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Nitric oxide donating anti-glaucoma drugs: advances and prospects

MAO Yu-Jie, WU Jian-Bing, YANG Ze-Qiu, ZHANG Yi-Hua, HUANG Zhang-Jian*

State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Drug Discovery for Metabolic Diseases, Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, China

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[ABSTRACT] Glaucoma is a disease that causes irreversible blindness. Reducing intraocular pressure (IOP) is the main treatment at present. Nitric oxide (NO), an endogenous gas signaling molecule, can increase aqueous humor outflow facility, inhibit aqueous humor production thereby reducing IOP, as well as regulate eye blood flow and protect the optic nerve. Therefore, NO donating anti-glaucoma drugs have broad research prospects. In this review, we summarize NO-mediated therapy for glaucoma, and the state of the art of some NO donating molecules, including latanoprostene bunod in market and some other candidate compounds, for the intervention of glaucoma, as well as prospects and challenges ahead in this field.

[KEY WORDS] Nitric oxide; Glaucoma; Intraocular pressure; Trabecular meshwork; Prostaglandin analogues
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Introduction

Glaucoma is the second degenerative eye disease in the world and the leading cause of irreversible blindness. There are nearly 79.64 million glaucoma patients worldwide, and this number is estimated to reach 111.8 million by 2040 [1]. Glaucoma is characterized by optic atrophy, visual field defects and eventually progress to blindness, dividing into open-angle glaucoma and closed-angle glaucoma. Among them, primary open-angle glaucoma (POAG) is the most common one, accounting for about 74% of all glaucoma patients [2]. Increased intraocular pressure (IOP) is one of the causes of glaucoma progression. The level of IOP is controlled by the balance of aqueous humor production and outflow. Aqueous humor is produced by the ciliary epithelial cells and outflowed *via* two ways. One is the conventional outflow pathway, in which aqueous humor exits through the anterior ciliary vein *via* the trabecular meshwork (TM) and the Schlemm's canal (SC). This pathway is pressure-dependent, and elevated IOP promotes aqueous humor drainage to maintain homeostasis. The other is the unconventional outflow pathway, also known as the uveoscleral pathway, which is non-pressure-dependent. Aqueous humor enters the ciliary body and suprachoroidal space through ciliary muscle bundle space, and passes through scleral collagen or neurovascular space. At present, there are three main therapeutic methods for the intervention of glaucoma: topical medication, laser treatment and surgical intervention. Among them, topical medication is the most basic and important therapeutic mean [3]. To date, the main drugs in clinics include prostaglandin analogues, β-adrenergic receptor antagonists, α-adrenergic receptor agonists, carbonic anhydrase inhibitors and cholinergic receptor agonists [4]. Rho kinase inhibitors, as a novel class of anti-glaucoma and ocular hypertension drugs, can reduce IOP by increasing trabecular meshwork drainage and decreasing aqueous humor production, when treating alone or in combination with other anti-glaucoma drugs [5].

Nitric oxide (NO), as a colorless, odorless gasotransmitter, and a highly active free radical molecule, plays extremely important physiological and pathological functions in cardiovascular, immune and nervous systems. NO is synthesized by three isoforms of NO synthases (NOS). Generally, under normal physiological conditions, endothelial NOS (eNOS) and neuronal NOS (nNOS) synthesize a small amount of NO (pmol·L^{-1}–nmol·L^{-1}) [6], while under pathological conditions, inducible NOS (iNOS) produces a large

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[*Corresponding author] E-mail: cpudahuang@163.com
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amount of NO and maintains the high concentrations of NO (μmol·L^{-1}–mmol·L^{-1}) for a longer time [7]. TM, SC cells, the ciliary body and iris sphincter of anterior chamber angle express nNOS and eNOS, which are associated with the regulation of blood flow and outflow of aqueous humor [7-8]. NO can promote the outflow of aqueous humor from TM and reduce IOP by activating soluble guanylate cyclase (sGC)-cyclic guanosine monophosphate (cGMP) pathway [8-9], as well as directly reduce the formation of aqueous humor via modulation of ion transporters [10]. Additionally, NO is involved in the regulation of ocular blood flow to protect visual field, and possesses neuroprotective function [11-14]. Furthermore, elevated anterior chamber perfusion pressure induces iNOS transcription to produce a large amount of NO in TM cells, which can promote the outflow of aqueous humor and downregulate IOP [15]. Notably, excessive NO may lead to pathophysiological actions such as inflammation and optic nerve degeneration *via* peroxynitrite and other reactive nitrogen species (RNS) which are derived from the reactions of NO with superoxide free radical or other reactive oxygen species (ROS).

