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REVIEW ARTICLE

Prostaglandin analogues and nitric oxide contribution in the treatment of ocular hypertension and glaucoma

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In patients with ocular hypertension or glaucoma, all treatments aim to lower intraocular pressure (IOP) by modulating aqueous humour (AH) production and/or uveoscleral and trabecular meshwork/Schlemm's canal AH drainage. PG analogues are considered to be the 'gold standard' treatment and are the most frequently used IOP-lowering agents. Recent data support an important role for NO in regulating IOP. Thus, novel PG analogues carrying a NO-donating moiety were recently advanced. Latanoprostene bunod (LBN) and NCX 470, NO-donating derivatives of latanoprost and bimatoprost, respectively, are examples of such compounds. LBN ophthalmic solution, 0.024% (Vyzulta™), showed greater IOP-lowering efficacy compared with that of Xalatan® (latanoprost ophthalmic solution, 0.005%) or 0.5% timolol maleate in clinical settings. NCX 470 was found to be more effective than bimatoprost in animal models of ocular hypertension and glaucoma. Selective EP$_2$ receptor agonists (i.e. taprenepag isopropyl, omidenepag isopropyl and aganepag isopropyl) and non-selective prostanoid receptor agonists (i.e. ONO-9054, sepetaprost isopropyl) that concomitantly stimulate FP and EP$_3$ receptors have also been shown to hold promise as effective IOP-lowering agents.

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Abbreviations

AH, aqueous humour; IOP, intraocular pressure; LBN, latanoprostene bunod; SC, Schlemm's canal; TM, trabecular meshwork

Introduction

Prostaglandins elicit various biological effects including regulation of smooth muscles, inflammation and immune response. Pioneer studies characterizing the effects of PGs in ocular compartments mostly describe significant ocular irritation and an initial intraocular pressure (IOP) elevation following topical ocular dosing. Later studies, however, demonstrated that the topical administration of **$PGF_{2\alpha}$** and **PGE_2** to rabbits decreases IOP in a dose-dependent fashion, thus establishing, for the first time, that stimulation of the respective $PGF_{2\alpha}$ receptors (**FP receptor**) and PGE_2 receptors (mainly **EP_2** and **EP_3**) could be novel approaches to effectively treat ocular hypertensive and glaucomatous patients (Camras *et al.*, 1977). These initial findings led several independent laboratories and worldwide pharmaceutical companies to devote large research efforts to the identification of potent and selective synthetic compounds that are able to stimulate FP receptors and, more recently, EP_2 and EP_3 receptors.

The first $PGF_{2\alpha}$ analogue, **latanoprost**, received Food and Drug Administration (FDA) approval in 1996 (Xalatan®, latanoprost ophthalmic solution, 0.005%) as the second-line treatment for patients with open-angle glaucoma who are intolerant or unresponsive to other IOP-lowering products. **Bimatoprost** (Lumigan®, bimatoprost ophthalmic solution, 0.03%), **travoprost** (Travatan®, travoprost ophthalmic solution, 0.004%), **tafluprost** (Zioptan®, tafluprost ophthalmic solution, 0.0015%) and the partial agonist, **unoprostone** (Rescula®, unoprostone isopropyl ophthalmic solution, 0.15%), were subsequently approved. Latanoprost, travoprost and tafluprost are ester prodrugs of their more active respective de-esterified free carboxylic acid derivatives. Controversy exists for bimatoprost since it is an amide derivative and less prone to release the respective carboxylic free acid in biologically active environments (Woodward *et al.*, 2008). All of these compounds exert a robust and sustained IOP-lowering effect in animal models and affected patients, primarily by stimulation of aqueous humour (AH) outflow through the interstitial spaces of the ciliary muscle into the suprachoroidal space of the eye, where AH drains into the ciliary body capillaries and lymphatic vessels. This pathway is known as the unconventional or uveoscleral outflow pathway and, in normal individuals, usually accounts for 20–40% of the total AH drainage from the eye. The remaining (60–80%) AH drainage flows out of the eye *via* the conventional pathway where AH is conveyed into the trabecular meshwork (TM), Schlemm's canal (SC) and endothelium, lumen and aqueous veins (Gabelt and Kaufman, 2005; Alm and Nilsson, 2009; Winkler and Fautsch, 2014). This latter pathway is considered pressure-dependent and is poorly affected by PGs. However, it seems highly sensitive to **NO**, a key signalling molecule responsible for mediating a wide array of physiological roles across multiple biological systems including the eye (Aliancy *et al.*, 2017) where it stimulates soluble **GC** activity and **cGMP** formation, thereby leading to ciliary muscle relaxation and enhanced conventional outflow facility (Cavet *et al.*, 2014; Muenster *et al.*, 2017). Of note, NO also regulates ocular blood flow and may promote retinal ganglion cell survival in the eye (Pasquale, 2016).

The complementary mode of action of PGs and NO in the eye is best evidenced in recently identified compounds combining FP agonistic activity and NO-mediated GC activation/cGMP formation that are being developed to treat ocular hypertension and glaucoma. The recently (November 2017) FDA-approved drug Vyzulta™ [**latanoprostene bunod** (LBN) ophthalmic solution, 0.024%], which is an NO-donating derivative of latanoprost, is the most advanced compound from this class. Similar technology is applied to the less advanced compound, NCX 470, which is an NO-donating derivative of bimatoprost.

