LEARNING OBJECTIVES
1. Understand the indications and methods for using topical cholinergic agents to differentiate causes of efferent pupil defects that result in anisocoria being greater in bright light conditions.
2. Understand the advantages and disadvantages of using cocaine and apraclonidine to diagnose oculosympathetic defects.
3. Explain why use of hydroxyamphetamine for localization of the site of an oculosympathetic lesion is helpful, rather than imaging both pre and post-ganglionic sympathetic pathways over the entire neuro-axis.

CME QUESTIONS
1. Which of the following conditions will produce under-sensitivity to topical pilocarpine?
   a) Giant cell arteritis with no subsequent light–near dissociation
   b) High intraocular pressure the night after cataract surgery
   c) Carotid artery stenosis
   d) b and c
   e) all of the above
2. Which factor would account for equivocal results of cholinergic super–sensitivity testing for a unilateral efferent pupil defect, comparing the unaffected and affected eye response?:
   a) Testing after only 7 days following a preganglionic parasympathetic lesion from a p–com aneurysm
   b) Genetic differences in corneal permeability to drugs
   c) Applanation tonometry prior to testing
   d) Lack of light–near dissociation
   e) Recording pupil diameters before and after drops in darkness
3. Which of the following situations would not adversely affect the interpretation of a hydroxyamphetamine test?
   a) Testing 2 days prior with apraclonidine.
   b) Testing 2 days prior with cocaine.
   c) Testing 2 days after an internal carotid artery dissection.
   d) Testing 2 years after the discovery of Horner syndrome in an infant from old pictures.
   e) Testing 2 days after the discovery of metastatic breast carcinoma in a patient with anisocoria.

KEY WORDS
1. Horner Syndrome
2. Super–sensitivity
3. Hydroxyamphetamine
4. Apraclonidine
5. Cocaine
6. Pilocarpine

EVIDENCE PRESENTATION
1. Disorders of the iris sphincter that may require pharmacologic testing — cholinergic super–sensitivity vs. under–sensitivity:
   When evaluating an anisocoria that increases in bright light and decreases in dim light, attention usually shifts toward the pupil that is larger in bright light to determine if a paresis of the iris sphincter paresis is present. The causes are either from denervation of the iris sphincter, blockade of the cholinergic receptors, or damage to the iris sphincter itself.

   Cholinergic Super–sensitivity Testing — Over–utilized:
   In most cases where denervation of the parasympathetic nerves to the iris sphincter is suspected, the use of dilute topical cholinergic agents for determining super-sensitivity of the iris sphincter and ciliary body is not necessary. This is because most postganglionic causes of cholinergic denervation of the iris are obvious on clinical examination. The most common finding is sectoral sphincter palsy where some clock–hour segments of the iris sphincter contract to light when observed under high magnification of a slit lamp or with infrared transillumination1 and adjacent sphincter segments do not react. Sectoral paresis interposed with sectors that do react to light is a hallmark feature of postganglionic
parasympathetic denervation, but this may also occur with direct damage to the iris sphincter muscle (see below). Rarely, all iris sphincter segments can be equally paretic in the case of a complete postganglionic parasympathetic lesion, but it is the rule with preganglionic parasympathetic lesions.

I have yet to see a case of acute preganglionic denervation from pathology of the oculomotor nerve that caused a sectoral paresis with some intervening sectors spared. Usually the sphincter weakness is symmetric over all clock-hour segments with acute pupil involving third nerve palsies. However, in the case of chronic palsies, when aberrant regeneration is present from a preganglionic parasympathetic lesion, sectoral re-innervation may occur, but only after a period of at least 8 weeks following denervation. 8 weeks is the minimal time required for re-growth of damaged preganglionic axons to reach the ciliary ganglion in the orbit.

Another important sign that aids in the diagnosis of iris sphincter denervation that circumvents the use of dilute cholinergic agents is light–near dissociation, a form of aberrant regeneration, signifying that denervation as occurred and has been followed by re-growth of postganglionic parasympathetic cholinergic fibers that were previously only innervating the ciliary body and have now found their way to the iris sphincter muscle. The presence of light near dissociation is often missed, either because it was inadvertently omitted from the evaluation or because it was not properly tested. Constriction of the pupil to a near effort in this setting takes longer to develop than does a normal near pupil contraction; the patient needs to make a concerted near effort for at least 10–15 seconds. I often click my thumb and index fingernails together in front of the patient’s nose as I cheer them on to make a forceful near effort — the additional auditory stimulus helps them focus and converge at my finger and thumb. If done correctly, the near pupil constriction will be slow to develop, but will often exceed the normal near constriction in the opposite eye and it will also be slow to dilate after looking off in the distance (the classic “tonic” near response). It should also be noted that once postganglionic nerves have grown back into the denervated sphincter segments, receptor sensitivity goes down, so it is not unusual to lose cholinergic super-sensitivity once aberrant regeneration has taken place. To signify a light–near dissociation, the near contraction of the pupil in dim light should exceed the amount of miosis to bright light, rather than an added constriction to near effort on top of a simultaneous bright light stimulus.

Various factors can confound the interpretation of super-sensitivity testing, discouraging its use in most situations. That being said, when cholinergic super-sensitivity testing is performed using dilute 0.1% pilocarpine, the drop should always be given to both eyes, comparing the response of the affected eye relative to the opposite eye in darkness after 30 minutes. However, the results can still be equivocal. For example, applanation tonometry and topical corneal anesthetics can disrupt the epithelial barrier and influence the penetration of the dilute topical agent through the cornea and its subsequent effect. In addition, there appears to be a wide range of miotic effect of dilute pilocarpine in normal eyes, owing to corneal permeability differences among individuals, some of which may be genetically determined. When denervation does take place, super-sensitivity usually only takes 4–5 days to develop, which should also be kept in mind; super-sensitivity occurs whether the lesion is pre or postganglionic, so its presence is not localizing.

From the discussion above, when should pharmacologic testing be used in the setting of an anisocoria that increases in bright light, signifying an efferent pupil defect from dysfunction of the iris sphincter?

The main clinical diagnoses that need to be differentiated in the setting of an efferent pupil defect due to sphincter dysfunction include the following:

a. Cholinergic denervation due to
   i. post-ganglionic parasympathetic lesions e.g., Adie pupil, ischemia, orbital inflammation, compressive lesion, traumatic denervation or iatrogenic denervation from laser, cryotherapy, or orbital surgery
   ii. pre-ganglionic lesions e.g., oculomotor nerve compression, inflammation or trauma

b. Pharmacologic mydriasis due to either anticholinergic blocking of cholinergic receptors or sympathomimetic stimulation of the dilator muscle. When pharmacologic mydriasis is starting to wear off, there may be observable pupil contraction, but less than the unaffected eye.

c. Direct damage to the iris sphincter from ischemia e.g., occlusive artery diseases, spikes in intraocular pressure, vasculitis from herpes Zoster, intraocular surgery, pseudoexfoliation syndrome, hereditary mydriasis, or scarring (synechiae) of the iris to the lens

d. Blocking of cholinergic receptors by antibodies i.e. anti Hu cancer associated antibodies

Cholinergic Testing for Under-sensitivity — Under-utilized: From the differential diagnosis of efferent iris sphincter paresis outlined above, the main question is not necessarily if super-sensitivity is present, but rather whether under-sensitivity is present, indicating a problem with the iris sphincter muscle itself. The causes listed above are due to either denervation or direct damage/dysfunction of the iris sphincter muscle; both sites of damage commonly show elements of segmental sphincter paresis at the slit lamp, so this feature does not help in differentiating the cause.

The question of whether there is cholinergic under-sensitivity of the iris sphincter muscle is best resolved by administering 0.5% pilocarpine to both eyes and evaluating the response of the involved pupil relative to...
the opposite pupil after 30 minutes. This is the pharmacologic test that is most indicated when it is not clear whether there is direct damage to the iris sphincter muscle or whether cholinergic denervation has occurred. Since the objective of such pharmacologic testing is to assess the direct effect of a cholinergic agonist on the iris sphincter muscle, it is best to assess the effect of a cholinergic in conditions of dim light or darkness, allowing only the pharmacologic effect on the sphincter to be determined and not confounded by any coincident light effect on pupil size. Under these conditions, pathology of the iris sphincter muscle itself will result in less pupil contraction to 0.5% pilocarpine relative to the opposite, normal eye. Another approach is to first give dilute 0.1% pilocarpine to both eyes, observe after 30 minutes, and then follow this with 0.5% pilocarpine OU if there was less reaction of the involved eye to the dilute pilocarpine test.