Based on the therapeutic potential of NO, NO donating compounds have attracted many attentions in the arena of glaucoma treatment. It has been reported that some typical NO donating drugs including nitroglycerin, isosorbide dinitrate, sodium nitrite, and sodium nitroprusside, can lower IOP after topical administrations [16]. Additionally, novel NO donating compounds, which could decompose to NO and the other active fragment for synergistic IOP management, have been recently developed. A representative drug Latanoprostene bunod (LBN), as a NO-donating prostaglandin F2*α* analogue, had been approved for the treatment of glaucoma by FDA in the USA in November 2017 [17]. Nipradilol, as a NO-donating *β*-adrenoceptor antagonist, was marketed in Japan in 1988 [18].

This review addresses NO-mediated therapy for glaucoma (Fig. 1), and summarizes the state of the art of some NO donating molecules, including latanoprostene bunod in the market and some other candidate compounds, for the intervention of glaucoma, as well as prospects and challenges ahead in this field.

NO and Glaucoma

NO promotes conventional aqueous humor drainage

As a lipophilic molecule, NO can rapidly penetrate through the phospholipid bilayer and bind to targets in cytoplasm, of which sGC is the main target [19-20]. sGC is a heterodimeric protein with heme, consisting of an α subunit and a *β* subunit. Binding with Fe^{2+} center of heme in sGC, NO activates sGC to convert guanosine triphosphate (GTP) into second messenger cGMP, which regulates the functions of many downstream effectors, such as protein kinase G (PKG), cyclic nucleotide phosphodiesterase (PDEs) and cyclic nucleotide-gated ion (CNG) channels, triggering a series of cascade reactions, such as vascular smooth muscle relaxation, platelet aggregation inhibition, myocardial contractility inhibition, and nerve excitation. In eyes, elevated PKG through NO/sGC/cGMP pathway can directly activate myosin light chain phosphatase (MLCP), which causes dephosphorylation of myosin light chain (MLC), bends protein conformation and decreases bind ability with actin, thus leading to cell relaxation [21]. In the aqueous humor outflow conventional pathway, NO induces the TM and SC cells relaxation, decrease cells volume, enlarges cells gap, decreases outflow resistance

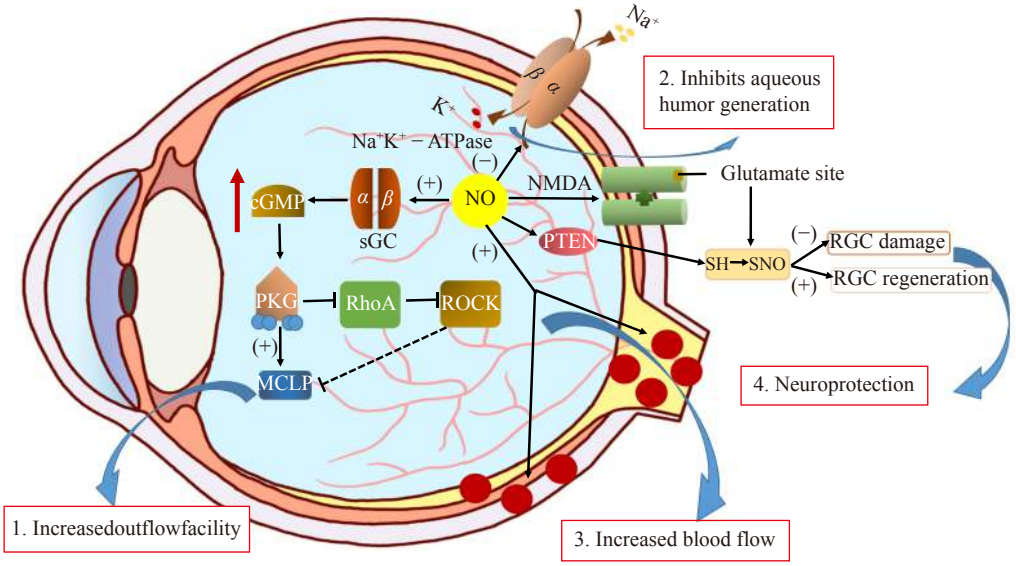

Fig. 1 Summary scheme of NO-mediated therapy for glaucoma. (1) NO targets sGC to convert GTP into cGMP. cGMP activates PKG, which directly activate MLCP. Meanwhile PKG blocks the activation of RhoA to prevent inhibition of myosin phosphatase by ROCK. These actions lead to TM and SC relaxation, and increase outflow facility. (2) NO inhibits Na^+ K^+-ATPase in the ciliary process, and reduces aqueous humor secretion. (3) NO and downstream effectors such as cGMP participate in blood flow autoregulation. (4) NO induces S-nitrosylation of NMDA type glutamate receptors and PTEN, thus protecting RGC and axons

and eventually lowering IOP. Another aspect, Rho belongs to the Ras super family of low molecular weight GTPases, which consist of Rho A, Rho B, and Rho C. Rho associated coiled-coil forming protein kinase (ROCK) is one of the most typical effectors of Rho A. Activation of ROCK promotes MLC phosphorylation and increases the contractility of actin-myosin. Thus, inhibition of Rho/ROCK pathway may promote relaxation of the TM to increase aqueous outflow [22]. Sauzeau *et al*. found that NO can block the activation of Rho A *via* NO/cGMP/PKG pathway, inhibiting trabecular network contraction and promoting aqueous humor outflow [23].