Somewhat interesting are also EP_2 receptor-selective agonists, which have recently been reported to effectively lower IOP (Prasanna *et al.*, 2011; Schachar *et al.*, 2011; Aihara *et al.*, 2017). From this class, the most advanced is omidenepag isopropyl (DE-117), which is being co-developed by Santen and Ube Industries and is currently in phase 3 clinical trials. Likewise, the EP_3/FP receptor agonist, ONO-9054, is currently being developed by Santen and ONO Pharmaceutical for the reduction of IOP in patients with ocular hypertension and glaucoma (Miller Ellis *et al.*, 2017).

Prostanoid receptors in the eye

Nine **prostanoid receptors** localized in the cell membrane and nuclear envelope have been described, namely, the FP receptor (most potent endogenous ligand: $PGF_{2\alpha}$); **EP_1**, EP_2, EP_3 and **EP_4** receptors (most potent endogenous ligand: PGE_2); **DP_1** and **DP_2** receptors (most potent endogenous ligand: **PGD_2**); **IP** receptor (most potent endogenous ligand: **PGI_2**); and **TP** receptor (most potent endogenous ligand: **TxA_2**) (Alexander *et al.*, 2017a). In addition, there is pharmacological evidence for the existence of an as yet unidentified specific prostamide receptor (Woodward *et al.*, 2008). The molecular structure of all the prostanoid receptors identified is typical of that for GPCRs and consists of seven α-helical transmembrane domains, three loops and an amino terminus lining in the extracellular compartment and three loops and a carboxyl terminus intracellularly.

In ocular tissues, only FP and EP_{1-4} receptors have been demonstrated in most compartments. The EP_{1-4} and FP receptors are mostly localized in uveoscleral tissues where they are found in ciliary body and sclera. In these tissues, FP receptors seem the most abundant and are specifically localized in the circular portion of the ciliary muscle (Schlötzer-Schrehardt *et al.*, 2002). The FP and EP_{1-4} receptors are also found within the TM and SC (Anthony *et al.*, 1998; Schlötzer-Schrehardt *et al.*, 2002). In the TM, the gene expression of the EP_2 receptor is the most abundant, followed by FP, EP_1 and EP_4 receptors with EP_3 receptors having the lowest levels. EP_1 receptor staining is predominantly found in TM cells and in cells lining the SC, while EP_2 receptors seem mainly to be localized within the wall and periphery of the SC. EP_3 and EP_4 receptors are present throughout the entire meshwork. FP receptor protein expression is found in the outer portion of the TM and in SC endothelial cells, collector channels and aqueous vein (Kamphuis *et al.*, 2001).

In more superficial structures, EP_1 receptor protein is found in the epithelia of the cornea and in the conjunctiva. Likewise, EP_2 receptor labelling is also prominent in the corneal epithelium and choriocapillaris, while EP_3 and EP_4 receptors are primarily observed in the corneal endothelium and keratocytes, as well as in conjunctival and iridal stroma cells. The FP receptor protein is mainly present in the corneal epithelium (Schlötzer-Schrehardt et al., 2002).

NO signalling in the eye

Since the discovery of NO in 1987, research on this molecule has expanded in many directions. Most recently, NO and its second messenger cGMP were shown to be important for multiple functions in the eye including IOP homeostasis (Cavet et al., 2014; Aliancy et al., 2017; Muenster et al., 2017) and blood flow modulation (Schmidl et al., 2013). Under physiological conditions, NO is synthesized by the constitutive NOS isoforms, neuronal NOS (**nNOS**) and endothelial NOS (**eNOS**) that catalyse the oxidation of L-arginine to form NO and L-citrulline in a Ca^{2+}/calmodulin-dependent manner. NO produced in low nM concentrations by nNOS and eNOS activates soluble GCs, thereby leading to cGMP formation and activation of protein kinases that ultimately produce biological functions (Ignarro, 1991). Other NO-mediated activities may occur through cGMP-independent pathways such as post-translational modification of proteins by S-nitrosylation or transcriptional modulation of protein synthesis via regulation of the transcription factor NF-κB (Colasanti and Suzuki, 2000). In pathological conditions, where inflammation or ischaemia is prominent, NO is produced by a third NOS isoform, namely, the inducible NOS (**iNOS**); iNOS works in a Ca^{2+}-independent manner and generates large amounts of NO (μM range) that are generally considered detrimental to exposed tissues.

While nNOS is mainly found in ciliary processes and in nerve endings, intense nNOS expression is found in the anterior segment of the eye, not only in the vascular endothelium and perivascular nerve fibres but also in the non-pigmented epithelium of ciliary processes, ciliary muscle, TM, SC and collecting channels (Nathanson and McKee, 1995). These structures efficiently respond to NO and play a pivotal role in regulating AH dynamics and IOP homeostasis in physiological and in pathological conditions such as ocular hypertension and glaucoma (Aliancy et al., 2017).

Ocular hypertensive patients were shown to have reduced NO formation compared with healthy subjects (Nathanson and McKee, 1995), and exogenously administered NO was shown to lower IOP in these patients (Nathanson and McKee, 1995). Moreover, recent genetic studies showed that polymorphisms in eNOS are associated with a higher risk of glaucoma (Kang et al., 2010). Further confirming the importance of NO signalling in regulating IOP, eNOS-deficient mice (Lei et al., 2015) or animals with impaired GC activity (Buys et al., 2014; Muenster et al., 2017) were shown to have higher IOP compared with their respective wild-type littermates.