2. Disorders of the iris dilator muscle requiring pharmacologic testing:
When evaluating an anisocoria that increases in dim light and decreases in bright light, attention usually shifts toward the pupil that is smaller in dim light to determine if an oculosympathetic paresis is present. The most common cause of anisocoria in this setting is physiologic anisocoria, which is thought to be due to asymmetric supranuclear inhibition to the Edinger–Westphal Nucleus. The main causes of anisocoria in dim light are:

a) Oculosympathetic paresis (Horner syndrome)
b) Physiologic anisocoria
c) Topical drug-induced Horner syndrome (brimonidine)
d) Pseudo Horner syndrome caused by direct damage to the dilator muscle due to:
   i. Pseudoexfoliation syndrome
   ii. Pigmentary dispersion syndrome
   iii. Little old Adie pupil
e) Trauma resulting in prostaglandin mediated transient miosis

Most of the time, a relative miosis caused by an oculosympathetic palsy can be diagnosed by observing dilation lag and also by the company it keeps, namely ptosis of the upper and/or lower eyelid and in a smaller number of cases, anhidrosis and conjunctival injection (acutely). With that being said, why would one need pharmacologic confirmation of Horner syndrome?

The answer lies in two basic concepts, not readily appreciated, which often interfere with the ability to diagnosis Horner syndrome without the aid of pharmacologic testing. First, most oculosympathetic lesions are incomplete or partial, similar to other nerve palsies. In the case of partial lesions with less fibers being affected, the degree of dilation lag and ptosis may be subtle. This may also cause pharmacologic testing to be equivocal, overlapping with the degree of response that may occur in a normal eye. Second, in oculosympathetic lesions, each end organ (i.e. dilator muscle, Mueller muscle, sweat glands) may not be affected in the same proportion due to the topographic segregation of axons within the sympathetic nerve. This may cause one of the signs of Horner syndrome to predominate, such as anisocoria without noticeable ptosis, providing a greater challenge to the clinical diagnosis of Horner syndrome without the aid of pharmacologic testing.

The indication for pharmacologic diagnosis of Horner syndrome is when there is relative uncertainty in the clinical signs of dilation lag, Mueller’s muscle paresis, and anhidrosis, as noted above.

Cocaine Testing — On the Decline: Cocaine testing has lost its luster as the gold standard for diagnosing oculosympathetic paresis in recent years owing mainly to the increased difficulty in maintaining its easy availability because it is a controlled substance. Also, cocaine is not a perfect diagnostic test; a positive test depends on demonstrating a relative lack of mydriasis in the more miotic eye compared to the expected mydriasis in the other, normal eye. When the normal eye does not show significant cocaine–induced dilation due to reduced sympathetic activity (the patient may be partly napping during the 50–60 minute wait after the drop), the differential effect of cocaine on the two eyes becomes minimal. As noted above, a partial Horner syndrome also can confound the cocaine test; the more remaining intact sympathetic fibers there are, the greater the cocaine–induced mydriasis. Hence, there are some patients in which the cocaine test result can be equivocal. In 1990, we reported on what constitutes a positive cocaine test using logistic regression statistics and odds ratios.

Beware of a False Positive Cocaine Test: A false positive cocaine test can occur when other factors besides oculosympathetic denervation interfere with pharmacologic mydriasis. These include a little old Adie pupil, iris synchia, pseudoexfoliation and pigmentary dispersion syndrome. Often when cocaine fails to dilate the miotic pupil and it also resists dilation in darkness, even after 30–45 seconds, I am suspicious for a false positive cocaine test. Addition of 2.5% neosynephrine to both eyes at the end of the cocaine test (i.e. 60 minutes after topical cocaine) can help sort this out. If it really is a true positive cocaine test, then direct sympathomimetic stimulation of the dilator muscle after cocaine should cause mydriasis, often more in the affected eye, due to super–sensitivity. However, if direct acting sympathomimetics do not dilate the abnormal pupil as much as the opposite normal eye, then a false positive cocaine test should be suspected and not an oculosympathetic paresis.

Apraclonidine (Iopidine) Testing — Popularity Contest Winner: Apraclonidine testing for the diagnosis of Horner syndrome is rapidly becoming the new standard for pharmacologic testing to diagnose Horner syndrome. Based on what has been published in the literature and a prospective trial that we have conducted comparing cocaine with apraclonidine in the same patients on two separate test dates, I favor apraclonidine use in the
A diagnosis of Horner syndrome with only a few exceptions (infants and lesions occurring within the first 3 days of pharmacologic testing).

**Apraclonidine Factoids:**

- Apraclonidine was designed as a strong alpha-2 adrenergic agonist to lower intraocular pressure by suppressing aqueous formation. Alpha-2 receptor stimulation on the presynaptic adrenergic nerve terminal inhibits the release of norepinephrine, suppressing sympathetic nerve action on its end organ.

- Apraclonidine also has weak alpha-1 adrenergic agonist activity, but in normal eyes this is completely overshadowed by the strong alpha-2 agonist effect which suppresses sympathetic induced effects on the end organ. In contrast, brimonidine was developed as a pure alpha-2 agonist and does not have any alpha-1 activity. Therefore, brimonidine should not be used to diagnose Horner syndrome. In fact, it will produce a pharmacologic Horner syndrome, like apraclonidine, in many cases of a “reversal of anisocoria” after apraclonidine.

- Apraclonidine easily penetrates the cornea and does not readily penetrate the central nervous system as brimonidine does (Warning: 3 reports of transient CNS depression in children after topical apraclonidine drops\(^5\)\(^7\)\(^9\), so either one should consider monitoring this age group for hours after apraclonidine or substitute cocaine testing).

- When super-sensitivity is present, apraclonidine’s weak alpha-1 agonist activity predominates, causing smooth muscle contraction, resulting in mydriasis (after 30 minutes) and reversal of ptosis (after less than 5 minutes) in the eye with sympathetic paresis. In the normal eye, the alpha-2 agonist activity predominates, causing a pharmacologic Horner with the pupil becoming smaller in darkness, thus resulting in many cases of a “reversal of anisocoria” after apraclonidine.

- Apraclonidine is very useful for diagnosing Horner syndrome, but not for localization of the site of the lesion. When sympathetic nerve stimulation is interrupted, decentralization takes place within 3–7 days, causing super-sensitivity at the distal end organ, even 1–2 synapses downstream. Therefore, super-sensitivity of the dilator muscle will occur with lesions of the first, second or third order neurons. Super-sensitivity does not require denervation — only a decrease in sympathetic nerve activity — for example, a sympathectomy ganglionic blocking agent given to animals (hexamethonium) causes super-sensitivity.

Therefore, apraclonidine has unique advantages in the diagnosis of Horner syndrome. Its diagnostic effects can be easily monitored in two end-organs; the pupil and the eyelid. This gives it extra added value in interpreting the results of testing. It also is unique because it is a positive test in the affected eye with oculosympathetic paresis by actively dilating the pupil on the affected side and reversing the ptosis owing to its effect on the sympathetically innervated Mueller muscle. At the same time, it has a negative effect in the normal eye, causing a pharmacologic Horner syndrome with the pupil becoming miotic in dim light and the upper eyelid falling slightly. The opposite effects in the normal and affected eye give it an added diagnostic advantage over cocaine testing. There have been reports of false negative testing with apraclonidine\(^40\)\(^41\), but this may have been due to the expectation that a reversal of anisocoria occurs, which is not always the case. In milder sympathetic lesions, there may only be a decrease in anisocoria after testing without a reversal. Since it was designed to easily penetrate the cornea, inter-individual differences in corneal permeability have minimal effects.

**Hydroxyamphetamine testing — do I really need to localize the oculosympathetic paresis?**

Once the diagnosis of oculosympathetic palsy is made, what is next? What is the cause and potential morbidity? Does it require treatment (e.g. tumor, carotid dissection, lateral medullary stroke, disc herniation within spinal cord)? Unless a clear history reveals the cause, an imaging study is usually indicated. MRI has become the imaging study of choice, but what kind of MRI is required and where does one image?