NO inhibits aqueous humor generation

The ciliary process is a double-layer structure composed of pigmented epithelium (PE) at one side and non-pigmented epithelium (NPE) cells at the other side, which are coupled by gap junctions. PE cells connect with choroid and is responsible for the absorption of solute from blood, while NPE cells mainly secrete aqueous humor. The blood-aqueous humor barrier is located at the NPE side, preventing the backflow of aqueous humor and limiting the diffusion of proteins and other macromolecules, as well as sodium ions. Na^+, K^+-ATPase, as a carrier protein consisting of α and β subunits, locates on cell membrane in the ciliary process and controls the transportation of Na^+ and K^+. The α1 subunits, existing in the basolateral side of PE cells, play an important role in controlling the overall sodium ion secretion, while the α2 subunits, mostly concentrated on the side of NPE facing aqueous humor, control the sodium ion to enter the ciliary epithelial layer through PE. Under normal physiological conditions, Na^+, K^+-ATPase moves 3 sodium ions out of cell and moves 2 potassium ions into the cell to maintain the high potassium and low sodium ions liquid environment and suitable cell osmotic pressure, providing the necessary ion gradient for aqueous humor secretion and transportation. Therefore, inhibition of Na^+, K^+-ATPase in the ciliary process can significantly reduce the production of aqueous humor [24-25]. Early studies had demonstrated that NO and NO donor sodium nitroprusside (SNP) inhibited Na^+ K^+-ATPase in the choroid plexus [26], trachea [27], and kidney tubule [28]. Shahidullah *et al*. reported in 2001 and 2005 that SNP reduced the secretion of aqueous humor *via* directly acting on the ciliary epithelium (CE) cells and decreased IOP in the eyes of cattle and pigs [29-30]. In 2013, it was revealed that SNP inhibited the activity of Na^+ K^+-ATPase in the isolated NPE epithelial cells from pig eyes in a dose-dependent manner. Further studies found that PKG inhibitors significantly abrogated the inhibitory activity of NO donors or cGMP analogues on Na^+ K^+-ATPase, suggesting that the NO/sGC/cGMP/PKG pathway is involved in Na^+ K^+-ATPase inhibition by NO [10, 31].

NO regulates eye blood flow

At present, there are two main mechanisms of optic nerve damage in glaucoma. One is mechanical compression, that is, optic nerve damage is caused by elevated IOP. However, at least one third of glaucoma patients possess normal IOP, suggesting that high IOP may be a symptom of glaucoma, but may not be a decisive factor. For these patients, blood flow disorders, as the other mechanism, could be particularly prominent in the process of glaucoma. Flammer *et al*. believed that long-term insufficiency of blood supply to the optic nerve head would lead to damage of the ganglion cells [32]. Additionally, Bata *et al.* observed abnormal automatic regulation of optic nerve head blood flow (ONHBF) in POAG patients. Nevertheless, there is no direct evidence that insufficient blood flow can cause glaucoma [33]. Dorner *et al*. found that L-NMMA, an NOS inhibitor, significantly reduced the diameter of retinal artery and vein, and decreased blood flow in choroid and retina, resulting in high IOP. These results indicated that NO can maintain the basic blood flow in eye tissue, regulate the tension of retinal blood vessels and promote the relaxation of blood vessels [11]. In addition, L-NMMA can also affect the response of ocular vascular system to the change of ocular perfusion pressure. Lasta *et al*. found that inhibition of NOS by L-NMMA altered the response of the retinal vein to isometric exercise, suggesting that NO participates in retinal blood flow regulation during isometric exercise [34]. Furthermore, the detailed mechanism of NO involved in ocular blood flow regulation remains to be elucidated, which may be related to the regulation of intracellular Ca^{2+} *via* by NO-sGC-cGMP pathway [35].

