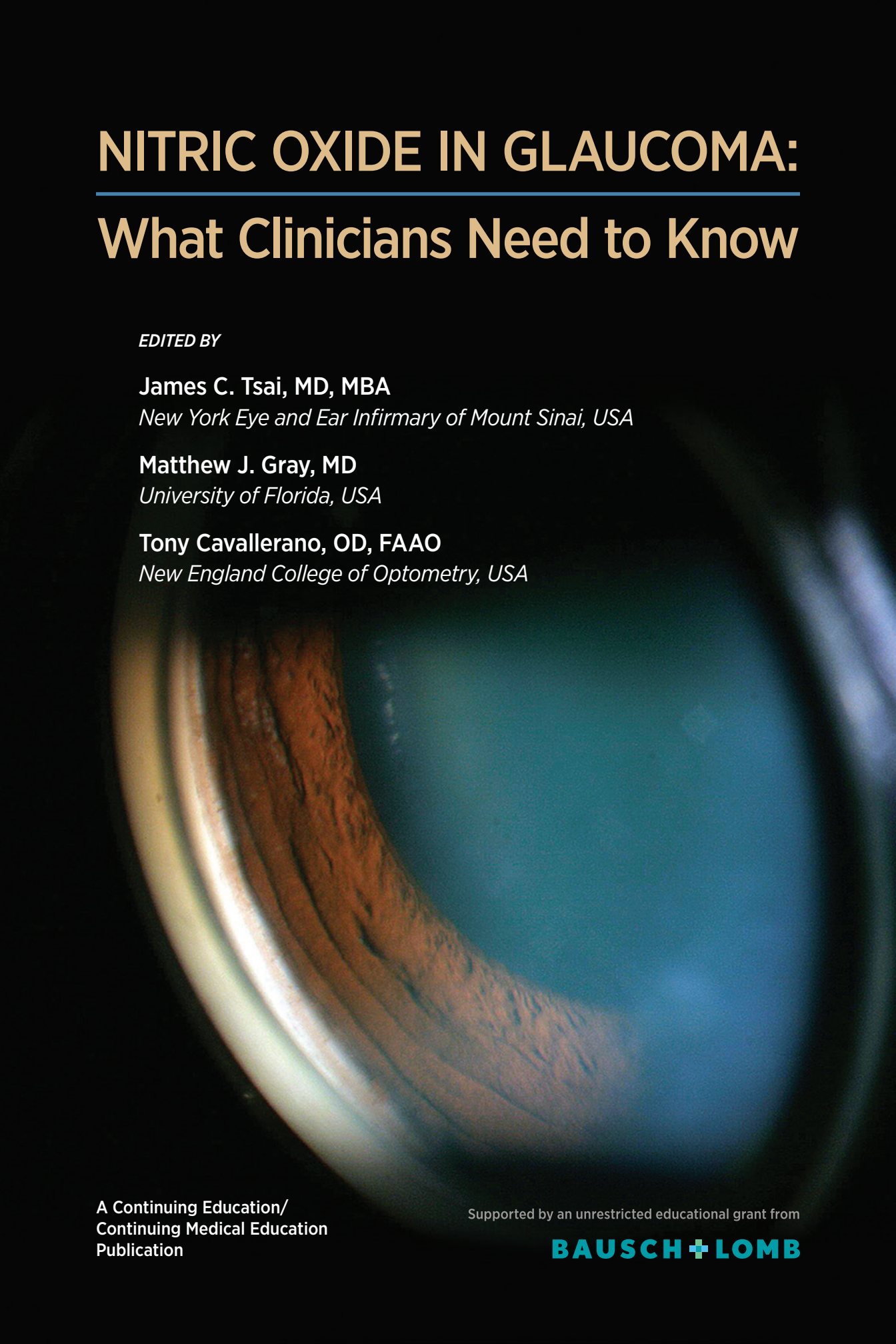
NITRIC OXIDE IN GLAUCOMA:
What Clinicians Need to Know

EDITED BY

James C. Tsai, MD, MBA
New York Eye and Ear Infirmary of Mount Sinai, USA

Matthew J. Gray, MD
University of Florida, USA

Tony Cavallerano, OD, FAAO
New England College of Optometry, USA



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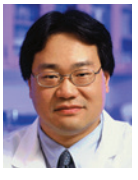
PROFILES

Chairperson/Activity Director: **James C. Tsai, MD, MBA**
Dr. Tsai is the president of New York Eye and Ear Infirmary of Mount Sinai and system chair of ophthalmology for the Mount Sinai Health System. He also serves as the Delafield-Rodgers Professor of Ophthalmology at the Icahn School of Medicine at Mount Sinai. He is a consultant for Aerie Pharmaceuticals, Inotek Pharmaceuticals, EyeNovia, and Shire.

CME Reviewer: **Matthew J. Gray, MD**
Dr. Gray is a professor at the University of Florida College of Medicine department of ophthalmology. He states that in the past 12 months, he has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients relevant to this manuscript.

CE Reviewer: **Tony Cavallerano, OD, FAAO**
Dr. Cavallerano is the executive director of clinical training and patient care, director of professional relations, and adjunct professor at New England College of Optometry in Boston, Massachusetts. He states that in the past 12 months, he has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients relevant to this manuscript.



Author: **Richard K. Lee, MD, PhD**
Dr. Lee is associate professor of ophthalmology, cell biology, and neuroscience at the Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, in Miami, FL. He states that he has received research grants from National Eye Institute and BrightFocus Foundation, and has served as a consultant for Aerie Pharmaceuticals.

Author: **Tony Realini, MD, MPH**
Dr. Realini is a tenured professor of ophthalmology at West Virginia University, in Morgantown, WV, where he serves as clinical research director, glaucoma fellowship director, and glaucoma service director. Dr. Realini has received grant/research support from Alcon, Aerie Pharmaceuticals and National Institutes of Health. He is active in eye research, has received numerous research grants (including three from the National Eye Institute), and has published widely in the ophthalmic medical journals. He is also a consultant for Alcon, Bausch + Lomb, and Inotek Pharmaceuticals.

Author: **Leo Semes, OD, FAAO**

Dr. Semes is a former professor of optometry at the UAB School of Optometry in Birmingham, Alabama. Since retiring recently from full-time practice, Dr. Semes provides volunteer ophthalmic services in Northeast Florida. He is a consultant for Allergan, Bausch + Lomb, OptoVue, Shire, and ZeaVision, and is also on the speaker's bureau for Alcon, Allergan, OptoVue, Regeneron, Shire, and ZeaVision. Dr. Semes is also a stock shareholder of High Performance Optics.

Author: **W. Daniel Stamer, PhD**

Dr. Stamer is the Joseph A.C. Wadsworth professor of ophthalmology and professor of biomedical engineering at Duke University, Durham, NC. Dr. Stamer is a consultant for Aerie Pharmaceuticals. He has also received grant/research support from Allergan, Aerie Pharmaceuticals, Ironwood, and Inotek.

Author: **Anne L. Coleman, MD, PhD**

Dr. Coleman is the Fran and Ray Stark Foundation professor of ophthalmology in the Stein Eye Institute of the David Geffen School of Medicine at UCLA as well as professor of epidemiology at the UCLA Fielding School of Public Health in Los Angeles, CA. She is director of the Stein Eye Institute Center for Community Outreach and Policy, UCLA Mobile Eye Clinic and the vice-chair for academic affairs for the department of ophthalmology. She states that in the past 12 months, she has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients relevant to this manuscript.

Glaucoma, a group of ocular diseases characterized by progressive damage to the optic nerve, is the second leading cause of blindness worldwide, affecting a significant and growing portion of the US population.

Much remains to be understood about the pathophysiology of glaucoma, but intraocular pressure (IOP) has been identified as an important causative factor and modifiable risk factor. As demonstrated in several large clinical trials, IOP reduction can prevent progression of optic nerve damage and visual field loss in both early and late stages of the disease.

Latanoprostene bunod, a nitric oxide (NO)-donating prostaglandin F2α receptor agonist, is a novel glaucoma drug with a unique dual mechanism of action, achieved by chemically fusing two moieties—latanoprost and an NO donor—into one molecule. While latanoprost increases uveoscleral outflow like other PGAs do, the NO donor contributes to IOP lowering by increasing aqueous outflow through the trabecular meshwork.

To give their glaucoma patients the full benefit of treatment advances, clinicians require clear, actionable insights from knowledgeable subspecialists and researchers. *Nitric Oxide in Glaucoma: What Clinicians Need to Know* will distill and organize findings about the role of NO in glaucoma and the role of NO donation in glaucoma therapy in order to make them accessible to ophthalmologists and medical optometrists who want to optimize their decision-making in glaucoma.

LEARNING OBJECTIVES

Upon completion of this activity, participants should be able to:

- Review theorized mechanisms of optic nerve damage in glaucoma and recent advances in the understanding of the pathophysiology of glaucomatous optic neuropathy.
- Outline aqueous humor dynamics and the control of IOP in healthy and glaucomatous eyes.
- Identify sites of action for available IOP-lowering agents and recognize current deficiencies in medical treatment of glaucoma.
- Summarize the physiologic function of NO in various bodily systems and identify various NO-donating agents across medicine.
- Explain what is known about NO and its function in the eye.
- Describe the mechanism of action and therapeutic benefit of enhancing NO signaling in glaucoma patients.
- Discuss the potential role of emerging NO-donating therapeutics in glaucoma therapy.

INTRODUCTION

Glaucoma is one of the most common causes of blindness, affecting nearly 70 million people worldwide. Vision lost from glaucoma is not reversible since the disease causes progressive degeneration of the optic nerve and death of retinal ganglion cells (RGCs). No cure for glaucoma currently exists, but different treatment modalities—topical eye drops, laser therapy, and conventional surgery—are available to lower intraocular pressure (IOP) and stabilize disease and reduce the risk of further vision loss. Elevated IOP is the main risk factor for glaucomatous damage, even though other pathophysiologic mechanisms may also be involved. Generally, the IOP elevation in glaucoma is caused by increased resistance to aqueous humor outflow.

A topical IOP-lowering eye drop is typically the first option for glaucoma therapy. Since the introduction of beta-blockers in the 1970s, the number and types of glaucoma medications have increased remarkably. While therapeutic choices expanded, it became recognized that individualizing each patient's treatment regimen is necessary to maximize benefit and safety. Thus, glaucoma remains a complex disease and a challenge to treat. Many patients require more than one type of agent to achieve control of IOP. The current medications, though generally effective, do not work in every case. For glaucoma patients as a whole, the likelihood of preserving functional vision diminishes in the long term despite treatment, and the risk of developing blindness over time is considerably high.

To fulfill the need for additional glaucoma therapies, a tremendous amount of effort has been invested in the development of drugs with novel mechanisms of action. The desired features of an optimal IOP-lowering agent are evident: a high degree of effectiveness, minimum undesirable effects, and convenient dosing. A less obvious but equally important consideration is where and how an IOP-lowering agent works. The current medications either reduce the inflow of aqueous humor or increase the outflow through the uveoscleral pathway. The tissue compromised in glaucoma that leads to increased IOP—the trabecular outflow system—remains an important yet underexploited therapeutic target.

Nitric oxide (NO), an essential biological messenger, has emerged as a potential new therapeutic agent for IOP lowering in glaucoma. Simple yet versatile, this gaseous signaling molecule plays an important role in many physiological processes in the human body. Within the eye, NO is produced endogenously in various tissues, including the trabecular aqueous outflow pathway, where it participates in the regulation of IOP. An activator of soluble guanylate cyclase (sGC), NO is thought to alter IOP via modification of trabecular outflow facility.

This handbook provides a comprehensive overview of NO and its potential therapeutic application in the treatment of glaucoma. We first look at longstanding and new theories of how increased IOP and other factors may contribute to the pathophysiology of primary open-angle glaucoma (POAG), the most prevalent form of glaucoma in the US. A review of current and emerging therapeutics for glaucoma

follows next. We then outline the history of NO's discovery as an endogenous signaling molecule and its physiological functions in nonocular systems. The next and last two chapters concentrate on the links between NO and glaucoma. After examining NO's physiologic effects in the eye, we present available evidence that impaired NO signaling is implicated in the pathophysiology of POAG and discuss NO-based therapies being developed to treat glaucoma.

James C. Tsai, MD, MBA
Chairperson/Activity Director

CHAPTER 1

Pathophysiology of Primary Open-angle Glaucoma

RICHARD K. LEE, MD, PHD

Primary open-angle glaucoma (POAG) is a chronic optic neuropathy that causes progressive loss of retinal ganglion cells (RGCs) and their axons.[1] Clinically, the disease is often recognizable by its characteristic features including optic disc cupping, retinal nerve fiber loss, and correlated visual field defects (Figures 1 and 2). The many risk factors that have been associated with POAG include older age, higher levels of intraocular pressure (IOP), African race or Latino/Hispanic ethnicity, family history of glaucoma, and thinner central cornea.[1] To this day, we still do not fully understand how these factors are tied to susceptibility and, most importantly, what pathophysiologic processes underlie the onset and progression of the disease.

CHANGING VIEWS

Elevated IOP is a major causative risk factor for POAG. This association of elevated pressure with POAG has been identified in a number of population-based studies.[2-4] Additionally, large clinical trials over the past two decades have provided overwhelming evidence that lowering IOP can either delay or halt visual field progression in patients with POAG.[5-10] The therapeutic effect of IOP reduction has been observed not only in patients with elevated IOP but also in those with normal pressures. Assuming that any effective therapy for a given disease has a pathophysiologic basis, the fact that IOP reduction continues to be the gold standard treatment for glaucoma patients reinforces the notion that IOP is a major contributor to the course of the disease.

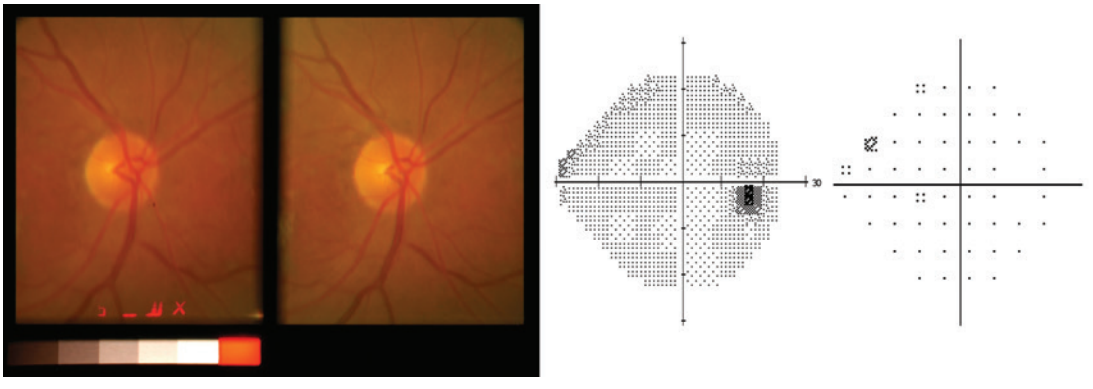


FIGURE 1 Stereophotograph of healthy optic nerve (right eye) and corresponding normal visual field (right eye). *(Images courtesy of Dr. Lee.)*

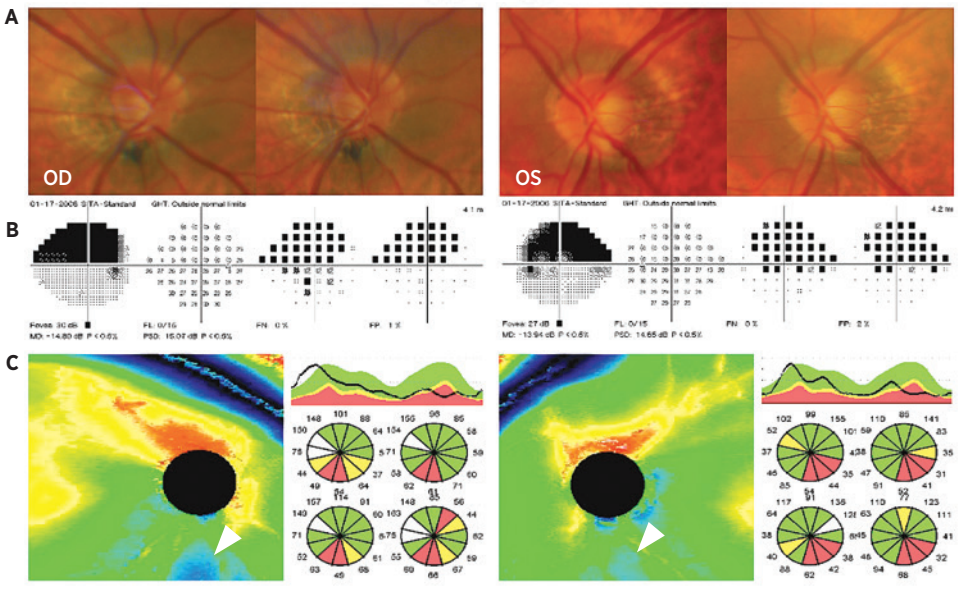


FIGURE 2 Glaucomatous optic neuropathy for right eye (OD) and left eye (OS). **A.** Stereophotograph of glaucomatous optic nerve heads. Note the enlarged optic disc cupping with inferior notches in the optic nerve in both eyes. **B.** Corresponding superior arcuate glaucomatous visual field defects in both eyes. **C.** Corresponding inferior loss of retinal nerve fiber layer in both eyes in color-coded en face topographic thickness maps (arrowheads) and sectorial nerve fiber layer maps (red wedges) from OCT imaging. Note: thicker nerve fiber layer appears in red and yellow in the topographic thickness maps. *(Images courtesy of Dr. Lee.)*

Despite its strong association with glaucoma, however, IOP elevation has been removed from the official definition of the disease.[1] Compelling evidence exists that IOP is a principal but not the sole factor contributing to glaucomatous structural and functional damage. In the Ocular Hypertension Study (OHTS), the majority of individuals with raised IOP did not go on to develop glaucoma within 5 years—whether treated or not by IOP lowering.[7] In contrast, at least one-third of POAG patients were found to have pressures within the normal limits.[2,3] Furthermore, control of IOP is not always effective in the treatment of glaucoma. Reducing IOP alone fails to halt disease progression and vision loss in some patients with POAG. In the Low Pressure Glaucoma Treatment Study (LoGTS), which compared twice-daily brimonidine 0.2% with twice-daily timolol 0.5%, patients on brimonidine had less visual field loss than those on timolol despite a similar IOP reduction from both agents.[11] Collectively, these clinical observations suggest that factors other than IOP contribute to the pathophysiology of POAG.