PG analogues as ocular hypotensive agents

Early studies analysing the effects of PG analogues in the eye reported substantial irritation and IOP elevation following topical dosing. However, later work demonstrated that the administration of low doses of $PGF_{2\alpha}$ consistently lowered IOP after an initial transient increase (Camras et al., 1977). More recently, the introduction of knockout animals for various prostanoid receptors has been pivotal in the documentation of the specific contribution of each receptor subtype to IOP regulation. As a result of these studies, various PG analogues have been discovered that either act by stimulating FP receptors (below referred to as first-generation PG analogues), carry residues that are able to either activate other prostanoid receptors (i.e. EP_2 and EP_3) or are processed into two active metabolites each able to activate independent signalling pathways (below referred as modified PG analogues).

First-generation PG analogues

Unoprostone isopropyl and latanoprost, an isopropyl ester analogue of $PGF_{2\alpha}$ (Figure 1A–C), were initially approved by the FDA for the treatment of elevated IOP in patients
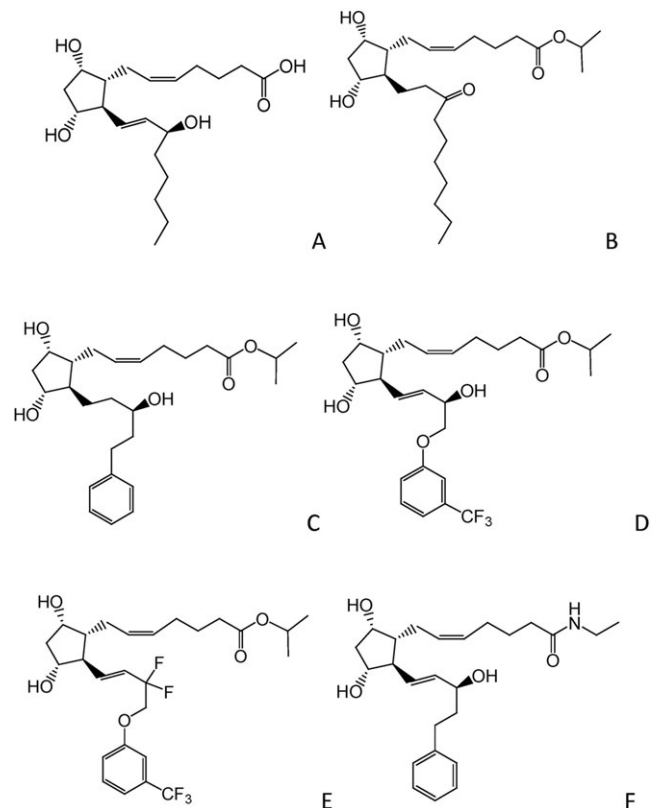

Figure 1

Molecular structures of $PGF_{2\alpha}$ and the synthetic PG analogues used to lower IOP in patients with ocular hypertension and glaucoma. (A) $PGF_{2\alpha}$, (B) unoprostone, (C) latanoprost, (D) travoprost and (E) tafluprost are prostanoids metabolized to active free acids in target ocular tissues, while (F) bimatoprost is a prostamide acting as such and via the release of the respective free acid.

with open-angle glaucoma or ocular hypertension who are intolerant of other IOP-lowering medications or insufficiently responsive to other IOP-lowering agents (Rescula, unoprostone isopropyl ophthalmic solution, 0.15%, and Xalatan, latanoprost ophthalmic solution, 0.005%) followed several years later by bimatoprost (Figure 1F) (Lumigan, bimatoprost ophthalmic solution, 0.03%), travoprost (Figure 1D) (Travatan, travoprost ophthalmic solution, 0.004%) and, more recently, tafluprost (Figure 1E) (Zioptan, tafluprost ophthalmic solution, 0.0015%). Latanoprost, travoprost and tafluprost are prostanoid analogue ester prodrugs with similar modes of action. These compounds are cleaved by tissue esterases to the respective free acid carboxylic derivative and bind, albeit with different affinity and selectivity, to FP receptors (Nakajima *et al.*, 2003). The free carboxylic acid form of tafluprost also binds with slightly less affinity to the EP_3 receptor subtype (IC_{50} = 67 nM) (Takagi *et al.*, 2004; Ota *et al.*, 2007).

Bimatoprost is the amide of 17-phenyl-$PGF_{2\alpha}$ and has been classified by some as a prodrug because low concentrations of the respective free acid, which are sufficient to stimulate the FP receptors, are found in AH following its topical ocular application (Faulkner *et al.*, 2010) and by others as a compound acting on an unidentified prostamide receptor having a unique pharmacological profile (Woodward *et al.*, 2008).

All these compounds produce a similar increase in uveoscleral outflow in animal models of ocular hypertension and glaucoma, with the exception of rabbits in which anatomical constraints make the uveoscleral outflow pathway less functional than in other species. These compounds also have been shown repeatedly to be highly effective IOP-lowering agents in ocular hypertensive and normotensive glaucomatous patients (Alm and Nilsson, 2009; Winkler and Fautsch, 2014). However, the pharmacodynamic efficacy of this class of molecules in patients seems to be less at night-time because of physiological changes in uveoscleral outflow during the night (Orzalesi *et al.*, 2006; Gulati *et al.*, 2012).

