Review Article

Nitric oxide: an old drug but with new horizons in ophthalmology—a narrative review

Soomin Lee, Choul Yong Park

Department of Ophthalmology, Dongguk University, Ilsan Hospital, Goyang, South Korea

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Correspondence to: Choul Yong Park, MD, PhD. Department of Ophthalmology, Dongguk University, Ilsan Hospital, Donggukro 27, Ilsan-dong-gu, Goyang, Gyunggido 10326, South Korea. Email: oph0112@gmail.com.

Background and Objective: Based on basic knowledge and prior research on nitric oxide (NO), the potential of NO for treating eye diseases is reviewed, and the possibility of NO-based eye drops in clinical practice and the future potential of NO in ophthalmology are discussed.

Methods: A PubMed search was performed for English-language original reports and reviews using the following key words: nitric oxide, eye, ocular, and drug.

Key Content and Findings: NO is synthesized in the human body by NO synthase (NOS) from L-arginine or through enzyme-dependent reduction of dietary nitrate. Three types of NOS (eNOS, nNOS, and iNOS) are abundantly expressed in the eye under normal physiologic or pathologic conditions. The biological effect of NO in the eye is dose dependent. Low intraocular NO concentrations, produced by eNOS or nNOS, have various cellular effects, including vasodilation, intraocular pressure (IOP) regulation, and neuroprotection. iNOS induced under pathologic ocular conditions produces high NO concentrations in the local environment and mediates tissue inflammation, ocular cell apoptosis, and neurodegeneration. In particular, increased iNOS has been reported in glaucoma and retinal ischemic or degenerative diseases. NO plays a vital role in ocular injury. NO can facilitate ocular surface wound healing while eradicating pathogens such as bacteria and Acanthamoeba in chemical burns or infectious keratitis. Furthermore, NO has antifibrotic activity via the cyclic guanosine monophosphate (cGMP) signaling pathway. NO causes smooth muscle relaxation, which can be used to inhibit myopia progression in children. NO can be a stem cell modulator and may help in treating ocular stem cell disorders.

Conclusions: Because of its diverse biologic effects, NO can be a key player in regulating ocular inflammation in various ocular diseases, aiding ocular surface wound healing, controlling IOP in glaucoma, alleviating retinal disease, and suppressing myopia progression. Although there remain limitations to the effective use of highly unstable state, gaseous NO, the role of NO in the field of ophthalmology can be greatly expanded through the development of novel NO donors and effective delivery platforms.

Keywords: Nitric oxide (NO); eye; ocular inflammation; glaucoma; retina

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Introduction

Nitric oxide (NO) is an essential biological gas for homeostasis. This small-molecule gas is ubiquitous in our bodies and plays various roles in cellular functions. Physiologic NO concentrations modulate vasodilation, platelet function, and neurotransmission (1,2).

Because NO is a gas and disappears from the site of action very quickly, its effective therapeutic application

Table 1 The search strategy summary

Items	Specification
Date of search	October 25th, 2022
Databases and other sources searched	PubMed
Search terms used	Nitric oxide, eye, ocular, and drug
Timeframe	November 1980–October 2022
Inclusion and exclusion criteria	Inclusion: English publications that discuss nitric oxide, eye, ocular and drug
	Exclusion: non-English publication; Not discussing nitric oxide, eye, ocular and drug
Selection process	The authors reviewed the papers involved and agreed to review them based on the relevance of the articles

has been challenging in ophthalmology. However, NO-releasing antiglaucoma topical medication, latanoprostene bunod (Vyzulta, Bausch & Lomb), has been recently introduced. Although much basic research on NO has been conducted, it has not often translated into commercial NO-based ophthalmic drugs. The introduction of latanoprostene bunod has thus strengthened the ophthalmologist's arsenal.

NO has long been used for patients with coronary artery disease due to its vasodilatory effect (3). It was initially identified as an endothelium-derived relaxation factor in the 1980s. However, subsequent studies demonstrated other important roles of NO, such as a potent cytotoxic substance for pathogens and a neurotransmission modulator (1,4). Three scientists, Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad, were awarded the Nobel Prize in Physiology or Medicine for 1998 for their discoveries concerning the NO as a signaling molecule in the cardiovascular system (5).

Here, we review the basics of NO, its relevant underlying mechanisms in ophthalmology, and its potential for treating ophthalmic diseases. We present this article in accordance with the Narrative Review reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-5634/rc).

Methods

We searched the PubMed database for English-language original studies and reviews between November 1980 and October 2022 using the following keywords: nitric oxide, eye, ocular, and drug. The detailed search strategy is presented in *Table 1*.

Discussion

NO biosynthesis in living organism

In living organisms, NO can be produced in several ways. The most well-known source is L-arginine through the action of NO synthase (NOS) (6). This process consumes reduced nicotinamide adenine dinucleotide phosphate ($NADPH^+$) and oxygen and converts L-arginine to citrulline with NO production (*Figure 1A*).

NOS exists in three isoforms: type 1 or neuronal NOS (nNOS), type 2 or inducible NOS (iNOS), and type 3 or endothelial NOS (eNOS) (6). They are expressed in many types of cells. nNOS and eNOS are calcium dependent, are constitutively expressed in numerous cells in the body, and produce small amounts of NO, usually in picomolar or nanomolar concentrations.

iNOS is also expressed in many cell types, but it is induced in immunologic or inflammatory conditions, generates NO in large amounts (micromolar or millimolar concentration), and has a long duration of action (4,7). Inflammatory cytokines, such as interleukin 1β, tumor necrosis factor α, and lipopolysaccharides, are strong inducers of iNOS. In contrast to the constitutive expression of nNOS or eNOS, the full expression of iNOS is usually delayed for 2–8 h after stimulation because its synthesis requires cofactors (8).

