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Drug Discovery Stories

Discovery to Launch of Anti-allergy (Emadine; Patanol/Pataday/Pazeo) and Anti-glaucoma (Travatan; Simbrinza) Ocular Drugs, and Generation of Novel Pharmacological Tools Such as AL-8810

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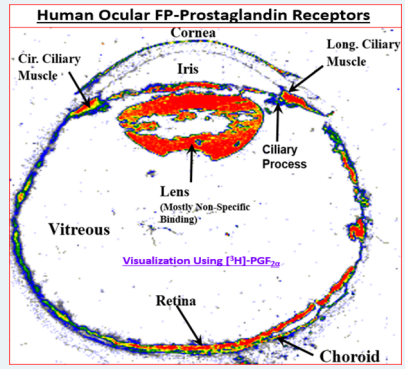
ABSTRACT: The eye and eyesight are exquisitly designed and are precious, and yet we often take them for granted. Good vision is critical for our long-term survival and for humanity's enduring progress. Unfortunately, since ocular diseases do not culminate in life-and-death scenarios, awareness of the plight of millions of people suffering from such eye ailments is not publicized as other diseases. However, losing eyesight or falling victim to visual impairment is a frightening outlook for most people. Glaucoma, a collection of chronic optic neuropathies, of which the most prevalent form, primary open-angle glaucoma (POAG), is the second leading cause of irreversible blindness. POAG currently afflicts >70 million people worldwide and is an insidious, progressive, silent thief of sight that is asymptomatic. On the other hand, allergic conjunctivitis (AC), and the associated rhinitis ("hay-fever"), frequently victimizes a huge number of people worldwide, especially during seasonal changes. While not life-threatening, sufferers of AC soon learn the value of drugs to treat their signs and symptoms of AC as they desire rapid relief to overcome the ocular itching/pain, redness, and tearing AC causes. Herein, I will describe the collective efforts of many researchers whose industrious, diligent, and dedicated team work resulted in the discovery, biochemical/pharmacological characterization, development and eventual launch of drugs to treat AC (e.g., olopatadine [Patanol/Pataday/Pazeo] and emedastine [Emedine]), and for treating ocular hypertension and POAG (e.g., travoprost [Travatan] and Simbrinza). This represents a personal perspective.



KEYWORDS: *allergic conjunctivitis, olopatadine, Patanol, Pataday, ocular hypertension, glaucoma, travoprost, Travatan*

It was a humbling but exalting feeling to be invited to chart the journey of my colleagues and I in the processes of establishing, validating, and implementing a few drug discovery/development platforms and projects that eventually yielded drugs to treat two different types of eye diseases. The first of these was allergic conjunctivitis (AC), and in particular seasonal AC (SAC), a bothersome ocular disorder, the hallmark signs and symptoms of which are undeniable (intense ocular itching, hyperemia (redness), tearing and swelling of the eyelids with possible pain that people of all ages suffer from every few months, some even more frequently.[1−3] The second disease was ocular hypertension (OHT)/primary open-angle glaucoma (POAG),[4−9] that involves a slowly progressing, symptomless but unrelenting demise of retinal ganglion cells (RGCs) and their axons that connect the eye to the brain. The net result of OHT/POAG is loss of peripheral vision that eventually leads to pan-visual impairment culminating in blindness if the patient remains undiagnosed and untreated. While SAC is an acutely debilitating eye disorder, OHT/POAG is achronic disease that mainly affect older individuals across our planet. As can be imagined, the research strategies, tactics, molecule design/syntheses, screening paradigms, go/no-go criteria stage-gates, *in vitro* assays, and *in vivo* animal models were totally different for each target disease, including the target product profiles for each drug.[6−8] Needless to say, both drug classes required a high therapeutic index for patient tolerability, acceptance, and eventual introduction into clinical practice following health authority approvals in different geographic jurisdictions.

Prior to discussing the etiologies of the ocular diseases of interest and our drug discovery strategies, it is worth elaborating on the overall incidence, impact on patient quality of life (QoL), and the economic burden associated with management of ophthalmic diseases in order to provide a contextual perspective. Based on the data provided by the World Health Organization (WHO, 2018)[9] and other key organizations such as National Eye Institute (US), globally greater than 2 billion people are

