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Effects of Prostaglandin Analogues on Aqueous Humor Outflow Pathways

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Abstract

Elevated intraocular pressure (IOP) is the most prevalent risk factor for glaucoma. All treatments, whether surgical or pharmaceutical, are aimed at lowering IOP. Prostaglandin analogues are a first line therapy for glaucoma due to their ability to reduce IOP, once-daily dosing, efficacy, and minimal side-effect profile. Whereas prostaglandin analogues have been known to alter aqueous humor outflow through the unconventional (uveoscleral) pathway, more recent evidence suggests their action also occurs through the conventional (trabecular) pathway. Understanding how prostaglandin analogues successfully lower IOP is important, as this information may lead to the discovery of new molecular targets for future therapeutic intervention. This review explores the current understanding of prostaglandin analogue biology as it pertains to IOP reduction and improved aqueous humor outflow facility.

Introduction

Glaucoma is a neurodegenerative disease that damages the optic nerve. There are several risk factors for the disease (age, race, family history, diabetes, and reduced cerebrospinal fluid pressure). However, elevated intraocular pressure (IOP) remains the most prevalent risk and causal factor for glaucoma. If left untreated, elevated IOP will lead to loss of retinal ganglion cells resulting in optic nerve head cupping and irreversible vision loss. At present, all treatments for glaucoma are geared toward reducing elevated IOP to slow progression of the disease.

In the normal eye, IOP is maintained through a balance between the amount of aqueous humor produced in the ciliary body epithelium and the amount drained from the anterior chamber. Whereas secretion of aqueous humor remains normal in glaucoma, the main cause of elevated IOP is the reduced ability to drain aqueous humor from the anterior chamber. In humans, between 60% and 80% of aqueous humor is drained through the conventional (trabecular) outflow pathway, which consists of the trabecular meshwork, a multilayer tissue that filters aqueous humor as it flows from the anterior chamber, and Schlemm's canal, a tubule that drains the trabecular meshwork and moves the aqueous humor through collector channels into aqueous veins and into the episcleral venous system. A portion of aqueous humor (20%–40%) is also removed through the unconventional (uveoscleral) outflow pathway by diffusion through the interstitial spaces of the ciliary muscle into the suprachoroidal space. Fluid removal through the unconventional pathway decreases with age resulting in the conventional outflow pathway becoming the primary route of aqueous humor drainage in the elderly.[1] In the glaucomatous eye, impaired drainage of aqueous humor is caused by an increased resistance to fluid drainage at the interface between the trabecular meshwork and the inner wall of Schlemm's canal within the conventional outflow pathway.[2] This leads to reduced outflow facility, large diurnal IOP fluctuations, and elevated IOP, which eventually will lead to glaucoma.[3–5] At present, treatment aimed at lowering IOP is the only method to slow glaucoma progression and prevent irreversible blindness.

Identifying Prostaglandins as Ocular Hypotensive Agents

Prostaglandins elicit many effects throughout the body, including constriction and relaxation of smooth muscles and regulation of the immune response.[6,7] Early studies analyzing the effects of prostaglandins in the eye revealed significant irritation and initial IOP elevation. Whereas the prevailing thought in the early 1970s was that prostaglandins elevate IOP, it was not until 1977 when Drs. Camras, Bito, and Eakins published the seminal article showing that administration of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and E2 (PGE_2) to uncannulated rabbit eyes lowered IOP in a dose-dependent

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manner following an early ocular hypertensive effect.[8] This groundbreaking research led to the FDA approval in 1996 of latanoprost, an isopropyl analogue of $PGF_{2\alpha}$ (Fig. 1). In 2001, bimatoprost and travoprost, followed by tafluprost in 2012, joined latanoprost as commercially available prostaglandin analogues in the United States for the treatment of glaucoma. Latanoprost, travoprost, and tafluprost are prostanoids, while bimatoprost is classified as a prostamide. All these agents are prodrugs of $PGF_{2\alpha}$.