Sometimes the history, clinical signs, and symptoms alone provide a good road map as to the likely site of the oculosympathetic paresis. For example, the pattern of anhidrosis, if present, can be very localizing, or accompanying neurologic signs. However, most of the time, the location of the lesion to the 1st, 2nd, or 3rd order neuron is not apparent. On the surface, the easy answer is to forego the hydroxyamphetamine localization step and just image the entire oculosympathetic pathway, because “I don’t want to miss anything” Is this really the best approach?

Why not just image the whole sympathetic pathway and skip the hydroxyamphetamine test? Anyone who reads their own patient’s images has come to realize that structural lesions can be missed by even the most experienced reader. Many times this is due to spread of attention over too large of an area of anatomy and not knowing where to direct one’s focus. This is why a neuro-ophthalmologist sometimes has an advantage in knowing the patient’s history and presentation to helps narrow the area of interest on an imaging study. Structural lesions causing Horner’s syndrome can also be missed on imaging scans due to suboptimal imaging protocols with inadequate sequencing and incomplete plane of section because of trying to scan the entire neuro-axis. To this end, tailoring the MRI scan to the pre or postganglionic location improves the sensitivity of detecting lesions most commonly found at certain sites\(^42\)\(^43\). In addition, imaging both the pre and post-ganglionic sympathetic pathway results in a 50% increase in cost and scan time.

**A vote for hydroxyamphetamine localization:** Usually, the patient will be returning for an imaging study, and it is relatively easy to perform the hydroxyamphetamine test.
on the day of the imaging study so that the result can be used to tailor the scan. It is important to remember that hydroxyamphetamine releases any norepinephrine stored in vesicles in the presynaptic nerve terminal of the 3rd order postganglionic neuron, causing the pupil to dilate, if this neuron is intact. If the lesion has damaged the postganglionic neuron, then the nerve degenerates and the stores of norepinephrine have been depleted. The result is very little mydriasis compared to the normal eye. If the change (increase) in anisocoria is greater than 0.5mm in room light (anisocoria measured at 50 minutes post hydroxyamphetamine minus baseline anisocoria in room light), then the probability is relatively high that it is a postganglionic lesion.

Warning — Conditions that may cause a false localization result of the hydroxyamphetamine test:

No pharmacologic test is bullet-proof and hydroxyamphetamine is no exception. Here are some examples of how the test can go off the rails and lead to wrong interpretation:

a) It is important to remember that a postganglionic lesion of less than 7 days duration (i.e. a carotid dissection) may still give a false localization to the preganglionic site because not enough time has elapsed for the norepinephrine stores to be released.

b) Cocaine blocks the reuptake of noradrenergics back into the presynaptic nerve terminal. It will also block the uptake of hydroxyamphetamine, thereby reducing its effect. It has been shown that a full 3 days is needed to elapse before the confounding effects of cocaine on the hydroxyamphetamine test can be dismissed. This effect does not occur with apraclonidine and the test can be performed the following day.

c) Oculosympathetic preganglionic lesions that occur congenitally or during the first year of life can lead to secondary anterograde degeneration of the postganglionic fibers, causing a false postganglionic localization result after hydroxyamphetamine. This does not prevent me from doing the hydroxyamphetamine test in a child, but if the result is postganglionic, I do not rely on it.

d) Pathology of the iris that prevents dilation with either direct sympathomimetics or cocaine will also prevent mydriasis with hydroxyamphetamine. That is why when one suspects this at the time of cocaine testing, a drop of 2.5% phenylephrine OU will help identify these eyes (they fail to dilate to direct acting sympathomimetics), preventing false interpretation of hydroxyamphetamine test results.

Here are the main reasons why I recommend pharmacologic testing with hydroxyamphetamine for localization to preganglionic vs. postganglionic sympathetic lesions:

a) Hydroxyamphetamine localization compartmentalizes the attention to a region both for optimizing the scan and for focusing attention of the person reviewing the scan.

b) Hydroxyamphetamine localization helps in the vigilance of follow-up when imaging is negative. For example, a pre-ganglionic lesion is usually more worrisome and would be followed more closely than a post-ganglionic lesion.

c) The cost difference for imaging the entire sympathetic anatomy vs. a directed scan to either the pre or post-ganglionic pathway is significant.

In summary, hydroxyamphetamine testing for localizing the oculosympathetic lesion to a pre-ganglionic vs. post-ganglionic lesion is useful in the proper context and when one knows how to interpret the results. Its main clinical advantage is that it helps to direct the proper imaging to a confined area of the neuro-axis and helps focus the review of the images to the likely site along the sympathetic pathway. Since hydroxyamphetamine is widely available again, there is no significant impediment to its use.

REFERENCES


27. Chen PL, Hsiao CH, Chen JT, Lu DW, Chen WY. Efficacy of apraclonidine 0.5% in the diagnosis of Horner syndrome in pediatric patients under low or high illumination. Am J Ophthalmol. 2006 Sep;142(3):469–74.


EXPERT OPINION & COMMENTARY

Dr. Kardon has capsulized the issues with logic, science, rich clinical experience, and practical advice. I have few — and mostly minor — disagreements.

1. Iris cholinergic disorders. This is where the affected pupil constricts relatively poorly to direct light because of efferent dysfunction.

Dr. Kardon believes that testing with topical use of dilute (0.1%) pilocarpine is not essential in this setting. I agree, and will summarize the points.

a. Preganglionic vs postganglionic parasympathetic dysfunction. I concur with Dr. Kardon in saying that postganglionic lesions should be easily distinguished from preganglionic palsies because post-ganglionic lesions will uniquely have SECTORAL SPHINCTER PALSY, LIGHT–NEAR DISSOCIATION, AND A LACK OF OTHER manifestations of intracranial disease (the anisocoria is “isolated”). Preganglionic lesions, he states, never produce sectoral palsies in the acute phase.; with reinnervation of the ciliary ganglion, sectoral palsy might occur (news to me). I wonder if he is completely right about sectoral palsies not occurring in acute preganglionic lesions. Acute “midbrain corectopia” has been reported and I have seen it. But midbrain — and extra-axial — preganglionic lesions will always display other distinctive neurologic features, making the distinction from a postganglionic lesion easy. Be careful here, as those features may be subtle.

Dr. Kardon notes that in postganglionic lesions, iris sphincter supersensitivity to topical cholinergic agents develops within 4–5 days (valuable but unpublished information) and reminds us that such supersensitivity can be seen in preganglionic lesions (well–published information), presumably because some preganglionic fibers do not synapse in the ciliary ganglion. That such supersensitivity can occur in preganglionic and postganglionic lesions is reason enough not to depend on dilute pilocarpine testing in differentiating between these two sites.

There are other problems with using dilute pilocarpine in this setting. As Dr. Kardon points out, interocular differences in corneal penetration can mess up the results. Some patients have small pupils on both sides and may not have enough supersensitivity to allow the medication–induced miosis to become apparent. Our hospital pharmacy no longer concocts the dilute solution, leaving me or my assistants to make the dilutional errors. For all these reasons, I have stopped using it except as a demonstration to trainees of a nifty but antique approach to practicing neuro-ophthalmology.

b. Postganglionic vs. iris sphincter dysfunction. This distinction can be tough. Fortunately, it is rarely vital. Dr. Kardon points out that lesions in either place can produce sector palsy. Denervation supersensitivity should not, of course, occur in sphincter dysfunction. In fact, the dysfunctional sphincter should be SUBSENSITIVE to cholinergic agents. So, as Dr. Kardon suggests — and I agree — why not take advantage of that feature and test with a pilocarpine concentration that SHOULD CONSTRIC THE NORMALLY FUNCTIONING SPHINCTER IN THE FELLOW EYE BUT NOT THE ABNORMALLY FUNCTIONING SPHINCTER IN THE AFFECTED EYE.

That pilocarpine concentration is 0.5%, not 0.1%. After instillation, the anisocoria will increase in cases of postganglionic palsy, decrease in cases of iris sphincter dysfunction, and remain the same in cases of pre-ganglionic palsy.