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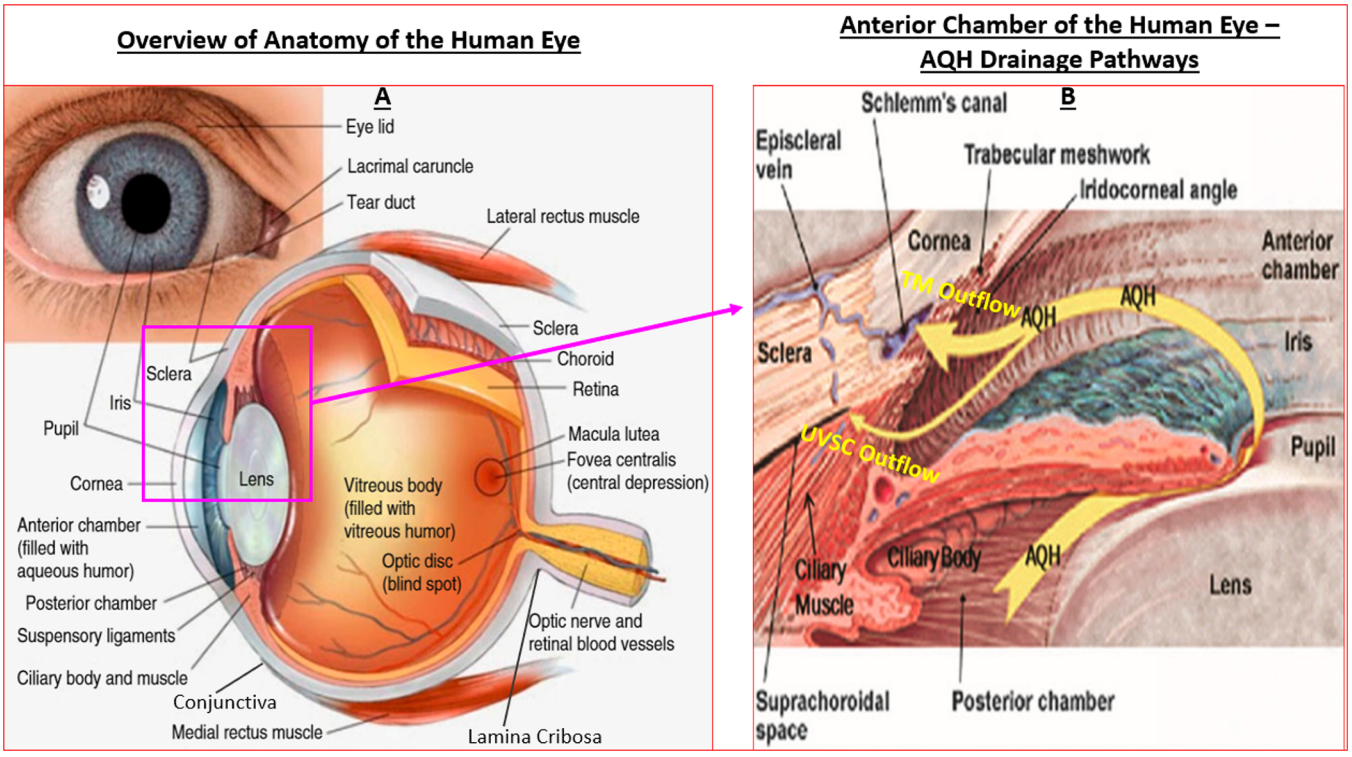

Figure 1. Basic anatomy and structural elements of the human eye to illustrate key features discussed in this article. Panel A reproduced and modified with permission from ref 8. Copyright 2020 Springer Publishing Company. Panel B reproduced and updated with permission from ref 7. Copyright 2018 Mary Ann Liebert Publishing Inc.

dealing with visual impairment or blindness. Sadly, up to 1 billion of these impairments were preventable.[9] Currently, there are 196 million patients suffering from age-related macular degeneration (AMD), greater than 76 million with glaucoma, 146 million with diabetic retinopathy, and several billion people who have myopia and near-sight problems (presbyopia) around the world. With an aging world population, the number of people afflicted with AMD is projected to rise to 243 million, and for glaucoma is expected to increase to greater than 95 million by 2030. Others estimate that by 2040, there may be as many as 112 million patients with glaucoma, with the highest prevalence in Asia and Africa.[5,9,10] As for allergic conjunctivitis (AC), as much as 40% of the population is affected by some symptoms of AC, with the majority of the cases (up to 95%) ascribed to SAC or perennial AC (PAC).[1−3,11,12] Therefore, ophthalmic disorders and diseases represent a significant healthcare issue since our society, communities, education systems, economies, sports, media, and every facet of our waking lives are incredibly reliant on the ability to see. Sight is important from infant to mother bonding, to continued learning/educational achievements over the course of life, development of social skills/personality and character, and is critical for physical development and health, and for mental health and self-esteem development and maintenance. Hence, eyesight is critical for survival and life progression, and undoubtedly people with visual impairment are frightened of losing their sight. Economically, the annual cost to society from ophthalmic diseases runs into billions of dollars when considering the lost productivity, decrease in QoL, disability, and morbidity associated with them. For instance, it is estimated that visual impairment (mild to severe) in the US costs greater than $16 billion, while the global financial burden associated with uncorrected myopia and short-sight vision impairment alone is $244 billion and $25 billion, respectively (WHO, 2018).[9] The various eye diseases mentioned above have recently been discussed in the literature.[6,8,13,14]