Over the past few decades, physiologic and molecular—and more recently proteomic, metabolomic, and genetic—research has helped better define glaucoma and understand its mechanisms. In particular, it has become increasingly clear that vascular dysregulation plays a pathogenic role in glaucomatous optic neuropathy,[12] probably more so in certain patient populations such as patients with normal tension glaucoma (NTG). Today, POAG is considered a multifactorial disease in which optic nerve damage and consequent vision loss are the end result of a range of pathological processes influenced by genes and perhaps environmental factors.[1,13,14] It affects a variety of tissues in both the anterior and the posterior segment of the eye.

IMPAIRED AQUEOUS HUMOR OUTFLOW

Aqueous humor dynamics are important to the pathophysiology of glaucoma: the course of the disease can be altered by altering the dynamics of aqueous circulation. The level of IOP is determined by the balanced production of aqueous humor from the ciliary body and its drainage through two independent outflow tracts: the trabecular and uveoscleral pathways (Figure 3). It has long been suggested that elevated pressure in POAG results mainly from increased resistance to aqueous outflow through the trabecular meshwork (TM),[15,16] although the exact site and nature of the pathology responsible for elevated outflow resistance have not been clearly identified.

The trabecular pathway—consisting of the TM, Schlemm's canal, collector channels, and episcleral veins—is the primary route for aqueous humor to exit the human eye. Resistance to aqueous outflow is critical in generating IOP, and the juxtacanalicular tissue (JCT) region of the TM and the inner wall of Schlemm's canal are thought to form the main site of outflow resistance in the normal eye.[16] The molecular mechanisms controlling pressure-dependent trabecular outflow are not well understood. Recent studies have suggested that mechanotransduction—the mechanisms by which mechanical stimuli such as shear stress are converted into biochemical signals to elicit specific cellular responses—in TM and Schlemm's canal endothelial cells is critical.[17-20]

The additional outflow resistance found in glaucomatous eyes is believed to also reside in the JCT area,[15] although the presence of other sources of increased resistance cannot been ruled out. Because patients with POAG exhibit no visible morphological abnormalities in the trabecular outflow pathway by gonioscopy,

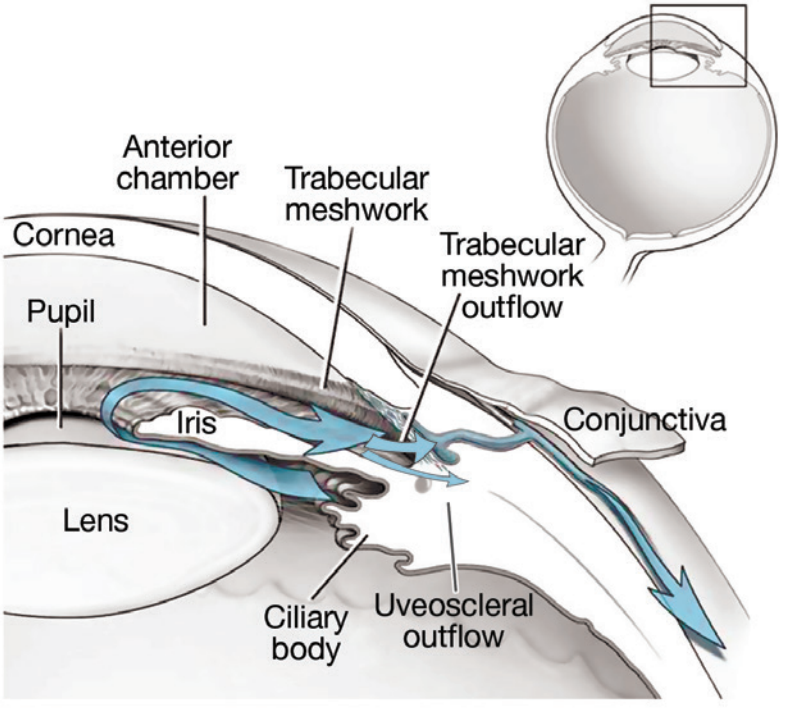


FIGURE 3 Schematic image demonstrating aqueous outflow pathways. ***(Image courtesy of National Eye Institute, National Institutes of Health.)***

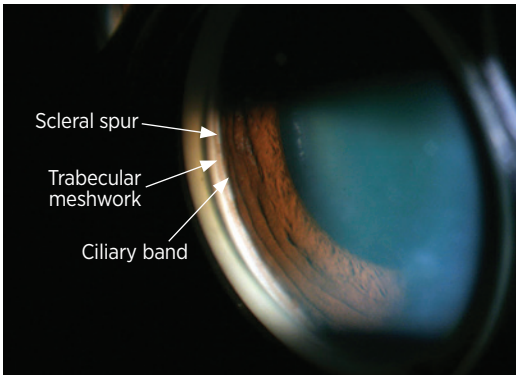


FIGURE 4A The anterior chamber angle. An open angle viewed through the goniolens. The ciliary body band, scleral spur, and trabecular meshwork are clearly visualized. *(Courtesy of Dr. Lee.)*

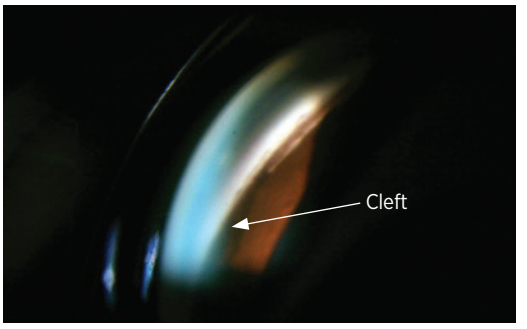


FIGURE 4B A cyclodialysis cleft is noted in the right half of the angle (visualized via goniolens). The ciliary body is disinserted from the scleral spur, allowing flow of aqueous humor from the anterior segment into the suprachoroidal space. *(Courtesy of Dr. Lee.)*

[Figures 4A and 4B] it is logical to think the pathologic damage causing increased outflow resistance occurs at the cellular or the molecular level or distal to the TM. Indeed, many studies have shown that glaucomatous TM tissue is altered in both its cells and extracellular material. Eyes with POAG were found to have decreased cellularity in the TM compared to age-matched normal controls.[21] TM cells cultured from glaucomatous eyes demonstrated higher levels of cross-linked actin networks (CLANs), a cytoskeletal change that may contribute to decreased aqueous outflow.[22] More recently, studies have reported increased cellular stiffness in glaucomatous TM and Schlemm's canal cells.[18] This change in the mechanical properties of the cells is believed to be at least partly responsible for increased outflow resistance in POAG.

Furthermore, there is evidence that the extracellular matrix (ECM) of TM tissue, which helps regulate aqueous outflow through mechanical sensing mechanisms,[23,24] differs between POAG and normal eyes.[25,26] The profile of TM glycosaminoglycans (GAGs) in glaucomatous eyes is altered in a way that may increase aqueous outflow resistance.[27] Glaucomatous TM tissue was also found to contain higher levels of the mechanosensitive molecule cochlin.[28,29] It has not been determined whether cochlin plays a causal role in IOP elevation related to POAG; however, absent in normal TM, the extracellular protein may disrupt the TM ECM and impair the TM's ability to allow aqueous humor transit.[30]

GLAUCOMATOUS NEURODEGENERATION

The retinal pathology of POAG is characterized by death of RGCs and atrophy of the optic nerve with the loss of axons. The extent of nerve tissue loss largely determines the clinical appearance of glaucomatous optic neuropathy in a given patient. While diffuse loss of nerve tissue results in cupping of the optic disc and an increased cup-to-disc ratio, focal retinal ganglion cell loss usually manifests as localized notching of the optic nerve rim (Figure 5). Cupping and notching often coexist, although some POAG patients present with a focal notch only and a subjectively normal cup-to-disc ratio.

RGCs are thought to die by apoptosis in POAG,[31] but the molecular path-

ways for the progressive optic nerve damage characteristic of the disease has not been clearly defined. A group of divergent theories have been proposed to account for the initiation and progression of glaucomatous neuronal damage, including blockade of axonal transport, neurotrophic factor deprivation, activation of intrinsic and extrinsic apoptotic signals, mitochondrial dysfunction, excitotoxic damage, hypoxia (or ischemia), oxidative stress, dysfunctional reactive glia, and loss of synaptic connectivity.[32] Because POAG is a complex disease with a multifactorial etiology, it is likely that more than a few pathways are involved and that signals from these pathways converge to induce RGC death.

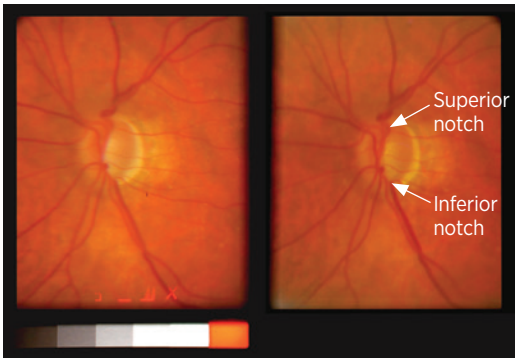


FIGURE 5 Stereophotograph of a glaucomatous optic nerve head. Note the notching of the superior and inferior disc rim. ***(Courtesy of Dr. Lee.)***

Substantial experimental evidence suggests elevated IOP contributes to glaucomatous neuropathy by directly affecting the posterior structures of the eye, particularly the lamina cribrosa. Perforated to allow optic nerve fibers to exit, the lamina cribrosa is biomechanically the weakest region in the scleral wall and the putative site of most direct axonal damage to RGCs in POAG.[33] According to the mechanical theory, one of the longest-standing theories of glaucomatous optic nerve damage, elevated pressure can cause mechanical stress and strain on the lamina cribrosa,[33] leading to tissue compression and distortion and interruption of axoplasmic flow.[34,35] Eventually, the supporting connective tissues within the optic nerve head collapse and axonal transport system become further damaged to deprive the RGC soma of nutrients and survival signals—two pathophysiological changes that are considered central to glaucomatous optic nerve head damage and subsequent RGC dysfunction and then death.

VASCULAR AND PERFUSION FACTORS

In addition to IOP, impaired blood supply in the optic nerve is believed to be an important factor in the pathogenesis of glaucomatous optic neuropathy. The retina and optic nerve head are energy-demanding tissues that rely on auto-regulatory mechanisms to optimize blood supply to meet their high metabolic needs.[36] The vascular theory holds that chronic hypoxia or ischemia due to decreased optic nerve perfusion can create stress conditions leading to demise of RGCs.[37-39] Individuals who have poor vascular supply to the optic nerve head, as predicted by the theory, are predisposed to glaucomatous changes.

Hemodynamics studies support that optic nerve head and retinal blood flow are reduced in glaucomatous eyes.[38,40-44] This disturbance in ocular blood flow may occur secondary to elevated IOP, as mechanical compression caused by high IOP may restrict blood flow and reduce perfusion of the retina and other ocular neuronal tissues. A possible trigger for ischemia in the development of glaucomatous neuronal damage is deficient or impaired vascular tone regulation. As is the case with NTG, glaucomatous damage can occur in the absence of elevated IOP. Patients

with NTG have been observed to have reduced ocular blood flow.[45,46] Moreover, an association has been found between NTG and systemic disorders that may correlate with decreased autoregulation of optic disc blood flow, such as Raynaud's phenomenon, migraine, and peripheral vasospasm.[1,47]

Over the past decade, evidence has accumulated to support the theory of vascular dysregulation as an independent contributor to glaucomatous optic neuropathy. Population-based studies have identified an adverse association between OAG and low ocular perfusion pressure or low systemic blood pressure.[12] One immunochemistry study found upregulation of the transcription factor hypoxia inducible factor 1-α—a marker of ischemia—in the retina and optic nerve head of glaucoma patients.[48] In a monkey model of optic neuropathy, chronic optic nerve ischemia induced by endothelin-1 caused regional axonal damage similar to that found in glaucoma.[49]

More recently, findings of several genetic studies have strengthened the notion that vascular risk factors are implicated in the pathogenesis of POAG. Several genome-wide association studies have identified significant associations between POAG and genomic variants of CAV1 and CAV2, genes involved in vascular regulation.[50-52] Another study of nearly 200 vascular tone-regulating genes found that eight such genes, including CAV1, are associated with POAG.[53]

A CONVERGING POINT

Genes CAV1 and CAV2 code for caveolins, membrane proteins that are known to interact with endothelial nitric oxide synthase (eNOS), an enzyme that produces nitric oxide (NO). Acting as an endogenous signaling molecule, NO is a major regulator of vascular tone and vessel homeostasis. Abundant in vascular endothelium, caveolins take part in vascular tone regulation by controlling the activity of eNOS and production of NO.[54] Of the eight vascular tone-regulating genes that were associated with POAG, six have influences on eNOS activity.[53] This finding supports a role for dysregulated eNOS activity in glaucoma pathogenesis.

The potential role of caveolin/eNOS interactions in glaucoma pathophysiology is intriguing because these interactions have been implicated in IOP homeostasis, in addition to vascular tone regulation. Caveolins are abundantly expressed in the TM and Schlemm's canal,[16,55,56] and gene association studies have repeatedly identified polymorphisms of the caveolin genes CAV1 and 2 as a genetic factor influencing IOP.[57-59] In mice lacking caveolins, IOP was significantly higher than in age-matched controls.[55] The exact role of caveolins in IOP homeostasis is not well understood but likely involves mechanotransduction pathways. Recent evidence in the eye suggests that these membrane proteins respond to mechanical stimuli and protect the trabecular outflow tract cells from mechanical stress induced by higher IOPs.[55] Additionally, caveolins may influence IOP regulation by mediating mechanical activation of eNOS,[55] as eNOS activity increases trabecular outflow facility and can help lower IOP.[17]

PRESSURE AND PRESSURE DIFFERENCES

It has been more than 100 years since the mechanical and vascular theories were initially proposed to explain glaucomatous damage. Our understanding of the pathophysiology of POAG is still evolving as scientific research continues to uncov-

er new pieces to the puzzle. One factor that was recently identified as a potentially important contributor to POAG is decreased cerebrospinal fluid (CSF) pressure.

This new theory is based upon the concept that tissues of the optic nerve head and lamina cribrosa anatomically separate the intraocular and retrobulbar orbital compartments and bear the pressure gradient between the two spaces. Normally, IOP is slightly higher than orbital CSF pressure. It is hypothesized that pressure imbalance across the lamina cribrosa directly affects the pressure against the optic nerve head, and glaucomatous optic nerve damage can occur when an elevated posterior-directed translaminar pressure gradient—from increased IOP and/or decreased CSF pressure—compresses the lamina cribrosa and damages optic nerve structures, blood supply, and axonal transport.[60,61]

According to this two-pressure model, low CSF pressure, as the counter pressure against the IOP at the lamina cribrosa, is an independent risk factor for glaucomatous optic neuropathy. This provides a plausible explanation for the existence of NTG: patients with NTG may indeed have IOPs within the normal range, but they may also have abnormally low orbital CSF pressure and, as a result, an increased posteriorly directed translaminar pressure affecting RGC axons. Similarly, relative protection from higher CSF pressures may be the reason why many patients with ocular hypertension do not develop glaucomatous damage.

The evidence consistent with the hypothesis has been slowly but steadily mounting in recent years. Population-based studies have found that glaucomatous optic neuropathy associates better with lower CSF pressure as compared to IOP.[62,63] Retrospective and prospective observational studies have reported that POAG patients had lower CSF pressure than normal individuals and that patients with NTG had lower CSF pressure compared to either POAG or normal controls.[64-66] Low CSF pressure is also associated with variables such as low blood pressure, low IOP, and low body mass index.[66,67]

Despite available evidence, CSF pressure's significance in the pathogenesis of glaucomatous optic neuropathy warrants further research. Currently, the lack of a simple and reliable method to non-invasively measure orbital CSF pressure is a major barrier to understanding the dynamic aspects of CSF pressure and its relationship to other relevant pressure parameters including IOP, blood pressure, and ocular perfusion pressure. Lumbar CSF pressure can be used for estimation of orbital CSF pressure, but it is impractical to perform an invasive lumbar puncture on every patient. More importantly, it remains a question whether lumbar CSF pressure is directly related to orbital CSF pressure. Even if low CSF pressure proves to be an important pathogenic factor for POAG, its therapeutic importance would be uncertain because there is no clinically effective method at present known to alter CSF pressure in a non-invasive manner (although oral carbonic anhydrase inhibitors may have a potential role). Currently, the potential utility of the CSF pressure lies more in the screening and diagnosis of POAG than treatment.

FROM PATHOPHYSIOLOGY TO THERAPY

A better understanding of the cellular and molecular mechanism of POAG will open new avenues for treatment. Because caveolins play a potentially critical role in the regulation of IOP and aqueous humor outflow, they may represent a viable molecular target for developing IOP-lowering therapeutics in POAG. Several molecules

with novel IOP-lowering mechanisms have recently made their way into clinical trials, including an NO-donating prostaglandin analog, a Rho kinase (ROCK) inhibitor, and an adenosine receptor agonist. These agents work through different mechanisms of action, but they all reduce IOP by enhancing physiologic trabecular outflow, presumably through direct effects on the cells and/or ECM of the TM and Schlemm's canal. New drugs targeting direct trabecular outflow would represent a breakthrough in glaucoma therapy: until now such agents have been largely lacking, even though IOP has been known to originate from outflow resistance at the TM.

Currently, IOP reduction remains the goal of all clinically available treatment modalities for glaucoma and the gold standard by which the FDA approves glaucoma medications. However, elevated IOP does not directly cause irreversible loss of visual field; rather, the primary problem in POAG and other forms of glaucoma is optic nerve dysfunction and damage and neuronal death. For the prevention or even the cure of glaucoma, the ultimate solution is to preserve or, better, restore the structure and function of the RGCs and the optic nerve. Neuroprotection, a therapy that is by definition directed at the RGCs rather than at IOP, has so far not been realized—but it continues to be the focus of intense investigation. A number of strategies aimed at promoting neuronal survival are being explored, including enhancement of neurotrophic support, apoptosis inhibition, improvement of blood flow, blockade of excitotoxicity, immunomodulation, and retinal stem cell transplantation (not as direct neuronal replacements but as neuroprotective therapies mediated by stem cell secretion of trophic factors).

For the most part, current efforts in the development of neuroprotective strategies for glaucoma are directed at rescuing dying neurons or, in the case of cell-based therapy, transplanting cells to aid in the function and survival of injured RGCs. A more practical and promising approach, however, may be one that focuses on the living neurons, rather than the damaged ones, to enhance vision. Known as neuro-rejuvenation, the idea is to strengthen the activity and communication among existing healthy RGCs so that retinal signals can be enhanced to produce a better image.[68] Unlike cell-based RGC replacement therapy concepts, which has been hindered by difficulties such as establishment of correct neuronal circuits along the visual pathway to the brain, neuro-rejuvenation therapy targets existing neurons and neuronal networks at the same time.[68] Identifying molecules that can enhance the intrinsic functional capacity of RGCs, therefore, may provide new therapeutic targets for POAG and other forms of OAG.