2. Iris adrenergic disorders. This is where the iris dilator is dysfunctional, so that the anisocoria is greater in darkness than in light, and the affected pupil has a dilation lag as room illumination is quickly reduced. The challenge here is much more important than to separate physiologic anisocoria from Horner syndrome. As Dr. Kardon emphasizes, this distinction can be difficult because Horner syndrome may have subtle clinical manifestations. Ptosis may be absent. Anisocoria may be absent. At best, each finding is minimal. And each finding can be generated by other causes. Dr. Kardon states that dilation lag is usually present in Horner syndrome, but I have not figured out a reliable way to bring out that phenomenon. Because I cannot count of clinical findings, I depend on topical pharmacologic testing for confirmation of Horner syndrome. But what kind?

I have stopped using topical cocaine except in children under the age of one year (because apraclonidine, my choice, may cause severe autonomic side effects in that age group). With cocaine testing, you depend on finding reduced dilation in the affected eye, but this substance — even in a 10% concentration — is a weak dilator of the normal iris. Moreover, it is a controlled substance; our hospital regulations require that it remain in a locked safe!

Why bother with cocaine when apraclonidine 0.5% has so far never let me down. There are now reports that even a central Horner syndrome will show reversal of anisocoria within three days. The false positive rate appears to be low. More data would be helpful here, especially to ascertain the false negative rate. I’m already a believer, but I am pleased to learn that Dr. Kardon’s formal comparison of cocaine and apraclonidine showed that the two agents are comparable in detecting denervation supersensitivity in Horner syndrome.
As Dr. Kardon acknowledges, apraclonidine does not allow localization of a Horner syndrome. For that purpose, he recommends using hydroxyamphetamine. This is our major point of disagreement. First, I do not think pharmacologic localization of Horner is reliable enough to serve as a useful guide to imaging. Second, we will not break the health care fund by non-targeted imaging in Horner syndrome. In the March 2010 issue of Journal of Neuro-Ophthalmology, Almog et al reviewed 52 cases of Horner syndrome examined in Israel over the past 14 years. In 2/3s of cases, the location of the lesion was already known by the time of the first visit. In half of the remaining 1/3 of cases, clinical features allowed targeted imaging which usually located the lesion. In 1/6 of cases, the Horner syndrome was isolated, and imaging from mid cranium to mid thorax was negative except in one case, in which it disclosed a thyroid neoplasm.

Based on these facts, I suggest using clinical clues rather than pharmacologic testing to target imaging in Horner syndrome. In the few cases where there are no such clinical clues, run the imaging gamut from skull base to mid thorax. If you suspect carotid dissection, scanning is urgent because some type of anti-thrombotic treatment may be helpful in reducing the risk of stroke (anecdotal evidence only). Stroke risk dissipates rapidly over 2 weeks, so starting anti-thrombotic treatment after that time is probably not useful. Although MRI/MRA has often been recommended, our neuroradiologists prefer CT/CTA because it is quicker, provides more reliable images of the cervical carotid artery, and very adequate images of the chest, neck, and subcranial tissues.

REFERENCES

CME ANSWERS
1. E) all of the above; all of these causes directly affect the sphincter muscle due to ischemia and would make the iris under–sensitive to 0.5% pilocarpine.
2. C) applanation tonometry may disrupt the corneal barrier and may affect the penetration of dilute pilocarpine in one eye more than the other.
3. A) apraclonidine will not affect the uptake and results of hydroxyamphetamine testing performed the next day.
**LEARNING OBJECTIVES**

1. To examine the wide variability of the disorder worldwide.
2. To discuss some of the key clinical manifestations of NMO.
3. To discuss the current management of NMO.

**CME QUESTIONS**

1. How many spinal segments are typically involved in a patient with Neuromyelitis optica?
   - a. 1
   - b. 2
   - c. 3 or more

2. What percent of patients are legally blind in at least one eye with Neuromyelitis Optica?
   - a. 10
   - b. 20
   - c. 30
   - d. 40
   - e. 50

3. Which are of the following agents is an accepted treatment for NMO?
   - a. Interferon beta 1-a
   - b. Interferon beta 1-b
   - c. Glatiramer Acetate
   - d. Azathioprine

**KEY WORDS**

1. Optic neuritis
2. Neuromyelitis optica
3. Multiple Sclerosis

**EVIDENCE PRESENTATION**

Neuromyelitis Optica (NMO) is a demyelinating disorder with predilection for the optic nerves and brainstem. Devic initially defined “neuromyelitis optique” as acute monophasic disorder characterized by coincident demyelination of the optic nerves and spinal cord; however, subsequent clinical observations expanded the spectrum of disease to include relapsing cases of optic neuritis and transverse myelitis. In 1999, Wingerchuk and colleagues proposed diagnostic criteria based on clinical, radiologic, and laboratory criteria. The intention was to provide a framework for future prospective studies and to distinguish NMO and multiple sclerosis (MS) patients. The subsequent identification of a specific serum auto-antibody against the aquaporin−4 (AQP4) water channel in a majority of NMO patients has lead to a modified set of diagnostic criteria based on clinical, radiologic, and serologic data. The specificity of AQP4 autoantibody for NMO has renewed the debate over the relationship between NMO and MS. Are the two disorders distinct or related? And, does it matter?

**Distinguishing NMO and MS**

**Clinical:** Optic nerve and spinal cord demyelination are common clinical events in both NMO and MS. While visual recovery from a single episode of optic neuritis (ON) in NMO appears to be slightly poorer than in MS, a systematic study is lacking. Wingerchuk et al. reported an average visual acuity better than 20/30 following monophasic ON in NMO, whereas the average recovery in the Optic Neuritis Treatment Trial was better than 20/20. Merle et al. noted that 30% of their NMO patients had severe vision loss after a single episode of ON compared to only a single individual in the MS comparator group. Unfortunately, the number of patients evaluated in both studies was rather limited and the treatment regimens were not standardized. The data on monophasic transverse myelitis is even less clear. While NMO patients typically have more longitudinally extensive spinal cord lesions, the correlation between the extent of the lesion and clinically symptoms is far from uniform. On funduscopic exam, the proportion of papillitis and retrobulbar optic neuritis is similar in NMO and MS suggesting that anatomic distribution of inflammatory disease in the anterior and retrobulbar nerves is not distinct between the two conditions. The natural history of visual function in NMO, however, appears to be
significantly worse than in NMO\(^5\). How recovery relates to attack frequency and therapy remains poorly defined.

**Imaging:** The initial modern criteria for NMO required a normal brain MRI. Recently, studies have found that 60\% of patients with NMO demonstrate brain white matter abnormalities with 10\% of individuals showing a distribution highly suggestive of MS\(^6\). While a small subset of intracranial lesions in NMO patients were observed in regions of high AQP4 expression in rodent brain, the common predilection of MS and NMO lesions to the spinal cord and optic nerve remains unexplained.

While MRI appears unable to distinguish between optic nerve inflammation arising from NMO or MS, ocular coherence tomography (OCT) has identified accentuated retinal nerve fiber layer (RNFL) and macular volume loss in NMO eyes when compared to MS eyes\(^7\)-\(^10\). On average, a single episode of ON in an NMO patient was estimated to cause an additional 24 \(\mu\)M of RNFL loss when compared to an ON event in MS. The difference in RNFL loss remained significant after controlling for the frequency of ON\(^10\) and visual acuity\(^9\). No study, however, has investigated whether the changes are purely axonal or represent the loss of Mueller cell endfeet.

**Laboratory:** Recent studies have repeatedly found that serum IgG antibodies to AQP4 are associated specifically with NMO\(^2\)-\(^4\). The test for anti–NMO–IgG, however, is far from a diagnostic gold standard. A significant fraction of NMO patients do not have serum NMO–IgG, indicating that either AQP–4 antibodies are not necessary for clinical disease or that alternative target antigens are sufficient for pathogenesis. Despite the use of multiple sensitive assays, 10–39\% of patients with clinically definite NMO are seronegative for AQP–4 IgG, and serum AQP–4 antibody titers do not reliably correlate with clinical activity\(^12\),\(^11\). In addition, NMO patients do not have increased amounts of CSF IgG1 protein\(^14\), and only 10\% of affected individuals demonstrate intrathecal antibody production\(^13\),\(^15\). Interestingly, more than 70\% of adult NMO patients are positive for antinuclear antibodies\(^16\), and 42\% of children with NMO have a coexisting autoimmune disorder\(^17\).