Several molecular mechanisms have been proposed for their IOP-lowering effectiveness. Remodelling of the extra-cellular matrix within the ciliary muscle and sclera *via* direct activation of the FP receptors or indirectly, by *de novo* synthesis of an endogenous PG *via* PLA_2 stimulation, is the most commonly described mechanism involved in their IOP lowering effects. Stimulation of FP receptors induces an increase in metalloproteinase (MMP) enzymes (i.e. **MMP1**, **MMP2** and **MMP3**) in the ciliary muscle, as well as in the surrounding tissues. MMPs dissolve collagenase types I and III within the connective tissue promoting specific rearrangements of the ciliary muscle and sclera that favour a more efficient uveoscleral drainage of the AH from the eye.

Less consistent are reports suggesting an effect of this class of molecules on TM/SC outflow. While it seems that all PG analogues result in a marginal yet consistent increase in TM/SC facility in the human eye, animal studies, with the exception of those in canine and primate models, have shown either no change or an increase depending on the specific PG analogue, the animal species or the experimental design. The mechanism of action for this effect may differ from that involved in the regulation of uveoscleral outflow.

For example, travoprost was reported to affect TM contractility and conventional outflow by decreasing the secretion of endothelin-1 (Thieme *et al.*, 2006), while latanoprost and bimatoprost were shown to promote the release of endogenous NO (Chen *et al.*, 2005).

Modified PG analogues

Novel NO-donating PGs with dual mechanism of action. $PGF_{2\alpha}$ analogues and NO provide robust IOP-lowering activity by concomitantly activating two independent mechanisms: uveoscleral outflow and TM/SC conventional outflow facility as a result of FP receptor activation and soluble GC/cGMP stimulation in target tissues respectively (Figure 2). Moreover, enhanced vasodilatation, anti-inflammatory effects and antiplatelet activity have been attributed to NO making this class of molecules particularly interesting as impaired ocular blood flow and inflammation seem to also contribute to glaucoma progression (Resch *et al.*, 2009; Costa *et al.*, 2014). Various NO-donating PGs have been described in recent years for their enhanced IOP-lowering activity in animal models of ocular hypertension and glaucoma compared with standard of care treatments (Borghi *et al.*, 2010; Impagnatiello *et al.*, 2011; Krauss *et al.*, 2011; Impagnatiello *et al.*, 2015). Among others, LBN, a compound discovered at Nicox and later licensed by Bausch + Lomb, has been advanced through phase 3 and recently received FDA approval for the reduction of IOP in patients with open-angle glaucoma and ocular hypertension (Vyzulta™, LBN ophthalmic solution, 0.024%,). NCX 470 is a follow-up compound fully owned by Nicox and for which an Investigational New Drug application is expected to be submitted during 2018.

Vyzulta™ (latanoprostene bunod ophthalmic solution, 0.024%). LBN is an NO-donating $PGF_{2\alpha}$ analogue (Figure 3A) that is rapidly metabolized *in situ* by esterases into latanoprost free carboxyl acid and the NO-donating moiety, butanediol mononitrate (BDMN). BDMN is further metabolized to 1,4-butanediol and NO (Krauss *et al.*, 2011). Latanoprost acid reduces IOP by increasing AH outflow primarily through the uveoscleral pathway (unconventional route), whereas NO facilitates AH outflow through the TM and SC pathways (conventional route) (Cavet *et al.*, 2015; Garcia *et al.*, 2016; Cavet and DeCory, 2017).

LBN reduced IOP to a greater degree than equimolar concentrations of latanoprost in various experimental animal models of ocular hypertension and glaucoma (Krauss *et al.*, 2011). Furthermore, LBN ophthalmic solution, 0.024%, was shown to be more effective at reducing IOP compared with latanoprost ophthalmic solution, 0.005%, in a phase 2 dose-ranging study in patients with open-angle glaucoma or ocular hypertension (Table 1) (VOYAGER study, Weinreb *et al.*, 2015). Moreover, a phase 2, open-label, randomized trial (CONSTELLATION study) found that, compared with timolol maleate ophthalmic solution, 0.5%, LBN 0.024% resulted in similar diurnal efficacy and a greater reduction in nocturnal IOP in patients with open-angle glaucoma or ocular hypertension (Table 1) (Liu *et al.*, 2016; Kaufman, 2017).

The APOLLO (Weinreb *et al.*, 2016) and LUNAR (Medeiros *et al.*, 2016) studies were nearly identically designed phase 3 safety and efficacy studies of LBN 0.024% in patients with open-angle glaucoma or ocular hypertension. Each study
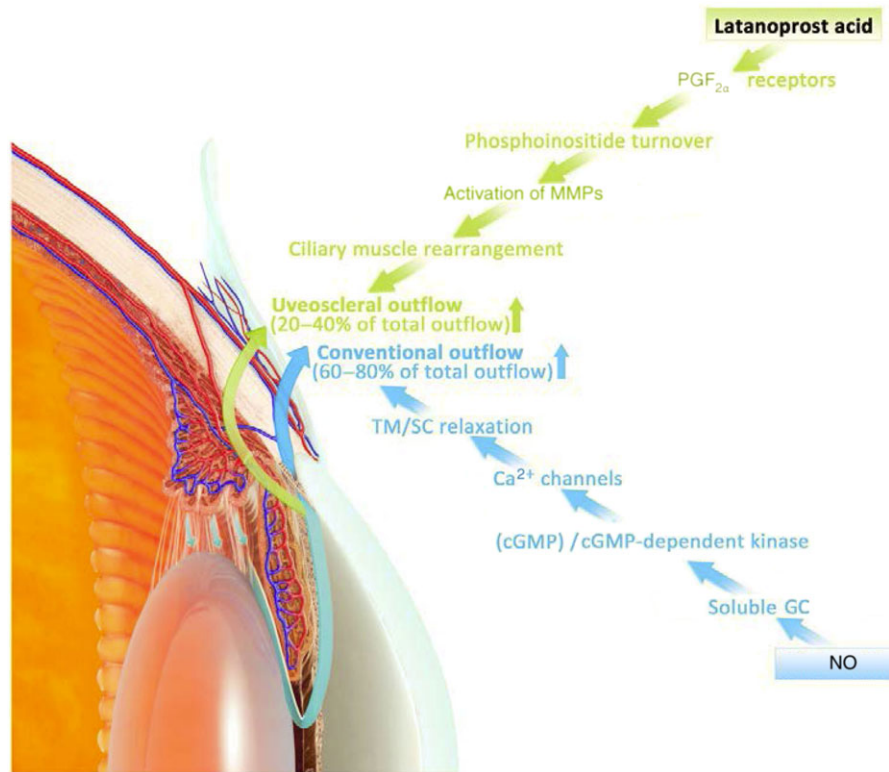
Figure 2