THE EYE AND SOME MAJOR OCULAR DISEASES

Since the ocular drug discovery to be described in what follows depends on having at least a basic understanding of the structural and functional components of the visual system, I believe a short introduction to the eye anatomy and physiology would be helpful to the readers. Because of their physical location on the face, the eyes are susceptible to injury, and during waking hours are exposed to light and other radiation, and all kinds of pollutants in the air around us. Preserving and protecting visual function is therefore a major challenge and requires attention.

The human eye is made up of three layers of which the outermost layer, known as the fibrous tunic, is composed of the cornea and sclera which give the eye its shape and which provide support for the deeper structural elements (Figure 1A). The thin membranous tissue that stretches on from the cornea, covers part of the sclera and then forms the underside lining of the eyelids is the conjunctiva, formed by highly vascularized tissues. The middle layer, known as the uvea or vascular tunic, encompasses the choroid, ciliary body, pigmented epithelium, and the iris. Lastly, the innermost layer is represented by the retina, which is composed of several layers of highly specialized cells that receive their nutrients and oxygen from the choroidal circulation at the back of the eye, while other retinal vessels supply the anterior parts of the retina. The clear fluid in the anterior segment of the eye (aqueous humor (AQH), residing between the cornea and lens) and the jelly like substance (vitreous humor [made up of water and numerous classes of proteins], located behind the lens) that fills the posterior segment of the eye also help maintain the eyeball shape in

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addition to the scleral outer covering of the eyeball (Figure 1A/1B). Since the anterior segment of the eye is avascular, the cells lining the anterior chamber receive their nutrition and oxygen from the circulating AQH made by the ciliary processes (nonpigmented ciliary epithelium [NPCE] cells) within the ciliary body (composed of the ciliary processes and ciliary muscle [CM]) (Figure 1B). The lens is connected to the ciliary body by hundreds of fine transparent fibers (suspensory ligaments) which help change the shape of the lens through muscular forces to aid image focusing (accommodation). Under normal circumstances the VH turns over very slowly, whereas the AQH is constantly produced, flows through the anterior chamber, delivers nutrients and removes toxic waste, and exits the latter area through the trabecular meshwork (TM)/Schlemm's canal (SC) (Figure 1B). Again, in the normal situation the light enters the eye via the cornea, passes through the pupil, is focused by the lens and is projected onto the retina. At a macrolevel, complex biochemical reactions in the photoreceptors convert the received information into signals that the retinal ganglion cells (RGCs) then convert into electric impulses which are transmitted down the RGC axons, that are bundled together to form the optic nerve, to the thalamic region of the brain (superior colliculus/lateral geniculate nucleus) from where they are relayed to the visual cortex in the brain. The visual cortex decodes the information to form the visual images the person sees.

Briefly, ocular surface disorders comprise allergic conjunctivitis, dry eye, corneal perforation, and corneal and conjunctival pain.[8] Within the anterior chamber (ANC), dysfunctional corneal endothelial cells cause corneal dystrophies, while AQH drainage disorders due to blockage of the TM raise intraocular pressure (IOP) to cause ocular hypertension (OHT) that is frequently linked to glaucoma.[5,6,10] Aggregation of proteins in the lens, due to excessive exposure to sunlight or due to smoking and diabetes, results in cataract formation.[8] When the iris repeatedly brushes up against the lens, cellular debris and iridial pigment are shed into the AQH. Eventually these materials arrive at the TM and they obstruct the latter tissue causing exfoliation/pigmentary glaucoma due to elevation of IOP. Even though VH acts as a cushion to the surrounding tissues and vascular elements in the back of the eye (Figure 1A/1B), many retinal diseases exist or develop due to defects in the cellular machinery of the many specialized cells within the retina-choroid. These include dry and wet age-related macular degeneration (AMD), retinitis pigmentosa, diabetic retinopathy, glaucomatous optic neuropathy [GON], that require specific treatment modalities.[5−10] Since I will be focusing on glaucoma and glaucomatous optic neuropathy (GON), the features of this disease and its treatment will be discussed further ahead and have recently been reviewed.[6−8,13,14]