Several features of the cerebrospinal fluid (CSF) may help to distinguish NMO from MS. A significant pleocytosis (white blood cell count \(\geq 50 \times 10^3/\mu\)L) and the absence of oligoclonal banding are more prevalent in NMO than MS\(^1\),\(^5\),\(^15\). Nevertheless, the CSF profiles show significant overlap. Indeed, we have recently demonstrated that autoantibodies against AQP4 may be intrathecally synthesized in NMO (Bennett et al., Ann Neurol, in press).

**Histopathology:** Both NMO and MS are inflammatory demyelinating disorders that affect patients of similar age. Similar to observations in MS\(^1\),\(^8\), NMO pathology may exhibit differing subtypes\(^19\),\(^20\). Japanese patients with opticospinal myelitis (OSMS) have only a few eosinophils and neutrophils compared to cases of Western NMO. However, similar to Western NMO lesions and acute MS plaque, OSMS lesions uniformly demonstrate T–cell and macrophage infiltration. While immunoglobulin and complement deposition are common in MS plaque and NMO lesions, the distribution is frequently different\(^18\),\(^21\). Interestingly, recent studies have demonstrated early astrocyte loss in MS plaques (Parrott J. and Prineas, J.W., ECTRIMS 2009, P585; Konig et al., ECTRIMS 2009, P879).

**Pathogenesis:** Recently, three laboratories have independently demonstrated that AQP4 autoantibodies contribute to the pathogenesis of NMO\(^2\) (Bennett et al., Ann Neurol, in press; Brandl et al., Ann Neurol, in press). The infusion of a single AQP4–specific, human plasmablast–derived, recombinant antibody was sufficient to reproduce NMO–specific pathology: perivascular astrocyte depletion, polymorphonuclear and lymphocyte infiltration, myelinolysis, and immunoglobulin and complement deposition (Figure 1). While the data confirm a direct role of AQP4 autoantibodies in NMO pathogenesis, the reproduction of NMO pathology is dependent on the concurrent induction of experimental autoimmune encephalomyelitis. This suggests that NMO and MS may share a common underlying pathogenic mechanism. Further identification of antigenic targets in MS may yield critical information on the etiologic and pathogenic relationship of these demyelinating disorders.

**Treatment:** MS and NMO patients show both similarities and differences in their response to immunomodulatory therapies. While a double masked clinical trial in NMO has yet to be performed, open label trials and case reports have documented clinical improvement with a variety of agents: glatiramer acetate, mitoxantrone, azathioprine, and rituximab\(^23\)-\(^26\). Lack of clinical effect has been reported in NMO patients treated with interferon\(^27\). This could be secondary to differential cytokine signaling in the two disorders\(^28\). Similar to MS patients with severe demyelinating attacks, acute exacerbations in NMO have demonstrated improvement with plasma exchange\(^29\). This may indicate a common mechanism of acute immune–mediated injury in these two disorders.

**SUMMARY**

Is NMO a distinct entity from MS? In order to answer this question, we need to address a more difficult issue, what does it mean for two disorders to be distinct? Do the two conditions require distinct semiologies, differing pathogenesis, or alternative etiologies? Or alternatively, should the distinction be pragmatic? That is, is it only important that the two conditions be distinguished diagnostically and therapeutically? At the present time, no matter how we try to distinguish NMO and MS, the two disorders maintain an intriguing degree of overlap. So, are you a “lumper” or a “splitter”?
FIGURE 1: Prototypic NMO pathology induced by intravenous transfer of an AQP4-specific recombinant antibody into guinea pig MBP72-85-induced EA E rats. (H&E), Hematoxylin-eosin staining showing perivascular polymorphonuclear and lymphocytic cellular infiltrates with associated tissue destruction. (GFAP), glial fibrillary acidic protein staining demonstrates perivascular astrocyte loss. (LFB), Luxon fast blue staining shows perivascular myelin loss. (MBP) Myelin basic protein immunohistochemistry shows relative preservation of myelin basic protein staining.

REFERENCES
A hallmark clinical feature of NMO is its severity, providing yet another argument for its clinical and phenomenological separation from other forms of demyelinating disease. Wingerchuk and colleagues have reported that within five years of disease onset, fully 50% of patients are blind in at least one eye and require assistance with ambulation.

While distinct differences have supported defining NMO as an independent entity, in practice there is considerable variation in the presenting clinical phenotypes. As each new case series emerges in the literature, it is clear that no single characteristic is adequate to define the broad scope of NMO. Each individual case needs to be considered individually, and it is the constellation of clinical features that allows the best differentiation of NMO from other demyelinating disorders.

In distinguishing NMO from MS an expert panel agreed that:

- NMO should be distinguished from MS because of its different prognosis and its response to immunomodulatory therapy.
- NMO is most often a relapsing disorder, and therefore this characteristic is not helpful to distinguish it from MS.
- The key clinical characteristics that distinguish NMO from MS are the predilection in NMO to severe episodes of myelitis often, but not always, manifest as a complete transverse myelitis, and to severe episodes of optic neuritis, often but not always with incomplete recovery. The myelitis, unlike that which occurs in MS, is usually accompanied in the acute phase by a T2-weighted spinal cord lesion extending over three or more spinal segments (longitudinally extensive transverse myelitis, LETM) which may be hypointense on T1-weighted MRI and also associated with varying degrees of gadolinium enhancement.
- Brain involvement in NMO is uncommon clinically and brain MRI is often normal particularly early in the disease. When present, brain lesions generally do not fulfill typical Barkhof criteria for dissemination in space.

### NMO-IGG TESTING

In 2004, a serum autoantibody (NMO-IgG) was identified as a potential specific biomarker for NMO. The NMO-IgG autoantibody selectively binds to aquaporin-4 (AQP4), the predominant water channel in the central nervous system that is principally expressed in astroglial foot processes in the blood–brain barrier. This serum marker was reported to be 73% sensitive and 91% specific for NMO in patients with an initial optic–spinal syndrome, and was not commonly found in patients with conventional MS. Despite a similar phenotypic presentation, there may be geographic variability in the detection of this antibody. Furthermore, the absence of the antibody does

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**TABLE 1: Clinical Features of NMO versus Multiple Sclerosis**

<table>
<thead>
<tr>
<th>Neuromyelitis Optica</th>
<th>Multiple Sclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe myelopathy, visual loss unilateral early in course, often bilateral late</td>
<td>Mild to moderate myelopathy, visual loss is usually unilateral</td>
</tr>
<tr>
<td>Attacks more severe with less complete remission</td>
<td>Attacks mild to moderate with initially good recovery</td>
</tr>
<tr>
<td>Secondary progressive course is uncommon</td>
<td>Secondary progressive course is common</td>
</tr>
<tr>
<td>Patients may not respond to conventional MS therapy, often require immunosuppression</td>
<td>Patients often respond to conventional MS therapy, immunosuppressive drugs often not needed</td>
</tr>
</tbody>
</table>
not imply a different therapeutic response. Patients with and without the antibody respond similarly to immunosuppressive therapy and other therapeutic modalities.\textsuperscript{17}

The NMO IgG antibody may be observed in other connective tissue disorders such as systemic lupus erythematosus or Sjogren’s syndrome, particularly those with evidence of neuro–myelitis optica. A survey of patients with relapsing transverse myelitis and positive NMO antibody tests indicated that about 50% also had positive test results for antinuclear antibodies, and approximately 75% of patients with recurrent demyelination demonstrated the presence of autoimmune antibodies.\textsuperscript{18} These other autoimmune conditions such as lupus are most reliably distinguished from NMO when there are clinical and biopsy features that suggest a systemic inflammatory process. However, some patients with the NMO phenotype will just have other autoimmune antibodies without evidence of a systemic inflammatory condition.\textsuperscript{19} Further study of these patients will help elucidate the autoimmune overlap that may exist between these conditions.

CSF TESTING

Cerebrospinal fluid analysis has been advocated as an additional means of differentiating NMO from MS. The presence of CSF pleocytosis (white blood cell count of greater 50 cells, often with neutrophils) is very rare in MS, but may be seen in up to one-third of cases of NMO. Furthermore, oligoclonal banding may be detected in 70 to 90% of patients with MS, but in only 20 to 30% of patients with NMO.