NO can be generated from dietary nitrate, which is abundant in leafy greens such as spinach and cabbage, via the nitrate-nitrite-NO pathway (*Figure 1B*) (9). Nitrate can be reduced to nitrite by nitrate reductase produced by oral cavity bacteria (9). Nitrite can then be further reduced to NO by nitrite reductases, such as xanthine oxidoreductase, deoxyhemoglobin, and deoxymyoglobin (3). Because NO generation by nitrite reductase is oxygen independent, it is

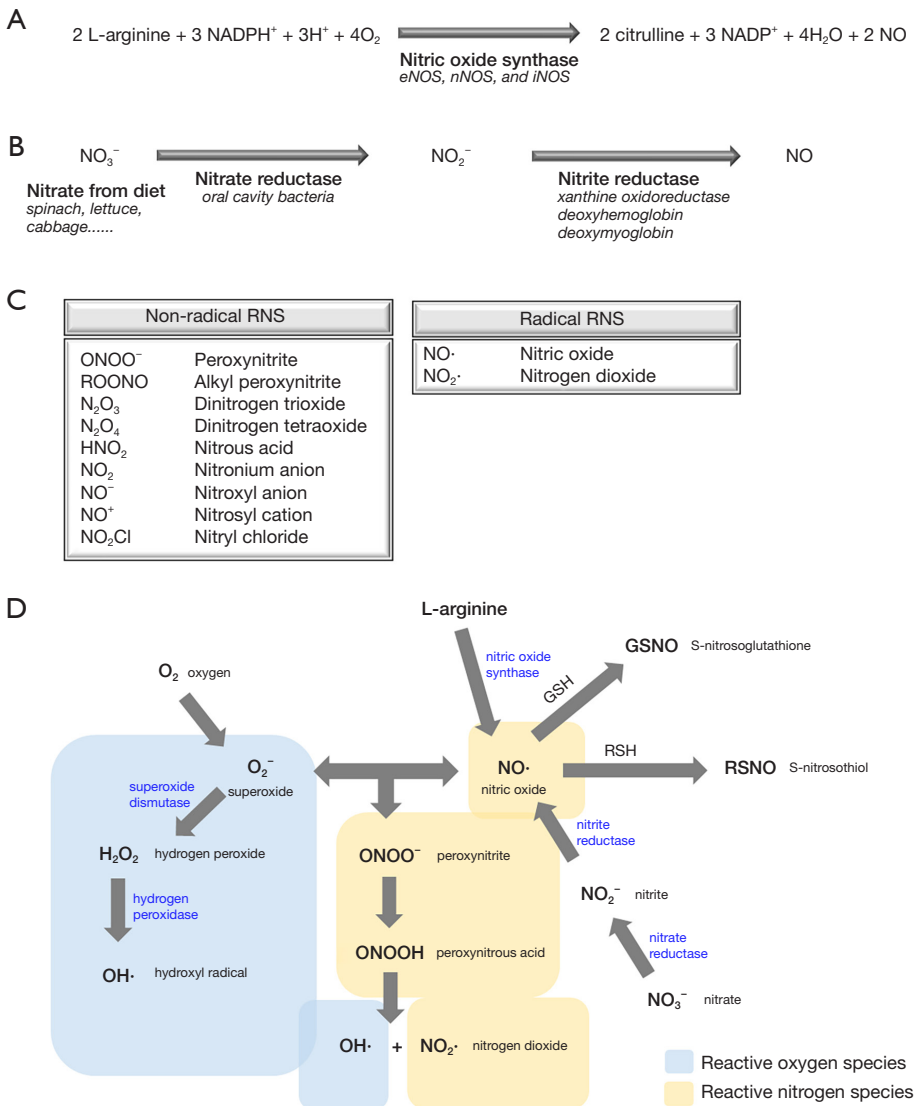

Figure 1 NO synthesis and RNS generation. (A) NO synthase produces NO from L-arginine; (B) the nitrate-nitrite reduction pathway is another source for NO production; (C) various RNS are secondary products of NO and have diverse biological actions; (D) ROS and RNS are closely linked to each other. NO reacts with superoxide to produce a strong RNS, peroxynitrite, but in this process, it scavenges the potent ROS, superoxide. NO, nitric oxide; RNS, reactive nitrogen species; GSH, glutathione; GSNO, S-nitrosoglutathione; RSH, thiol; RSNO, S-nitrosothiol; ROS, reactive oxygen species.

a major NO producer in mammalian cells under hypoxic conditions (10). NO generated by nitrite reductase induces vasodilation in the cardiovascular system, thus helping the survival of cells by increasing blood flow even under hypoxic conditions (3).

Reactive nitrogen species

As mentioned, iNOS generates large amounts of NO for a prolonged period. In the human body, high NO concentrations are an essential source of reactive nitrogen species (RNS) (*Figure 1C*). RNS are various and highly active nitrogenous products, including S-nitrosothiols, peroxynitrite, dinitrosyl iron complexes, and nitrosonium cations, which induce reactions such as nitrosylation of sulfhydryls (S-nitrosylation) or metals and nitration of tyrosine residues (11). Through these reactions, RNS regulates various functions in our body.