Diagram describing the dual mode of action of the NO-donating derivative of latanoprost, LBN. AH, produced by the ciliary body, exits the eye through the TM/SC, also known as the conventional pathway (blue arrows), and the uveoscleral pathway, also known as the unconventional pathway (green arrows). The balance between AH production and outflow *via* the conventional (pressure-dependent) and unconventional pathway determines IOP. PG analogues mostly affect the uveoscleral, unconventional outflow while NO increases the TM/SC, conventional outflow pathway.
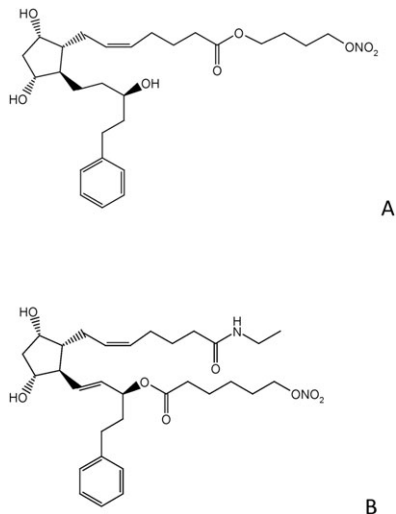

Figure 3

Chemical structures of (A) LBN and (B) NCX 470.

consisted of a 3 month active (timolol) comparator, double-masked efficacy phase followed by a 9 month (APOLLO) or 3-month (LUNAR) open-label safety extension (Table 1). At the conclusion of the 3 month phase of the studies, pooled data from the studies showed significantly lower IOP in the LBN group compared with the timolol group at all time points (least squares mean IOP in the study eye ranged from 17.8 to 18.9 mmHg and from 19.0 to 19.7 mmHg, in the LBN 0.024% QD group and timolol 0.5% BID group, respectively) (Table 1). Furthermore, the proportion of patients reaching an IOP of ≤18 mmHg was greater in the LBN group compared with the timolol group (Medeiros *et al*., 2016; Weinreb *et al*., 2016; Kaufman, 2017). Pooled results from the APOLLO and LUNAR open-label safety extension phases demonstrated that the IOP reduction reached with LBN 0.024% was maintained up to 12 months, with no apparent loss of activity over time (Vittitow *et al*., 2016). In all studies, the compound was well tolerated; the percentages of patients experiencing treatment-related adverse events, primarily eye irritation and conjuntival hyperaemia, were similar in the LBN- and timolol-treated groups. However, as might be expected for a

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Table 1

Summary of clinical studies performed with Vyzulta™ (LBN ophthalmic solution, 0.024%)

Trial features (ID)	Phase subjects (n)	Design	Treatment			Results	Reference
			Duration	Arms	Dosage	Mean change in IOP in mmHg (±SD, where noted)	
Kronus[a] (NCT01895985)	I (n = 24)	Single-centre, controlled, open-label	14 days	LBN	0.024% qPM	3.6 ± 0.8, treated eye	Araie et al. (2015)
						3.5 ± 0.9, fellow eye	
VOYAGER[b] (NCT01223378)	II (n = 413)	Multicentre, randomized, controlled, investigator-masked, dose-ranging	28 days	LBN	0.006% qPM	6.8, day 7; 7.6, day 14; and 7.8, day 28	Weinreb et al. (2015)
					0.012% qPM	7.7, day 7; 7.9, day 14; and 8.3, day 28	
					0.024% qPM	8.3, day 7; 8.9, day 14; and 9.0, day 28	
					0.040% qPM	8.5, day 7; 8.6, day 14; and 8.9, day 28	
				LAT	0.005% qPM	7.3, day 7; 7.7, day 14; and 7.8, day 28	
CONSTELLATION[c] (NCT1707381)	II (n = 25)	Single-centre, randomized, controlled, open-label, crossover	8 weeks, crossover at 4 weeks	LBN	0.024% qPM	1.1–1.2, diurnal/wake	Liu et al. (2016)
				TIM	0.5% BID	2.3 ± 3.0, nocturnal/sleep	
APOLLO[d] (NCT01749904)	III (n = 420)	Multicentre, randomized, controlled, double-masked	3 months	LBN	0.024% qPM	1.2, 1.4 and 1.1, week 2	Weinreb et al. (2016)
				TIM	0.5% BID	1.0, 1.3 and 1.3, week 6	
						1.0, 1.3 and 1.3, 3 months	
LUNAR[d] (NCT01749930)	III (n = 420)	Multicentre, randomized, controlled, double-masked	3 months	LBN	0.024% qPM	0.4, 0.8 and 0.7, week 2	Medeiros et al. (2016)
				TIM	0.5% BID	0.9, 0.8 and 1.0 week 6	
						0.9, 1.3 and 1.3, 3 months	
JUPITER[e] (NCT01895972)	III (n = 130)	Multicentre, open-label	1 year	LBN	0.024% qPM	4.3, 4 weeks	Kawase et al. (2016)
						5.3, 1 year	