Neuroprotection of NO

As a progressive neurodegenerative disease, glaucoma causes the death of retinal ganglion cells (RGC) and axons, eventually leading to visual field defects and vision loss. The underlying mechanism is complicated with many influential factors. Direct regulation of intracellular redox state and protection of RGC from injury or death may be a new treatment for glaucoma. Mizuno *et al*. reported that the neuroprotective effect of nipradilol (a NO donating β receptor blocker) involved NO. This is because that NO induced *S*-nitrosylation of *N*-methyl-D-aspartate (NMDA) type glutamate receptors, thus downregulating the receptors activity and inhibiting NMDA-induced RGC damage [12]. In addition, Watanabe and Koriyama *et al*. found that nipradilol also induced *S*-nitrosylation of phosphatase and tensin homolog deleted on chromosome ten (PTEN), which activated mammalian target of rapamycin (mTOR)/protein kinase B (Akt) pathway to promote RGC axon regeneration in cat eyes [13-14]. However, as a double-edged sword, NO could aggravate RGC death when the concentration increases. Olney *et al*. reported that increased IOP lead to excessive glutamate production which induced retinopathy, also known as "excitotoxicity" [36-37]. During this pathological process, over activation of NMDA receptor stimulate NOS to synthesize a large amount of NO, meanwhile, high IOP also decrease the activity of antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GSH PX), catalase (CAT) and thioredoxin (TRX), inducing oxidative stress in RGC. The reaction of NO with superoxide free radical forms toxic peroxynitrite anion ($ONOO^-$), accelerating RGC apoptosis *via* nitration mechanism [38]. Nevertheless, once glutamate concentration increases, neurons may downregulate NMDA receptors, reducing cell sensitivity to glutamate [12]. Therefore, exogenous NO release for the treatment of glaucoma should consider dosing, avoiding side effects induced by excess of NO.

NO Donating Anti-glaucoma Drugs

Based on the NO-mediated therapy for glaucoma, NO donating anti-glaucoma drugs have been attracted many attentions and achieved great advances. Most of NO-donating anti-glaucoma drugs consist of a conventional anti-glaucoma drug to which a NO-releasing group is attached covalently *via* a linker. After administration *via* eye drops, NO-donating anti-glaucoma drugs would decompose to regenerate parent anti-glaucoma drugs and NO, generating additional or synergistic anti-glaucoma effect. Importantly, with limited dosing, few of NO could pass blood-aqueous humor barrier to distribute whole body, avoiding side effects and enhancing druggability.

NO-Prostaglandin analogs

Prostaglandins (PG), a kind of unsaturated fatty acids, play an important physiological regulatory role in blood vessels, smooth muscle, gastrointestinal tract, nervous system and respiratory system by binding with specific receptors. In eyes, among 9 subtypes of prostaglandin receptor, FP and EP_{1-4} are mainly distributed in the ciliary body and sclera, and less distributed in TM and SC [39]. It is found that prostaglandin F2α (PGF2α) can activate FP and EP receptors on the uveoscleral outflow pathway, relax ciliary muscle and increase aqueous outflow, thus effectively reducing IOP [40]. Latanoprost, as a PGF2α analogue, is the first anti-glaucoma drug approved by FDA in USA, which is suitable for the patients with open-angle glaucoma who are intolerant to other IOP lowering drugs. Other PGF2α analogues such as bimatoprost, travoprost and tafluprost were later approved for the treatment of glaucoma. These molecules, as ester prodrugs, activate the PG receptor in the form of free acid after hydrolysis to exert physiological activity.

Since NO can promote aqueous outflow via the conventional pathway and prostaglandin analogues increase aqueous outflow via uveoscleral outflow pathway, NO donating prostaglandin analogs could possess two different IOP-lowering mechanisms with synergistic anti-glaucoma effect, which have attracted many attentions. Based on the structure of latanoprost, NicOx SA developed a NO donating latanoprost derivative vyzulta (latanoprostene bunod, LBN eye drops, 0.024%, Fig. 2) for the treatment of glaucoma, which was approved by FDA in 2017. After administration, LBN can be rapidly hydrolyzed in eyes to latanoprost acid (LA) and butanediol mononitrate (BDMN) (Fig. 2). LA binds to FP receptor in the eyes, upregulates the release of matrix metalloproteinase (MMP), enhances extracellular matrix digestion, and improves the outflow via uveoscleral pathway. Meanwhile, BDMN, as an NO donor moiety, is further metabolized to inactive 1, 4 butanediol and NO. It was demonstrated that LBN can increase intracellular cGMP levels by activating sGC in primary human TM cells, and LBN-mediated relaxation of TM cells was also found to be more significant than equivalent of latanoprost [41].

