I'm Dr. James Tsai of the New York Eye and Ear Infirmary and Mount Sinai Health System. This Continuing Medical Education activity is supported by an unrestricted educational grant from Bausch & Lomb, Incorporated, and brought to you by Candeo Clinical / Science Communications and the University of Florida College of Medicine.

Join me as we take a Closer Look at Nitric Oxide in Glaucoma.

Control of intraocular pressure, or IOP, is the primary goal in the management of glaucoma. For about two decades, prostaglandin analogues, or PGAs, have been the preferred choice for initial glaucoma therapy, thanks to their exceptional IOP-lowering efficacy and systemic safety.

Most recently, the class of topical PGAs has expanded to include a new addition: latanoprostene bunod, or LBN, 0.024%. A unique PGA with a nitric oxide, or NO, component, LBN is approved for IOP reduction in the treatment of open-angle glaucoma or ocular hypertension.

In this video, we will closely examine the relationship between NO and glaucoma: reviewing the current understanding of NO’s functions in the eye, explaining the mechanism of NO-donating therapy for IOP reduction, and discussing how the availability of NO-based agents may change the treatment of glaucoma.

NO is endogenously synthesized in the human body as a signaling molecule and an important regulator of multiple organ systems; and has been implicated in a wide range of physiological events, from vascular tone regulation to neurotransmission to inflammatory response.

NO performs its physiological functions via complex intra- and intercellular signaling. Within the cell, NO directly stimulates soluble guanylate cyclase, or sGC, to increase the
production of cyclic guanosine monophosphate, or cGMP. This leads to the activation of cGMP-dependent protein kinases and downstream phosphorylation of proteins.\(^1\) As a second messenger, cGMP can also regulate cation channels and some isoforms of phosphodiesterase.

Small and highly lipophilic, NO can readily diffuse across cell membranes and is therefore able to induce changes in neighboring cells in a paracrine manner. Depending on the direction of NO release and the site of cGMP production, different biological effects can result from the NO / cGMP signaling cascades. In the cardiovascular system, for instance, NO plays a central role in the regulation of vascular tone.

**NO synthase, or NOS, is the enzyme that generates NO from the amino acid L-arginine. NOS has three isoforms that differ in expression site and physiological role.\(^2\)**

The discovery of endogenous NO’s beneficial effects in the cardiovascular, nervous, and immune systems has triggered substantial interest in therapeutic strategies to increase NO levels or modulate NO signaling. It is worth noting that some lifestyle factors known to be strongly associated with cardiovascular protection, such as physical exercise and healthy diet, have been found to increase NO bioavailability.\(^3\)
All three isoforms of NOS are expressed in the eye, suggesting that low-level intraocular NO production is fairly ubiquitous.

As in other organ systems, NO has a vital role in many ocular homeostatic processes. It is likely that NO facilitates aqueous outflow by relaxing the juxtacanalicular TM, wherein most outflow resistance resides.

The TM and Schlemm’s canal are presumed to be enriched sites of NO synthesis mediated by eNOS. It is likely that NO facilitates aqueous outflow by relaxing the juxtacanalicular tissue, wherein most outflow resistance resides.

The TM and Schlemm’s canal cells are highly contractile in nature, similar to vascular smooth muscle cells. Evidence suggests that NO alters the contractility and volume of these cells, leading to relaxation of the juxtacanalicular TM.

Separately, endogenous NO produced in the posterior segment of the eye is involved in vascular, immunological, and retinal physiology.
In the optic nerve head, the site of initial axonal damage in glaucomatous optic neuropathy, there is evidence that NO is essential for maintaining basal blood flow and participates in the autoregulation of blood flow during IOP elevations.11,12

The pathophysiology of POAG is not yet fully elucidated, but it has been established that elevated IOP is a major causative risk factor in its development and progression.13,14 In eyes with POAG, there is increased resistance to aqueous outflow through the TM, and the resulting increased IOP is a direct cause of the retinal ganglion cell death characteristic of glaucomatous optic neuropathy.15

In addition to elevated IOP, poor vascular supply to the optic nerve head has gained wide support over the past few decades as a potential mechanism contributing to glaucomatous neuronal damage.

Studies examining ocular blood flow show that glaucomatous eyes have inadequate ocular perfusion compared with normal eyes,16,17 and it is plausible to assume that an optic nerve with poor circulation would be more sensitive to increased IOP levels.