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CHAPTER 2

Therapeutic Strategies for Open-angle Glaucoma

TONY REALINI, MD, MPH

Glaucoma is the second leading cause of blindness worldwide.[1] Patients diagnosed with primary open-angle glaucoma (POAG) or other chronic forms of OAG face significant lifetime risk of visual disability and functional impairment.[2] Adequate treatment is critical to reduce the visual impact of glaucoma and conserve patients' quality of life. At present, the only treatment that has clinically proven to be effective for the management of glaucoma is reduction of intraocular pressure (IOP). Elevated IOP is the strongest causal risk factor for the onset of POAG, and a higher IOP is associated with greater disease progression and a greater lifetime risk of blindness.[3-5] Current treatment modalities for glaucoma, from medications to laser procedures to incisional surgery, are all aimed at the greatest possible reduction of IOP with a minimum of adverse effects.

TREATMENT GOALS

For most glaucoma patients, the ultimate goal of therapy is to stop or slow the rate of disease progression to prevent visual field loss. Some patients require more vigorous treatment than others in order to reduce their IOP enough so that no further damage occurs. The American Academy of Ophthalmology (AAO) Preferred Practice Pattern for POAG recommends an initial 25% reduction of IOP from baseline for patients with early to moderate disease.[6] If the patient is at particularly high risk for sight-threatening progression, then a greater initial pressure reduction, eg, 40% or even 50%, may be necessary. Multiple factors need to be considered in weighing the patient's risk for disease progression (Table I). Advanced disease, identifiable by more severe optic nerve damage and greater visual field loss, and higher IOP are two important predictors of further glaucomatous damage.[6] Blindness or severe damage in one eye is indicative of an increased risk of damage in the other eye. Young patients may also benefit from a more aggressive target IOP, because they will live with the disease for a longer time.

TABLE I
Risk factors for disease progression in patients with POAG

Older age
Higher IOP
Greater visual field damage
Large cup-to-disc ratio
Beta-zone peripapillary atrophy
Decreased corneal hysteresis
Disc hemorrhage
Thinner central cornea*
Pseudoexfoliation
Lower ocular perfusion pressure

*Mixed evidence

Currently, no clinically validated algorithms are available for determining a given patient's future risk of progressive glaucomatous damage. Target pressure is essentially a clinical guesstimate based on analysis of known risk factors such as disease severity and IOP level; it is no guarantee that progression will be prevented. Patients who have achieved the desired pressure reduction need to be regularly monitored with clinical examinations, visual field tests, and optic nerve imaging to assess the adequacy of the target pressure. If progression continues to occur despite reduction of IOP to the target level, then a new, lower target pressure needs to be set.

CURRENT OPTIONS

There are three treatment modalities for lowering IOP in patients with OAG: medications, laser procedures, and incisional surgery. IOP is determined by the balance between aqueous humor production and aqueous drainage from the eye. Reduction of IOP can thus be achieved by decreasing aqueous production, increasing aqueous outflow of aqueous humor, or both. Several classes of available glaucoma medications, including beta-blockers, carbonic anhydrase inhibitors (CAIs), and alpha-2 adrenergic agonists, act by suppressing aqueous humor production; others, such as prostaglandin analogs (PGAs), help open the drainage tracts to enhance outflow of the intraocular fluid.

Similarly, different laser procedures target different components of aqueous humor dynamics to lower IOP. Laser trabeculoplasty works by increasing aqueous outflow through the trabecular meshwork (TM), whereas cyclophotocoagulation is aimed at decreasing aqueous humor formation at the ciliary body. All surgical procedures available for managing OAG are designed to facilitate aqueous outflow, either by creating a new anatomic pathway to bypass the TM outflow system or by installing drainage devices.

Overall, the treatments available today for reducing IOP in glaucoma patients are effective and well tolerated. Each treatment modality, however, has its own advantages and disadvantages. As expected, more invasive therapies, such as incisional surgeries, carry higher risks of complications.

MEDICATIONS

Among available glaucoma drops, the PGA class of drugs are the most effective and well tolerated (Table II).[6] In addition, they have a simple dosing schedule—once daily in the evening. Rarely does one drug class excel in both efficacy and safety in the way the topical PGAs do. Because of their efficacy, safety, and convenience, PGAs became the drug of choice for treatment of glaucoma soon after their introduction in the 1990s.

Currently, there are four PGAs that are approved for clinical use: latanoprost, bimatoprost (available in different strengths of 0.03% and 0.01%), travoprost, and tafluprost. Among these, latanoprost, travoprost, and bimatoprost 0.03% are available in generic form. Tafluprost, the latest addition to the PGA family, is the only preservative-free PGA drop.

The PGAs lower IOP by increasing aqueous outflow primarily through the uveoscleral pathway, presumably by relaxing the ciliary body and increasing spaces between ciliary muscle bundles as well as altering the extracellular matrix (ECM) of

TABLE II Glaucoma medications: classification, mechanism, therapeutic and side effects

Classification	Example	Mechanism	Efficacy	Side effects
PGAs	Latanoprost Travoprost Bimatoprost Tafluprost	Enhanced outflow (primarily uveoscleral)	++++	Conjunctival hyperemia Periocular hyperpigmentation Eyelash growth Increased iris pigmentation Allergic conjunctivitis Periorbital fat atrophy
Beta-blockers	Timolol Levobunolol Carteolol Betaxolol	Decreased aqueous production	+++	Bronchoconstriction Bradycardia Hypotension Depression Impotence Allergic conjunctivitis
Alpha-adrenergic agonists	Apraclonidine Brimonidine	Decreased aqueous production Enhanced uveoscleral outflow	+++	Allergic conjunctivitis Contact dermatitis Dry mouth and nose Fatigue
Miotics	Pilocarpine Carbachol Echothiophate	Enhanced trabecular outflow	+++	Decreased vision Eye ache Increased myopia Cataract
CAIs				
Topical	Dorzolamide Brinzolamide	Decreased aqueous production	++	Allergic conjunctivitis Metallic taste
Oral	Acetazolamide Methazolamide	Decreased aqueous production	+++	Malaise Weight loss Depression Kidney stones

ciliary muscle cells.[7,8] Large clinical studies suggest that agents of the PGA class significantly reduce IOP from baseline by 25% to approximately 35%, and their effects last throughout the 24-hour dosing interval.[9-12] The adverse effects of the PGAs are largely confined to the eye, with some cosmetic but no serious consequences. The most common ones include conjunctival hyperemia, increased iris or periocular skin pigmentation, and growth and darkening of eyelashes.

LASER TRABECULOPLASTY

Laser trabeculoplasty directly treats the anterior chamber angle to reduce resistance and improve aqueous outflow through the TM and Schlemm's canal. Argon laser trabeculoplasty (ALT), the original form of the procedure, was found in

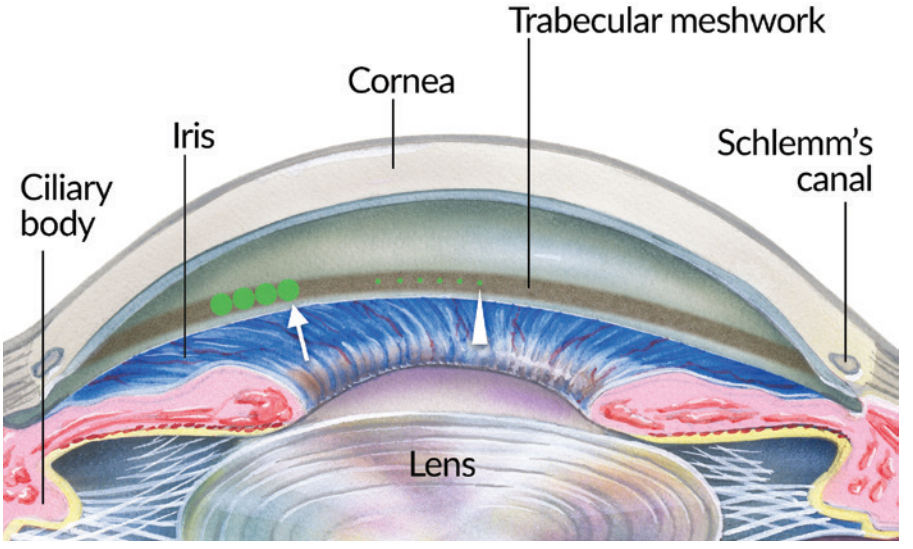

FIGURE 1 Laser spots of SLT (arrow) and ALT (arrowhead) at the anterior chamber angle.

the Glaucoma Laser Treatment Trial (GLT) to be at least as efficacious as initial treatment with timolol in patients with OAG.[13] However, ALT produces significant coagulative damage to tissues of the TM and potentially necrotic death of the nonpigmented cells in the area.[14] Repeat ALT, therefore, has limited effectiveness and may eventually lead to synechial angle closure and decreased outflow facility.[14]

Selective laser trabeculoplasty (SLT) utilizes a lower-energy, frequency-doubled Nd:YAG nonthermal laser. It selectively targets pigmented cells, sparing adjacent cells and tissues.[15] Because the laser spot covers the entire width of the TM, a size much larger than that of ALT, SLT is relatively easier to perform (Figure 1). First described in 1995 and approved by the FDA in 2001, the procedure is equally safe and effective as ALT in lowering IOP in OAG.[16,17] In recent clinical studies, SLT safely produced IOP reduction comparable to that of a PGA over one year.[18,19] It has also been shown that SLT is effective as primary therapy with few complications in a wide range of OAG patients, including patients with NTG, pigmentary glaucoma, pseudoexfoliation syndrome, or corticosteroid-induced glaucoma.[20-23] Although its precise actions remain unestablished, SLT appears less destructive to the TM structures than ALT. It is thought likely that SLT works by eliciting certain cellular and biological responses in the TM, such as altered gene expression, increased cell permeability, and cell repopulation.[24-26] Regardless of the mechanism behind SLT's therapeutic effect, the milder tissue response implies greater potential for the treatment to be repeated. Once controversial, the safety and effectiveness of repeat SLT are now well established.[27-32]

INCISIONAL SURGERY

At present, traditional incisional surgery, including trabeculectomy and the placement of aqueous tube shunts, remains the most effective IOP-lowering treatment available. In the Tube versus Trabeculectomy study, the average IOP 5 years

TABLE III Complications associated with traditional filtering surgery

Trabeculectomy	Aqueous shunt implantation
Conjunctival buttonhole	Flat anterior chamber
Scleral flap tear	Hypotony
Intraoperative bleeding	Choroidal effusion
Flat anterior chamber	Diplopia
Low filtration	Hyphema
Hypotony	Corneal decompensation
Choroidal effusion	Tube blockage
Bleb leaks	Tube erosion and exposure
Bleb failure from fibrosis	
Cataract	
Blebitis and endophthalmitis	

after surgery was below 15 mm Hg in both the tube shunt and the trabeculectomy groups.[33] These surgical procedures, however, have high complication and failure rates (Table III).[33,34] Even though substantial IOP reduction is often achieved, many patients require supplemental medications or reoperation for long-term control.[33] Application of an antifibrotic agent to the surgical site reduces conjunctival scarring after filtering surgery and the likelihood of surgical failure; however, the risk of complications such as hypotony and infection may increase (Figure 2).[6]

In the past 10 years, a new group of ab interno procedures known as micro invasive glaucoma surgeries (MIGS) have been developed as safer alternatives to traditional glaucoma surgery. These procedures are conjunctiva-sparing and aimed at bypassing the juxtacanalicular portion of the TM (Figure 3), the site of increased outflow resistance in most patients with OAG.[35] The MIGS procedures available today offer a better safety profile than filtering surgery and have been shown to be efficacious in patients with mild to moderate OAG.

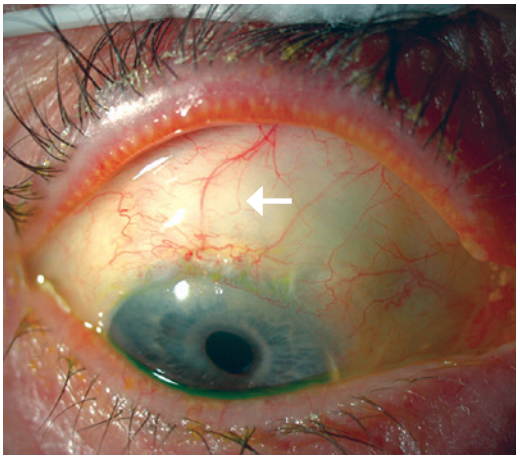

FIGURE 2 A functional bleb after trabeculectomy. *(Courtesy of James Tsai, MD.)*

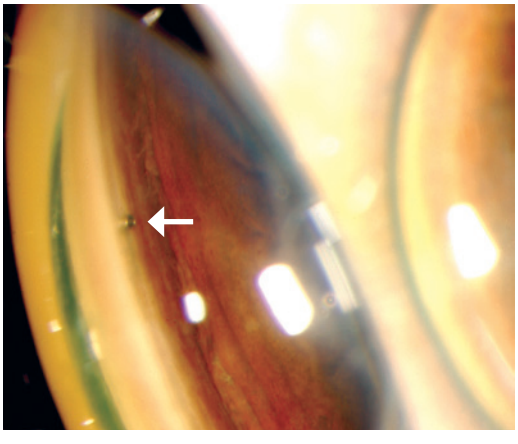

FIGURE 3 A trabecular micro-bypass stent inserted in Schlemm's canal, viewed under gonioscopy. *(Courtesy of Glaukos Corporation.)*

THE TREATMENT PARADIGM

Conventionally, the management of patients with newly diagnosed OAG follows a stepwise regimen, with medications being the first choice, laser treatment the second, and filtering surgery an option of last resort for cases where medical and laser therapy have insufficiently lowered IOP.[36] In recent years, however, a paradigm shift has been taking place, albeit at a slow pace. Clinicians are beginning to realize there may be advantages to SLT as opposed to topical drops in early intervention. SLT is now moving from after the second or third medication to after the first medication or, in some cases, replacing medications altogether as initial therapy, as evidenced by the growing number of studies comparing SLT to PGA therapy as first-line treatment for glaucoma.[18,37,38]

Although the current IOP-lowering medications are effective, well tolerated, and generally available in relatively inexpensive generic form, their therapeutic benefit comes with considerable risk of adverse effects and burden of costs in the long run. Furthermore, the outcome of medical therapy relies heavily on patient adherence; and poor adherence has been shown to lead to disease progression and visual loss in patients with glaucoma.[39] One major challenge with medical glaucoma treatment is to get patients to understand the importance of therapy and to take their drops on a daily basis. As revealed in many studies, inadequate adherence to treatment regimen is prevalent among glaucoma patients.[40,41]

The beneficial effects of laser therapy, by contrast, are not reliant upon patient adherence. Patients who attain target IOP after SLT alone can be entirely free of the responsibility for daily dosing, and those who attain target IOP with a combination of SLT and medications benefit from the simpler regimen. One frequently cited reason for opposing SLT's role as primary therapy for OAG is that its effect may fade over time. Diminishing effectiveness of SLT is a valid concern, but the procedure is repeatable, and repeat SLT is effective and safe. Since the PGAs are instilled on a daily basis, SLT will still have a dosing advantage even if it has to be repeated every 6 months.

THE NEED FOR ALTERNATIVE THERAPIES

Given the effectiveness of available treatment options, it is possible to attain target pressure in most glaucomatous eyes. However, control of IOP and glaucoma often requires aggressive steps. In reality, surgical rates and prevalence of blindness remain high among glaucoma patients. The number of glaucoma surgeries performed in the US of 2006 was estimated to be nearly 85,000.[42] Worldwide, nearly 4.5 million people were estimated to have bilateral blindness from OAG in 2010; and 6 million are projected by 2020.[43] There remains an unmet need for noninvasive or minimally invasive treatments that can safely produce substantial IOP reductions.

In contrast to advances in laser and surgical therapy, such as SLT and MIGS, glaucoma pharmacology has been in an innovation lull for almost 20 years. The last time a new class of drugs was added to the glaucoma management toolbox was in 1996, when latanoprost was introduced. The latest IOP-lowing drop was tafluprost, a fourth-in-class therapeutic approved by the FDA in 2012. Several fixed-dose combination drops have become available in the US in recent years, including dorzolamide 2.0%/timolol 0.5%, brimonidine 0.2%/timolol 0.5%, and brinzolamide 1%/

brimonidine 0.2%. There are now more topical drops to choose from than two decades ago, but the mechanisms by which they lower IOP are not new.

The PGA class of drugs has set a high standard for first-line glaucoma medications: high effectiveness, low dosing frequency, and a favorable side effect profile. Surpassing a drug that combines these features would be very difficult, which may be one reason why the medical treatment paradigm for glaucoma has not changed much for two decades. A PGA, however, is not the best treatment choice for every patient. Because they have the potential to aggravate intraocular inflammation, PGAs should be used with caution in patients with uveitis. Other rare complications, such as macular edema and the reactivation of latent herpes keratitis, have not been conclusively established;[44] use of PGAs in these eyes may be reasonable if the risks of alternative therapies are significant. Clinicians should also be cautious about using a PGA as the first choice when the circumstance calls for unilateral therapy, such as when a patient has OAG in only one eye or has had surgery in the other eye. The cosmetic side effects of PGAs (red eyes, lash changes, or orbital fat atrophy) can be more noticeable and more disconcerting to patients when they occur unilaterally.

As with all ocular therapeutics, treatment response to PGAs varies. Some patients simply cannot achieve a meaningful IOP reduction with these otherwise effective agents. For these suboptimal responders and those for whom PGAs are contraindicated, there is a great need for an alternate therapy. Beta-blockers can be a reasonable alternate first-line therapy for some patients, but these drugs also have limitations. They have limited efficacy in lowering IOP during the nocturnal period.[45,46] They also tend to be less effective in patients that are already on a systemic beta blocker for hypertension,[47] which is a large portion of the glaucoma patient population. Moreover, they are associated with significant systemic side effects and often require twice-daily dosing.

In principle, for a drug to replace the PGAs as the first-line option for most patients, it will have to offer either better efficacy with a similar safety profile and tolerability to a PGA, or similar efficacy with better safety and tolerability. A drug that is as effective as a PGA but causes less hyperemia, for instance, would be a very attractive option. So would be a drug that has the same efficacy and safety as a PGA but is dosed even less frequently.