NEURO-IMAGING

Characteristically in NMO, there is a longitudinally extensive spinal cord lesion that extends over three or more vertebral segments (Table 2). As opposed to MS the NMO lesion in the cord tends to be central. In addition, the lesion is often hypointense on T1 imaging reflecting axonal degeneration\textsuperscript{3} Studies have shown that up to 60% of patients will have nonspecific white matter findings. However, it is uncommon for them to fulfill the Barkof criteria for MS. Finally, atypical lesions that involve the central medulla and hypothalamus also may suggest the presence of an NMO phenotype. We have seen large asymptomatic lesions of the brain spontaneously remit in this disorder. Optical coherence tomography shows more severe loss of retinal nerve fiber layer loss (RNFL) after a bout of optic neuritis in NMO patients. In general, a loss of greater than 15 microns of RNFL after optic neuritis should suggest the possibility of NMO.\textsuperscript{25}

### TABLE 2: Radiographic Features of NMO versus Multiple Sclerosis

<table>
<thead>
<tr>
<th>Neurmyelitis Optica</th>
<th>Multiple Sclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain MRI: often normal, particularly early in the disease course.</td>
<td>Brain MRI: typically show periventricular and subcortical lesions</td>
</tr>
<tr>
<td>Cord MRI: long, central lesions (&gt; 3 vertebral segments), hypointensity on T1 images</td>
<td>Cord MRI: peripheral lesions, usually short in length (2 vertebral segments or less)</td>
</tr>
<tr>
<td>Lesions involving central medulla, hypothalamus and diencephalon</td>
<td>Brainstem lesions involve the medial longitudinal fasciculus or peripheral brainstem</td>
</tr>
</tbody>
</table>

TREATMENT STRATEGIES IN NMO

It is important to emphasize that we are unlikely to have a large randomized control trials of NMO patients, so our treatment protocols are likely to be based on expert opinion and anecdotal evidence. It is useful to think of treatment as both acute and chronic.

For acute therapy, we use intravenous methylprednisolone 1 gram for 5 days and pheresis for severe attacks.\textsuperscript{20} The severity of attacks may be defined as an acuity of 20/200 or less and an EDSS score of 4.0 or less when spinal cord dysfunction predominates. It is also important to emphasize that patients with the phenotype of NMO respond to therapy regardless of the antibody status. This includes the response to steroids, pheresis and rituximab. Likewise, there are patients who going to fail even rituximab and may require a combination of therapies including immunosuppressive drugs such as azathioprine. Monthly IVIG or every 6 week therapy remains a consideration, and a report of two patients\textsuperscript{21} suggested that relapses may be minimized by this approach. Because of its availability and cost, I usually start patients with the NMO phenotype on azathioprine.\textsuperscript{22} I have no trouble with those that initiate therapy with rituximab, but it can be an enormous hassle to get the insurance company to approve it. There are a number of ways rituximab can be used. One is to get a local rheumatologist or oncologist to administer the drug through their infusion center. The other major mechanism to get rituximab administered is to admit the patient for their infusion. There are several different regimens for rituximab including 375 mg/m2 weekly for a month followed by a repeat dose in 6 months. Alternatively, you could give a 1000 mg dose followed two weeks later by another 1000 mg dose. A repeat dose could be given in 6 months.\textsuperscript{23,24}

It is not uncommon for NMO to be associated with other autoimmune conditions such as Sjogrens, Lupus and even myasthenia gravis. By consensus, if a patient fulfills the criteria for Lupus or another autoimmune condition, they have that condition. Nonetheless, these patients tend to respond to the treatments advocated for NMO.
REFERENCES


CME ANSWERS

1. C

2. E

3. D
LEARNING OBJECTIVES

1. Know the natural history of the two most common forms of hereditary optic neuropathy, Leber hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA).

2. Know the available evidence in the literature on the treatment of the mitochondrial diseases in general and of the hereditary optic neuropathies in particular.

3. Be familiar with the future options for treatment of the hereditary optic neuropathies.

4. To know the range of presentations and course for Leber's hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA).

5. To understand the possible pathophysiological mechanisms by which LHON and DOA may cause RGC loss.

6. To adequately weigh the evidence for treatment of LHON and DOA.

CME QUESTIONS

1. True or False? Natural history studies have shown that all males who have homoplasmic mtDNA mutations for LHON will have visual loss some time during their lifetime.

2. True or False? The evidence supports treatment of acute LHON with ubiquinone, vitamins and anti-oxidants.

3. True or False? Gene therapy in LHON involves direct insertion of replacement mtDNA into the mitochondria.

4. True or False? All cases of LHON begin with an acute or subacute loss of vision.

5. True or False? Patients with LHON who have visual loss and optic atrophy do not recover any substantial level of visual acuity or visual fields.

KEY WORDS

1. Leber Hereditary Optic Neuropathy

2. Dominant Optic Atrophy

3. Mitochondrial Disease

4. Hereditary optic neuropathy

5. LHON

EVIDENCE PRESENTATION

The past two decades have witnessed remarkable advances in our understanding of the clinical presentation, genetics and even the pathophysiology of the hereditary optic neuropathies, specifically Leber hereditary optic neuropathy (LHON) and dominant optic atrophy (DOA). We now know that most of the hereditary optic neuropathies, including LHON and DOA, have a pathophysiology reflecting a final common pathway in mitochondrial dysfunction, despite their genetic origin in two different genomes (LHON a result of point mutations in the mitochondrial DNA, and DOA a consequence of mutations in nuclear chromosomes). However, investigations into potential therapies for these and other mitochondrial disorders are still in their nascentcy. Before reviewing the evidence currently available on the treatment of these disorders, it is important to discuss the natural history of visual loss in these clinical settings, specifically the prognosis for spontaneous visual recovery.

NATURAL HISTORY OF VISUAL LOSS IN LHON AND DOA

LHON

In most patients with LHON, vision loss is devastating and permanent, with visual acuities typically worse than 20/200 in both eyes. Approximately 50% of patients with visual loss from LHON will recognize sequential eye symptoms, with intervals between affected eyes ranging from days to months, with a typical interval of 2 to 4 months. At least 97% of patients with visual loss in one eye will have second eye involvement within 1 year. In some patients, visual recovery may occur after acute visual loss, sometimes manifested as a “fenestration” within a visual field defect (the so-called donut or bagel scotoma) or with more diffuse return of central visual acuity and color vision, usually bilaterally. Visual recovery, when
Symptomatic treatments should be considered in all patients with LHON and DOA. Although 10% of patients in that single study had vision loss, visual acuity can range from 20/20 to light perception. Although not as rapid or as devastating as LHON, DOA may nevertheless significantly impair quality of life in the majority of patients.

DOA

Visual loss in DOA is detected between ages 4 and 6 in the majority of patients, and 58–84% of patients with DOA report visual impairment by age 11. Compared to LHON, vision loss is typically mild in DOA, with a mean visual acuity of 20/80 to 20/120. More than 80% of patients retain vision of 20/200 or better, although visual acuities can range from 20/20 to light perception. Although not as rapid as or as devastating as LHON, DOA may nevertheless significantly impair quality of life in the majority of patients.

Progressive decline in visual acuity occurs in 19–67% of DOA patients. The rate of progression varies considerably among and within families; however, in general, disease progression in DOA follows a relatively indolent course, and is independent of visual acuity at diagnosis. In one long-term follow-up study of 69 patients with a confirmed DOA-causing mutation, of whom 58 (84%) were symptomatic, 43 (62%) had stable visual acuity in at least one eye at 10 year follow-up. Although 10% of patients in that single study had improvement in their vision, this may reflect improved testing as children age; true substantial spontaneous improvement of vision does not appear to be a feature of DOA. In a more recent study of DOA patients in the north of England, visual function worsened in 29 of 43 patients (67.4%) for whom there was longitudinal follow-up (mean follow-up time of 15 years).