LAT, latanoprost; TIM, timolol maleate; qPM, every evening; BID, twice a day.
[a]Data reported refer to IOP changes from baseline (mean ± SD) over 24 h in study and fellow-treated eyes.
[b]Data reported refer to least mean changes from baseline diurnal IOP at day 4, day 14 and day 28 study visits.
[c]Data reported refer to IOP changes between treatments during diurnal/wake period taken in sitting and supine conditions (reported as range differences) or nocturnal/sleep period taken in supine condition (reported as mean difference ± SD).
[d]Data reported refer to IOP changes between treatments recorded during different visits (week 2, week 6 and 3 months) at various time points (8:00 h, 12:00 h and 16:00 h).
[e]Data reported refer to IOP changes from baseline.

PG analogue-containing molecule, the percentage of patients with moderate to severe hyperaemia tended to be greater in patients treated with LBN compared with those treated with timolol 0.5% (Medeiros *et al.*, 2016; Weinreb *et al.*, 2016; Kaufman, 2017).

Factors other than IOP seem to be involved in glaucoma progression. Among others, defective ocular perfusion pressure has been reported in patients with glaucoma (Tielsch *et al.*, 1995; Resch *et al.*, 2009; Costa *et al.*, 2014). Of interest, in a crossover trial in patients with early open-angle glaucoma or ocular hypertension, LBN improved ocular perfusion pressure to a greater extent than timolol (Liu *et al.*, 2016). This effect is particularly important in light of the well-established effect of NO on vascular reactivity. Although additional work is needed, these findings suggest that Vyzulta™ treatment might ultimately result in a protective effect on the head of the optic nerve, which is independent of the reduction in IOP.

NCX 470.   NCX 470 is a new molecular entity composed of the prostamide bimatoprost that is structurally related to $PGF_{2\alpha}$, with the hydroxyl group in position 15 esterified with the NO-donating moiety 6-(nitrooxy)hexanoic acid (Figure 3B).

Nonclinical pharmacological studies have demonstrated that the IOP-lowering efficacy of NCX 470 is greater than that of equimolar doses of bimatoprost in well-established animal models of glaucoma and ocular hypertension. In particular, NCX 470 was shown to lower IOP in transient ocular hypertensive rabbits (previously shown to respond poorly to FP receptor agonists, Borghi *et al.*, 2010) probably by inducing the release of NO (Impagnatiello *et al.*, 2015). Additionally, NCX 470 0.042% lowered IOP more effectively than equimolar doses of bimatoprost (0.03%) in ocular normotensive dogs (Figure 4A, Impagnatiello *et al.*, 2015) and in laser-induced ocular hypertensive non-human primates (Figure 4B, Impagnatiello *et al.*, 2015). This compound holds promise to become the best in class as it adds NO-mediated efficacy to that of bimatoprost, whose Lumigan 0.03% formulation was considered to be the most efficacious PG analogue among those approved to date.

However, it remains to be seen whether the IOP-lowering effects of NCX 470 observed in animal models will be confirmed in patients.

EP$_2$ and EP$_3$/FP receptor agonists.   In recent years, several attempts have been made to find compounds affecting IOP by modulating PG signalling on multiple receptor subtypes including EP_2 or concomitantly FP and EP_3 receptors. Several of these compounds are currently in clinical development.

PF-04217329 (taprenepag isopropyl), DE-117 (omidenepag isopropyl) and AGN 210961 (aganepag isopropyl).   Recent studies have defined EP_2 receptors as an attractive target for compounds intended for treatment of open-angle glaucoma and ocular hypertension. Prasanna *et al.* (2011) reported the IOP-lowering effects of **PF-04217329** (taprenepag isopropyl, Figure 5A), a prodrug of the respective carboxylic free acid (CP-544326) in rabbit, dog and non-human primate models of ocular hypertension and glaucoma. CP-544326
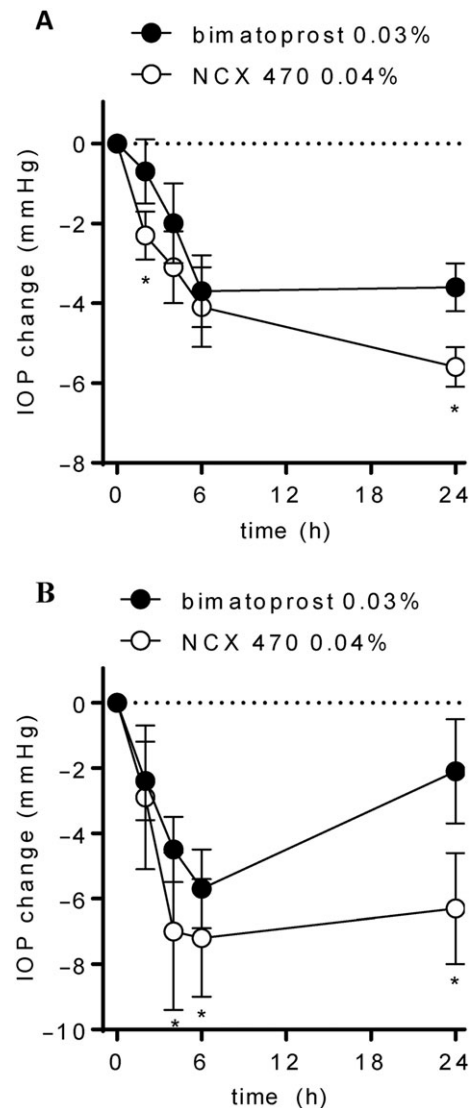