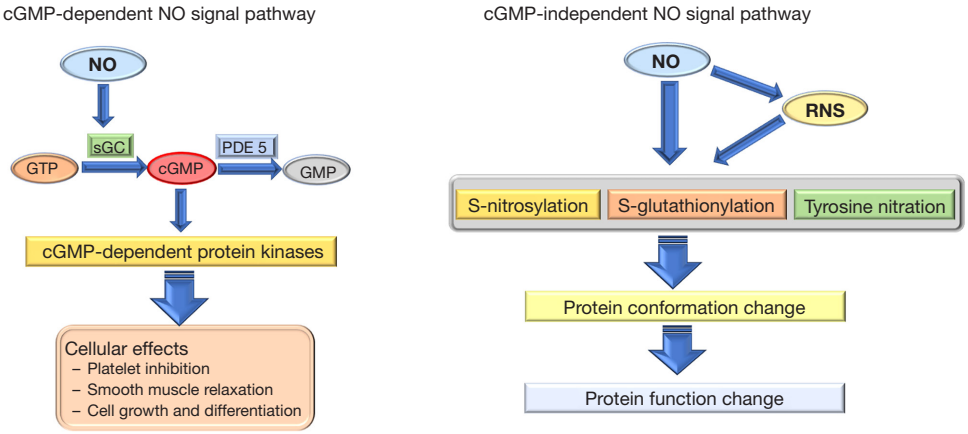

Figure 2 cGMP-dependent and cGMP-independent pathways of nitric oxide signals. In the cGMP-dependent pathway, the signal of NO is converted to increased cGMP concentration by activation of soluble guanylate cyclase. Increased cellular cGMP activates various protein kinases in the cell. The major effect of NO via the cGMP-dependent pathway includes inhibition of platelet aggregation, smooth muscle relaxation, and cell growth and differentiation. In the cGMP independent pathway, the NO signal modifies the protein structure via S-nitrosylation, S-glutathionylation, and tyrosine nitration. Confirmation change in cellular protein results in the alteration of functional activity. cGMP, cyclic guanosine monophosphate; NO, nitric oxide; sGC, soluble guanylate cyclase; GTP, guanosine triphosphate; GMP, guanosine monophosphate; PDE5, phosphodiesterase 5; RNS, reactive nitrogen species.

Peroxynitrite (ONOO-) can be generated in the reaction between superoxide and NO. Peroxynitrite is an unstable isomer of nitrate (NO3-) and affects various intracellular signal pathways such as mitogen-activated protein kinase (MAPK), receptor tyrosine kinase (RTK), p38/Jun-N-terminal kinase, and extracellular signal-regulated kinase (ERK) by oxidizing and nitrating proteins (12). *In vivo*, reactive oxygen species (ROS) and RNS are closely linked (*Figure 1D*). ROS can induce RNS, and many detrimental effects of NO occur by forming peroxynitrite from the reaction between NO and superoxide (13). However, RNS plays a vital role in converting ROS to RNS and thus decreasing excessive ROS signals (14).

Biology of NO

The half-life of NO is approximately 2 ms in the blood and 2 s in the tissues. Its steady state concentration is a key determinant of its biologic function (15). It has dichotomous actions in cellular biology. In general, low NO concentrations promote cell survival and proliferation, while high NO concentrations induce apoptosis and cell damage (15). NO-mediated toxicity is due to the production of cytotoxic RNS, whereas the protective effect of NO has been suggested by the activating antioxidant mechanism.

Different NO concentrations have different cellular targets (15). For example, cyclic guanosine monophosphate (cGMP) mediated processes are activated when NO concentration is less than 1–30 nM, while Akt phosphorylation occurs when NO concentrations are between 30 and 100 nM. Furthermore, hypoxia inducible factor-1α (HIF-1α) is stabilized when NO concentrations are between 100 and 300 nM, phosphorylation of p53 takes place when NO concentrations are approximately 400 nM, and nitrosative stress occurs when NO concentrations are over 1 μM.

Downstream pathway of NO signal

In vivo, NO effects are manifested through cGMP-dependent and cGMP-independent pathways (*Figure 2*).

cGMP dependent pathway

The NO/cGMP signaling pathway is the most well studied, and it regulates various physiological parameters such as smooth muscle relaxation, inhibition of platelet aggregation, neuronal transmission, and wound healing response.

NO activates cytoplasmic soluble guanylate cyclase (sGC), which contains a heme structure in the Heme Nitric oxide/Oxygen binding (HNOX) domain. Binding of NO to the heme structure increases the enzyme catalytic rate by 300 folds (16). sGC converts GMP to cyclic GMP and

propagates the signal to various effector gene expressions. Increased cGMP itself relaxes vascular smooth muscle by decreasing the intracellular calcium concentration.

The majority of cGMP is degraded by phosphodiesterases such as phosphodiesterase 5 (PDE5); therefore, the balance between sGC and PDE5 determines the duration and potency of NO signals via the cGMP-dependent pathway (16).

cGMP independent pathway

NO and RNS can directly modulate the structure and function of proteins through posttranslational modifications, which mainly include S-nitrosylation, S-glutathionylation, and tyrosine nitration (11,12). S-nitrosylation (or S-nitrosation) of thiols converts the thiol group (R-SH) of amino acids, such as cysteine, to S-nitrosothiol (R-SNO). S-glutathionylation of protein is a two-step process and requires S-nitrosoglutathione (GSNO) produced through S-nitrosylation, and then glutathione is added to the cysteine sulfhydryl residues of proteins. Tyrosine nitration is also a two-step process: the oxidation of tyrosine by peroxynitrite generates a tyrosyl radical, which then reacts with nitrogen dioxide (NO_2) to form 3-nitroso-tyrosine (11).

These processes modify the conformation of proteins and change functional activity. Protein modification by NO is an important process of signaling and can control the transcription of several important gene expressions (12).

NO donors

NO is in a gaseous state and disappears very quickly, therefore NO donors are used to supply NO to the target tissue. Various chemicals and polymers can be used as potential NO donors.

N-diazeniumdiolate and S-nitrosothiols are the most commonly used NO donors for biomedical applications (17). S-nitrosothiols are a group of molecules in which a nitroso group is bound to a sulfur atom. S-nitrosothiols release NO spontaneously or when catalyzed by light, enzymes, and metal ions. NO release from S-nitrosothiols resulted in the formation of a disulfide bond between two S-nitrosothiol molecules. N-diazeniumdiolate is generally termed NONOates due to the functional group [N(O)NO]-. N-diazeniumdiolate releases two molecules of NO when it undergoes protonation.

However, these NO donors tend to burst-release NO, making it difficult to expect a long and steady effect of NO. Researchers have been attempting to combining these NO donors with various polymers or nanoparticles for the stable release of NO (17).

NO production in the eye

The expression of various types of NOS in ocular tissues implies the endogenous roles of NO in ocular homeostasis (*Figure 3*).

nNOS is expressed in the inner retina; outer and inner plexiform layer, cell bodies of bipolar cells, outer and inner nuclear layer, ganglion cells, amacrine cells, and retinal pigment epithelial cells (18,19). nNOS is also present in the limbus, cornea cells (endothelium and epithelium) and lens epithelium (4). iNOS is inducible in inner nuclear layer, outer segments of the photoreceptors, macrophages, and microglia in inflammatory conditions. Moreover, iNOS is also detected in the iris, ciliary body, and vessels after stimulation (4). eNOS was detected in photoreceptors, horizontal cells, bipolar cells, amacrine cells, Muller cells, and ganglion cells. eNOS is also present in the uveal vascular endothelium, trabecular meshwork, Schlemm's canal, and ciliary body (4,19). During ischemia/reperfusion injury, eNOS increased in retinal vessels and the ganglion cell layer.