The popularity of prostaglandin analogues as first line pharmaceuticals for glaucoma treatment stems from their ability to effectively reduce IOP by increasing outflow facility, once-daily dosing, and minimal side effects.[9] However, the responsiveness of individuals to prostaglandin analogues in the clinical setting can be highly variable.[10,11] Some patients respond well to treatment using only a single prostaglandin analogue. Others may not observe a clinically significant decrease in IOP. These patients may require cotreatment with another family of drugs (β-adrenergic receptor inhibitor, timolol maleate, carbonic anhydrase inhibitor, or dorzolamide hydrochloride) to elicit pressure reduction to the normal IOP range of 12–15 mmHg.[11–15] There is also a small subset of patients who are refractory to one prostaglandin analogue, but responsive to another.[16–18] For example, bimatoprost has been found to be effective for patients who do not respond to latanoprost treatment.[17] Clinically, bimatoprost appears slightly more effective in lowering pressure than latanoprost and travoprost, but has a more significant side-effect profile, causing greater hyperemia and eyelash growth than latanoprost, but less iridial hyperpigmentation.[9,11,19–24]

Prostaglandin Receptors

Prostaglandin analogues elicit their effect by binding to specific receptors localized in the cell membrane and nuclear envelope.[25] There are 9 prostaglandin receptors: PGE receptor 1–4 (EP1–4), PGD receptor 1–2 (DP1–2), PGIP receptor, PGFP receptor, and thromboxane A2 receptor (TP), their designation based mainly on the prostaglandin for which binding is most specific.[7,26,27] $PGF_{2\alpha}$ binds the FP, EP1, and EP3 receptors with significant affinity, while travoprost binds the FP receptor with highest affinity among the prostaglandin analogues, with minimal affinity for DP, EP1, EP3, EP4, and TP receptors.[26,28] Pharmacologic and pharmacokinetic data suggest the existence of a unique bimatoprost receptor, distinct from the known FP receptors; however, this receptor is yet to be cloned and bimatoprost has not been shown to work independent of FP receptor activation.[29–31] In the eye, FP and all 4 EP receptors (1–4) have been identified in several ocular tissues, including the trabecular meshwork and the cells of Schlemm's canal.[25,32–38] Investigation with mice lacking the FP receptor gene suggests that the FP receptor is critical for IOP reduction caused by latanoprost, bimatoprost, and travoprost, as all 3 treatments had no effect in knockout mice.[39]