Given NO’s role in the regulation of outflow facility and IOP, it is not surprising that research has linked an altered NO signaling system with POAG.

Analysis of a specific histochemical marker for NOS found that NO production is markedly decreased in the TM and the Schlemm’s canal of eyes with POAG.18

Patients with POAG also demonstrated lower levels of NO markers in their aqueous humor and plasma compared with control subjects.19,20
Collectively, these findings suggest that an altered NO system is present in POAG and may play an important role in the pathophysiology of the disease. Impaired NO signaling could influence IOP via a direct effect on conventional aqueous outflow; it may also alter perfusion of the optic nerve head, making the neural tissue vulnerable to pressure-induced damage and degeneration.21

Given the evidence that impaired NO signaling is a contributing mechanism in the pathophysiology of POAG, intervention targeting the NO system should be beneficial in the treatment of the disease.

Nipradilol, a nonselective beta-blocker with an NO-donating nitroxyl moiety, is available in Japan for use as a topical IOP-lowering agent. In randomized clinical trials, nipradilol demonstrated comparable IOP-lowering efficacy to timolol in patients with POAG or ocular hypertension.22

In POAG, elevated IOP results primarily from pathologies in the trabecular outflow pathway. However, the vast majority of commercially available glaucoma therapies do not act on the trabecular outflow pathway.

In the US, prior to the approval of LBN, the only glaucoma medication targeting aqueous outflow through the TM has been pilocarpine, a cholinergic drug that works by inducing contraction of the ciliary muscle and thus, widening the anterior chamber angle. Though fairly effective in IOP reduction, pilocarpine requires frequent dosing, and has been largely
abandoned in favor of newer treatment options with fewer side effects.

This gap in the mechanisms of glaucoma drugs’ therapeutic action is beginning to fill with the introduction of NO-based treatments like LBN.

With two entities—latanoprost and an NO-donating moiety—chemically fused into one molecule, LBN is an NO-donating PGA and is thought to lower IOP through different actions on aqueous humor outflow.

Upon topical administration, LBN is rapidly metabolized by esterases to produce latanoprost acid and, via the NO-donating moiety butanediol mononitrate, NO.

Latanoprost, as a PGA, contributes to LBN’s efficacy chiefly by increasing uveoscleral aqueous outflow. Separately, NO released in the anterior chamber exerts its hypotensive effect by activating the NO/cGMP pathway, relaxing the juxtacanalicular tissue, and enhancing outflow through the TM.

Preclinical studies support the role of NO in LBN’s IOP-lowering activity.

In three animal models of hypertensive glaucoma, including cynomolgus monkeys with laser-induced ocular hypertension, LBN produced IOP reduction of great magnitude relative to vehicle, while latanoprost at equimolar doses showed minimal effect.23

The safety and efficacy of LBN have been evaluated in randomized, prospective clinical trials of patients with OAG or ocular hypertension.
clinical trials that compared the diurnal IOP-lowering effect of LBN against that of timolol in patients with OAG or ocular hypertension.

LBN dosed once daily showed noninferiority to twice-daily timolol for IOP reduction and diurnal IOP in both studies, producing a mean IOP reduction of 7.5 to 9.1 millimeters of mercury from baseline over 3 months of treatment. The mean IOP in the LBN group was significantly lower than in the timolol group at all nine timepoints in APOLLO, and at all but one timepoint in LUNAR.

During the 3-month efficacy phase of both studies, the percentage of patients with at least a 25% IOP reduction was significantly higher in the LBN group than in the timolol group. In APOLLO, a greater proportion of patients achieved consistent IOP of less than or equal to 18 millimeters of mercury for all nine timepoints.

LBN’s IOP-lowering efficacy was maintained through the entire duration of both studies, which was one year for APOLLO and six months for LUNAR.

In general, LBN treatment was well tolerated in the APOLLO and LUNAR studies, and adverse events—ocular or nonocular—were uncommon. The most common ocular adverse events were conjunctival hyperemia, eye irritation, and eye pain.