Several PGA-based implants and other sustained drug delivery platforms are currently being developed for glaucoma, with the goal to lower IOP for up to several months with one application. The place of such therapies in the current treatment regimen for glaucoma is not yet clear. Switching to an intraocular implant, for instance, may not add much benefit for patients well controlled with drops. The best candidates for sustained-release therapy, perhaps, are those who are known or suspected to be poorly adherent. Such patients, in fact, constitute a significant fraction of the total patient population with OAG.

NEW IOP-LOWERING MECHANISMS

Aside from sustained-release therapy, several novel IOP-lowering agents are expected to emerge over the next few years. Latanoprostene bunod (LBN), a nitric oxide (NO)-donating PGA, has completed phase 3 testing and will likely receive market approval in the US in 2017. Netarsudil mesylate, a Rho kinase (ROCK) inhibitor,

has also been through several phase 3 studies and may be under consideration by the FDA for approval some time in 2017. The adenosine receptor agonist trabodenoson is also in late-stage development, but disappointing results of a recent phase 3 trial may affect further development.[48]

All three drugs have one thing in common: they help recover the physiologic function of the TM and Schlemm's canal, the primary pathway for aqueous outflow and the site of pathology responsible for IOP elevation in POAG.[49,50] LBN acts through two metabolites released during its hydrolysis inside the eye: latanoprost and NO. Latanoprost—identical to its monotherapy form—reduces IOP primarily by enhancing uveoscleral aqueous outflow, whereas NO is thought to increase outflow facility across the TM.[51] Through inhibition of the Rho pathway, netarsudil mesylate causes cytoskeletal changes and relaxes cells of the TM and Schlemm's canal. Additional mechanisms, such as reduction of episcleral venous pressure,[52] might contribute to the ROCK inhibitor's IOP-lowering effect but need further elucidation. Much less is known about the mechanism of trabodenoson, though adenosine agonists are thought to increase ECM turnover in the TM.

These novel agents could help fill the gap in glaucoma therapeutics. Of the several classes of glaucoma medications now in common clinical use, none directly targets the trabecular outflow pathway. Miotics (eg, pilocarpine and echothiophate iodide), one of the earliest types of glaucoma medications, reduce IOP by contracting the ciliary muscle to pull open the TM to increase aqueous outflow.[53] Their clinical use, however, has become limited today owing to availability of drops that are safer and require less frequent instillation.

How these drugs might fit into our stepped approach to glaucoma medical management is not knowable at this time. It is worth noting, however, that LBN is the only molecule that has demonstrated statistically significant superiority over latanoprost in terms of efficacy. In a phase 2 study of patients with OAG or ocular hypertension, LBN therapy led to an additional IOP reduction of about 1.2 mm Hg over latanoprost with no additional side effect issues.[54] Cost is likely to be a key difference setting LBN and latanoprost apart. Whether the incremental efficacy within the range of 1 to 2 mm Hg will be worth the added cost of a branded product over a generic will largely be determined by market forces.

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CHAPTER 3

Nitric Oxide: Historic Perspective and Recent Developments

LEO SEMES, OD, FAAO

Nitric oxide (NO), as indicated by an increasing body of experimental and clinical evidence, has important etiological and therapeutic implications for primary open-angle glaucoma (POAG).[1-5] In the healthy eye, NO has been recognized as an important regulator of regional blood flow and as one of the key local mediators that modify trabecular outflow facility to maintain physiological intraocular pressure (IOP).[6-10] Reduced NO production may contribute to IOP elevation in POAG through dysregulated trabecular outflow; it may also increase the risk of glaucomatous damage by affecting optic nerve head perfusion.

Glaucoma is in fact only one of many human diseases that involve insufficiency or dysregulation of NO (Table I; Table II). A ubiquitous endogenous signaling molecule with diverse biological effects, NO is a crucial player in a myriad of physiological and pathological processes of the human body. Blood pressure regulation, vascular homeostasis, control of smooth muscle tone, antimicrobial defense, and learning and memory are various examples of the gaseous messenger's biological effects.[11,12] To provide an appropriate context for understanding NO's role in ocular physiology and specifically the pathophysiology of glaucoma, this chapter will review the molecule's history and non-ocular functions.

TABLE I
Processes and diseases with NO participation

Neurotransmission, memory, stroke
Glaucoma and other neurodegenerative disorders
Vasodilation, blood pressure, blood-flow
Pulmonary hypertension
Penile erection/dysfunction
Angiogenesis, wound healing
Atherogenesis
Inflammation, arthritis, nephritis, colitis, etc.
Cytotoxicity of tissues, pathogens, tumors
Asthma
Tissue transplantation
Septic shock, dialytic hypotension
Platelet aggregation
Gastrointestinal motility
Hormone secretion
Gene regulation
Hemoglobin delivery of oxygen
Stem cell proliferation and differentiation
Bronchodilation

Republished from: Murad F. Nitric oxide: the coming of the second messenger. *Rambam Maimonides Med J.* 2011;2(2):e0038.

DISCOVERY MILESTONES

NO was first discovered in the 1770s by Joseph Priestly, an English chemist and theologian.[13] For much of the following two centuries, NO was deemed merely a reactive free radical and an air pollutant. Although nitroglycerin was synthesized and found to have a vasodilatory effect in the 1840s and, by the 1870s had been widely used along with related nitrate compounds to treat patients with angina pectoris and hypertension,[14] NO's chemical effects on vascular tissues were not realized for another century.

In 1977, Ferid Murad discovered that NO release accounts for the vasodilatory effect of nitroglycerin and its relatives.[15,16] As it turned out, the free-radical gas is also a biologically active molecule when released from these compounds during metabolic processes. The discovery of exogenous NO's vasodilatory effect raised, for the first time, the question about endogenous NO's potential role in cardiovascular physiology.

Several years later, while studying vasodilation and a drug called acetylcholine, Robert Furchgott observed that, in the absence of the endothelial lining, the smooth muscle cells of vessels were unable to relax in response to acetylcholine.[17] He postulated that some substance produced by the endothelium was required for the relaxation of vessels, setting off an intense search in the scientific field to identify this substance, known as endothelium-derived relaxing factor (EDRF) at the time.

In 1987, nearly a decade after the elucidation of NO's role in nitrate-induced vasodilation, Salvador Moncada and Louis Ignarro independently discovered that NO is in fact the mystery EDRF that has long been sought after by researchers.[18,19] To honor their pioneering work "concerning nitric oxide as a signaling molecule in the cardiovascular system," the 1998 Nobel Prize in Physiology or Medicine was awarded to Furchgott, Ignarro, and Murad.[20]

TABLE II
NO Isoforms in Some Diseases

Disease	NO Isoform
Cardiac diseases	↑iNOS
Myocardial ischemia and reperfusion injury	
Myocarditis	
Heart failure	
Vascular diseases	
Atherosclerosis	↑eNOS
Hypertension	↑eNOS
Aging	↓eNOS
Neurodegenerative disorders	↑iNOS
Parkinson's disease	
Alzheimer's disease	
Huntington's disease	
Multiple sclerosis (MS)	
Amyotrophic lateral sclerosis (ALS)	
Traumatic brain injury (TBI)	
Local inflammation	↑iNOS
Chronic arthritis	
Inflammatory bowel diseases	
Tissue inflammation from toxic origin	
Cancer	↑iNOS, eNOS, nNOS
Metabolic syndrome	↑iNOS
Diabetes	↑eNOS
Obesity	↑iNOS
Dyslipidemias (particularly hypercholesterolemia and hypertriglyceridemia)	↑eNOS

Republished from: Khazan M, Hdayati M. The Role of Nitric Oxide in Health and Diseases. *Scimetr*. 2015;3(1): e20987

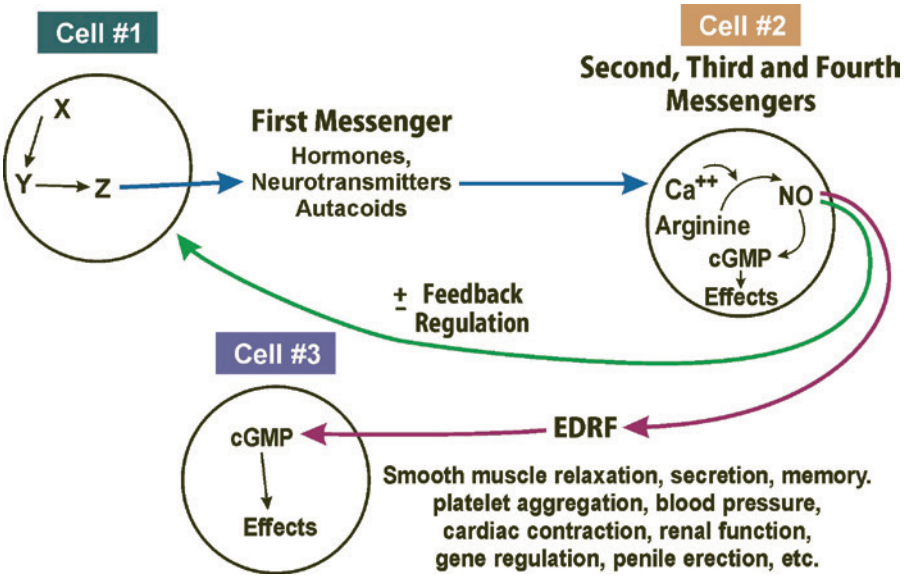

FIGURE 1 The NO (ie, EDRF) and cGMP signaling system for intracellular and intercellular communication. Abbreviations: cGMP, cyclic guanosine monophosphate; EDRF, endothelium-derived relaxing factor; NO, nitric oxide. (Republished from: Murad F. Nitric oxide: the coming of the second messenger. *Rambam Maimonides Med J.* 2011;2(2):e0038.)

The discovery of endothelial-cell-derived NO's critical role in vasodilation marked a major advancement in the history of physiology. Thereafter, researchers began to explore the messenger molecule's functions beyond the vessel wall, and they soon recognized that NO was a remarkably versatile messenger playing a complex role in physiological activities of multiple human body systems.[11] In 1992, NO was named the "Molecule of the Year" by the journal *Science*.[21]

THE PATHWAY

The chemical properties of NO—highly lipophilic and highly volatile—make it a distinct and elusive intracellular messenger in the human body. Unlike other second messengers, NO consumes no energy in transporting itself between cells.[22] Owing to its lipophilic nature, the gaseous molecule is able to readily diffuse across cell membranes to function as a paracrine messenger that induces changes in adjacent cells. The classic NO pathway begins with a hormone, first messenger, or ligand binding to its receptor, which then signals production of NO (Figure 1).[22] Within the cell, NO directly stimulates the soluble guanylate cyclase (sGC) to increase the production of cyclic guanosine monophosphate (cGMP), a second messenger.[15,23] Low nanomolar concentrations of NO are sufficient to activate purified sGC with a 200- to 400-fold increase in the enzyme's maximum reaction rate.[22]

Increased cGMP levels lead to activation of cGMP-dependent protein kinases and subsequent phosphorylation of proteins downstream (Figure 2). Cyclic GMP can also regulate cation channels and some isoforms of phosphodiesterase. The signaling cascades produce different biological effects depending on the direction of NO release and site of cGMP production. In vascular smooth muscle cells, for

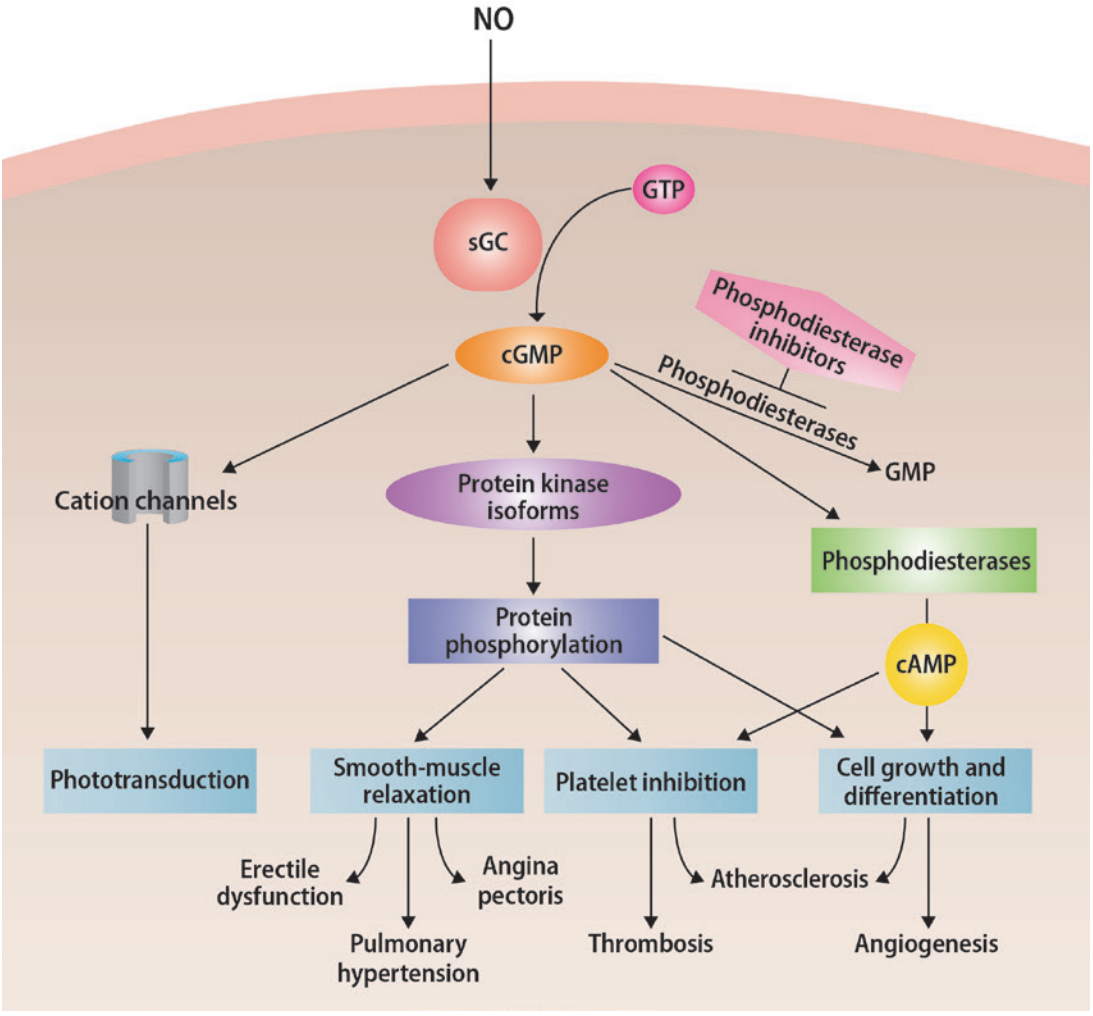

FIGURE 2 Downstream events and multiple effects of NO/cGMP cellular signaling. Abbreviations: cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate; NO, nitric oxide; sGC, soluble guanylate cyclase.

example, NO derived from endothelial cells causes activation of cGMP-dependent protein kinase G, which then relaxes the muscle cells by lowering intracellular calcium.[24] Near the surface of the vascular lumen, NO/cGMP signaling inhibits platelet aggregation and adhesion to the vascular wall.[25]

NITRIC OXIDE SYNTHASE

Endogenous NO is derived from the amino acid L-arginine by NO synthase (NOS).[26] The enzyme has three isoforms: endothelial NOS (eNOS or NOS-3), neuronal NOS (nNOS or NOS-1), and inducible NOS (iNOS or NOS2) (Table III). With about 50% to 60% homology with each other, these isoforms are encoded by three different genes located on different chromosomes.[23] All three isoforms of NOS require cofactors such as oxygen and nicotinamide-adenine-dinucleotide phosphate (NAPH) to produce NO. They convert L-arginine to L-hydroxy-arginine through

TABLE III NOS isoforms

Isoform	Type	Calcium-dependence	Expression sites	Functions	Notes
NOS-1 (155 kD)	Neuronal	Yes	Central and peripheral neurons Nonadrenergic and noncholinergic neurons Islets Endometrium Skeletal muscle Others	CNS: Synaptic plasticity, learning and memory formation Blood pressure regulation PNS: Gut peristalsis Vasodilation Sexual arousal	
NOS-2 (125 kD)	Inducible	Bound to calmodulin in a calcium-independent fashion	Macrophages Liver Heart Smooth muscle Endothelium Others	Nonspecific immune defense Mediation of inflammation	Regulated by lipopolysaccharides, cytokines, and glucocorticoids; can be induced in almost any cell type.
NOS-3 (135 kD)	Endothelial	Yes	Endothelium Brain Heart Other sites	Vasodilation Inhibition of platelet aggregation and adhesion Vasoprotection Prevention of atherosclerosis	Subject to acylation and phosphorylation

NOS, nitric oxide synthase; CNS, central nervous system; PNS, peripheral nervous system.

hydroxylation and then oxidize the intermediate to NO and citrulline. While eNOS and nNOS are constitutive and calcium-dependent, iNOS is—as its name suggests—inducible and calcium-independent.[27,28]

As their expression site varies, the three NOS isoforms differ in their physiological functions. Endothelial NOS is found mainly in endothelial cells.[27] NO derived from eNOS is the main endothelial-derived vasodilator that acts to decrease blood pressure and improve circulation (Figure 3). It also inhibits platelet aggregation to prevent undesired blood clotting and obstruction to blood flow. Additionally, eNOS-derived NO plays a pivotal role in suppressing leukocyte adhesion and migration, control of vascular smooth muscle proliferation, and regulation of cerebral blood flow.[27,29] Neuronal NOS is constitutively expressed in central and peripheral neurons as well as some other cell types.[27] NO produced by nNOS contributes to synaptic plasticity, learning and memory formation, and central regulation of blood pressure; in the peripheral nervous system, it acts as an atypical neurotransmitter that mediates peristalsis (the involuntary wavelike movements of the digestive tract during digestion), vasodilation, and sexual arousal.