Early studies found that transient ocular hypertensive (tOHT) rabbits were sensitive to most IOP-lowering drugs,

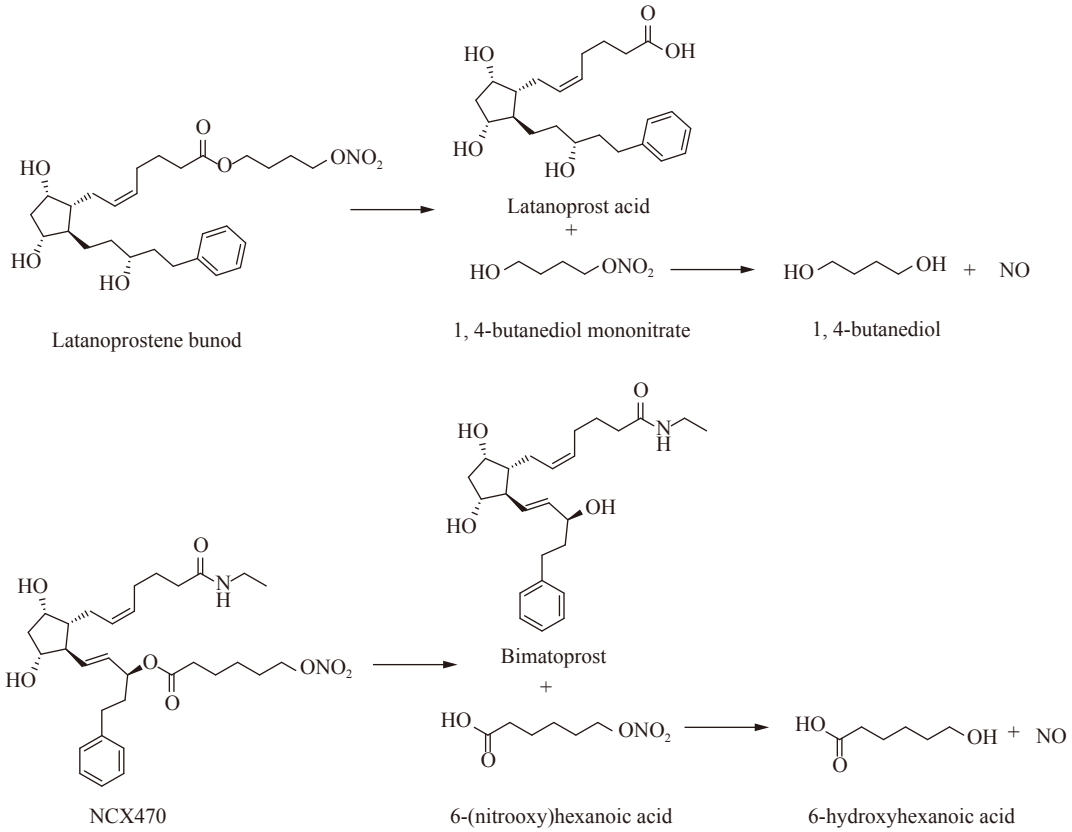

Fig. 2 The structures of latanoprostene bunod, NCX 470 and the metabolic pathway

except PGF2α receptor agonists, so the role of NO in LBN could be directly observed by using this model. In the tOHT rabbit model, LBN (0.036%) significantly decreased IOP for 13.2 mmHg during 0.5–1.5 hours after administration, while equivalent latanoprost had no effect on IOP. In both a laser-induced ocular hypertension model in nonhuman primates and a glaucoma dog model, LBN exhibited more potent IOP lowering effect than equivalent latanoprost [17]. Furthermore, in a prostaglandin FP receptor knockout mouse model, it was found that the IOP lowering effect in LBN-treated group was significantly greater than latanoprost group [42]. These experimental data in different animal models suggested that the additional IOP-lowering effect of LBN may be attributed to NO *via* the conventional aqueous humor outflow pathway.

In VOYAGER phase II clinical studies, LBN (0.024%) reduced IOP more 1.23 mmHg than latanoprost (0.005%). During 28 days, LBN (0.024%) was well tolerated without aggravating ocular hyperemia, which was similar with the side effects of latanoprost (0.005%) [43-44]. In APOLLO and LUNAR Phase III studies, once-daily dosing LBN (0.024%) exhibited more potent IOP-lowering effect than twice-daily dosing timolol (0.5%) at all 9 time points over the three-month period [45-46]. In the JUPITER study, LBN was evaluated through one year of treatment among Japanese patients with OAG or OHT. The data showed that LBN treatment resulted in a significant reduction in IOP from week 4 to week 52. At week 52.9% of treated eyes had an increase in iris pigmentation [47].