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<tr>
<th>Week 2</th>
<th>Week 6</th>
<th>Month 1</th>
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<tbody>
<tr>
<td>8 am</td>
<td>12 pm</td>
<td>4 pm</td>
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<tr>
<td>LBN mean CFB (mm Hg)</td>
<td>22.9</td>
<td>11.3</td>
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<tr>
<td>Timolol mean CFB (mm Hg)</td>
<td>39.2</td>
<td>39.5</td>
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<tr>
<td>Treatment difference</td>
<td>-15.3</td>
<td>-28.2</td>
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<tr>
<td>P-value</td>
<td>&lt;0.001</td>
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During the 3-month efficacy phase of both studies, the percentage of patients with at least a 25% IOP reduction was significantly higher in the LBN group than in the timolol group. In APOLLO, a greater proportion of patients achieved consistent IOP of less than or equal to 18 millimeters of mercury for all nine timepoints.
**CONSTELLATION** was a randomized, open-label, crossover phase 2 study that compared the diurnal and nocturnal IOP-lowering effects of LBN and timolol in patients with OAG or ocular hypertension.

After 4 weeks of treatment, LBN significantly reduced IOP during both the diurnal and nocturnal period, while timolol only reduced IOP during the diurnal period. In addition to better 24-hour IOP control, LBN treatment also improved nocturnal ocular perfusion pressure compared to timolol.

Taken together, these results establish that LBN has a favorable IOP-lowering and comparable adverse event profile to timolol.

**VOYAGER** study was a randomized, investigator-masked, multicenter, dose-ranging phase 2 study evaluating the efficacy and safety of LBN at several concentrations against latanoprost, in patients with OAG or ocular hypertension. In this study, LBN 0.024% showed greater IOP reductions at multiple timepoints (days 7, 14, and 28) compared with latanoprost.

At day 28, the primary endpoint, the LBN 0.024% group demonstrated a greater reduction in mean diurnal IOP versus the latanoprost group, with a difference of 1.23 millimeters of mercury.

Since its introduction, I have started substituting LBN for latanoprost in patients who will likely benefit from one or two more millimeters of mercury in pressure reduction. This seemingly small additional and consistent reduction of IOP could have a significant impact—the Early Manifest Glaucoma Trial suggests that each decreased millimeter of mercury of IOP is associated with a 10% reduction in the risk of visual field progression across the population, a clinically meaningful difference.

I may also consider prescribing LBN when latanoprost monotherapy has produced a...
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<th><strong>pressure response but over time proved insufficient to lower the IOP to the target range.</strong></th>
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<td>In JUPITER, an open-label phase 3 study conducted in Japanese patients with OAG or ocular hypertension, LBN was well tolerated and produced robust, sustained IOP reduction for up to 1 year. As we accumulate more of this data and clinical experience of LBN’s efficacy and safety in the long term, I can foresee its wide adoption as a first-line therapy.</td>
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<tr>
<td><strong>[ON-SCREEN OVERLAY COPY OR TITLE SLIDE] The Future of NO-based Glaucoma Treatments</strong></td>
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<td><strong>ON SCREEN: DR. TSAI</strong></td>
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<td>The NO signaling system is rich in potential therapeutic targets, and more medications acting through the pathway are expected to emerge.</td>
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<td><strong>ON SCREEN: DR. TSAI</strong></td>
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<td>Netarsudil, a rho kinase inhibitor, has recently been approved in the US for IOP lowering in patients with OAG or ocular hypertension. This new drug targets the trabecular outflow as well, but it bypasses the sGC portion of NO signaling to directly relax the cells of the TM and inner walls of Schlemm’s canal.</td>
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<td><strong>ON SCREEN: DR. TSAI</strong></td>
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<td>As mentioned, an NO-donating therapy may have the potential to enhance perfusion of the optic nerve and the retina while lowering IOP. Further exploration of this potential added benefit of NO-donating therapies is needed. Improving blood flow to the optic nerve could be especially beneficial in certain patient populations, such as those with POAG and baseline pressures slightly above 21 millimeters of mercury; those who progress despite pressures that are controlled in the high teens; and, most importantly, patients with normal-tension glaucoma. Abnormal ocular blood flow is believed to play a more significant pathogenic role in normal- than high-tension glaucoma.</td>
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<td><strong>ON SCREEN: DR. TSAI</strong></td>
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<td>An important signaling molecule throughout the body, NO is also a key regulator of</td>
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aqueous outflow and IOP, as well as optic nerve head blood flow. A wealth of laboratory and clinical evidence supports the NO signaling system as a therapeutic target in glaucoma, and our accumulating experience with NO-donating drugs like LBN is beginning to reshape our approach to this disease.

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