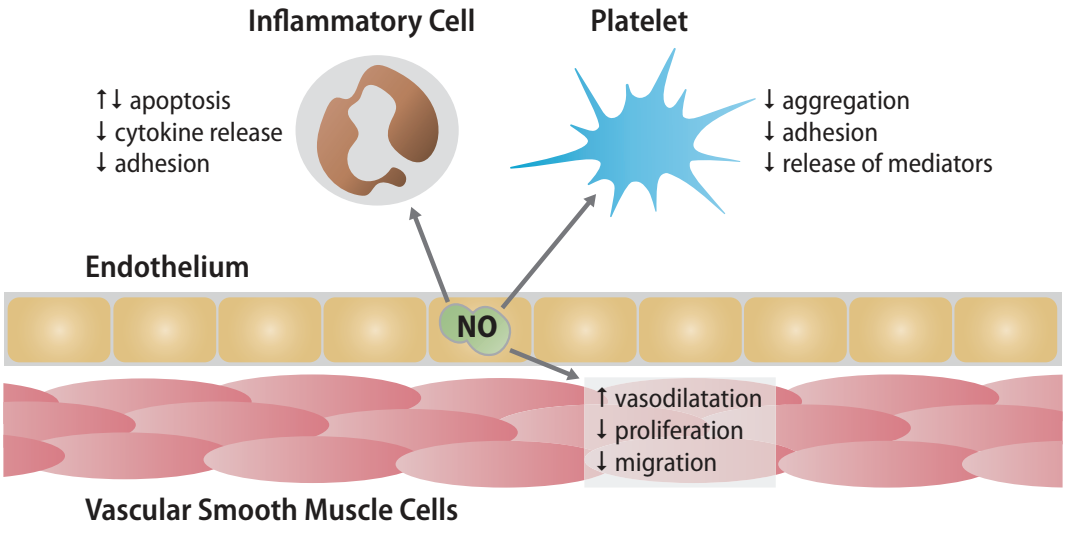

FIGURE 3 Diverse effects of NO in the vascular system.

Unlike eNOS and nNOS, iNOS is not expressed under physiological conditions. Its expression—primarily in macrophages but potentially in any cell type—is usually induced by inflammatory cytokines or other agents such as bacterial endotoxin (ie, lipopolysaccharide).[27] Independent of intracellular calcium levels, iNOS is constantly active once expressed. It can produce NO in relatively large amounts (up to the micromolar range, 100 to 1000 times greater than eNOS- and nNOS-derived NO) for prolonged periods—as a defensive mechanism.[30,31] With microbicidal, antiviral, and antiparasitic effects, NO generated in this fashion contributes to pathogen eradication. It also plays a critical role in regulating antiinflammatory pathways involved in wound healing and tissue repair.[32]

NITRIC OXIDE IN SYSTEMIC DISEASES

Since NO provides many cardiovascular benefits, it is not surprising that impaired NO signaling has been implicated in a number of cardiovascular conditions, including atherosclerosis, systemic hypertension, myocardial ischemia, heart failure, and stroke.[33] It is thought that the risk factors for these conditions, including high blood pressure, hypercholesterolemia, diabetes mellitus, tobacco smoking, and aging, promote pathological processes—such as endothelial stiffening and mechanotransduction responses—leading to a reduction of bioactive NO.[34-37] Multiple mechanisms underlie such NO insufficiency: excessive oxidative stress, inhibition of eNOS expression and activity, and deficiency of eNOS cofactors. Decreased NO synthesis and bioavailability in turn leads to oxidization of low density lipoproteins (LDL) and a cascade of vascular oxidative stress and inflammatory responses that contribute to formation and progression of atherosclerotic lesions.[38] Lifestyle factors known to prevent cardiovascular disease, such as physical exercise and a healthy diet, are associated with increases in the bioavailability and signaling of NO.[36]

Notably, patients with obstructive sleep apnea have been found to have reduced bioavailability of NO, among other signs of increased oxidative stress.[39] In

these patients, serum levels of nitrite and nitrate are reduced, while levels of asymmetric dimethylarginine, an eNOS inhibitor, and arginase, an enzyme that metabolizes L-arginine to L-ornithine and urea, are elevated. Obstructive sleep apnea is a known risk factor for cardiovascular diseases such as atherosclerosis and hypertension. Several recent studies also suggest that the sleep disorder may also increase one's risk for glaucomatous damage,[40,41] presumably by compromising optic nerve head perfusion and perhaps decreasing oxygen levels in the trabecular meshwork cells to alter aqueous dynamics.

Altered NO signaling has been associated with a range of neurodegenerative diseases that are characterized by progressive neuronal damage and death. These include Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and multiple sclerosis.[42] The central nervous system is particularly vulnerable to oxidative stress, the result of increased levels of reactive oxygen and nitrogen species; oxidative damage is believed to be an important contributor to the pathogenesis of neurodegenerative diseases.[42,43] At present, the specific roles of NO—neuroprotective or neurotoxic—in the pathophysiology of the neurodegenerative disorders remains to be elucidated, and no consensus exists on each NOS isoform's role.[44] There is, however, consistent evidence that iNOS expression in activated glial cells and neurons under pathological conditions produces high levels of NO, which can interact with superoxide under oxidative stress to produce peroxynitrite and other cytotoxic molecules that can cause neuronal death.[27,44,45] Indeed, much of NO-related toxicity lies in formation of peroxynitrite, which inhibits mitochondrial respiration and triggers cell death.

Excessive NO production by iNOS is also implicated in many chronic inflammatory and autoimmune disorders, including rheumatoid arthritis, asthma, and inflammatory bowel disease.[42] These conditions are generally characterized by presence of abundant activated macrophages and neutrophils. High levels of NO produced by these inflammatory cells might not just kill invading microbes or tumor cells but also harm healthy cells and tissue. The cell or tissue damage is attributable to the NO radical itself as well as other reactive nitrogen species generated from NO metabolism and reactivity, such as peroxynitrite.[27]

ENCHANCING NITRIC OXIDE SIGNALING

Given NO's multifaceted beneficial effects, delivery of exogenous NO makes an attractive therapeutic option for the treatment of a wide variety of conditions. Nitric acid has indeed had a long history of being used as a therapeutic agent: organic nitrates such as nitroglycerin, which were first used to treat angina pectoris nearly 150 years ago, act through release of NO. Since NO's physiological functions began to come to light in the 1980s, there has been intense interest in broadening the molecule's therapeutic applications. However, efforts to develop novel NO-targeting drugs has so far been relatively unsuccessful, and few candidate compounds designed to increase NO bioavailability have become marketed products.

Today, the organic nitrates remain one major type of NO-donor drugs used in clinical practice.[46] Nitroglycerin (ie, glyceryl trinitrate) is mainly used for acute relief of chest pain associated with angina; slower release preparations, such as isosorbide mononitrate, are used to manage chronic angina. In 2005 a fixed dose combination of the organic nitrate isosorbide dinitrate and the vasodilator hydralazine was

approved for treatment of heart failure in African-American patients. Another type of NO-donor drug is sodium nitroprusside, which has been used as a treatment for hypertensive crises and conditions such as acute myocardial infarction and pulmonary hypertension. Besides these NO-donating compounds, inhaled NO has been used in newborns with persistent pulmonary arterial hypertension and in adult respiratory distress syndrome.

Although no new NO-donor drugs have emerged yet, strategies to increase cGMP levels have led to identifications of therapies that target NO signaling. A notable advance with such strategies is the development of drugs that inhibit phosphodiesterase (PDE), a family of enzymes that catalyze the degradation of cGMP. By preventing cGMP breakdown and increasing intracellular cGMP levels, PDE inhibitors enhance downstream NO signaling. Currently, these agents are used in the management of a variety of conditions, including erectile dysfunction, pulmonary arterial hypertension, severe chronic obstructive pulmonary disease, asthma, and bronchitis. A different group of drugs that have also been available for use in pulmonary arterial hypertension are the sGC activators, which stimulate downstream NO pathway by directly binding to and activating sGC to increase cGMP production.

As a cardiovascular protective agent and an antiinflammatory and neuroprotective agent, NO has enormous therapeutic potential. There is a continuous search for novel therapeutics that are capable of enhancing NO signaling, either through release of NO, inhibition of NO breakdown, or directly stimulating downstream signaling pathway.[36] Because of its role in IOP regulation, NO holds therapeutic promise for glaucoma. Most recently, an NO-donating glaucoma drug, latanoprostene bunod (LBN), has been developed with the aim of delivering two IOP-lowering moieties through one molecular entity. The design of LBN is based on the concept of attaching an NO-releasing moiety to a pre-existing drug—in this case latanoprost, a prostaglandin analog (PGA)—to enhance the latter's efficacy. In the anterior eye, LBN is metabolized into latanoprost acid and an NO-donating moiety, both of which contribute to the drug's pharmacological activities. Currently, the NO-donating PGA has advanced through Phase 3 clinical trials and is awaiting FDA approval.

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CHAPTER 4

Nitric Oxide in Ocular Physiology

W. DANIEL STAMER, PHD

Over the past several decades, extensive research has elucidated the role of nitric oxide (NO) as an important intracellular and intercellular messenger in the cardiovascular, nervous, and immune systems. The gaseous molecule is synthesized from the amino acid L-arginine by the family of NO synthase (NOS); its physiological functions vary widely from vasodilation to neurotransmission to inflammatory responses.[1] (See Chapter 3, Tables I and II.)

Considering NO's many functional roles in the nonocular systems in the body, it is plausible that this ubiquitous messenger molecule is an important regulator in ocular physiology—after all, vasculature, neural tissues, and immune cells are integral components of the eye. Indeed, mounting evidence from the past several decades supports that NO signaling in the eye, as seen in other organs and tissues, is involved in varied homeostatic processes, such as IOP regulation and control of blood supply.

SITES OF SYNTHESIS

Both the anterior and posterior tissues of the healthy human eye have the capacity to generate NO, as evidenced by the presence of the three isoforms of NOS, ie, endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS). (See Chapter 3, Table III.) The enzyme eNOS is constitutively present in the endothelium of ciliary and retinal vessels (Table I).[2,3] Neuronal NOS, also constitutive, is found in the ciliary non-pigmented epithelium and optic nerve head.[3] Inducible NOS has been detected in the iris and ciliary body and vessels following cytokine and endotoxin exposure and also in astrocytes of the human optic nerve head in response to hypoxia in vitro,[2,4] but under physiological conditions it is not usually expressed in cells.

Apart from vascular endothelium, eNOS is found in tissues involved in the maintenance of intraocular pressure (IOP), including the ciliary muscle and cells of the conventional outflow pathway (Table I).[2,5] Although early studies suggest that the trabecular meshwork (TM) is an important site of NO synthesis mediated by constitutive eNOS,[2,5] newer evidence indicates otherwise.[6] While the TM is probably a major site of NO action, eNOS expression is predominantly localized in Schlemm's canal cells,[6-8] which are vascular in origin.[9,10] Nitric oxide generation in the TM, if any, is likely mediated by inducible NOS (iNOS) instead—under stimulated conditions. For example, raised perfusion pressure in anterior segments of human donor eyes has been reported to significantly increase iNOS expression and NO production in the TM.[11] In addition, upregulation of iNOS expression and activity has been found in the TM of eyes with POAG.[12]

TABLE I
Ocular distribution of NOS isozymes and sGC

Gene	Species	Cell or tissue type
NOS1	Human	Ciliary non-pigmented epithelium
	Human	OHN astrocytes, lamina cribosa
	Monkey	Amacrine cells, rod and cone photoreceptors, RGC
	Canine	RGC
	Rabbit	Amacrine cells, rod and cone photoreceptors, RGC
	Rat	Ciliary process epithelium
	Murine	Retinal amacrine cells
	Murine	Retinal amacrine cells, RGC layer somata; IPL puncta
	Murine	Müller cells
NOS2	Human	Macrophages in stroma and ciliary processes
	Human	Astrocytes
NOS3	Human	Longitudinal CM fibers, TM, SC
	Human	Retinal vasculature
	Human	TM
	Murine	SC
sGC	Human	RGC, IPS, ONL
	Human	TM cells
	Rabbit	Amacrine cells, bipolar cells, cone photoreceptors, RGC
	Murine	Somata in the INL, ONL, IPL, and OPL
	Murine	RGC, IPL, ONL
	Turtle	Amacrine cells; bipolar cells, RGC layer, IPL

CM, ciliary muscle; INL, inner nuclear layer; IPL, inner plexiform layer; NOS, nitric oxide synthase; ONH, optic nerve head; ONL, outer nuclear layer; OPL, outer plexiform layer; RGC, retinal ganglion cell; SC, Schlemm's canal; sGC, soluble guanylate cyclase; TM, trabecular meshwork.

Republished with modification from Buys ES, Potter LR, Pasquale LR, Ksander BR. Regulation of intraocular pressure by soluble and membrane guanylate cyclases and their role in glaucoma. *Front Mol Neurosci*. 2014:May 19;7:38.

AQUEOUS HUMOR DYNAMICS AND IOP

In a similar way as the central nervous system, to function well, requires cerebrospinal fluid circulation, the eye has its own aqueous humor flow that serves to provide nutrients to and remove waste products from avascular ocular tissues such as the cornea, the crystalline lens, and the TM. The clear intraocular fluid is secreted by the bilayered epithelium of the ciliary processes in the posterior chamber. It flows from behind the iris, through the pupil, into the anterior chamber.

The aqueous humor exits the eye at the iridocorneal angle via two pathways. The conventional or "trabecular" pathway, composed of the TM, Schlemm's canal, collector channels, intrascleral vessels and episcleral veins, accounts for the bulk of aqueous outflow in healthy human eyes. The uveoscleral pathway, which consists of the uveal meshwork, the anterior face of the ciliary muscle, and the spaces between the longitudinal muscle fibers and drains into the supraciliary and suprachoroidal spaces, contributes to a minor portion of total aqueous outflow (from less than 10%

to more than 30% based on reported estimate).[13] As the rigidity and functionality of ocular tissues change with age, both trabecular and uveoscleral outflow decreases; coincident with a decrease in inflow.[14]

The aqueous humor secretion and uveoscleral outflow are largely pressure-independent. For the most part, they are not affected by varying IOP levels. Conventional outflow, on the other hand, is pressure-dependent. What drives aqueous flow through this route is the pressure gradient resulting from the difference between IOP and the episcleral venous pressure. Given an IOP of 16 mm Hg and mean episcleral venous pressure of 8 mm Hg, there will be an 8 mm Hg pressure drop across the aqueous outflow system.[15]

The IOP of any given eye arises mainly from outflow resistance in the conventional outflow pathway. Greater resistance means lower outflow facility (the reciprocal of resistance) and, as a result, higher IOP. Most outflow resistance in the normal eye is generated in the juxtacanalicular portion of the TM and the inner wall of Schlemm's canal.[16,17] Pathology in the conventional outflow pathway—presumably in the juxtacanalicular region—is believed to be responsible for extra outflow resistance and pressure elevation in POAG.[17-19]

NITRIC OXIDE IN IOP REGULATION

The complex mechanisms of physiological IOP regulation are not yet fully understood. IOP is a dynamic variable that fluctuates throughout the day. Cyclic IOP changes are largely attributable to aqueous humor production[20,21] and, as recent evidence indicates, pulse-dependent TM motion[15,22] due to choroidal volume changes as a result of the heartbeat. Apart from diurnal variations, routine daily activities such as eye movements, eye rubbing, and exercise contribute to short-term IOP fluctuations.[23,24] Dampening such pressure oscillations is an essential component of IOP regulation. In glaucomatous eyes, IOP dysregulation typically manifests itself not just in elevated pressure but in exaggerated diurnal pressure variations as well.[25-27]

Although its underlying mechanisms remain to be specified, IOP regulation under physiological conditions depends largely on control of outflow through the conventional outflow pathway. Current concept holds that resistance generation (and thus IOP regulation) occur in the juxtacanalicular region, where the trabecular and Schlemm's canal cells interact physically and hydrodynamically.[17,19] In this process, endogenous NO appears to play a pivotal mechanoregulatory role.[8] When pressure increases in the eye, the TM tissue is pushed outward toward Schlemm's canal, causing the latter to narrow or collapse. This leads to increased shear stress in the lumen of the canal—up to levels that are comparable to those in the large arteries (Figure 1).[28] The shear forces act on Schlemm's canal cells, increasing expression and activity of eNOS and production of NO. Nitric oxide then works to restore IOP homeostasis—by relaxing the juxtacanalicular cells to increase pressure-dependent outflow.

The cellular mechanisms that mediate NO's IOP-lowering action, discovered only recently, are analogous to those by which NO regulates vascular tone and blood pressure. The TM has multiple tissue layers, each of which is composed of a complex extracellular matrix architecture that is maintained by resident trabecular cells. Schlemms's canal is completely lined with an endothelial layer, a structure resembling that of a vein.[29] The TM and Schlemm's canal cells are highly contractile in

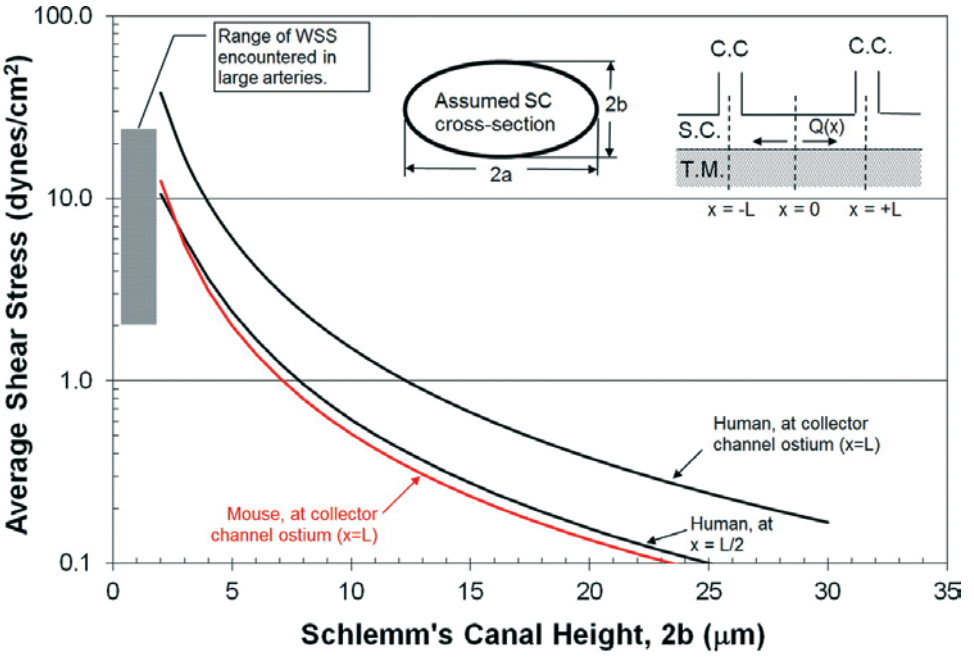

FIGURE 1 **Theoretical shear stress in Schlemm's canal of mouse and human eyes. The red line indicates predicted values of shear stress in Schlemm's canal over a range of canal heights for the mouse eye, while black lines show predicted values of shear stress for human Schlemm's canal over a range of canal heights at CC ostium (top line) or at midpoint between ostia (bottom line). Inset shows the assumed cross-sectional shape of the canal and the terminology for computing the flow rate as a function of position in the canal, Q(x).** ***(Stamer WD, Lei Y, Boussommier-Calleja A, Overby DR, Ethier CR. eNOS, a pressure-dependent regulator of intraocular pressure.*** Invest Ophthalmol Vis Sci. ***2011;52(13):9438-44. Copyright ©2011 Association for Research in Vision and Ophthalmology.)***

nature (Figure 2), similar to vascular smooth muscle cells.[30,31] Evidence suggests that NO relaxes the juxtacanalicular TM by altering contractility and cell volume of TM and Schlemm's canal cells.[6,32-34]

In addition to the conventional outflow pathway, NO as a ubiquitous signaling molecule may have physiological effects in other tissues involved in aqueous humor dynamics. In the ciliary body, an enriched site of NO synthesis, NO generated in the vasculature may influence aqueous secretion through regulation of blood flow. Likewise, NO derived from eNOS in the ciliary muscle may have a role in regulating uveoscleral aqueous drainage via relaxation of the smooth-muscle fibers, although this still needs to be tested directly.