NO as a novel antiglaucoma treatment

Glaucoma is a condition in which the visual field gradually narrows, with progressive thinning of the retinal nerve fiber layer. Because increased intraocular pressure (IOP) is closely related to disease progression, lowering IOP is critical in glaucoma treatment. NO may lower IOP by regulating aqueous humor dynamics through changes in ciliary muscle contraction and aqueous humor outflow pathway resistance (4,7).

Evidence that NO is involved in IOP regulation was first presented in animal models. Behar-Cohen *et al.* (20) reported that intraocular administration of NO donors induced significant IOP reduction in rabbits. In addition, some NO donors reduced aqueous humor production from the ciliary epithelium (21,22). IOP of transgenic mice expressing human eNOS is lower than wild type control, and treatment with NO synthesis inhibitor, N-nitro-L-arginine methyl ester (L-NAME), in these mice normalized the reduced IOP (23). In a normotensive rabbit model, various NO donors such as nitroglycerin, ISDN, sodium nitrite, S-nitrosothiol, sodium nitroprusside, spermin NONOate, 3-morpholinosydnonimine, and S-nitroacetylpenicillamine successfully lowered IOP (20,24,25). In a nonhuman

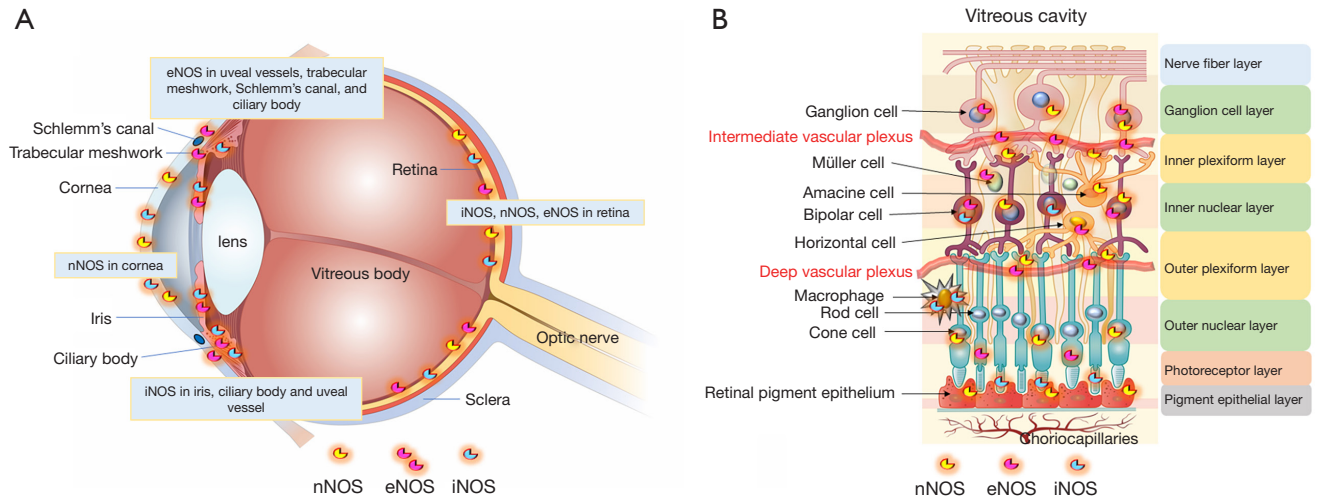

Figure 3 NOS expression inside the eye. (A) Three types of NOSs are constitutively expressed or induced in various ocular tissues and cells. NO is involved in all vital ocular phenomena. eNOS and nNOS are expressed constitutionally in both the anterior and posterior parts of the eye, and iNOS can be actively induced in inflammatory conditions. (B) Different types of cells in the whole retinal layer express eNOS and nNOS. iNOS can be induced in the retina in inflammatory conditions, and infiltrating macrophages also express iNOS. NO, nitric oxide; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase.

Table 2 Human evidence of NO regulation of aqueous humor dynamics

Authors	Key findings
Nathanson *et al.* (32)	eNOS decreased in trabecular meshwork, Schlemm's canal and ciliary body in primary open angle glaucoma patients
Doganay *et al.* (33)/Galassi *et al.* (34)	NO decreased in aqueous humor of primary open angle glaucoma patients
Kang *et al.* (35)/Magalhães da Silva *et al.* (36)	eNOS gene variation was associated with primary open angle glaucoma in women
Chuman *et al.* (37)	Intravenous injection of L-arginine reduced intraocular pressure in an average of 2 mmHg
Chang *et al.* (38)	Aqueous humor NO level was higher in the glaucoma patients than in the cataract patients

NO, nitric oxide; eNOS, endothelial nitric oxide synthase.

primate study, repeated application of topical nitroglycerin or SNP induced IOP reduction up to 20% (26,27). NO-releasing prostaglandin analogs reduce IOP more than their prostaglandin counterparts (28-30).

In human, high nitrate consumption from green leafy vegetables in the diet lowered the risk of primary open angle glaucoma by 21% in a large population-based cohort study (31). Other studies have also provided evidence that NO regulates IOP in humans (*Table 2*).

Notably, glaucomatous optic nerve damage can be aggravated by impaired blood flow to the optic nerve. Given that NO is a potent vasodilator, the findings that NOS is expressed in the vascular endothelium of the retina and choroid suggest its role in the regulation of blood flow to these tissues. The experimental NOS inhibition or sGC decreased both ocular and retinal perfusion (39,40). By contrast, exogeneous NO can dilate intraocular and extraocular vessels. Therefore, the dilatation of optic disc vessels can protect the optic nerve from ischemic damage when it is exposed to high IOP in glaucoma. Furthermore, the dilatation of episcleral vessels can reduce the outflow resistance of aqueous humor and decrease IOP.