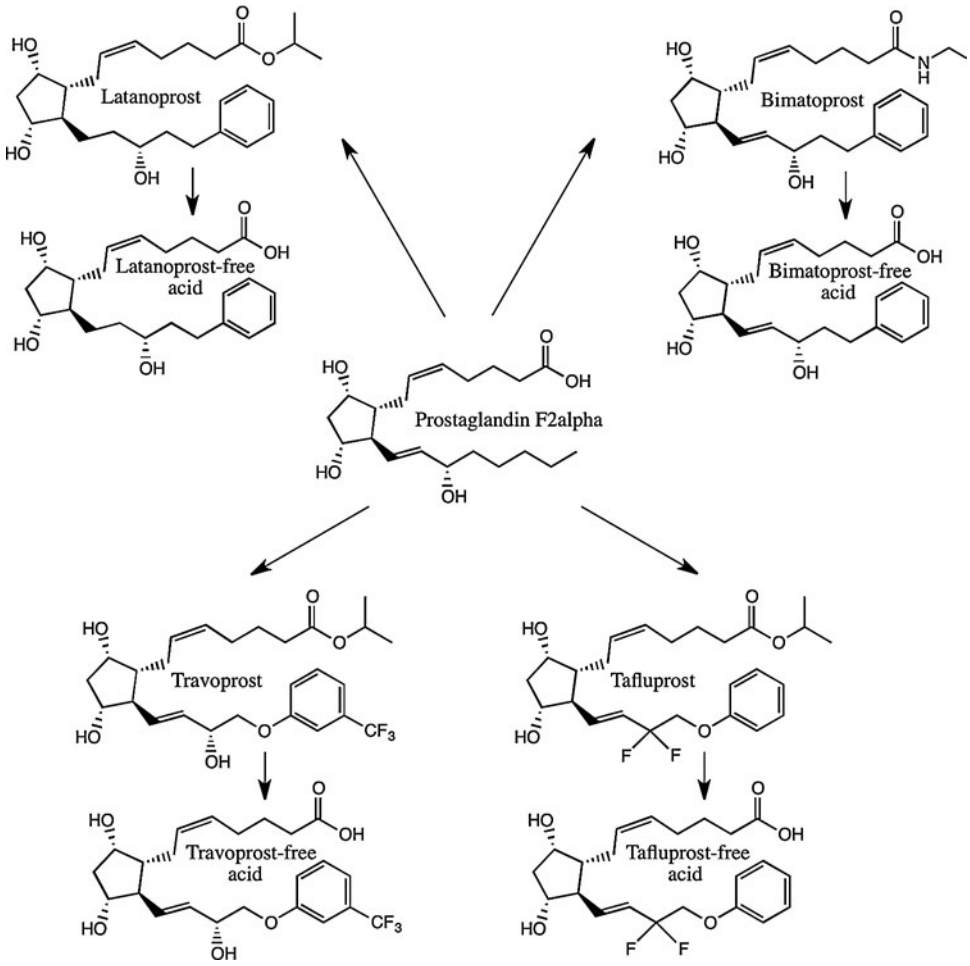

FIG. 1. Molecular structure of prostaglandin analogues used to treat ocular hypertension. Latanoprost, bimatoprost, travoprost, and tafluprost are prostanoids, while bimatoprost is a prostamide. Associated physiologically active free acid forms are also shown.

The importance of the FP receptor was further confirmed in human nonpigmented ciliary epithelial and trabecular meshwork cells, where use of the FP receptor antagonist AL-8810 inhibited phosphoinositide turnover.[40,41] The absence of EP3 receptors in mice reduced the IOP lowering effect of latanoprost, bimatoprost, and travoprost, but mice lacking EP1 and EP2 receptors showed inconclusive results.[42] These studies in mice suggest that FP and EP3 are the primary receptors that trigger downstream signaling pathways and the eventual physiologic response following treatment with latanoprost, bimatoprost, and travoprost. However, in primates, the story is slightly different. EP2 receptor stimulation has been shown to increase uveoscleral outflow,[43] and EP4 receptor activation reduces IOP by increasing outflow facility without effecting uveoscleral outflow.[44] These results in mice and primates suggest that species-specific mechanisms may exist.

Mode of Action: Unconventional Pathway

As mentioned previously, IOP is regulated by the balance between production and removal of aqueous humor from the anterior chamber through the conventional and unconventional pathways. Several studies suggest that prostaglandins do not reduce the amount of aqueous humor production in humans,[45–50] but instead have a minimal increase in aqueous humor production, although the amount is not clinically significant.[51] At present, all evidence supports the role of prostaglandin analogues increasing aqueous humor outflow. In the unconventional pathway, $PGF_{2\alpha}$ and prostaglandin analogues bind to EP and FP receptors in the ciliary muscle, resulting in ciliary muscle relaxation and increased aqueous humor outflow.[43,52–55] Binding of prostaglandins and prostaglandin analogues to ciliary muscle FP receptors also disrupts extracellular matrix turnover.[56–60] The rate of turnover is dependent on the balance between the molecules that degrade and remodel the extracellular matrix [matrix metalloproteinases (MMPs)] and their inhibitors [tissue inhibitor of metalloproteinase (TIMPs)]. MMPs degrade and remodel the extracellular matrix in the ciliary muscle, iris root, and sclera, reducing outflow resistance to fluid flow.[59,61] Treatment with bimatoprost and latanoprost induced MMP-1, -3, and -9 expression in ciliary smooth muscle cells.[62] MMP-1 breaks down interstitial collagens, most notably types I, II, and III, while MMP-9 is most associated with the breakdown of collagens IV and V.[63–66] MMP-3 has a broad substrate specificity that includes degradation of fibronectin, laminin, elastin, proteoglycans, and several collagens, while also having the regulatory function of activating MMP-1 and MMP-9, making MMP-3 a critical molecule in connective tissue remodeling.[63,65] Only TIMP-3, which is associated with heparin sulfate and chondroitin sulfate containing proteoglycans in the extracellular matrix, was induced by bimatoprost and latanoprost.[62,67–69] Additionally, cynomolgus monkey ciliary muscle treated with $PGF_{2\alpha}$, latanoprost, or bimatoprost for up to 8 days showed increased space between muscle bundles indicating changes in extracellular matrix synthesis and turnover presumably increasing aqueous humor outflow.[70] Overall, it appears that treatment with $PGF_{2\alpha}$ and prostaglandin analogues increases the amount of MMPs, while maintaining TIMP expression. This shifts the balance in favor of degradation and remodeling of the extracellular matrix to enhance outflow facility.