Nonetheless, it appears that NO's primary IOP-regulatory function hinges mainly on its effect on outflow facility in the conventional pathway. This is supported by a large amount of experimental data, both in vitro and in vivo. In human anterior segments maintained under organ culture perfusion conditions, NOS inhibitors reduced levels of cGMP, the second messenger that mediates NO's biological actions (see Chapter III, Figure 1), and TM flow rate.[35] In monkey eyes, intracameral infusion of cGMP increased outflow facility in vivo.[36] Mice overexpressing eNOS were found to have lower IOP compared to wild-type mice (Figure 3A).[8] Enucleated eyes from these transgenic mice had higher TM outflow rates in perfusion studies that isolate the conventional outflow tract (Figure 3B). In both wild-type and eNOS-expressing mice, NOS inhibitors decreased outflow facility.[6,8] In contrast,

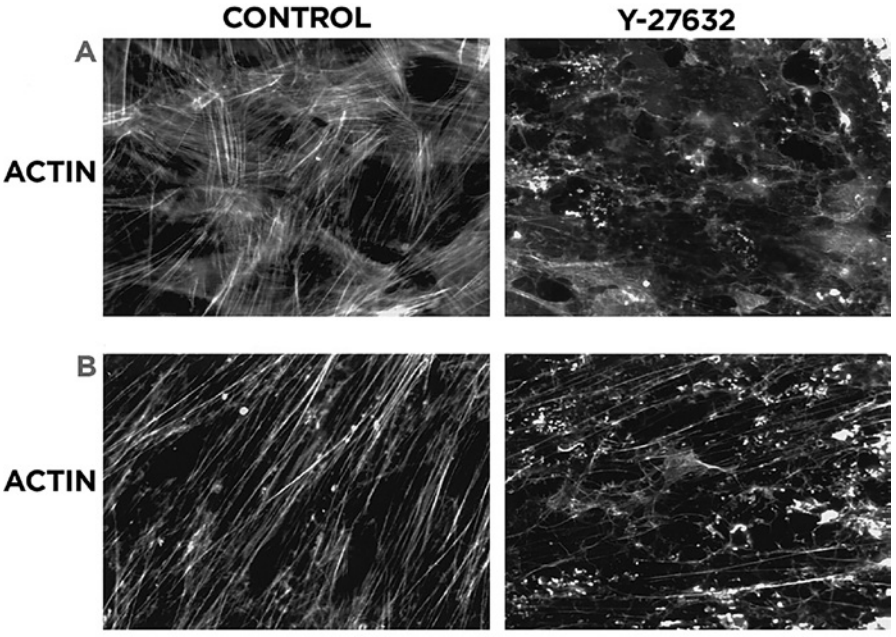

FIGURE 2 Changes in actin stress fibers in TM (A) and Schlemm cells (B) induced by Rho Kinase inhibitor Y-27632. *(Republished from Rao PV, Deng PF, Kumar J, Epstein DL. Modulation of aqueous humor outflow facility by the Rho kinase-specific inhibitor Y-27632.* Invest Ophthalmol Vis Sci. *2001; 42:1029–37. Copyright ©2001 Association for Research in Vision and Ophthalmology.)*

eNOS knockout mice have higher IOP and lower TM outflow rate compared to wild-type mice.[37] Knockout mice deficient in soluble guanylate cyclase (sGC), the downstream target of NO, have decreased aqueous humor outflow, elevated IOP, and also optic neuropathy and retinal vascular dysfunction.[38]

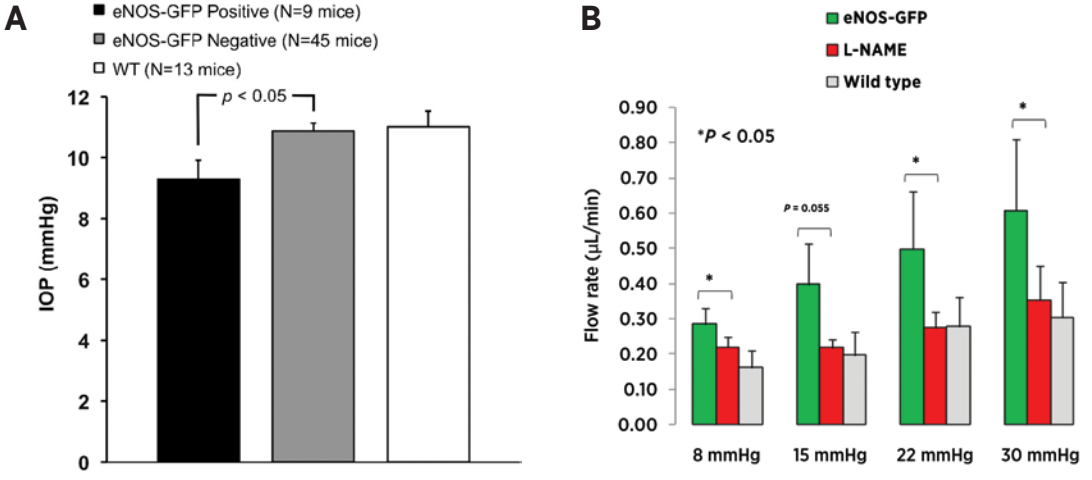

FIGURE 3 eNOS overexpression lowered IOP (A) and increased trabecular outflow (B) in the mouse eye. eNOS, endothelial nitric oxide synthase; GFP, green fluorescent protein; L-NAME, L-NG-nitroarginine methyl ester; WT, wild-type. *(Stamer WD, Lei Y, Boussommier-Calleja A, Overby DR, Ethier CR. eNOS, a pressure-dependent regulator of intraocular pressure.* Invest Ophthalmol Vis Sci. *2011;52(13):9438-44. Copyright ©2011 Association for Research in Vision and Ophthalmology.)*

NITRIC OXIDE AND OPTIC NERVE BLOOD FLOW

The optic nerve head—the site of glaucomatous axonal injury—is supplied by the posterior ciliary artery circulation (which also supplies the posterior choroid) and retinal circulation, the former being the main source of blood supply (Figure 4).[39] Posterior ciliary arteries, branching off the ophthalmic artery, divide later into a number of short posterior ciliary arteries that enter the globe around the optic nerve. Branches from the short posterior ciliary arteries contribute to the perfusion of the anterior optic nerve head (including prelaminar, laminar, and post-laminar neural tissue) except the surface nerve fiber layer of the retina, which is fed by arteriolar branches from the central retinal artery.

Blood flow in the optic nerve head depends on many factors (eg, blood pressure and IOP) and is autoregulated,[40] a phenomenon whereby local tissue blood flow is kept stable despite physiological or metabolic changes. Under physiological conditions, vasoactive factors produced by vascular endothelium—including the vaso-

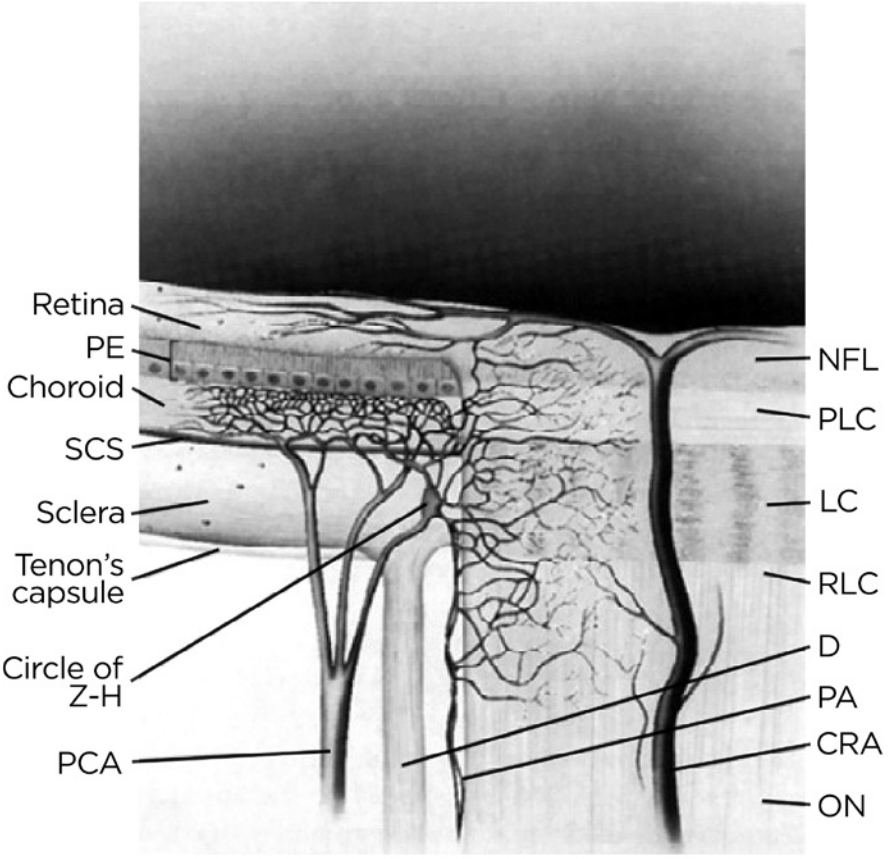

FIGURE 4 Arterial blood supply to optic nerve head. The anterior optic nerve is supplied mainly by branches from the short posterior ciliary arteries (PCA). Abbreviations: D, dura; CRA, central retinal artery; LC, laminar cribrosa; NFL, nerve fibre layer; ON, optic nerve; PA, pial arteries; PE, pigment epithelium; PLC, prelaminar cribrosa; RLC, retrolaminar cribrosa; SCS, suprachoroidal space; Z-H, Zinn-Haller. *(Republished from Canadian Journal of Ophthalmology, Vol. 2008 June. (43), Mackenzie PJ, Cioffi GA., Vascular anatomy of the optic nerve head., 308-12, Copyright (2008), with permission from Elsevier.)*

constrictor endothelin-1 and the vasodilator NO—play a major role in the control of ocular blood flow.[41,42] In the retina and optic nerve head, endogenous NO has been shown to be essential for maintaining basal blood flow.[43] This unique signaling molecule also appears to be a key mediator in the increases of optic nerve head blood flow observed with flickering light stimulation.[43]

Although its exact role remains to be identified, NO is likely involved in autoregulation of optic nerve head circulation during changes in IOP.[44,45] This has important therapeutic implications for glaucoma, as abnormalities in vascular autoregulation have been implicated in glaucomatous optic neuropathy, especially in patients with normal tension glaucoma (NTG).[46,47] When autoregulation is impaired, a sudden decrease in blood pressure or increase in IOP may reduce optic nerve head perfusion, causing ischemic injury to retinal ganglion cells (RGCs).

THERAPEUTIC IMPLICATIONS

Nitric oxide's function in the regulation of physiological IOP makes it a potential target for IOP-lowering therapeutic strategies. Patients with POAG are known to have increased rigidity of the TM and Schlemm's canal.[31,48] In theory, a stiffer TM may not get pushed into the Schlemm's canal as far as necessary to trigger shear stress increases and NO release when IOP is elevated.[7] In this case, it is presumably possible to relax the TM tissues to restore its physiological function via administration of exogenous NO, which should theoretically exert effects on TM cell contractility and outflow facility as endogenous NO does. Studies in multiple species including humans have provided indisputable evidence that exogenous NO delivered to the anterior eye indeed increases outflow facility and lowers IOP.[49-53]

To date, there has been a lack of IOP-lowering agents targeting the TM/Schlemm's canal, in part because of our limited understanding of the conventional outflow system and the nature of TM dysfunction in POAG. A new NO-based therapy would help fill this TM drug void in glaucoma therapy. Exogenous NO in the eye has the potential to exert effects not only on the TM but also on optic nerve blood flow. Relatively few studies, however, have examined blood flow alterations with exogenous NO delivered to the optic nerve head.[54] As imaging technology improves, it is expected that more studies will be performed to better understand optic nerve head blood flow and NO's effect.

Despite NO's diverse functions, its effectiveness is limited by its short lifespan, (~seconds in the eye).[55] Once produced, the gaseous molecule does not survive long before it is neutralized. The key to any drug strategy involving NO, therefore, is location. In the eye, the biggest challenge moving forward is developing technologies to deliver NO directly to the target tissue, be it the resistance-generating juxtacanalicular tissue[56] or the optic nerve head that is supplying nutrients to the susceptible RGCs. Furthermore, NO is a highly reactive free radical. Excessive NO particularly during bouts of ischemia can cause off target effects, toxicity and tissue damage, which is likely why it is a short-lived as a messenger molecule in the first place. To achieve the desired therapeutic effect with NO, finding the best window for the ephemeral molecule's functionality will be crucial.

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CHAPTER 5

Nitric Oxide and Glaucoma

ANNE L. COLEMAN, MD, PHD

As discussed in the previous chapters, nitric oxide (NO) is a crucial signaling molecule with wide-ranging physiological functions, and it acts by increasing the production of the second messenger cyclic guanosine monophosphate (cGMP). Soluble guanylate cyclase (sGC), the enzyme in cytosol that converts guanosine triphosphate (GTP) to cGMP, is directly activated by NO. The NO-sGC-cGMP signal transduction cascade is a major NO signaling pathway (see Chapter III, Figure 1). In the human eye, endogenous NO is recognized to be an important modulator of physiological IOP and optic nerve head blood flow. When the NO system becomes impaired, IOP dysregulation and reduced perfusion of the optic nerve head—two key pathophysiological characteristics of POAG—may occur as a result.

This chapter discusses: 1) the association between nitric oxide (NO) pathway deficiency and the pathogenesis or disease course of primary open-angle glaucoma (POAG); and 2) the therapeutic approach of exogenous NO delivery for POAG and other forms of chronic OAG.

IMPAIRED PATHWAY

There is significant clinical and experimental evidence that an endogenous insufficiency in NO bioavailability is linked with POAG, although the exact relationship between the two is unclear. In POAG eyes, local NO production (as indicated by reactivity of the NO-indicator marker NADPH-diaphorase) in the trabecular meshwork (TM) and Schlemm's canal has been found to be markedly decreased, alongside a marked reduction of anterior longitudinal ciliary muscle fibers that insert near the TM and are known to affect outflow resistance via contraction and relaxation.[1] When compared to normal controls, patients with POAG demonstrated lower levels of cGMP and the NO metabolites nitrite/nitrate in their plasma and aqueous humor (Figure 1A and B).[2,3] L-arginine, the amino acid precursor of NO, showed higher levels in the aqueous humor of POAG patients.[4] In patients with advanced glaucoma, serum concentrations of endogenous inhibitors of NO synthase (NOS) or L-arginine uptake were found to be significantly elevated.[5]

Furthermore, POAG and normal tension glaucoma (NTG) have both been associated with peripheral vascular endothelial dysfunction.[6,7] One key mechanism underlying vascular endothelial dysfunction is decreased NO bioavailability.[8-10] Since direct in vivo measurement of NO production in the eye is currently infeasible, one study investigated the response of ocular blood flow to an intravenously administered NOS inhibitor instead. Upon the systemic inhibition of NOS, POAG patients had reduced blood flow response in the optic nerve head and the choroid compared to healthy control subjects.[11] The result is consistent with the notion that POAG is associated with local alterations of the NO signaling system.

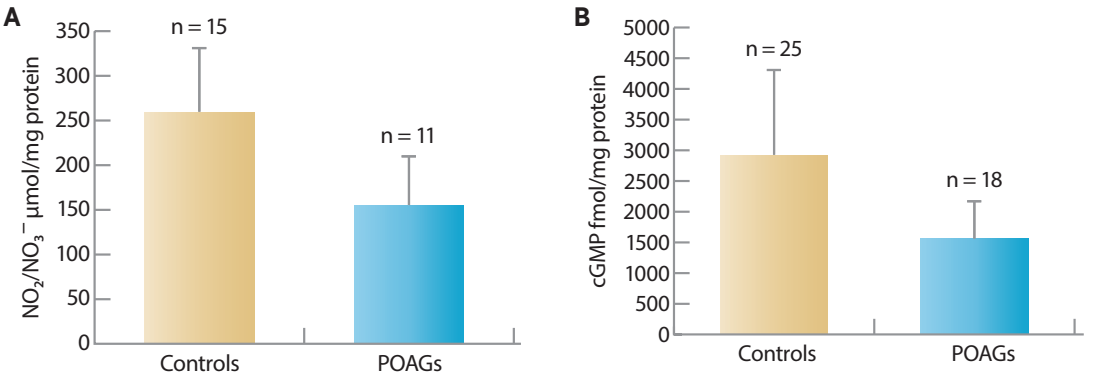

FIGURE 1 Nitric oxide (NO) markers nitrite (NO2−)/nitrate (NO3−) (A) and cyclic guanosine monophosphate (cGMP) (B) are reduced in the aqueous humor of eyes with primary open-angle glaucoma (POAG).

Additionally, evidence from genetic studies supports the theory of an altered NO signaling system in patients with POAG. Polymorphisms in NOS3—the gene encoding endothelial NOS (eNOS)—have been associated with the risk of POAG in a number of genetic studies.[12-17] Apart from NOS3, genes coding for local factors involved in the activity of eNOS have also been found to be associated with POAG.[18] Constitutively expressed in the human outflow pathway and ciliary muscle, eNOS catalyzes NO formation to regulate outflow facility.[19,20] Also, the endothelial isoform of NOS is expressed on all vascular endothelial cells; in the optic nerve head, NO derived from eNOS is thought to act as a key regulator of blood flow.[21] Since IOP and low optic nerve head perfusion are two major risk factors for POAG,[22] it is plausible that alterations in eNOS activity and NO production due to genetic variation affects one's risk of developing the disease.