In glaucoma, neuroprotection is also important because glaucoma eventually develops a visual field defect caused by irreversible damage to the retinal nerve fiber layer and retinal ganglion cells. Evidence has indicated that NO has neuroprotective effects. However, whether its exerts neuroprotective or neurodegenerative action depends on

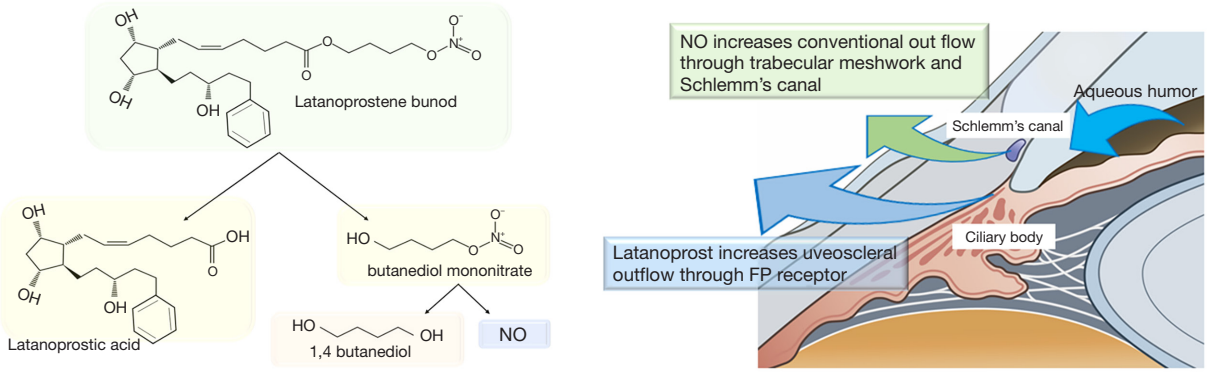

Figure 4 Latanoprostene bunod and its action. Latanoprostene bunod is hydrolyzed to latanoprostic acid and butanediol mononitrate. Butanediol mononitrate is further metabolized and NO is produced. Latanoprostic acid is similar to latanoprost and increases uveoscleral outflow of aqueous humor. NO relax trabecular meshwork and episcleral vessels and increase the conventional outflow of aqueous humor. Increases flow of aqueous humor via uveoscleral and conventional outflow pathway synergistically decreases intraocular pressure in glaucoma patient. NO, nitric oxide.

the dose of NO released by the NO donors. Higher NO concentrations (>50 μM) decreased neuronal cell survival, whereas lower NO concentrations (<1 μM) increased neuronal cell survival (41). In some animal models of retinal ischemia or elevated IOP, increased retinal iNOS expression has been reported (42). Localized and high NO concentrations can increase retinal ganglion cell loss due to the formation of high levels of peroxynitrite (43).

The most widely studied drug as a NO donor for glaucoma treatment is the nonselective beta blocker nipradilol. Topical administration of 0.25% nipradilol decreased aqueous flow rate by 20% and reduced IOP by a maximum of 4.2 mmHg in normal human volunteers (44). Nipradilol may have neuroprotective effects due to its NO-donating action (45). It also inactivates phosphatase and tensin homologue (PTEN) by S-nitrosylation and keeps activation of AKT/mTOR signal for cell survival (46). Arterial injection of nipradilol induced retinal arterial dilation in a canine model (47). Furthermore, its topical application can reach the retina at pharmacologically active levels (48,49).

Recently, latanoprostene bunod ophthalmic solution (0.024%, VYZULTA, Bausch and Lomb) has been introduced as a promising NO-donating prostaglandin $F_{2\alpha}$ analog (*Figure 4*). Several phase 3 clinical studies (APOLLO, LUNAR, and JUPITER) have concluded that 0.024% latanoprostene bunod was non-inferior to 0.5% timolol ophthalmic solution in terms of IOP-lowering efficacy (50-53). Latanoprostene bunod is hydrolyzed to latanoprostic acid (prostanoid FP receptor agonist) and butanediol mononitrate. Butanediol mononitrate is further metabolized inside the eye and produces 1,4-butanediol and NO (50).

NO in retinal diseases

The role of NO in retinal diseases remains controversial. In various retinal diseases, NO sometimes aggravates or alleviates the pathology. As discussed, high NO concentrations induced by iNOS are proinflammatory and generally aggravate retinal damage in ischemic or degenerative conditions. On the other hand, the constitutive level of NO produced by nNOS or eNOS has protective effects on retinal cells.

NO has downsides in retinal diseases. Becquet *et al.* (54) reported that NO decreased the phagocytic activity of retinal pigment epithelium (RPE), and NOS inhibition by L-NAME restored RPE phagocytosis in LPS/IFN-stimulated bovine RPEs. A decrease in RPE phagocytosis can lead to accumulation of ROS debris between photoreceptors and RPEs, and results in photoreceptor degeneration. In the ischemic injury model, iNOS mediates retinal apoptosis (55). In addition, iNOS inhibition can preserve retinal cells from ischemic injury (56,57). Hurst *et al.* (58) reported that iNOS inhibitor reduced the ischemic damage of retinal ganglion cells and bipolar cells in their CoCl2 induced retinal neurodegeneration model using porcine retina organ culture. CoCl2 also increases oxidative stress and active HIF-1α.

NO concentration in the aqueous humor is elevated

in patients with retinal vein occlusion (59,60). Under ischemia/reperfusion injury, elevated NO and superoxide can generate peroxynitrite, which rapidly oxidizes the sulfhydryl group and initiates lipid peroxidation for further retinal damage. Alternatively, peroxynitrite induces the formation of nitrotyrosine from tyrosine. Notably, physiologic concentration (nanomolar) of peroxynitrite may be beneficial with vascular relaxation and inhibition of platelet aggregation (8).