DISEASE-MODIFYING TREATMENTS

Therapies for mitochondrial disorders are very limited. A 2006 Cochrane review of 678 abstracts and articles found no evidence supporting any intervention in the management of mitochondrial disease. General therapies that have been studied in mitochondrial disease fall into four main categories: 1) vitamins and cofactors (Coenzyme Q10 (CoQ10), folic acid, vitamin B12, thiamine, riboflavin, L-carnitine, and creatine); 2) electron acceptors (vitamin C, menadiol); 3) free radical scavengers (CoQ10, idebenone, alpha-lipoic acid, and vitamin E); and 4) inhibitors of toxic metabolites (dichloroacetate (DCA)). Most of these general therapies are harmless at their usual doses, although some may be expensive. In the absence of any other proven therapy in mitochondrial disease, many clinicians resort, on theoretical or anecdotal grounds alone, to “mitochondrial cocktails” — various combinations of these agents — to treat their patients.

Coenzyme Q10 (CoQ10) is a lipophilic molecule found in the mitochondrial membrane that shuttles electrons from complex I and II to complex III. In patients with primary CoQ10 deficiency, OXPHOS is interrupted and ATP synthesis is impaired with consequent mitochondrial encephalomyopathy. In some of these patients, supplementation with exogenous CoQ10 has led to clear improvement in function, and doses of up to 3000mg/d of CoQ10 were tolerated without side-effects in other neurological populations. Because of its therapeutic usefulness in treating primary CoQ10 deficiency, exogenous CoQ10 therapy is frequently used to treat other diseases of the OXPHOS system, including LHON. Doses of greater than 400mg per day are typical. Similarly, exogenous riboflavin (100mg daily) and L-carnitine (3g daily) supplementation, useful in the treatment of multiple acyl-CoA dehydrogenase deficiency and primary carnitine deficiency, respectively, have had their use...
extended to mitochondrial disorders, (although not usually to the hereditary optic neuropathies) despite the absence of documented deficiency of these cofactors in primary mitochondrial diseases.

Vitamin C (4g daily) and menadiol diphosphate (40mg daily) were used as electron acceptors in patients with severe exercise intolerance and mitochondrial myopathy related to complex III deficiency to facilitate electron transfer from complexes I and II to complex IV.41 One patient had dramatic improvement initially on 31P MRS of muscle, but this effect was not sustained and was not seen in other patients with complex III deficiency.41,42

Because oxidative stress in mitochondrial disorders causes release of free radicals and can lead to apoptosis, free radical scavengers including CoQ10 (400mg daily), idebenone (up to 75mg/kg daily), alpha-lipoic acid (600mg daily), and vitamin E (400 IU daily), are often used in the treatment of mitochondrial disease.43,44 The combination of creatine (3g bid), CoQ10 (120mg bid), and alpha-lipoic acid (300mg bid) was shown to reduce serum lactate and markers of oxidative stress in patients with mitochondrial cytopathies in one randomized double-blind controlled trial, probably through a free-radical-scavenging mechanism.45

Idebenone, a short-chain benzoquinone structurally related to CoQ10, readily enters the brain and localizes to the mitochondria. It both stimulates net ATP formation and acts as a potent free radical scavenger protecting the mitochondrial membrane against lipid peroxidation. Compared to other analogs of coenzyme Q, idebenone is particularly suited for by-passing the functional impairment of mitochondrial complex I. Idebenone has been successfully used in Friedreich ataxia to improve both cardiac and neurological symptoms, especially at high doses.45,46 Neutropenia may be a rare side-effect of idebenone.

DCA, which reduces lactate levels by inhibiting pyruvate dehydrogenase, was recently studied in patients with MELAS in a randomized, placebo-controlled trial.47 This trial was terminated prematurely, however, because of an excessively high incidence of peripheral nerve toxicity, overshadowing any potential benefit in MELAS.

Allogenic stem cell transplantation has shown initial success in two MNGIE patients in partially replacing the deficient enzyme, thymidine phosphorylase, although further clinical followup is necessary.48 L-arginine has been shown in a prospective, unblinded, and unrandomized trial of 24 MELAS patients to reduce the frequency and severity of stroke-like episodes.49

DISEASE-SPECIFIC TREATMENT OF LHON

In light of the possibility for spontaneous recovery in some patients with LHON, any anecdotal reports of treatment efficacy must be considered with caution. The older literature includes attempts to treat or prevent the acute phase of visual loss with systemic steroids, hydroxycobalamin,50 and cyanide antagonists, none of which have proved effective.51-55 In the 1960s, reports from Japan advocated craniotomy with lysis of chiasmal arachnoid adhesions in patients with LHON, with 80% of more than 120 patients reporting visual improvement.56,57 Although the data are impressive, no further reports have followed, and it is difficult to support a surgical therapy logistically removed from the site of ocular neurovascular changes and of presumed primary involvement (the retinal ganglion cells). Optic nerve sheath decompression after progressive visual loss in two LHON patients resulted in no improvement.58,59

Mashima and colleagues60 reported the case of a 10 year old boy homoplasmic for the 11778 mutation who had early improvement in both eyes after 1 year of oral therapy with idebenone, but such an early age of onset certainly could have predisposed this child to spontaneous recovery. Other single case reports have also raised the possibility of a beneficial effect of idebenone on visual and neurologic recovery.61,62 In 2000, Mashima and colleagues63 reported on 28 LHON patients, 14 of whom were treated with idebenone combined with vitamin B2 and vitamin C. There was no significant difference in the number of eyes with visual recovery, although the authors claimed that the treatment seemed to speed recovery when it occurred. Huang and colleagues64 described a 21 year old man with visual loss from the 11778 mutation for 8 months who had substantial improvement of his vision within 4 months of starting CoQ10. Barnils and colleagues65 found no beneficial effects of large doses of idebenone and vitamin C and riboflavin in the prevention of second eye involvement in two LHON patients harboring the 11778 mutation.

Minocycline has been shown to have protective effects in various models of neurodegenerative disorders such as Parkinson’s disease, Huntington’s disease, spinal cord injury and amyotrophic lateral sclerosis.66 In one in vitro study, minocycline had a significant protective effect on the survival of LHON cybrid cells, presumably through anti-oxidant-mediated and megapore-inhibitor anti-apoptotic effects.66 No human LHON studies have been performed with minocycline to date, and this drug’s lack of efficient uptake into the central nervous system and its likely narrow range of useful concentration in the retinal ganglion cells may limit its usefulness in LHON.

Cyclosporin A has also been shown to be protective in cell culture analysis of oxidative stress due to the 11778 LHON mutation and of complex I toxin-induced apoptosis in neurons,67,68 as has exogenous glutathione.69 Brimonidine purite is an α-2 agonist used in the treatment of glaucoma which has been shown to have stabilizing effects on retinal ganglion cell survival in animal and human optic neuropathies, presumably partly through the promotion of antiapoptotic cell signals. Because brimonidine’s antiapoptotic properties likely occur through complex I, it seemed an obvious choice of agent to test in LHON, since the three primary mtDNA LHON mutations are located in protein coding genes of complex
Gene therapy shows significant promise in the treatment of mitochondrial diseases. Many ingenious strategies have been devised using transfected nuclear and mitochondrial genes to reduce the overall proportion of heteroplasmic mutant mtDNA in vitro, in yeast models, and in animal models — a strategy called “gene shifting”.

Although it is possible to introduce DNA into the cell nucleus using a variety of vectors, the techniques required to introduce genes directly into mitochondria have yet to be developed. Directly targeted repair or replacement of mutated mitochondrial genes is therefore not currently possible. However, “allotopic rescue” is one means of circumventing this barrier. With allotopic rescue, the nuclear genome is transfected by a genetically engineered adenovirus–associated virus (AAV) or other vector, to express a protein usually expressed by the mitochondrial genome. The transfected gene is engineered to attach a mitochondrial targeting polypeptide to the end of the transcribed protein, ensuring the nuclear protein is transported into the mitochondria. The nuclear protein, once in the mitochondria, may replace or complement a protein expressed by mutated mtDNA. This technique of allotopic rescue has been used to replace a mutated ND4 protein in a cybrid cell line homoplasmic for the 11778 LHON mutation, with consequent improvement in biochemical function and ATP synthesis.

Two independent groups have now demonstrated the proof–of–principle that allotopic expression can be effective treatment for LHON, one in a mouse model and one in a rat model. In both studies, an animal model of LHON–like optic neuropathy was induced by intravitreal injection of the human ND4 gene harboring the LHON 11778 mutation. Subsequent intravitreal injection of the wild–type ND4 prevented both retinal ganglion cell loss and impairment of visual function.