NCX 470 is another NO donating PGF2α analogue which is obtained by esterification of 15 hydroxyl in bimatoprost with 6-hydroxyhexanoic acid nitrate (Fig. 2). Similar with LBN, NCX 470 reduces IOP *via* uveoscleral outflow pathway mediated by bimatoprost as well as conventional aqueous humor outflow pathway triggered by NO. It was reported that NCX 470 significantly reduced IOP in tOHT rabbits, and this effect may depend on NO, not on bimatoprost and bimatoprostic acid. In both an ocular normotensive (ONT) dog model and an OHT monkey model, it was found that the IOP lowering effect of NCX 470 (0.014%) was even better than that of three equivalents of bimatoprost, suggesting the contribution of NO on IOP-lowering effect [48]. Bastia et al. reported a 28-day ocular toxicity study about NCX 470 [49]. It was showed that topical administration of NCX 470 (0.1%) to Beagle dogs in a GLP compliant condition twice daily for 28 consecutive days didn't cause any systemic or ocular adverse findings. The results suggested adequate safety and efficacy to support advancing NCX 470 into phase 2 clinical development.

NO donating β-adrenergic receptor antagonists

There are two kinds of adrenergic receptors: α receptors and β receptors. β receptors are divided into β1 and β2 receptor. In eyes, α receptors are mainly distributed in the ciliary processes and β receptors are mainly distributed in the pupillary sphincter and the ciliary muscle. β-Blockers decrease the production of aqueous humor *via* blocking ciliary epithelial sympathetic nerve terminals, thereby reducing IOP, and the representative drugs are non-selective β-blocker timolol and β1-selective blocker betaxolol. Nipradilol, which was marketed in Japan in 1988, is a NO-donating β-adrenoceptor antagonist (Fig. 3). It was reported that the lowering IOP effect of single administration of nipradilol (0.25%) was significantly better than timolol (0.5%) in rabbit eyes. Moreover, administration of nipradilol (0.25%) twice daily for eight weeks reduced IOP by about 4 mmHg in patients with ocular hypertension, without significant change in blood pressure and heart rate. The experiment demonstrated that nipradilol had the effect of increasing uveoscleral outflow of aqueous humor and reducing aqueous humor production [50-51]. In addition, the neuroprotective effect of nipradilol is because of the production of NO, which can reduce RGC damage and promote RGC axon regeneration, as described above.

Valerio *et al*. reported a novel group of NO-donating timolol derivatives which can release timolol and NO under physiologically relevant conditions. They adapted a triggering release strategy to designe new derivatives (representative compound **1**) in which the enzymatic hydrolysis triggered NO donor moiety release and the subsequent intramolecular cyclization produced timolol (Fig. 3). These conjugates combining timolol- and NO-mediated properties may have better IOP lowering activity *in vivo* [52].

NO donating carbonic anhydrase inhibitors

Carbonic anhydrase (CA) mainly exists in the ciliary process, playing a major role in the regulation of aqueous humor production. Inhibition of CA reduces the secretion of bicarbonate, thus reducing the production of aqueous humor and reducing IOP [53]. Due to the distribution of CA in multiple tissues throughout the body, the first generation of oral carbonic anhydrase inhibitors (CAIs) such as acetazolamide, methazolamide, and ethoxzolamide produced systemic side effects and their clinical utilities had been progressively decreased. The second generation of topical CAIs, represented by dorzolamide and brinzolamide, became available for the treatment of ocular hypertension since 1990s (Fig. 4).

Steele *et al*. designed and synthesized a series of NO donating CAIs (**2-3**, Fig. 4) by coupling the amine group of dorzolamide with NO-donor linkers bearing nitrate ester *via* amide or carbamate, respectively. Interestingly, this modification did not affect the binding of compounds **2-3** with CA, producing potent CA inhibitor activity similar with dorzolamide. With NO release, compounds **2-3** possessed dual aqueous humor production inhibitory and aqueous humor outflow promoting activities. In normotensive New Zealand white rabbits, compound **2** (−4.7 mmHg) exhibited more potent IOP lowering activity than dorzolamide (−3.8 mmHg) and NO donor drug isosorbide mononitrate (ISMN, −1.9 mmHg). Meanwhile, compound **3** (120 mins) showed prolonged Tmax than dorzolamide (60 mins) [54]. Based on the cocrystal structure of dorzolamide and dorzolamide with the CA enzyme, Huang *et al*. selected alkyl side chains, which pointing toward the solvent, to modify nitrate ester NO donor moieties to offer compounds **4** and **5** (Fig. 4). Expectedly, **4**–**5** displayed good CA enzymatic potency, comparable to dorzol-