On the other hand, NO may reduce the risk and severity of various retinal diseases. NO produced by eNOS of retinal vascular endothelial cells is important in the maintenance of vasomotor function of retinal microvasculature (61). Using an IOP-induced retinal ischemia model, acute retinal ischemia significantly increased ROS in the retina to a level enough to inhibit NO-mediated dilation of retinal arterioles (62). NOS inhibition by N^G-nitro-L-arginine during the initial phase of reperfusion of ischemia/reperfusion injury delayed retinal functional recovery in rats (63). NO donors such as molsidomine protected the retina from ischemia/reperfusion injury (64). Molsidomine was also reported to relieve radiotherapy induced retinopathy (65).

NO plays an important role in diabetic retinopathy (DR). There are some experimental evidences that excessive synthesis of NO can contribute to the development and aggravation of DR (66-68). Especially, high concentrations of NO produced by retinal iNOS are known as a major mediator in DR. In an immunohistochemical study using human retinas, iNOS was observed only in retina with DR (68). Several studies reported that intraocular NO concentration significantly increased in diabetic patients (69,70). As well known, increased leukostasis and vascular permeability is typical pathologic finding in DR. Interestingly, previous study has revealed that NOS inhibition by L-NAME or iNOS knockout in mice can reverse these pathologies (71). The role of eNOS in DR also appears to be important. One study revealed that eNOS knock out mice developed an accelerated DR in streptozotocin induced diabetes (72). In this study, although total retinal NO levels in non-diabetic eNOS knock out mice were similar to those in wild type mice, induction of diabetic in eNOS knockout mice resulted in highly increased total retinal NO level with increased iNOS mRNA expression (72). Furthermore, some human studies have reported interaction between eNOS gene polymorphisms and the development and progression of DR (73,74).

NO may play a role in the development of age-related macular degeneration (AMD). Bhutto *et al.* (19) studied NOS expression in the human retina and choroid by immunohistochemistry and found that nNOS expression was significantly lower in retinal ganglion cells, retinal vessels, and retinal pigment epithelial cells in eyes with AMD. In addition, eNOS in the choroidal arteries was also lower in AMD eyes. Gopinath *et al.* (75) reported that people who ate 100–142 mg of vegetable nitrates every day had approximately 35% lower risk of early AMD development during their 15 years of observation study. These findings suggest that the constitutional level of NO has a protective effect in preventing AMD.

Recently, the mainstay of treatment for AMD has been intravitreal injection of anti-vascular endothelial growth factor (VEGF) agents. De Cillà *et al.* (76) studied NO release from human retinal epithelial cells under the stimulation of antiVEGFs, such as ranibizumab and aflibercept. They reported that under normal conditions, anti-VEGF increased NO release from retinal epithelial cells; on the other hand, NO release under the stimulation of anti-VEGFs decreased with hydrogen peroxide pretreatment. This result implies that even the same cells have different NO responses to anti-VEGF depending on the pathologic state.

A specialized NO-based drug for treating retinal disease has not yet been introduced into clinical practice. These studies suggest that the pharmacologic role of NO in retinal diseases may differ depending on the types and stages of the disease (*Figure 5*). Therefore, elucidation of the exact role of NO in various retinal pathologies and the optimal concentrations should precede the development of therapeutic agents.

NO as a wound healing modulator

In general, normal wound healing has three important stages: inflammation, proliferation, and tissue remodeling (77). NO may play a multifaceted role in all these stages.

NO can facilitate wound healing while inhibiting bacterial infection after injury (78). High concentrations (μM) of NO generated by iNOS are important to eradicate bacterial pathogens, while low concentrations (pM to nM) of NO generated by eNOS are essential to modulate proliferative and tissue modeling stages (79). During wound healing, NO can decrease platelet aggregation, cytokine expression, leukocyte recruitment, and matrix metalloproteinase expression (80,81). In addition, dietary modulation of arginine can impair or enhance wound

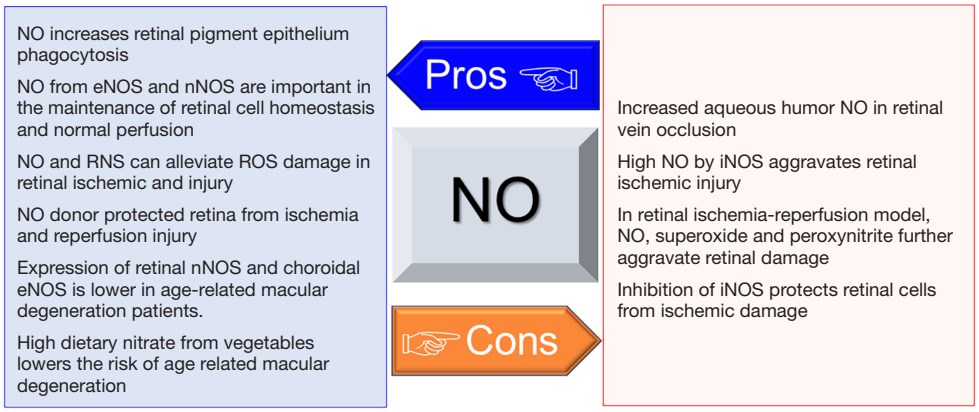

Figure 5 A double-edged sword action of NO in retinal pathology. NO, nitric oxide; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; RNS, reactive nitrogen species; ROS, reactive oxygen species; iNOS, inducible nitric oxide synthase.

healing in animals and humans (82).

iNOS inhibition can decrease collagen deposition and impair normal wound healing (82). On the other hand, exogenous NO supply can promote fibroblast migration and collagen deposition in skin and cornea wound (83-85). NO/cGMP signaling increased Rho-GTPases, important regulators of cytoskeletal dynamics, and enhanced cell proliferation, migration, and adhesion in the wound healing process (86). Cornea epithelial healing was also promoted with exogenous NO supply by activating the MAPK pathway (84).

NO has antifibrotic activity. Increased NO/cGMP signaling attenuated fibrotic responses in many organs, such as the liver, kidney, prostate, heart, skin, and lungs (87-89). Exogenous NO supply could attenuate myofibroblast differentiation of cornea keratocytes and decreased cornea opacity in murine chemical burn cornea (85) (*Figure 6*). Excessive fibrosis accompanying the ocular wound healing process can deteriorate vision by inducing corneal opacity or retinal fibrosis. Therefore, the antifibrotic effect of NO while facilitating wound healing response is a very attractive point as a wound healing modulator in the field of ophthalmology.