Another gene therapy strategy involves the in vitro transfection of homoplasmic 11778 LHON cells with an AAV vector containing the human mitochondrial superoxide dismutase (SOD2) gene. Superoxide dismutase, an antioxidant, is encoded by the nuclear SOD2 gene and detoxifies free radical species within the mitochondrial matrix, thereby acting as an anti–apoptotic agent. Although the SOD2 gene is expressed in LHON cells, superoxide dismutase activity is attenuated in cells homoplasmic for the LHON mutation. When LHON cells were transfected with the SOD2–AAV vector, superoxide dismutase was overexpressed, and three–day survival was increased by 89% in transfected LHON cells compared to non–transfected controls. This strategy of bolstering antioxidant mechanisms to prolong cell survival was also observed to protect against optic neuropathy in complex I–deficient mice, animals with similar histopathology to human LHON patients. These ground–breaking studies clearly open the door to future human clinical studies on patients with LHON.

In heteroplasmic mtDNA diseases, selective destruction of mutant mtDNA with a mutation–specific restriction endonuclease shifts heteroplasmic toward the wild–type state, allowing repopulation of mitochondria with wild–type mtDNA. This strategy has been shown to be effective both in vitro and in a murine model of the typically heteroplasmic mtDNA disorder known as neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP) which results from a point mutation at mtDNA position 8993. Unfortunately, LHON is only a heteroplasmic disease in a minority of pedigrees (probably less than 15%) and the risk of visual loss is reduced among those carriers who are heteroplasmic, making intervention in this manner probably unnecessary.
One form of gene therapy “treatment” for children of mothers with known mtDNA mutations would be the in vitro replacement of the entire mitochondrial genome of an oocyte which could then be fertilized in vitro and implanted for normal embryo development. This technique has been successfully demonstrated in primates, in which the nuclear contents from the mother’s egg is transferred by a technique known as “spindle replacement” to an enucleated, mitochondrial–replete donor cytoplast.81

Although gene therapy holds significant promise in human mitochondrial disease, its clinical use currently faces several challenges.32 Appropriate transfection vectors must be selected, and their delivery to affected tissues must be optimized. The duration of gene therapy effect must be improved, as current transfection methods have not resulted in prolonged and autonomous maintenance of transfected genetic material.84 Finally, patient safety from immunological and oncological side effects and from mtDNA depletion must be guaranteed,32,81 and efficacy must be shown in appropriate animal models before human trials can begin.

Plans for allotropic rescue studies in non–human primates are underway (John Guy, personal communication). In the meantime, identification of patients and carriers with the 11778 LHON mtDNA mutation are ongoing at the University of Miami in order to document feasibility for a clinical gene therapy trial and to develop standardized trial outcome measures (see http://www.bpei.med.miami.edu/site/disease/disease_neuro_LHON.asp#LHON).

Genetic counseling

One crucial aspect of the management of patients with hereditary optic neuropathies is genetic counseling, and knowledge of the basic principles of both nuclear and mitochondrial genetics is essential.

A patient with a DOA mutation has a 50% probability of transmitting the pathogenic allele to each of his or her children. Children with the mutant allele then have a 66–88% chance of developing DOA, in keeping with the known penetrance of the disease,20,85 although penetrance may be nearly complete when the parent manifests DOA themselves.21

Men with the LHON mtDNA mutations should be uniformly reassured that they have no chance of transmitting their mtDNA mutation to their children.86 Women with mtDNA mutations, on the other hand, always have a risk of transmitting mitochondrial disease to their children, and those with the LHON mtDNA mutations are no exception. If the mother is homoplasmic for the LHON mtDNA mutation, then all offspring will be homoplasmic as well. The risk of vision loss across all mutations is about 46% for men and 11% for women.87–90 Familial LHON may have a lower risk of vision loss — as low as 20% in men and 4% in women in one Australian study.91,92 The risk of vision loss in familial LHON has been observed to decline with successive generations, and this effect may relate to changes in environmental factors over decades of observation.91,92

The degree of risk for expression depends on several factors, including the presence or absence of heteroplasmy. The mutation load measured in a woman’s blood cells does not necessarily reflect the mutation load in her other cells, such as oocytes.93 Zygotcs may therefore begin embryogenesis with a mutation load quite different from the total mutation load in the mother. Replicative segregation during embryogenesis complicates matters further, as mutation loads may become magnified or diminished in various tissues of the developing fetus in an unpredictable way.32,86 Even asymptomatic heteroplasmic mothers with very low mutation loads in blood may have children with severe disease from very high mutation loads.93 The risk of transmission of disease with heteroplasmic mtDNA point mutations is therefore impossible to predict accurately. Prenatal testing with amniocentesis or chorionic villus sampling is confounded by heteroplasmy as well: amniocytes and chorionic villi may have mutation loads different from other fetal tissues and are unlikely to reflect the child’s ultimate phenotypic outcome, as large shifts in the proportion of mutant mtDNA may occur in developing tissue in utero or after birth as a result of replicative segregation.32

REFERENCES


67. Wong A Cortopassi G. mtDNA mutations confer cellular sensitivity to oxidant stress that is partially rescued by calcium depletion and cyclosporine A. Biochem Biophys Res Commun. 1997;239:139–145
68. Seaton TA, Cooper JM, Schapira AH. Cyclopisin inhibition of apoptosis by mitochondrial complex I toxins. Brain Res. 1998;809:12–17


EXPERT OPINION & COMMENTARY

Natural History of Visual Loss in LHON and DOA

LHON

• 11778
• 3460
• 14484
• Mixed mtDNA mutation and environment
  − Smoking
  − Ethanol
  − Antibiotics
• Men vs. Women – Menopause

DOA

• Classical
• Subclinical
• Plus age

Treatments for Mitochondrial Diseases

1. Vitamins
2. Electron acceptors
3. Free radical scavengers

Clinical Trials

1. Brimonidine
2. Idebenone

On the horizon

1. Gene therapy
2. Drug delivery trickery

CME ANSWERS

1. False
2. False
3. False
4. False
5. False
INTRODUCTION
Prostaglandin-J2 (PGJ2) has been proposed as a potential neuroprotective agent. We wanted to evaluate the toxicity/efficacy of a single intravitreal (IVT) injection of PGJ2 in a rodent model of nonarteritic anterior ischemic optic neuropathy (NAION).

METHODS
We used the laser-activated rose Bengal induction method to produce AION in Long-Evans rats. We evaluated IVT-PGJ2 retinal and ON toxicity. Following induction, PGJ2 was intravitreally injected in the treatment-group. IVT phosphate-buffered-saline (PBS) was used as a control. Functional studies (VEP) were performed at baseline and at 7 days post-treatment. Structural studies included immunohistochemical (IHC), electron microscopic (EM) analysis of the optic nerve (ON), and stereologic analysis of retinal ganglion cell (RGC) numbers 30 days post-induction.

RESULTS
Toxicity: IVT PGJ2 (5 eyes) did not induce any statistically-significant functional or structural changes in the retina or ON of treated animals compared with animals injected with PBS alone (5 eyes) 30 days post-injection.

Efficacy: Following a single IVT-injection, day 7 VEPs in the PGJ2 treatment group (n=7) had amplitudes 103.6% of baseline response, whereas the PBS-treated group (n=6) had VEPs that were 42.4% of the baseline response. 30 days post-stroke, EM visualization of ON from the treatment-group demonstrated significant preservation of axons and decreased demyelination. Stereological RGC counts confirmed significant (p<0.04) RGC preservation in PGJ2-treated animals (1462.6 cells/µm²) compared with the stroke+PBS group (1156.5 cells/µm²).

CONCLUSION
A single IVT-injection of PGJ2 produces no evidence of retinal or ON toxicity by functional/structural analysis in rats. The IVT-route of administration enables delivery of a high concentration of the drug in a low volume, with minimal risks from systemic side effects. A single IVT injection of PGJ2 preserves RGCs and their axons and provides sustained neuroprotection for at least 1 month following the initial ischemic event in a rodent model of NAION. Ongoing studies are conducted to evaluate whether a similar effect is observed in primates.

REFERENCES
None

KEY WORDS
Ischemic Optic Neuropathy, Neuroprotection, Prostaglandin, Retinal Ganglion Cell

FINANCIAL DISCLOSURE: NONE