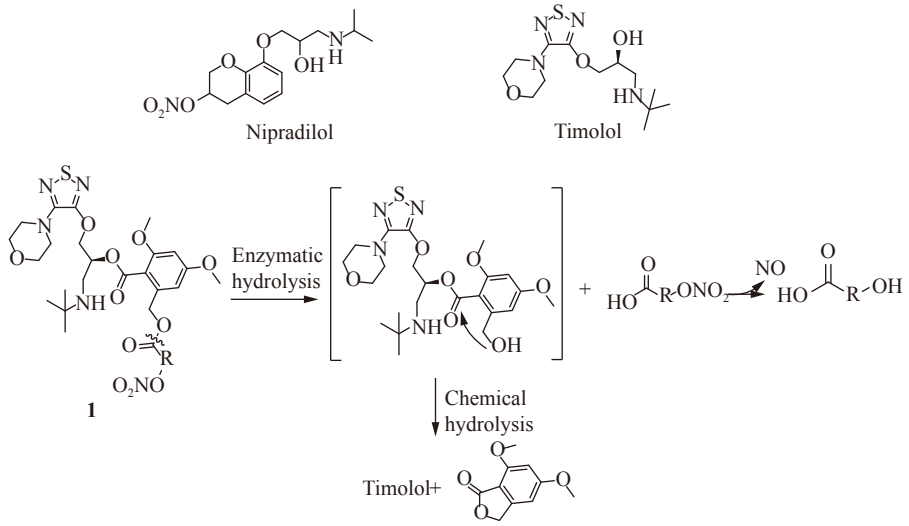

Fig. 3 Structures of nipradilol, timolol, and NO donating timolol derivative 1

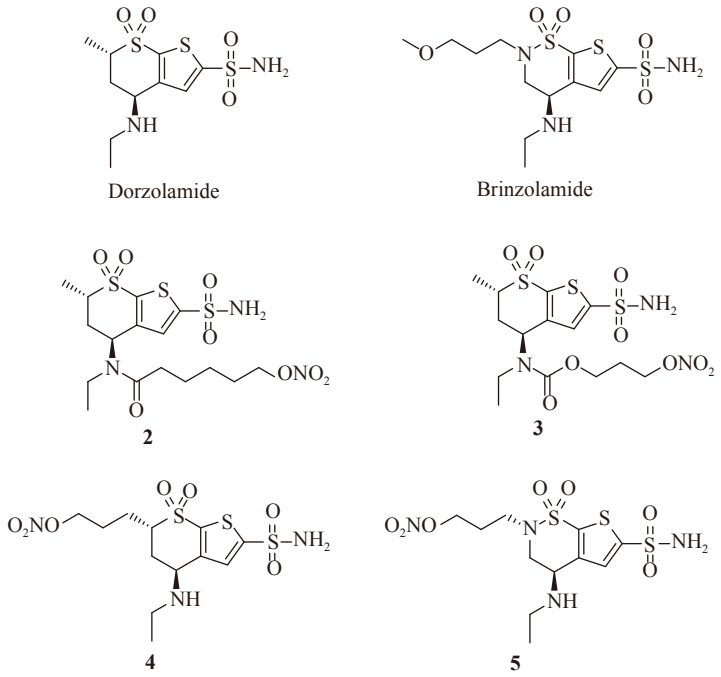

Fig. 4 Structures of dorzolamide, brinzolamide and NO donating CAIs 2-5

amide and brinzolamide. In normal rabbits, **4** and **5** exhibited improved IOP lowering activity than brinzolamide, while in OHT primate model, **5** significantly lowered IOP than brinzolamide, suggesting the potential synergistic effect of NO releasing and CA inhibition [55].

Other NO donating anti-glaucoma molecules

Oxidative stress and mitochondrial dysfunction generate superoxide radicals, which react with NO to generate $ONOO^-$, thus reducing NO bioavailability. Acharya et al. designed SA-2, a NO-donating antioxidant hybrid (Fig. 5), which can protect RGCs by releasing NO as well as scavenging free radicals to exert antioxidant activity and thus increase NO bioavailability. Cell experiments showed that SA-2 upregulated SOD enzyme levels and reduced H_2O_2-induced cell damage in photoreceptor cells, and enhanced the cell viability against the treatment of H_2O_2 [56]. It was also pointed out that SA-2 spontaneously released NO in a short time at pH 7.4, which can promote angiogenesis, inhibit the proliferation and migration of smooth muscle cells and protect endothelial cells under oxidative stress [57]. SA-2 showed promising therapeutic and preventive effects in three models of acute RGC injury: hypoxia, optic nerve crush (ONC), and ischemia-reperfusion (I/R), which was mediated by upregulation of SOD enzyme levels [58].