Figure 4

IOP-lowering effect of NCX 470 compared to that of equimolar concentrations of bimatoprost in (A) ocular normotensive beagle dogs and (B) ocular hypertensive non-human primates. Both drugs were dissolved in vehicle (0.25% Tween 80, 0.02% BAK, 2% glycerine and 0.1% EDTA) and administered (30 μL) to animals in a blinded fashion. Dog baseline IOP readings were 20.0 ± 0.5 and 19.9 ± 0.6 mmHg for NCX 470 and bimatoprost groups respectively. In non-human primates, baseline IOP readings for NCX 470 and bimatoprost were 26.8 ± 3.5 and 26.6 ± 3.6 mmHg respectively. One topical drop of proparacaine HCl 0.13% was applied to the eye before IOP readings that were taken with a pneumatonometer (Classic-30). IOP changes versus baseline were calculated as follows: ($IOP_{Tx} - IOP_{T0}$), where IOP_{Tx} and IOP_{T0} are respectively the IOP at the indicated time point and prior to dosing. *$P < 0.05$ versus bimatoprost at the respective time point (Impagnatiello *et al.*, 2015).

was demonstrated to be a highly selective and potent ($IC_{50} = 10$ nM) EP_2 receptor agonist that is found in AH after topical ocular dosing of PF-04217329. This latter compound, tested at concentrations ranging between 0.0025 and 0.03%
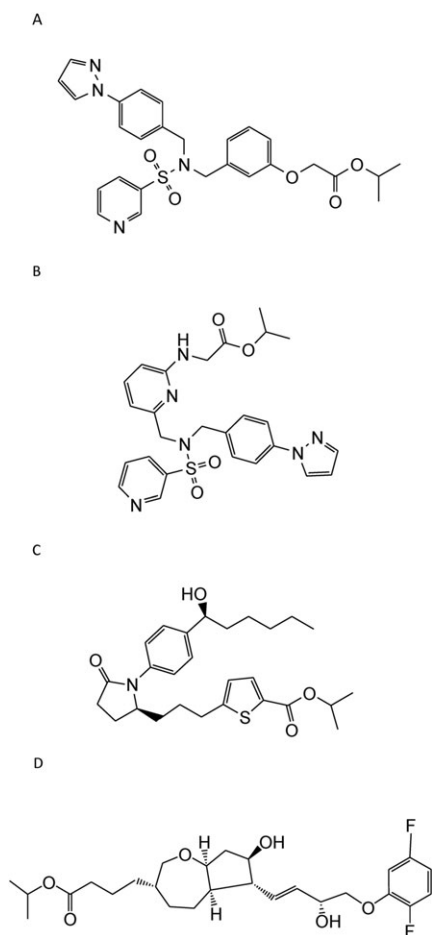
Figure 5

Molecular structures of representative EP$_2$ and EP$_3$/FP receptor agonists. (A) Taprenepag isopropyl, (B) omidenepag isopropyl (DE-117) and (C) aganepag isopropyl (AGN 210961), EP$_2$ receptor agonists, and (D) ONO-9054, EP$_3$/FP receptor agonist.

in a phase 2, 28 day clinical study, was found to lower IOP as effectively as 0.005% latanoprost (Schachar *et al.*, 2011).

DE-117 (omidenepag isopropyl, Figure 5B) is a non-prostanoid isopropyl ester derivative that is hydrolysed to the respective free carboxylic acid analogue in an enzymatically active environment such as the corneal epithelium. DE-117 and the respective free acid are selective EP$_2$ receptor agonists with little or no activity on EP$_4$ receptors (Kirihara *et al.*, 2015). The compound was reported to be very active in lowering IOP in various animal models of ocular hypertension and glaucoma (Kirihara *et al.*, 2015). DE-117 was also shown to be sufficiently safe in healthy volunteers when administered to subjects at 0.002% to warrant further clinical evaluation (Aihara *et al.*, 2017). In a randomized, investigator-masked, active-controlled multicentre phase 3 trial in Japanese patients comparing DE-117 versus latanoprost 0.005%, the compound wasshown to be similarly effective, although the IOP change from baseline in mean diurnal IOP for DE-117 and latanoprost was 0.63 mmHg in favour of latanoprost (Lu *et al.*, 2018). Attempts are also being

made to use it in long-term intracameral delivery devices (Kim *et al.*, 2016). The compound was also shown to be effective in Xalatan non-responsive patients (Ropo *et al.*, 2018).

AGN-210961 (Figure 5C) is likely to be an EP$_2$ agonist and has been formulated as a proprietary ophthalmic solution by Allergan. A sustained-release formulation is also being developed as AGN-210669. This compound showed similar IOP lowering activity as that of Lumigan 0.03% but produced a high occurrence of adverse events, including increased corneal thickness as has been reported for other EP$_2$ receptor agonists (Yanochko *et al.*, 2014).