Mode of Action: Conventional Pathway

Whereas increased aqueous humor outflow through the unconventional pathway has been the accepted mode of action after prostaglandin analogue treatment, studies have shown that prostaglandin analogues also play a significant role in modulating outflow facility through the conventional outflow pathway. Treatment with bimatoprost or latanoprost increased outflow facility in cultured human anterior segments, a model that contains only the conventional outflow pathway.[71,72] Histological analysis of latanoprost-treated anterior segments showed focal loss of Schlemm's canal endothelial cells, separation of inner wall cells from the basal lamina, and focal loss of extracellular matrix in the juxtacanalicular region.[71] Previous studies have confirmed that loss of extracellular matrix from the juxtacanalicular region and cell disconnection from the extracellular matrix increase conventional outflow.[73,74] Bimatoprost treatment of cynomolgus monkeys for 1 year showed disruption of the endothelial cell monolayer of Schlemm's canal, expansion of the juxtacanalicular region of the trabecular meshwork, loss of extracellular matrix, and in some samples, widening of intertrabecular spaces in the corneoscleral region of the trabecular meshwork.[75] Both collagen and the electron dense core of elastic-like fibers in the inner portion of the trabecular meshwork were lost, as well as connective tissue from the beams as a result of treatment. These changes appear to aid in increasing outflow facility through ways that are still not well understood. Prostaglandin treatment also increased outflow facility and hydraulic conductivity across human primary trabecular meshwork cell cultures.[72] More recent studies on EP receptor stimulation has shown that EP2 and EP4 activation results in increased cell contractility of the trabecular meshwork, and decreased cell contractility of the inner wall of Schlemm's canal, mediating IOP through the conventional pathway.[35]

Prostaglandins also alter the production of MMPs in human primary trabecular meshwork cells. Latanoprost increased expression of MMP-1, -3, -17, and -24.[68] Several MMPs and MMP inhibitors have been shown to increase outflow facility.[76–78] MMP-17 activates aggrecanase-1 (ADAMTS4), potentially causing degradation of glycosaminoglycans and extracellular matrix.[79,80] In monkeys treated unilaterally with latanoprost or bimatoprost for 12 months, significant IOP reduction was obtained. Histologically, treated eyes from these animals showed enlarged tissue space within the ciliary muscle and trabecular meshwork, confirming extracellular matrix remodeling within the conventional and unconventional outflow pathways as an effect of prostaglandin treatment.[75]

Molecular Targets of Prostaglandin Analogue Treatment

There is enough evidence to suggest that prostaglandin analogues lower IOP through tissue impedance changes and long-term remodeling of the extracellular matrix within the conventional and unconventional outflow pathways. However, this does not explain the early effects of prostaglandin analogue treatment in cell culture models.[35] IOP was found to be lowered within 2 h of treatment in mice[81] and human anterior segment culture.[71] Additionally, several prospective human studies have confirmed an ocular hypotensive effect

within a few hours of prostaglandin analogue treatment.[45,82–85] These early effects are most likely due to rapid changes in cell signaling.