A novel NO donor NCX 667 was reported by Bastia *et al*. (Fig. 5). It was effective in lowering IOP in ONT, OHT rabbit models and OHT nonhuman primate models, and multiple doses were well tolerated [59-60]. Combination of NCX

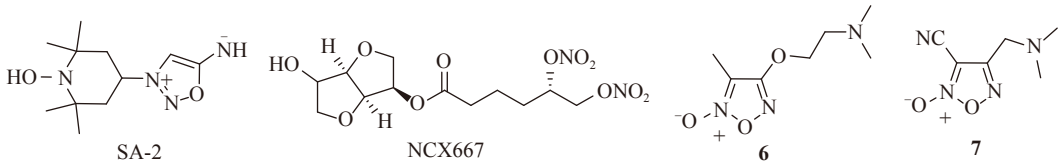

Fig. 5 Structures of SA-2, NCX-667, and furoxan derivatives 6 and 7

667 with the PGF2α analogue travoprost significantly reduced the IOP of normal rabbits within 5 hours after administration [61].

Furoxan can release NO under the action of mercaptan cofactor, and the amount of NO can be regulated by the 3-substituents on the ring. The 4-substituents mainly affect the hydrophilic-lipophilic balance of the molecules. Because of the special properties of furoxan, Blangetti *et al*. synthesized a series of furoxan derivatives, and detected the IOP reduction effect of the target molecule in the tOHT model. It was found that compounds **6** and **7** produced similar hypotensive effects to timolol after 60 minutes of dosing, and **7** had a longer duration of action and retained activity at 120 and 240 minutes after dosing. Interestingly, after comparing the NO-releasing ability and the hydrophilic-lipophilic balance of these molecules, it was found that the antihypertensive ability of these compounds may not be determined by the amount of NO released, but depending on the hydrophilic-lipophilic balance [62].

Besides NO donating small molecules, some researches focus on macromolecular NO delivery system to achieve sustained and prolonged NO release. Chandrawati *et al*. designed a liposome loaded with β-gal-NONOate, and delivered it to TM together with the carrier coated with β-galactosidase. β-gal-NOate released NO in time and dose-dependent manners through enzymatic reaction, generating IOP lowering effect [63]. In addition, based on mesoporous silica nanoparticles, Hu *et al*. designed a novel drug delivery system (SNP@MSNs) to deliver SNP to the TM and SC, releasing more NO over a prolonged period with IOP reduction for up to 48 hours [64]. The study of NO delivery system provides a new strategy to improve the bioavailability of NO, which also opens a new avenue for NO donating anti-glaucoma drug research.

Conclusion and Prospects

NO was first discovered in the 1870s, and had been ignored as a pollutant for a long time. Until 1986, Dr. Ignarro and Furchgott proposed that the endothelium-derived relaxing factor is NO, and NO became a "star molecule" and attracted more and more attentions. As outlined in Fig. 1, NO can increase aqueous humor outflow, inhibit aqueous humor generation, increase blood flow and protect RGC and axons, producing potent IOP lowering activities for the intervention of glaucoma. Compared with NO donating drugs for the treatment of other diseases, NO donating anti-glaucoma drugs possess the following advantages and features. 1) Eye drops administration could limit NO in eyes and avoid side effects mediated by wide distribution, enhancing druggability of this class of NO donating agents. As a matter of fact, LBN was the only NO donor drug approved by FDA in the last decade. Notably, naproxcinod, a NO donating nonsteroidal anti-inflammatory drug, which finished phase Ⅲ trials involving about 4 000 patients, was declined to approve by FDA in 2010, with oral administration and uncontrolled NO release as one concern. 2) It is easy to control the dose of NO released from NO donating agents by eye drops formulation, avoiding potential inflammation and optic nerve degeneration mediated by high concentrations of NO. 3) It is commonly accepted that in combination of two or three different topical anti-glaucoma drugs could realize better response of lowering IOP than monotherapy. Coupling NO donor moiety with other anti-glaucoma agents would supply more chances to achieve multiple mechanisms within one single small molecule.

Although some advances including FDA approved drugs were achieved in this field, there are some challenges ahead in this field. Current NO donating anti-glaucoma agents are nitrate ester, so nitrate tolerance could be a potential concern for this class of anti-glaucoma agents especially for long term use. Nitrate tolerance is a phenomenon that the organic nitrates lose their hemodynamic and clinical effectiveness during sustained therapy [65]. The mechanism of nitrate tolerance remains unclear, and the main hypothesis is the gradual reduction of nitrate bioactivation which involves mitochondrial ROS formation and the mitochondrial aldehyde dehydrogenase [66]. To this end, other NO donors including furoxans, diazeniumdiolates, and metal-NO complexes warrant further studies to investigate the therapeutic potential for glaucoma. Beside classic NO/sGC/cGMP pathway, protein S-nitrosylation (SNO) could be an important mechanism for NO signaling, which could be another challenge in this field. Proteomic technologies and new SNO probes will supply more improved methods for exploring this post-translational modification to understand the mechanisms of action underlying NO-based anti-glaucoma activity [67].

In summary, it is believed that with the deepening research on glaucoma and NO, more progress will be made in the study of NO donating anti-glaucoma agents and drugs.

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