A THERAPEUTIC TARGET

It has long been observed that nitrovasodilators, a group of NO-donating compounds with more than a century of successful clinical use in cardiovascular disease, have an IOP-lowering effect. In a study published in 1980, Wizemann and Wizemann described a dose-dependent decrease in IOP in both POAG patients and healthy subjects following intravenous or oral administration of organic nitrates nitroglycerin and isosorbide dinitrate.[23] Later, animal studies showed that topically applied nitrovasodilators, such as nitroglycerin and sodium nitroprusside, effectively lowered IOP and did so at least in part by increasing outflow facility (Figure 2).[24-27] Similarly, a topically administered cGMP analog lowered IOP and altered outflow facility in ocular normotensive and hypertensive rabbits and monkeys.[28-31]

Research has only recently shed light on how the NO/cGMP pathway influences outflow facility and IOP, and the precise cellular mechanisms require further elucidation. Changes in TM and Schlemm's canal cell volume appear to be correlated with rates of aqueous outflow such that a decrease in cell volume results in greater rate of outflow (ie, greater outflow facility) and lower IOP.[32] NO, based on current evidence, induces relaxation of TM and Schlemm's canal cells in the juxtacanalicular tissue and decreases cell volume to enhance outflow facility.[33-36] In any case, that exogenous NO and cGMP can decrease IOP raises the possibility of therapeutic use for

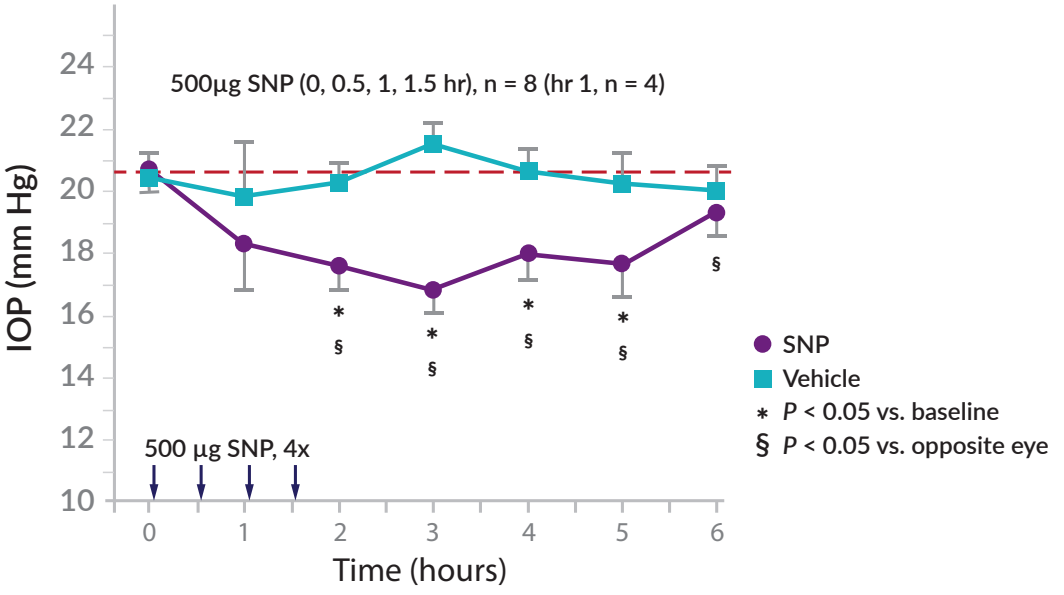

FIGURE 2 Topical NO donor SNP lowered IOP in nonhuman primates. Abbreviations: IOP, intraocular pressure; NO, nitric oxide; SNP, sodium nitroprusside. *(Heyne GW, Kiland JA, Kaufman PL, Gabelt BT. Effect of nitric oxide on anterior segment physiology in monkeys.* Invest Ophthalmol Vis Sci*. 2013;54(7):5103-10. Copyright © 2013 Association for Research in Vision and Ophthalmology. Adapted from Figure 1D.)*

NO donors in the treatment of glaucoma. Given that endogenous NO production appears to be deficient in POAG, supplying exogenous NO could enhance downstream NO signaling to provide the necessary signals to increase outflow facility and lower IOP.

Currently, IOP reduction remains the only proven method to preserve visual function in patients with glaucoma. While multiple classes of hypotensive drugs are available for lowering IOP, none directly modifies the trabecular outflow pathway, where most outflow resistance resides—in both normal and POAG eyes.[37,38] Working through a mechanism that enhances physiological aqueous outflow through the TM, NO donation represents a new pharmacological approach to glaucoma therapy.

Because of NO's vasodilating effect and likely role in optic nerve head blood flow regulation, an NO-based therapy that enhances optic nerve and retinal vascular NO signaling may have the potential to exert beneficial effects on injured retinal ganglion cells. This hypothetical neuroprotective effect, however, would be difficult to demonstrate clinically. Pressure-lowering by itself is neuroprotective in effect: as demonstrated in numerous clinical studies, sufficient reduction of IOP can slow or halt progression of visual field loss in glaucoma patients.[39-43] If an agent protects neurons from glaucomatous damage via vasodilation in addition to what pressure reduction does alone, teasing apart these effects will require larger, longer clinical studies. There is also the need to demonstrate vasodilation. However, changes in optic nerve head blood flow are extremely complicated to study, as the mechanisms underlying optic nerve head vascular autoregulation are largely unknown and many different confounding variables need to be controlled for.

ALTERNATE APPROACHES

Apart from NO-donating compounds, dietary nitrate supplementation is a practical and effective method to directly increase NO levels. Inorganic nitrate from dietary sources is metabolized to various bioactive nitrogen oxides including NO. As a naturally occurring NO precursor, dietary nitrate could provide an alternative source of NO to endogenous synthesis of NO from L-arginine. Certain leafy vegetables are particularly high in nitrate. One serving of spinach, lettuce, or beetroot, for instance, can produce more NO than that generated endogenously from L-arginine over a day.[44] Most recently, a large prospective study of 1483 POAG patients found that greater intake of dietary nitrate and green-leafy vegetables was associated with 20% to 30% lower risk for POAG.[45] The relation was particularly strong—40% to 50% lower risk—for POAG with early paracentral visual field loss at diagnosis, wherein ocular vascular dysregulation has been implicated.[46]

Other molecules located downstream of NO in the NO pathway, such as sGC and cGMP, offer additional potential targets for therapeutic approaches aimed at enhancing NO/cGMP signaling in POAG.[47] NO-independent sGC agonists, which have been approved for treating cardiovascular disease, are being investigated for their therapeutic potential as IOP-lowering agents. Preliminary studies have shown that IWP-953, a pharmacologic active sGC agonist, stimulates cGMP production in human TM cells and increases aqueous outflow facility in mouse eyes.[48] In theory, inhibition of cGMP-regulated phosphodiesterase (PDE), an enzyme that controls cGMP levels and is abundant in the eye, should also lead to enhanced cGMP signaling and altered outflow facility. However, PDE inhibitors such as sildenafil have largely failed to impact IOP in preliminary clinical testing.[49,50]

NO-DONATING THERAPIES

The increasing understanding of NO's IOP-lowering action and the relation between impaired NO signaling and the pathogenesis of POAG have recently rekindled the interest in the therapeutic potential of NO in glaucoma. At present, no NO-based therapeutics are available in the US for lowering IOP. But this may change soon, as several existing glaucoma drugs are being investigated as potential NO donors and one NO-donating agent is already under FDA review.

LBN

Latanoprostene bunod (LBN) is an NO-donating prostaglandin analog (PGA) that chemically combines an NO-donating moiety with latanoprost. The new molecule is thought to exert pharmacological effects through its two components: the PGA increases uveoscleral aqueous outflow via relaxation of the ciliary muscle and remodeling of the ciliary muscle's extracellular matrix, whereas NO enhances outflow through the TM by relaxing the TM and Schlemm's canal cells.

Now awaiting FDA approval, LBN ophthalmic solution 0.024% has been subject to extensive preclinical and clinical evaluation. The NO-donating PGA demonstrated significant IOP-lowering activity in multiple animal models of glaucoma;[51] it increased cGMP levels and induced cytoskeletal relaxation in vitro in human TM cells, while latanoprost had minimal effect (Figure 3).[52] In subjects with OAG or

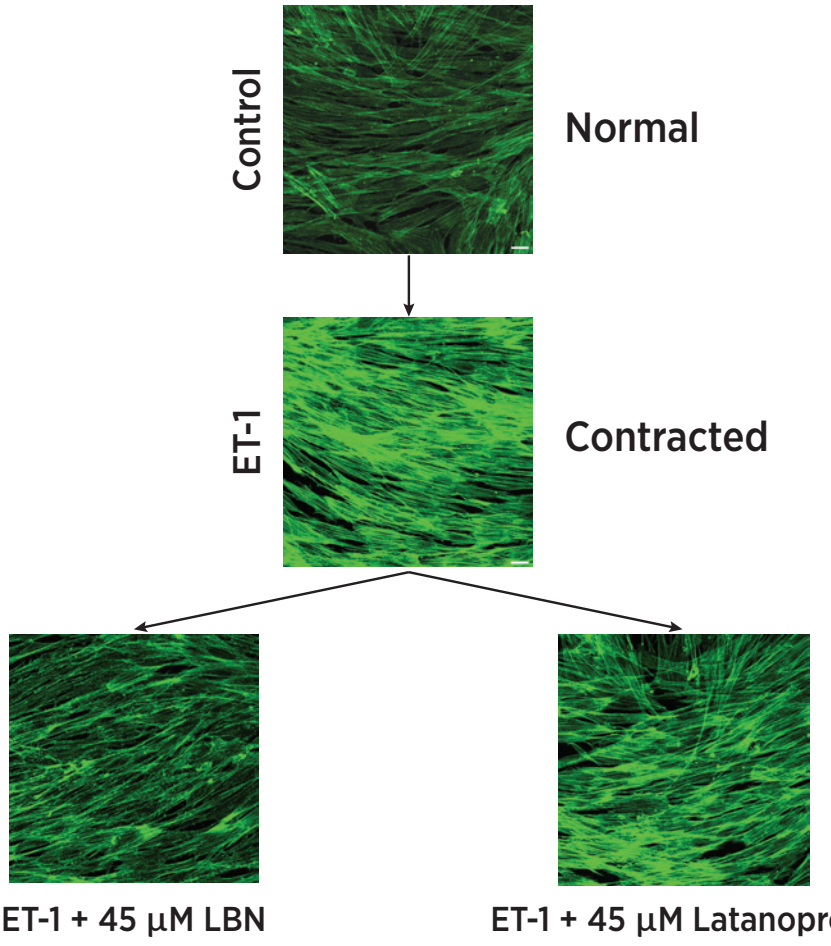

FIGURE 3 LBN relaxed endothelin-1 (ET-1) contracted human trabecular meshwork cells (HTMCs) by altering ET-1-induced actin stress fibers (green). Cells were treated with ET-1 (100 nM) alone or in combination with LBN (45 µM) or latanoprost (45 µM) for 1 hour before being stained with phalloidin and imaged by confocal microscopy. ***(Republished from Cavet ME, Vollmer TR, Harrington KL, VanDerMeid K, Richardson ME. Regulation of Endothelin-1-Induced Trabecular Meshwork Cell Contractility by Latanoprostene Bunod.*** Invest Ophthalmol Vis Sci. ***2015;56(6):4108-16. Copyright © 2015 Association for Research in Vision and Ophthalmology.)***

ocular hypertension, LBN 0.024% was noninferior to twice-daily timolol 0.5%, producing a mean IOP reduction of 7.5 to 9.1 mm Hg from baseline over three months of treatment in the randomized, multicenter phase 3 clinical trials APOLLO and LUNAR (Table I).[53,54] LBN met the criteria for superiority at all nine time points (8 AM, 12 PM, and 4 PM at Week 2, Week 6, and Month 3) in the APOLLO study and at all but one time point in LUNAR. In both studies, a significantly greater proportion of LBN-treated patients achieved an IOP ≤ 18 mm Hg or a ≥ 25% IOP reduction in a period of three months compared with those treated with timolol. Pooled data from the safety extension phases of APOLLO and LUNAR shows that IOP reduction with LBN 0.024% was maintained through 1 year.[55] Subjects who were initially treated with timolol and then crossed over to LBN after 3 months of treatment demonstrated an additional 6.3 to 8.3% IOP reduction.

In a third phase 3 clinical study, JUPITER, LBN 0.024% showed robust, sus-

tained IOP-lowering efficacy through 12 months of treatment in a group of subjects with low baseline IOP (19.6 mm Hg for the study eye and 18.7 mm Hg for the treated fellow eye).[56] Notably, in the phase 2 VOYAGER study, LBN treatment for 28 days resulted in a 1.2 mm Hg greater IOP reduction than latanoprost, one of the most effective topical IOP-lowering agents and one commonly used as first-line therapy for glaucoma.[57] In CONSTELLATION, a phase 2 study that assessed LBN's 24-hour effects on IOP and ocular perfusion pressure (OPP), LBN produced a greater reduction in IOP and a larger increase in OPP than timolol during the nocturnal period (Figure 4).[58] LBN was well tolerated and demonstrated a similar side effect profile to other PGAs in clinical testing.[53,54,57] This suggests that the NO donor's IOP-lowering efficacy comes with no additional safety issues.

These results, overall, support that LBN 0.024% as an IOP-lowering medication is effective and safe. If approved, LBN will become the first NO-donating PGA for OAG and ocular hypertension and also the first new IOP-lowering agent with a novel mechanism of action since the introduction of latanoprost in the 1990s. Given its efficacy in clinical studies, LBN as an NO-donating PGA is expected to expand first-line choices for glaucoma patients. In particular, it offers an alternative treatment option for patients who are already on PGA monotherapy yet cannot achieve target pressure or continue to show progression in the visual field or optic nerve. For these patients, adding another hypotensive eye drop to treatment runs the risk of complicating the therapeutic regimen and reducing patient adherence.[59] Switching to LBN, instead, may provide the desired additional IOP reduction while helping maintain proper adherence—like other PGAs, LBN is dosed once daily at night. For this same reason, LBN may also be a desirable option for patients cur-

TABLE I
Changes from baseline in mean IOP in the study eye in APOLLO and LUNAR

	Week 2			Week 6			Month 3		
	8 am	**12 pm**	**4 pm**	**8 am**	**12 pm**	**4 pm**	**8 am**	**12 pm**	**4 pm**
APOLLO									
LBN mean CFB (mm Hg)	–9.0	–8.5	–7.7	–9.1	–8.7	–7.9	–9.0	–8.7	–7.9
Timolol mean CFB (mm Hg)	–7.8	–7.2	–6.6	–8.0	–7.4	–6.7	–7.9	–7.4	–6.6
Treatment difference	–1.21	–1.37	–1.11	–1.03	–1.24	–1.26	–1.02	–1.27	–1.33
P-value	<.001	<.001	<.001	<.002	<.001	<.001	<.002	<.001	<.001
LUNAR									
LBN mean CFB (mm Hg)	–8.3	–8.1	–7.5	–8.8	–8.5	–7.8	–8.8	–8.6	–7.9
Timolol mean CFB (mm Hg)	–7.9	–7.3	–6.9	–7.9	–7.7	–6.8	–7.9	–7.4	–6.6
Treatment difference	–0.44	–0.76	–0.69	–0.92	–0.84	–0.98	–0.88	–1.29	–1.34
P-value	.216	.022	.025	.005	.007	.003	.006	<.001	<.001

Treatment difference = LBN - timolol. CFB, change from baseline; IOP, intraocular pressure; LBN, latanoprostene bunod.
Data compiled from references 53 and 54.

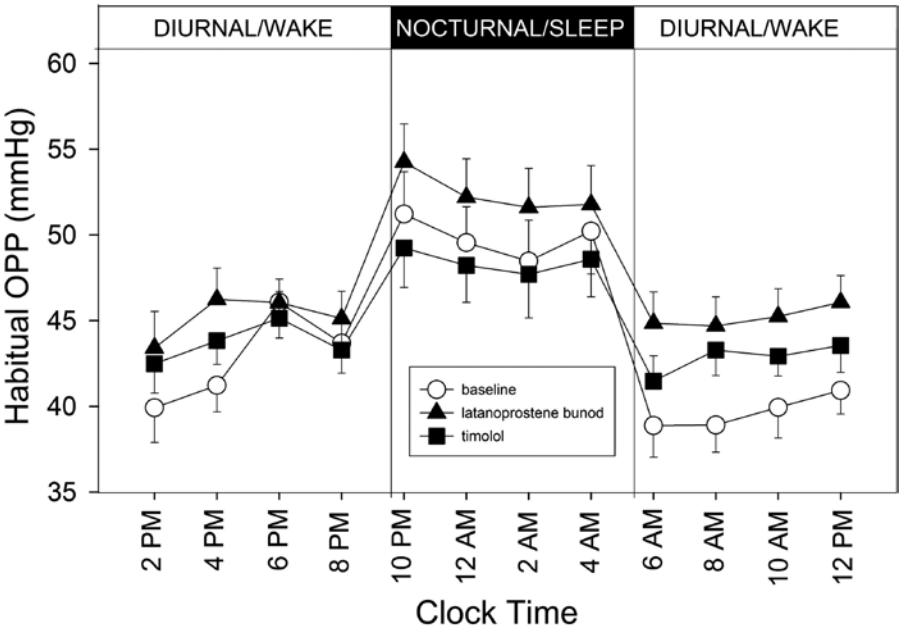

FIGURE 4 Habitual OPP over 24 hours after 4-week LBN or timolol treatment. *(Republished from* American Journal of Ophthalmology*, Vol. 169(Sep), Liu JH, Slight JR, Vittitow JL, Scassellati Sforzolini B, Weinreb RN. Efficacy of Latanoprostene Bunod 0.024% Compared With Timolol 0.5% in Lowering Intraocular Pressure Over 24 Hours. 249-57, Copyright (2016), with permission from Elsevier.)*

rently on combination therapy, who often have difficulty taking the medications consistently more than once a day. In the US, as many as half of glaucoma patients may require multiple drugs for adequate IOP control.[39,60]

For patients who are on maximal medical therapy for glaucoma—a patient-centered concept, but in general it refers to the combination of two or three medications—and still progressing, an eye drop like LBN should be a welcome addition to their limited choices moving forward. Because LBN has two different IOP-lowering mechanisms, it might give these patients a greater IOP reduction, which may make a clinically significant difference since each additional 1 mm Hg reduction of IOP has been associated with about 10% reduced risk of visual field loss.[41]

NIPRADILOL

Nipradilol is a nonselective beta-blocker with an NO-donating nitroxyl moiety that was developed in Japan for use as a topical IOP-lowering drug. Its hypotensive action has been shown to be attributable in part to NO in studies of normotensive rabbit eyes pretreated with the NO-trapping agent carboxy-PTIO.[61] In healthy normotensive subjects, nipradilol decreased the aqueous flow rate (determined fluorophotometrically) in the treated eye by 20% upon one single administration, presumably through its beta-blocking activity. (Beta-blockers lower IOP by decreasing aqueous humor production and the rate the aqueous flows into the anterior chamber.)[62] Randomized clinical studies in Japan indicate that nipradilol lowered IOP comparably to timolol in patients with POAG or ocular hypertension.[63]

In patients with NTG, the NO-donating beta-blocker effectively lowered IOP over a period of 5 years (baseline IOP: 17.0 ± 1.8 mm Hg, reduction of IOP after 5 years: 3.3 ± 2.0 mm Hg).[64]

Intriguingly, nipradilol has demonstrated a neuroprotective effect in animal models of optic nerve degeneration and promoted regeneration of transplanted cat retinal ganglion cells.[65-67] Such were further evaluated in a randomized multicenter clinical study by comparing the effects of nipradilol and timolol on visual field progression in Japanese NTG patients during a 3-year period.[68] If nipradilol confers neuroprotection beyond IOP lowering, the investigators hypothesized, patients treated with nipradilol should have less visual field progression compared with those treated with timolol because the two treatments had been shown to produce comparable IOP reduction. Initially, the study's results showed similar reduction of IOP and overall rates of visual field progression between the two groups.[68] A subsequent subgroup analysis, however, found that nipradilol was associated with slower visual field progression in patients with early visual field loss or those younger than 40 years.[69] Another recent Japanese study showed that nipradilol increases blood supply to the optic nerve head in normal and NTG eyes.[70] Thus, nipradilol might be neuroprotective in certain individuals or subgroups, but more research is needed.