NO as an ocular inflammatory mediator

NO is an important inflammatory mediator in autoimmune uveitis (90). In animal studies, NO production with increased iNOS expression (in the iris, ciliary body, and retina) was detected in endotoxin induced experimental uveitis and intraperitoneal injection of L-NAME reduced the severity of experimental uveitis (91-94). Wu *et al.* (95) observed the formation of peroxynitrite in photoreceptors, ganglion cells, nerve fiber layer, and retinal vessels in an experimental autoimmune uveitis model. The area with high production of peroxynitrite correlates with the area of severe pathologic change (95).

The different time points of NOS modulation by the NOS inhibitor, L-NAME, can result in controversial results in the endotoxin-induced uveitis model. While early intervention using L-NAME injection (at 0.5 h after endotoxin administration) inhibited uveitis, late L-NAME injection at 6 h after endotoxin administration exacerbated inflammation (96).

NO also regulates hypoxia-inducible factor 1α (HIF-1α). HIF-1α is increased in hypoxia and leads to increased expression of VEGF. An increase of HIF-1α was reported in AMD (97). Hypoxia and inflammation frequently coexist in various pathologic conditions (98). High concentrations (micromolar) of NO can increase HIF-1α stabilization in an oxygen-independent manner. Therefore, high NO production through iNOS activation can lead to HIF-1α activation even in normal oxygen conditions. On the other hand, low NO concentrations (nanomolar) can inhibit HIF-1α accumulation and stabilization (99). The dual actions of NO, which are dose and time dependent, can be the potential therapeutic target of ocular inflammatory and neovascular diseases (97).

NO as antimicrobial agent in cornea infection

NO is an endogenously produced antimicrobial molecule. At low concentrations, NO can stimulate the growth and

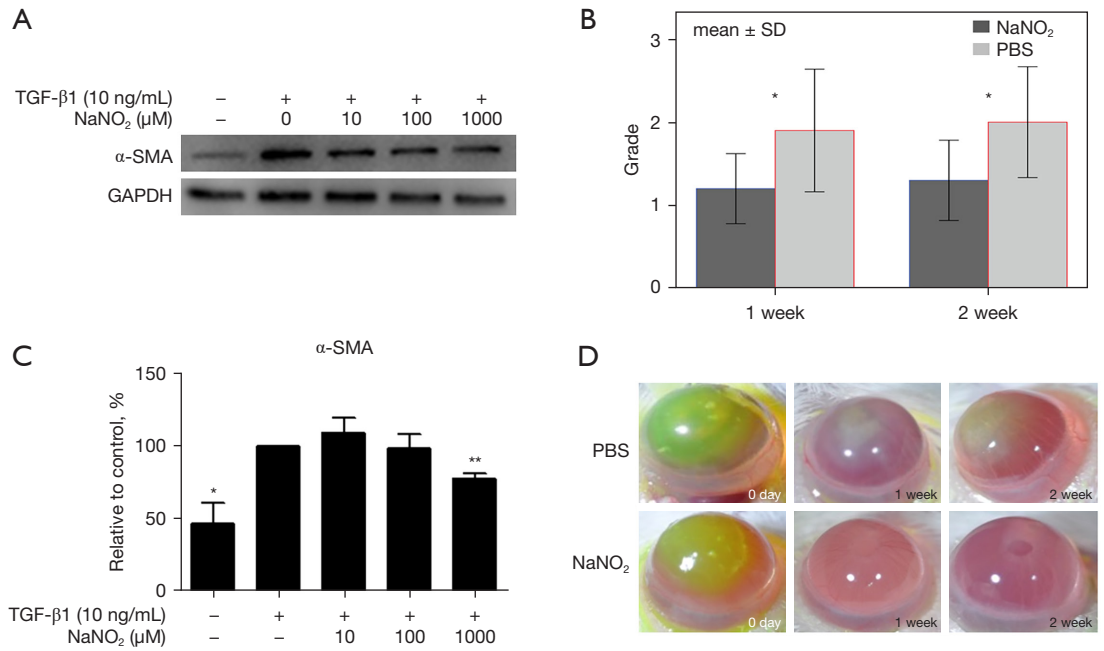

Figure 6 NO effect on corneal wound healing. $NaNO_2$ was used as an NO donor. (A) Cultured human keratocytes were stimulated by transforming growth factor β1 and myofibroblast differentiation was verified by increased α-SMA expression in protein blots. Addition of $NaNO_2$ in the culture media inhibited myofibroblast differentiation of keratocytes, as verified by decreased α-SMA expression. Approximately 20% decreased α-SMA expression was observed when 1,000 μM of $NaNO_2$ was added to the medium (unpublished data by the authors). (B) *In vivo* effect of NO in corneal chemical burn in Balb/c mice. Corneal opacity grade after healing from chemical burns significantly decreased with the topical treatment of $NaNO_2$ compared with PBS treatment. (C) The expression of α-SMA in keratocytes after TGF-β1 stimulation is attenuated by topical $NaNO_2$ treatment. (D) Representative ocular surface pictures of healing process of chemical burn shows more transparent cornea in $NaNO_2$-treated mouse compared with PBS control. Panels B and C are from *Fig. 6* in "Effect of Nitric Oxide on Human Corneal Epithelial Cell Viability and Corneal Wound Healing" Park *et al.* Sci Rep. 2017;7(1):8093 under Creative Commons Attribution 4.0 International License. *, P<0.05; **, P<0.01. TGF-β1, transforming growth factor beta-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SD, standard deviation; PBS, phosphate buffered saline; α-SMA, alpha smooth muscle actin; NO, nitric oxide.

maturation of immune cells, while at high concentrations, NO and RNS bind to microbial DNA, protein, and lipid, thereby damaging these structures (100). Bacterial DNA damage by NO occurs through deamination, oxidative damage, strand breaks, and DNA alterations (100).

