocentral scotoma develops. In other words, why should the papillomacular bundle be preferentially involved, and why should axons or their RGCs elsewhere in the retina be relatively spared?

The answer probably relates to the fact that the axons in the papillomacular bundle are smaller than average. Although there is controversy regarding this issue (2) there is strong evidence to suggest that: 1) RGCs in the papillomacular bundle are predominantly midtemporal cells with small axons; 2) the axons are small for most of their course towards the disc; 3) these features are different from fibers approaching the disc from other directions (26-28).

Sadun and colleagues have made convincing arguments that the size of RGC axons is relevant to the effects of a decrease in ATP production in the setting of LHON, with a greater mismatch between the ATP produced and the ATP needed in small versus large fibers of RGCs (29, 30). However, as indicated above in the section on LHON, a deficit in ATP production is unlikely to be the main causative factor in this disorder, and presumably, also in vitamin B₁₂ deficiency optic neuropathy or ethambutol optic neuropathy.

Instead, a parallel analytical process used by Pan and colleagues³⁰ for comparing small and large axons can be applied to the dynamics of superoxide production, as follows:

- 1) Most of the ATP needed for axon conduction is for renormalizing the sodium and potassium concentrations at the nodes of Ranvier, primarily after an action potential has occurred. Sodium enters the axon and potassium leaves the axon as the two major ionic fluxes associated with the formation of an action potential. The Na⁺-K⁺-ATPase renormalizes these concentrations by shuttling sodium and potassium ions to the extracellular and intracellular spaces, respectively.

- 2) The renormalization of sodium and potassium concentrations is an ATP-dependent step and, therefore, the amount of ATP being consumed will be proportional to the amount of ionic flux, which takes place at the surface of the axon, at the nodes of Ranvier.
- 3) Superoxide is a byproduct of ATP production in the mitochondrial electron transport chain, and therefore the amount of superoxide being produced will largely be proportional to the amount of ATP needed to support the ionic flux that takes place at the axon surface.
- 4) The detoxification of superoxide should be proportional to the axon volume, given that most superoxide is detoxified by intracellular superoxide dismutases.
- 5) The area/volume ratio is greater in small fibers than in large fibers because the surface area is proportional to the radius and the volume is proportional to the square of the radius.
- 6) Given that the amount of superoxide production is proportional to sodium and potassium flux at the axon surface area and that the superoxide detoxification is proportional to axon volume, there will be relatively more superoxide production than detoxification in small fibers compared with large fibers.

In other words, there should be a relatively greater mismatch between superoxide production (relative to ATP synthesis used to maintain axon conduction) and detoxification in small RGC axons than in large axons. This would support a preferential involvement of small axons in the papillomacular bundle. However, as pointed out by others (2), it is not fiber size alone that would explain the concentrated damage in this area. It is possible that the packing density of the axons in the retinal nerve fiber layer near the temporal disc or where the axons enter the disc that is the critical factors.

Chiasmal involvement

There are occasional cases of ethambutol optic neuropathy in which the visual field defects are suggestive of chiasmal involvement. There also is a pathological study in rats showing chiasmal axon damage (24). Interestingly, there are cases of LHON in which there is predominantly chiasmal involvement based on clinical, magnetic resonance imaging, or pathological evidence (31, 32).

These findings are not directly explained by aberrant superoxide levels causing death of RGCs. One possible reason for preferential involvement of the chiasm is that the crossing fibers within the chiasm theoretically undergo a small deformation as they cross under and over each other, in the same way that a weave deforms the fibers as they cross each other. Under those circumstances, the surface/volume relationship described above would be exacerbated in those locations where the chiasm is flattened. The surface/volume ratio of a flattened fiber is greater than one with a more circular cross-section. The factor of packing density and relation with adjacent fibers also may be relevant.

Other Optic Neuropathies with Cecocentral Scotomas

This hypothesis discusses three specific optic neuropathies, but there other optic neuropathies, such as dominant optic atrophy and methanol related optic neuropathy, in which the same clinical pattern occurs. The following is highly speculative and is included only in the interest of completeness.

Dominantly, inherited optic atrophy is associated most often with mutations in the OPA1 and OPA3 genes, both of which are important for mitochondrial function. Studies in *Drosophila* have shown evidence that OPA1 deficiency increases reactive oxygen species levels, causes a variety of ocular abnormalities, and is rescued by superoxide dismutase (33). It would not be surprising if OPA1 deficiency in patients with dominant optic atrophy leads to increased superoxide levels in most cells, with preferentially more toxicity in RGCs for the same reason that it occurs in LHON.

Methanol intoxication is another example in which there may be a role for superoxide. Methanol is metabolized to formate, which inhibits mitochondrial cytochrome c oxidase (complex IV). This inhibition leads to electrically reduced upstream complexes in the mitochondrial electron transport chain. The electron-rich reduced complexes can react with molecular oxygen to form superoxide. In experiments conducted in rodents, methanol intoxication does not cause the predominant RGC death that it does in humans, but instead results in primarily photoreceptor death (34). Therefore, it is unclear if studies in rodents can help clarify the precise mechanism by which methanol causes an optic neuropathy.

Implications for treatment of optic neuropathies associated with cecocentral scotomas

From the foregoing discussion, one might conclude that drugs that reduce superoxide levels within RGCs might be effective treatments for any or all of these diseases. This is a reasonable assumption, but there are several caveats. The most important is that it is the level of *intracellular* superoxide that is critical, and unless a superoxide scavenger is able to actually enter the RGC, it may not be effective. It also has to enter the cell at high levels. For example, loading patients with high levels of vitamin B₁₂ makes theoretical sense, but unless the intracellular concentration is maintained at a sufficiently high level for the long term, it may not be a sufficiently effective superoxide scavenger to be clinically beneficial. Drugs that our laboratory has studied, such as pegylated superoxide dismutase and metallocorroles, (5, 17, 18) are theoretically potent, but their use in humans would require a lengthy drug development process.

There has been much interest in drugs such as idebenone for LHON. A formal clinical trial was performed but did not meet its primary endpoint for preventing vision loss in LHON (35). However, much of the data from the trial was encouraging, as was a separate non-randomized study (36). Idebenone was designed as a drug that would serve to shuttle electrons, serving as a synthetic ubiquinone electron carrier. However, biochemical studies show that idebenone also is a superoxide scavenger (37, 38). It may actually be efficacious because it reduces intracellular superoxide and not because it restores mitochondrial electric transport in LHON. On this basis, one could speculate whether or not drugs such as idebenone could be used in other optic neuropathies associated with cecocentral scotomas.

Summary

I have presented above a hypothesis that ties together several disparate optic neuropathies, all characterized by a similar clinical presentation. The hypothesis is predicated on the formation of intracellular superoxide within retinal ganglion cells as a common pathological pathway for the type of cell death that occurs. The anatomical predisposition of the papillomacular bundle to have elevated superoxide levels is tied to the size of the fibers involved, a hypothesis that also implicates the crossing fibers of the chiasm. Much of this work is speculative and is an interpretation of several experimental studies that have been performed to date. Hopefully, this hypothesis will be developed further and its validity tested in both experimental models and, ultimately, in humans.

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