OTHERS

NCX 470, an NO-donating bimatoprost, is currently in development and expected to enter human clinical trials in 2018.[71] In multiple preclinical models of glaucoma including rabbits with transient hypertonic saline-induced IOP elevation, NCX 470 was well tolerated and reduced IOP more effectively than equimolar bimatoprost.[72] Because rabbits are known to be nonresponsive to prostaglandin F (FP) receptor agonists such as PGAs,[73] the IOP-lowering effect of NCX 470 observed in the study was most likely attributable to its NO-donating moiety. Likewise, other NO-donating analogs of PGAs or prostamides such as NCX 125 (comprising latanoprost acid and NO-donating moieties) and NCX 139 (comprising latanoprost amide and an NO-donating moiety) have been demonstrated to lower IOP in various animal species, with greater efficacies than the corresponding PGA or prostamide.[74,75]

Two NO-donating carbonic anhydrase inhibitors (CAIs), developed by introducing an NO donor into the alkyl side chain of dorzolamide and brinzolamide, are in preclinical development for IOP reduction. Because CAIs decrease aqueous secretion, these agents could lower IOP by modifying aqueous inflow as well as outflow. Animal studies have shown that these compounds reduce IOP more efficaciously than the CAI brinzolamide in rabbits and monkeys.[76]

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CME/CE EXAMINATION QUESTIONS

Directions: Select one answer to each question in the exam (questions 1–40).

- **Ophthalmologists (including those in residency and fellowship training):** Take the CME Exam Online at **http://cme.ufl.edu/ed/self-study/nitric-oxide-in-glaucoma-what-clinicians-need-to-know/**. Visit page 55 for general CME course information. **CME exam expires August 31, 2018.**
- **Optometrists:** Take the CE Exam Online at **http://www.neco.edu/academics/continuing-education/online-ce/nitric-oxide-in-glaucoma**. Visit page 55 for general CE course information. **CE exam expires July 28, 2020.**

1. In the human eye, IOP originates from aqueous outflow resistance localized to the:
   A. Trabecular meshwork
   B. Uveal tissues
   C. Episcleral veins
   D. Suprachoroidal/supraciliary space

2. Which of the following is NOT a vascular benefit of NO?
   A. Stimulates angiogenesis
   B. Inhibits platelet aggregation and adhesion
   C. Suppresses leukocyte adhesion
   D. Reduces vascular smooth muscle proliferation and migration

3. Which of the following outcomes of clinical studies evaluating efficacy and safety of latanoprostene bunod (LBN) is FALSE?
   A. > 1 mm Hg more IOP reduction vs latanoprost
   B. Sustained IOP reduction through 1 year
   C. Robust IOP reduction in subjects with low baseline IOP
   D. Increased hyperemia

4. Based on the presence of NOS, NO is generated in the following human ocular tissues except:
   A. Corneal endothelium
   B. Ciliary body
   C. Schlemm's canal endothelium
   D. Optic nerve head

5. The first known therapeutic application of NO and related nitrate compounds was in the treatment of:
   A. Angina pectoris
   B. Asthma
   C. Stroke
   D. Glaucoma

6. Nitric oxide is reduced by:
   A. Nitroglycerin
   B. Aging
   C. Physical exercise
   D. LBN

7. Which of the following statements about PGAs is false?
   A. They enhance trabecular aqueous outflow to lower IOP
   B. They lower IOP equally well during day and night
   C. They have few systemic side effects
   D. They may exacerbate intraocular inflammation

8. Which of the following agents lower IOP by enhancing both trabecular and uveoscleral aqueous outflow?
   A. Nitrovasodilators
   B. Latanoprostene bunod (LBN)
   C. Nipradilol
   D. Sildenafil

9. Neuro-rejuvenation therapy involves:
   A. Transplanting retinal stem cells
   B. Supplementing retinal cell-derived trophic factors
   C. Enhancing the intrinsic function of existing RGCs
   D. Stimulating axonal regeneration in injured optic nerve

10. When IOP increases, the following events are thought to lead to NO production in the juxtacanalicular TM region except:
    A. Physical interaction between the trabecular and Schlemm's canal cells
    B. Increased shear stress in Schlemm's canal
    C. Schlemm's canal dilation
    D. Expression of eNOS

11. Endogenous NO acts as a(n):
    A. Smooth muscle relaxant
    B. Inflammatory mediator
    C. Neurotransmitter
    D. All of the above

12. Which of the following abnormalities may occur in eyes with POAG?
    A. Reduced NO production in the TM
    B. Lower cGMP level in aqueous humor
    C. Higher L-arginine level in aqueous humor
    D. All of the above

13. Which of the following is true regarding the three NOS isoforms?
    A. They are all encoded by the same gene
    B. They are all expressed under normal conditions
    C. They all require cofactors to function
    D. They all depend on intracellular calcium

14. Which of the following statements about dietary nitrates is correct?
    A. They are not found in dairy and meat
    B. They cannot be metabolized to NO
    C. Greater intake of dietary nitrate may lower the risk for POAG
    D. Dietary nitrate supplementation can increase IOP

15. Which of the following NOS isoenzymes is the primary contributor to the regulation of conventional outflow and maintaining physiological IOP?
    A. eNOS
    B. nNOS
    C. iNOS
    D. All of the above

16. Which patients are expected to benefit most from the sustained-release therapies in development?
    A. Patients intolerant of current medications
    B. Patients with low baseline IOP
    C. Patients who do not adhere to their prescribed regimen
    D. Patients with advanced glaucoma

17. Which of the following is NOT a finding in the TM tissues from glaucomatous eyes?
    A. Decreased cellularity
    B. Decreased cellular stiffness
    C. Cochlin deposits
    D. Altered profile of GAGs

18. In the eye, NO participates in IOP regulation by:
    A. Decreasing aqueous production
    B. Decreasing episcleral venous pressure
    C. Increasing trabecular outflow
    D. Increasing uveoscleral outflow

19. The anterior optic nerve head is supplied by arterial branches from:
    A. The posterior ciliary artery
    B. The central retinal artery
    C. The choroidal circulation
    D. Both A and B

20. SLT is considered an attractive alternative for initial treatment of OAG because:
    A. It is as effective as a PGA in lowering IOP
    B. It is independent of patient adherence
    C. It is repeatable
    D. All of the above

21. What are the potential therapeutic benefits of NO in glaucoma?
    A. Enhanced aqueous outflow through the TM
    B. Improved ocular blood flow
    C. Inhibition of aqueous humor production
    D. Both A and B

22. With each decreased mm Hg of IOP, the reduction in the risk of visual field progression in glaucoma patients has been estimated to be about:
    A. 5%
    B. 10%
    C. 20%
    D. 30%

23. Which of the following about patients with obstructive sleep apnea is true?
    A. They are at increased risk for cardiovascular disease
    B. They are at increased risk for glaucoma
    C. They have increased serum levels of nitrite and nitrate
    D. Both A and B

24. Which of the following patients might have lower CSF pressure than normal individuals?
    A. Patients with NTG
    B. Patients with POAG
    C. Patients with ocular hypertension
    D. Both A and B

25. Caveolins have the potential of being an important contributor to the pathophysiology of POAG because they are involved in:
    A. IOP maintenance
    B. Axonal transport
    C. Vascular tone regulation
    D. Both A and C

26. Which of the following statements about the conventional aqueous outflow pathway is FALSE?
    A. It is the primary drainage route of aqueous humor
    B. It is the site of pathologies responsible for elevated IOP in POAG
    C. It is pressure-independent
    D. It is pressure-dependent

27. Which of the following is NOT a contributing factor to IOP fluctuation?
    A. Pulse
    B. Eye movement
    C. Flickering light
    D. Exercise

28. The most effective IOP-lowering treatment available is:
    A. Topical prostaglandins
    B. Laser trabeculoplasty
    C. Traditional filtering surgery
    D. MIGS

29. Translaminal pressure difference is the difference between:
    A. IOP and orbital CSF pressure
    B. Retinal arterial and venous blood pressure
    C. The arterial blood pressure and IOP
    D. Retinal arterial blood pressure and orbital CSF pressure

30. The initial target of IOP reduction recommended by the AAO practice guidelines for POAG is:
    A. 10%
    B. 25%
    C. 40%
    D. 50%

31. Abnormal NO signaling has been implicated in which of the following conditions?
    A. Atherosclerosis
    B. Alzheimer's disease
    C. POAG
    D. All of the above

32. Which of the following IOP-lowering therapies primarily targets the trabecular outflow pathway?
    A. PGAs
    B. SLT
    C. MIGS
    D. Both B and C

33. The risk of POAG has been associated with genetic polymorphisms in which of the following genes?
    A. NOS1
    B. NOS2
    C. NOS3
    D. NOS4

34. Experimental evidence supports glaucomatous cupping of the optic disc may result from damage to the:
    A. Optic nerve rim
    B. Lamina cribrosa
    C. Glial cells of the nerve head
    D. Retinal nerve fibers

35. Which part of the conventional outflow pathway does NO act on to regulate IOP?
    A. Juxtacanalicular TM
    B. Episcleral veins
    C. Uveal TM
    D. Ciliary muscle

36. NO produces its biological effects through upregulating which of the following?
    A. NAPH
    B. cGMP
    C. EDRF
    D. L-arginine

37. Other than elevated IOP, which of the following factors may increase the patient's risk for POAG?
    A. African race/ethnicity
    B. Impaired optic nerve perfusion
    C. Thin central cornea
    D. All of the above

38. In POAG patients, notching of the optic nerve rim is indicative of:
    A. Ischemia
    B. Focal RGC loss
    C. Compression of RGC axons
    D. Vascular distortion

39. Which of the following is an effect of aging on aqueous humor dynamics?
    A. Decreased trabecular outflow
    B. Decreased uveoscleral outflow
    C. Decreased aqueous inflow
    D. All of the above

40. Which of the following statements is FALSE about the topical IOP-lowering drug nipradilol?
    A. It is an NO-donating beta-blocker
    B. It is awaiting FDA approval
    C. It has shown comparable efficacy vs timolol
    D. It has shown neuroprotective effect in NTG patients

CME/CE COURSE CREDIT INFORMATION

Nitric Oxide in Glaucoma: What Clinicians Need to Know is jointly sponsored by Candeo Clinical/Science Communications, LLC, the University of Florida College of Medicine, and New England College of Optometry. This publication is administered by an independent editorial board and supported by an unrestricted educational grant from Bausch & Lomb, Incorporated.

LEARNING OBJECTIVES

Upon completion of this activity, participants should be able to:

- Review theorized mechanisms of optic nerve damage in glaucoma and recent advances in the understanding of the pathophysiology of glaucomatous optic neuropathy.
- Outline aqueous humor dynamics and the control of IOP in healthy and glaucomatous eyes.
- Identify sites of action for available IOP-lowering agents and recognize current deficiencies in medical treatment of glaucoma.
- Summarize the physiologic function of NO in various bodily systems and identify various NO-donating agents across medicine.
- Explain what is known about NO and its function in the eye.
- Describe the mechanism of action and therapeutic benefit of enhancing NO signaling in glaucoma patients.
- Discuss the potential role of emerging NO-donating therapeutics in glaucoma therapy.

TARGET AUDIENCE

This educational activity is intended for ophthalmologists and optometrists, including those in residency and fellowship training.

STATEMENT OF NEED

Glaucoma, a group of ocular diseases characterized by progressive damage to the optic nerve, is the second leading cause of blindness worldwide, affecting a significant and growing portion of the US population.[1,2]

Much remains to be understood about the pathophysiology of glaucoma, but intraocular pressure (IOP) has been identified as an important causative factor and modifiable risk factor.[3] As demonstrated in several large clinical trials, IOP reduction can prevent progression of optic nerve damage and visual field loss in both early and late stages of the disease.[4,5]

Despite multiple drug choices, however, current data shows that a significant proportion of glaucoma patients do not reach target IOP with a single-agent regimen.[6,7] Even if pressure is maintained within target levels, some patients may continue to develop progressive glaucoma damage and field loss. These treatment challenges highlight a continued need for more effective therapies.

The conventional trabecular meshwork pathway is the primary route of aqueous outflow in the human eye and the site of extra resistance that results in elevated pressure in primary open-angle glaucoma.[8] Even so, therapies targeting trabecular outflow have largely been lacking: the medications that are currently used to treat glaucoma reduce IOP primarily by modulating aqueous production or uveoscleral outflow.

Latanoprostene bunod, a nitric oxide (NO)-donating prostaglandin F2α receptor agonist, is a novel glaucoma drug with a unique dual mechanism of action, achieved by chemically fusing two moieties—latanoprost and an NO donor—into one molecule.[9] While latanoprost increases uveoscleral outflow like other PGAs do, the NO donor contributes to IOP lowering by increasing aqueous outflow through the trabecular meshwork.[10]

To give their glaucoma patients the full benefit of treatment advances, clinicians require clear, actionable insights from knowledgeable subspecialists and researchers. *Nitric Oxide in Glaucoma: What Clinicians Need to Know* will distill and organize findings about the role of NO in glaucoma and the role NO donation in glaucoma therapy in order to make them accessible to ophthalmologists and medical optometrists who want to optimize their decision-making in glaucoma.

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This work may discuss off-label uses of medications.

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Chairperson/Activity Director

James C. Tsai, MD, MBA, is the president of New York Eye and Ear Infirmary of Mount Sinai and system chair of ophthalmology for the Mount Sinai Health System. He also serves as the Delafield-Rodgers Professor of Ophthalmology at the Icahn School of Medicine at Mount Sinai. He is a consultant for Aerie Pharmaceuticals, Inotek Pharmaceuticals, EyeNovia, and Shire.

CME Editor

Matthew J. Gray, MD, is a professor at the University of Florida College of Medicine department of ophthalmology. He states that in the past 12 months, he has not had a financial relationship with any commercial organization that produces, markets, resells, or dis-

tributes healthcare goods or services consumed by or used on patients relevant to this manuscript.

CE Editor

Tony Cavallerano, OD, FAAO, is the executive director of clinical training and patient care, director of professional relations, and adjunct professor at New England College of Optometry in Boston, Massachusetts. He states that in the past 12 months, he has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients relevant to this manuscript.

AUTHORS

Richard K. Lee, MD, PhD, is associate professor of ophthalmology, cell biology, and neuroscience at the Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, in Miami, FL. He states that he has received research grants from National Eye Institute and BrightFocus Foundation, and has served as a consultant for Aerie Pharmaceuticals.

Tony Realini, MD, MPH, is a tenured professor of ophthalmology at West Virginia University, in Morgantown, WV, where he serves as clinical research director, glaucoma fellowship director, and glaucoma service director. Dr. Realini has received grant/research support from Alcon, Aerie Pharmaceuticals and National Institutes of Health. He is active in eye research, has received numerous research grants (including three from the National Eye Institute), and has published widely in the ophthalmic medical journals. He is also a consultant for Alcon, Bausch + Lomb, and Inotek Pharmaceuticals.

Leo Semes, OD, FAAO, is a former professor of optometry at the UAB School of Optometry in Birmingham, Alabama. Since retiring recently from full-time practice, Dr. Semes provides volunteer ophthalmic services in Northeast Florida. He is a consultant for Allergan, Bausch + Lomb, OptoVue, Shire, and ZeaVision, and is also on the speaker's bureau for Alcon, Allergan, OptoVue, Regeneron, Shire, and ZeaVision. Dr. Semes is also a stock shareholder of High Performance Optics.

W. Daniel Stamer, PhD, is the Joseph A.C. Wadsworth professor of ophthalmology and professor of biomedical engineering at Duke University, Durham, NC. Dr. Stamer is a consultant for Aerie Pharmaceuticals. He has also received grant/research support from Allergan, Aerie Pharmaceuticals, Ironwood, and Inotek.

Anne L. Coleman, MD, PhD, is the Fran and Ray Stark Foundation professor of ophthalmology in the Stein Eye Institute of the David Geffen School of Medicine at UCLA as well as professor of epidemiology at the UCLA Fielding School of Public Health in Los Angeles, CA. She is director of the Stein Eye Institute Center for Community Outreach and Policy, UCLA Mobile Eye Clinic and the vice-chair for academic affairs for the department of ophthalmology. She states that in the past 12 months, she has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients relevant to this manuscript.

Medical Writer

Ying Guo, PhD, MBBS, is a medical writer for Candeo Clinical/Science Communications, LLC. She assisted the authors in the preparation of the manuscripts in this supplement. She states that in the past 12 months, she has not had a financial relationship with any commercial organization that produces, markets, resells, or distributes healthcare goods or services consumed by or used on patients relevant to this manuscript.

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NITRIC OXIDE IN GLAUCOMA: What Clinicians Need to Know

EDITED BY

James C. Tsai, MD, MBA
Matthew J. Gray, MD
Tony Cavallerano, OD, FAAO

To give their glaucoma patients the full benefit of treatment advances, clinicians require clear, actionable insights from knowledgeable subspecialists and researchers. *Nitric Oxide in Glaucoma: What Clinicians Need to Know* will distill and organize findings about the role of NO in glaucoma and the role of NO donation in glaucoma therapy in order to make them accessible to ophthalmologists and medical optometrists who want to optimize their decision-making in glaucoma.

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