Prostaglandin FP receptors activate phosphatidylinositol metabolism through $G\alpha_q$ (G-coupled protein), leading to the formation of inositol triphosphate (IP_3) with mobilization of intracellular free calcium (Fig. 2).[32,54,86–88] Elevated intracellular calcium levels have been reported in primary open angle glaucoma trabecular meshwork cells as well as in a subset of trabecular meshwork cells after application of hydraulic pressure.[89,90] Additionally, calcium-regulated potassium channels, $BK_{(Ca)}$, affect trabecular meshwork and Schlemm's canal cell volume, further indicating a role of calcium signaling in IOP homeostasis.[91,92] $G\alpha_q$ is also linked to mitogen-activated protein kinase (MAPK) through calcium release and phospholipase Cβ (PLCβ)-induced activation of protein kinase C (PKC).[93] $PGF_{2\alpha}$ also activates $G\alpha_q$-independent ($G\alpha_i$) signaling leading to Rho-mediated cytoskeletal rearrangement and actin stress fiber formation.[94]

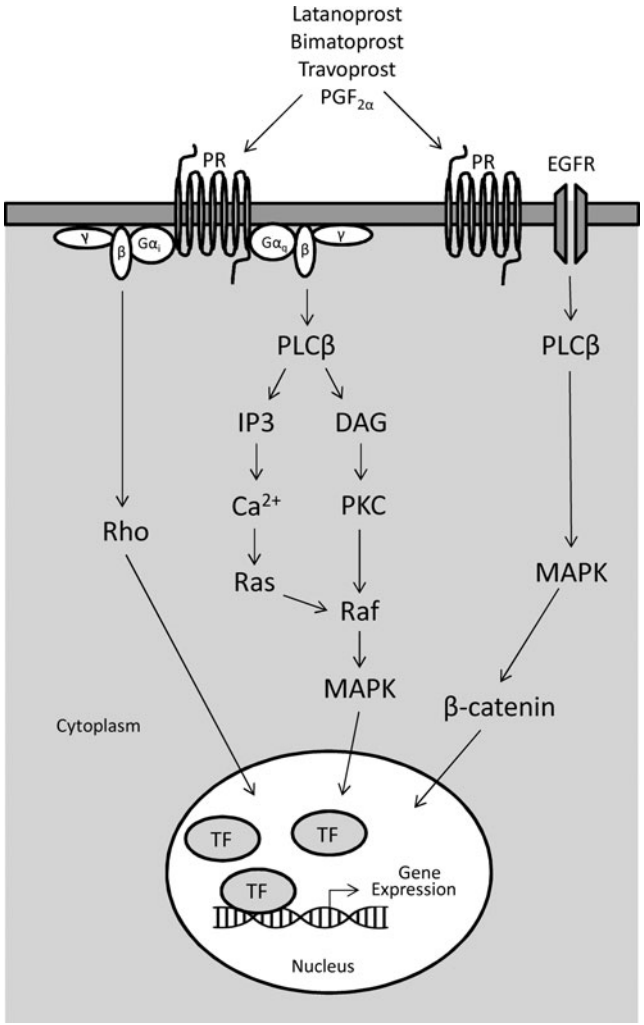

FIG. 2. Supported cell signaling cascades for activation of immediate early genes following prostaglandin analogue treatment. Ligand binding to prostaglandin receptor (PR) activates G-protein-dependent ($G\alpha_q$) and -independent ($G\alpha_i$) pathways. Activation of $G\alpha_q$-dependent pathway results in increased intracellular calcium stores and protein kinase C (PKC) induction leading to activation of the mitogen-activated protein kinase (MAPK) pathway. The $G\alpha_i$-independent pathway leads to activation of Rho signaling. PR activation can also lead to phospholipase Cβ (PLCβ)-dependent transactivation of the epidermal growth factor receptor (EGFR) followed by MAPK and β-catenin activation. MAPK, Rho, and translocation of β-catenin to the nucleus induce gene expression of immediate early genes (eg, early growth response-1) and molecules involved in cell migration and cytoskeleton remodeling. TF, transcription factor; DAG, diacylglycerol; IP3, inositol triphosphate.