ONO-9054. ONO-9054 (DE-127, sepetaprost) is an isopropyl ester derivative of the respective biologically active free acid ONO-AG-367 (Figure 5D). Once processed in a biologically active environment by local esterases, the resulting carboxylic acid derivative activates EP$_3$ and FP receptors (Yamane *et al.*, 2015). ONO-9054 has nanomolar affinity (16.8 nM) for the FP receptor while it lacks any meaningful binding affinity for most other PG receptor subtypes excluding the EP$_3$ receptor. Conversely, ONO-AG-367 is a more potent FP agonist compared with ONO-9054 (K_i = 0.72 nM) and, albeit with low potency, and binds the EP$_3$ receptor subtype with a K_i of 25 nM (Yamane *et al.*, 2015).

In animal studies, ONO-9054 was shown to lower IOP more effectively than other more selective FP agonists (Yamane *et al.*, 2015). Several studies tested this compound in healthy volunteers, as well as in ocular hypertensive and normotensive glaucoma patients (Harris *et al.*, 2016). The compound was generally found to be well tolerated, and when compared with latanoprost in phase 2b studies, ONO-9054 achieved a slightly greater diurnal mean IOP reduction (−7.2 and −6.6 mmHg, for ONO-9054 and latanoprost, respectively) (Miller Ellis *et al.*, 2017).

Discussion and conclusion

PG analogues have been the most widely used agents to lower IOP in patients with open-angle glaucoma and ocular hypertension because of their proven efficacy in most patients, long duration of action and relative safety profile.

The first generation of PG analogues (i.e. unoprostone, latanoprost, travoprost, bimatoprost and tafluprost) primarily lowers IOP by increasing AH drainage via the uveoscleral outflow pathway with minor, if any, effect on the conventional, TM/SC pressure-dependent pathway that accounts for about 60–80% of normal AH drainage from the eye (Gabelt and Kaufman, 2005; Alm and Nilsson, 2009; Winkler and Fautsch, 2014).

Several selective and non-selective agonists of EP$_2$ receptors are currently under development. All these compounds are being developed as the ester prodrug of their respective more active free carboxylic acid to enhance corneal penetration after topical application. Preclinical and clinical studies performed to date are encouraging; however, since this class of compounds appears to affect only uveoscleral outflow, it seems unlikely that they would perform better than any of the PG analogues already available. Some compounds under development were reported to have other activity in addition to acting as an FP receptor agonist (Schachar *et al.*, 2011)

making them of potential interest as alternative treatments for patients insufficiently responsive to FP receptor agonists.

More recently, dual-acting PG analogues were identified, namely, NO-donating PGs. These compounds affect both the uveoscleral (PG mediated) and TM/SC (NO mediated) outflow pathways (Cavet et al., 2015; Cavet and DeCory, 2017). Furthermore, given the well-known activity of NO on blood flow, these compounds may be expected to also improve ocular blood flow and optic nerve head oxygenation, which are otherwise impaired in glaucomatous patients (Resch et al., 2009; Costa et al., 2014).

Vyzulta™ (LBN ophthalmic solution, 0.024%), a NO-donating derivative of latanoprost approved by the FDA for the reduction of IOP in patients with ocular hypertension and glaucoma in November 2017, is the most advanced example from this class. Several clinical trials clearly demonstrate that LBN lowers IOP more than latanoprost (Xalatan), either directly in the VOYAGER trial or indirectly by comparing the approved range of IOP reduction for Xalatan (6–8 mmHg) with that shown in the APOLLO and LUNAR trials (7.5–9.1 mmHg). LBN acts by releasing latanoprost acid and NO that simultaneously activate FP receptors and the NO/soluble GC/cGMP signalling pathway, thereby increasing both uveoscleral and conventional outflow (Krauss et al., 2011; Cavet et al., 2015; Cavet and DeCory, 2017). Interestingly, LBN also increases ocular perfusion pressure in patients with open-angle glaucoma or ocular hypertension (Liu et al., 2016) likely by virtue of NO release. More rigorous long-term, randomized, placebo-controlled clinical studies using indicators of patient's disease-related visual impairments as primary outcome are needed for a better understanding of the neuroprotective ability of LBN in addition to its IOP-lowering effects.

NCX 470 is a NO-donating derivative of bimatoprost (Impagnatiello et al., 2015) holding greater expectations compared with LBN as it works, similarly to LBN, by activating bimatoprost-mediated uveoscleral outflow and NO-mediated conventional outflow. However, bimatoprost is considered the most effective PG analogue (Aptel et al., 2008; Quaranta et al., 2013): the added value of NO on top of that of bimatoprost may ultimately result in even greater IOP lowering with NCX 470 than with LBN. Initial exploratory findings in animal models of glaucoma and ocular hypertension support this concept. More direct confirmation will, however, only come after testing in human subjects.

In conclusion, the field of PG analogues for reducing IOP in patients with glaucoma and ocular hypertension has greatly expanded since their initial introduction in ophthalmic practice. Modified PGs analogues employ multiple mechanisms of action to maximize the IOP-lowering effects, targeting both outflow pathways (i.e. conventional and uveoscleral pathways). Furthermore, these compounds hold promise as they may also target mechanisms other than IOP reduction that are thought to play prominent roles in the pathogenesis of glaucoma.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander et al., 2017a,b).

Conflict of interest

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