Kim *et al.* (101) reported that the main sources of NO in ocular surface tissue are corneal epithelium, fibroblast, endothelium, and inflammatory cells in rabbit eyes. They also found that the concentration of NO increased in experimentally induced ocular surface pathology in rabbits. Normal tear level of NO increased in allergic conjunctivitis, LPS-induced inflammation, and corneal surgery (101).

NO can facilitate the dispersal of bacterial biofilm and NO can make bacteria more susceptible to other antibiotics (102,103). Kim *et al.* (104) reported that biofilm formation on contact lens surface by Pseudomonas aeruginosa and Staphylococcus aureus was successfully inhibited by NO donor such as sodium nitrite. NO releasing contact lenses were also investigated with promising *in vitro* data (105,106). Acanthamoeba keratitis is among the most intractable of corneal infectious diseases leading to blindness (107). Recently, Yim *et al.* (108) demonstrated the anti-Acanthamoeba effect using NO donors.

Studies have suggested that NO is not only a potential treatment for established bacterial keratitis but also an inhibitor of biofilm formation on contact lens and reduce the risk of bacterial keratitis.

NO and myopia

Ciliary muscle is a smooth muscle inside the eye and is controlled by parasympathetic nerve fibers arising from the

ciliary ganglion. The contraction of ciliary muscle increases the refractive power of the crystalline lens (which is called 'accommodation') and moves the vision focus to the near objects. Excessive near-working with sustained ciliary muscle contraction is one of the risk factors for myopia progression. Childhood myopia progression can be slowed down by topical application of atropine, an anticholinergic agent, and a smooth relaxant. Because NO also has a smooth muscle relaxing effect, the role of NO in myopia was previously investigated.

Whether NO is protective in myopia progression remains debatable. In an experiment using the chick myopia model by form deprivation process, Carr *et al.* (109) revealed that NO donors such as L-arginine and sodium nitroprusside enhanced the myopia inhibition effect of atropine whereas NO inhibitors decreased the atropine effect. However, Wu *et al.* (110) and Wen *et al.* (111) found that NOS activity and cGMP concentration increased in form deprivation myopia model in guinea pigs and this suggests that NO can also aggravate myopia progression. In addition, Fujikado *et al.* (112,113) injected L-NAME before inducing form-deprivation or lens induced myopia in chick eyes and found that NOS inhibition by L-NAME inhibited the development of myopia.

Because studies on this topic used animal models such as a bird or a guinea pig, animals such as birds have distinctive emmetropization processes such as choroidal thickening or thinning to compensate for defocus (114). This process has not been confirmed in humans. Moreover, inducing myopia in animals uses extreme methods, such as ocular occlusion. This is fundamentally different in the pathogenesis of human myopia. Therefore, further research should elucidate the exact role of NO in human myopia progression.

NO as a stem cell modulator

In studies using embryonic stem cells, depending on the concentrations, NO plays a dual role in preserving pluripotency and cell survival or inducing differentiation in a dose-dependent pattern (115,116). There has been evidence that the effects of NO on stem cell pluripotency and differentiation are independent of the sGC-cGMP pathway (117).

Limbal epithelial stem cells (LSCs) exist on the ocular surface and play important roles in supplying cornea epithelial cells. The deficiency of LSCs by various injuries impairs cornea transparency and leads to blindness. However, the role of NO in LSCs has not yet been studied. Bath *et al.* (118) reported that hypoxia increased stem cell markers with a low proliferate rate in LSCs compared with a normal oxygen environment. Because NO regulates HIF-1α, the key molecule in cellular response to hypoxia, the role of NO in maintenance and differentiation of LSCs is fully anticipated.

Future of NO in ophthalmology

Ocular diseases are divided into ocular surface diseases and intraocular diseases. In cases of ocular surface diseases, drugs can be delivered relatively easily in the form of eye drops, but in the case of intraocular diseases, various ocular drug barriers restrict effective drug penetration to the target tissue. This ocular drug barrier is the main obstacle to the development of new drugs in the field of ophthalmology.

As discussed, because various NO concentrations show various pharmacologic effects in ocular tissues, vigorous studies determining the concentration range of NO required for treating various ocular diseases should be preceded.

The instability of gaseous NO is the most problematic. Because NO cannot be delivered in gaseous form in clinical practice, there is a disadvantage that NO donors should be used as a prodrug. In addition, if excess NO is released too quickly, the therapeutic effect may not be sufficient, and the risk of toxicity increases. Therefore, future studies will be developing ideal platforms for the effective delivery of NO to the target. Methods for packaging a low molecular weight NO donor using macromolecular scaffolds, such as polymers or nanoparticles, as a support can be one of the promising options (119,120).

In that sense, it is very encouraging that latanoprostene bunod, which has been commercialized as an antiglaucoma medication, has been successfully introduced in clinical practice and is receiving positive responses.

Limitations of the review

This review describes the role of nitric oxide in a variety of ophthalmic diseases, from basic knowledge of nitric oxide to arousing interest in the development of therapeutics using nitric oxide in the field of ophthalmology. Therefore, the basic knowledge of nitric oxide cannot be covered too deeply, and it is limited in that it cannot provide lengthy explanations of eye diseases that are too specialized.

In addition, only researches published in English literature and full length articles retrieved from PubMed search were included in the review. This approach has a limitation in that

it does not include the latest researches presented as abstracts at recent academic conferences in the review.

Conclusions

NO was originally introduced as a treatment for cardiovascular disease and has since been shown to play a vital role in various organ diseases. Its potential as a therapeutic agent for various diseases in the field of ophthalmology is being discovered, and NO can be an all-round player from inflammation control, ocular surface wound healing modulator, IOP control in glaucoma, alleviating retinal disease, and myopia suppression.

In particular, ocular surface delivery of NO may be sufficient to produce the desired therapeutic effect can be obtained by in some ocular pathologies.

NO has long been studied in the medical field, but with the recent development of technology, its role in ophthalmic diseases has been more clearly identified, a more stable drug delivery method is being developed, and a new horizon as a future therapeutic agent will be open in the near future.

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Footnote

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