In addition to $G\alpha_q$-dependent and -independent signaling, the wingless/integrase 1 (Wnt) signaling pathway has been linked to FP receptor activation (Fig. 2). Activated β-catenin, produced through PLCβ-dependent epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase (ERK) phosphorylation, can translocate to the nucleus and act as a transcriptional activator.[95,96] In human trabecular meshwork cells, functional canonical and noncanonical Wnt signaling pathways are present.[97,98] Treatment of human trabecular meshwork cells with travoprost reduced the formation of dexamethasone-induced crosslinked actin networks through a β-catenin-dependent pathway.[99] Myocilin, a glaucoma-associated protein, increased cell migration through activation of focal adhesion kinase (FAK) and protein kinase B serine threonine kinase pathway (PKB/AKT).[100] Together, these fast responsive intracellular signaling mechanisms (MAPK, Rho, and β-catenin) may explain some of the early effects of prostaglandin analogue treatment (Fig. 2).

In addition to signaling mechanisms, changes in transcription following prostaglandin analogue treatment have been identified in ciliary smooth muscle and trabecular meshwork cells. $PGF_{2\alpha}$ upregulates several early response genes, including early growth response-1 (EGR-1), connective tissue growth factor (CTGF), hypoxia-inducible factor-1a (Hif-1a), c-fos, and Cyr61.[59,101,102] All of these genes are involved in extracellular matrix remodeling through regulation of cell adhesion, migration, and survival. Many of these gene inductions appear to be working through the activation of MAPKs, specifically ERK1/2 (Fig. 2).[101] Other transcriptional changes have also been reported, but with longer prostaglandin analogue treatment. Upregulation of insulin growth factor 1 (IGF1), fibroleukin, TNFSF10 (TRAIL), and promelanosome-concentrating hormone was identified in primary human trabecular meshwork cells treated with latanoprost for 9 days.[103] IGF1 has been linked to changes in metabolic activity and activation of the MMPs, stromelysin and gelatinase. Fibroleukin acts as a protease, and may digest extracellular matrix components. However, glaucoma-associated proteins myocilin, optineurin, and αβ-crystallin showed no change in expression indicating that these genes may not be involved in long-term prostaglandin analogue-mediated cell signaling. Detailed genome-wide *in vitro* studies analyzing short- and long-term effects of prostaglandin analogue treatment will be important to further identify candidate genes involved in prostaglandin analogue-mediated IOP reduction.

Conclusion

The identification of prostaglandin analogues as ocular hypotensive agents has had an enormous positive impact on

the treatment of glaucoma. Each mmHg drop in mean IOP decreases the risk of glaucoma progression by 10%.[104] However, some patients do not respond to treatment with one prostaglandin analogue, but find success with another.[16–18] Understanding how prostaglandin analogues can selectively lower IOP provides an avenue to understand how IOP is regulated in normal and glaucomatous conditions. Whereas prostaglandins have historically been thought of as unconventional outflow pathway modifiers, significant evidence supports their role in modifying the conventional outflow pathway, which is the site of increased outflow resistance in glaucoma. Understanding the physiologic responses of outflow tissues to prostaglandin analogue treatment will be an important area of research in the future. New analogues are being developed that show specificity for FP and EP receptors, providing new opportunities to improve IOP reduction and minimize side effects. Characterizing the molecular fingerprint of prostaglandin treatment will elevate our understanding of the signaling mechanisms leading to identification of the physiological responses associated with IOP reduction in the conventional outflow pathway. This will also lead to identification of better and more specific therapeutic targets for the treatment of glaucoma.

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Author Disclosure Statement

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