CHALLENGES AND STRATEGIES TO DISCOVER NOVEL DRUGS TO TREAT OCULAR ALLERGIC DISEASES

Up to 30% of the population is affected by allergic hypersensitivity, due to a hereditary component, and are prone to experience various atopic conditions including eczema, asthma, and allergic rhinitis. Atopic eye disorders encompass, SAC, PAC, keratoconjunctivitis, giant papillary conjunctivitis, and vernal conjunctivitis. SAC and PAC account for 80−98% of all cases of eye allergies, with 20−30% of the population succumbing to their symptoms throughout the year.[1−3,11,12,15−17] While SAC and PAC are somewhat acute self-limiting disorders, the other forms of conjunctivitis mentioned above can be chronic sight-threatening diseases. Most people would attest that one of the most annoying and malaise-causing problems they frequently experience is "allergies". Itchy, watery, swollen red eyes coupled with sneezing and runny nose and light-sensitivity herald the signs and symptoms of AC and "hay fever" (rhinitis), respectively.[12,15,16] Episodic misery from these ailments easily incapacitates us all many times in our lives for which we seek immediate relief.[17,18] The unbearable urge to rub the eyes due to itching is almost uncontrollable.[18−21] Herein, I will deal with the etiology, diagnosis, and treatment of AC, and more specifically SAC, since that was the target malady we deemed of highest importance when we began our drug discovery campaign in 1992/1993 at Alcon Laboratories Inc. (Fort Worth, TX), which later became Alcon Research, Ltd.

During the day we experience eye strain from computer/mobile device-related work, and in addition our eyes are constantly being assaulted by airborne allergens, pathogens, and other irritants. Blinking, use of artificial tears, and gently rubbing the eyes provide some relief and get rid of some of the offending substances. But this relief is short-lived. When the seasons change or at times of sustained high humidity the air quality sharply declines as the air gets filled with high levels of new allergens such as pollen (from grasses, trees, flowers, weeds), and with fungal spores and other pollutants. Furthermore, some people are highly sensitive to dust mites, pet dander, and dust that accumulates in the house. The irritants elicit the classic allergic/inflammatory cascade in the cornea/conjunctiva but also within the nasopharynx as we breathe in these allergens.

The pathophysiology of SAC unfolds mainly in the conjunctival epithelium underneath the eyelids which contains a large number of dendritic cells and macrophages along with a rich supply of blood vessels.[2,20,21] The latter cells are responsible for the innate and adaptive immunity of the conjunctiva. As an allergen (antigen) such as pollen binds to a B-lymphocyte and is cross-linked to the immunoglobulin-E (IgE) in a sensitized individual, the latter binds to the high-affinity IgE receptor on the mast cells in the conjunctiva and triggers the mast cell to immediately empty its content of preformed mediators such as histamine, bradykinin (BK), platelet activating factor (PAF), serotonin, cathepsin G, and tryptase onto the surrounding tissues.[1,22−25] The immediate actions of histamine (and probably that of BK and PAF) are to cause vasodilation of conjunctival blood vessels and to enhance vascular permeability.[1−3,22−25] This acute early phase response begins to subside but the damage has been initiated and the cascade of other deleterious events ensues. Over the next few minutes to hours, the mast cells release newly generated prostaglandins (PGs; mainly PGD_2), leukotrienes and cytokines (e.g., interleukin-3 [IL-3], IL-6, IL-8, and tumor neurosis factor-α [TNF-α]) as part of a delayed late-phase secondary response to the allergen.[2,3,21,24] The cytokines in turn induce IgE synthesis/release by B-cells and cause inflammatory white blood cells (e.g., eosinophils) to infiltrate the conjunctiva, and to cause leukocyte adhesion, migration, and activation, thereby amplifying and exacerbating the situation.[2,23−25] By now the clinical manifestation of the allergic inflammation in the eye is readily observable and the patient feels the swollen eyelids that are itchy, red, and increasingly becoming painful and further irritated.[1−3,20−25] Since the human conjunctival epithelial (HCE),[26−32] human corneal epithelial (HCEPI),[33−38] and human corneal fibroblast (HCF)[26,27,29,30] cells express functionally active receptors for histamine (H_1-type), for BK (B_2-type, and perhaps the B_1-type

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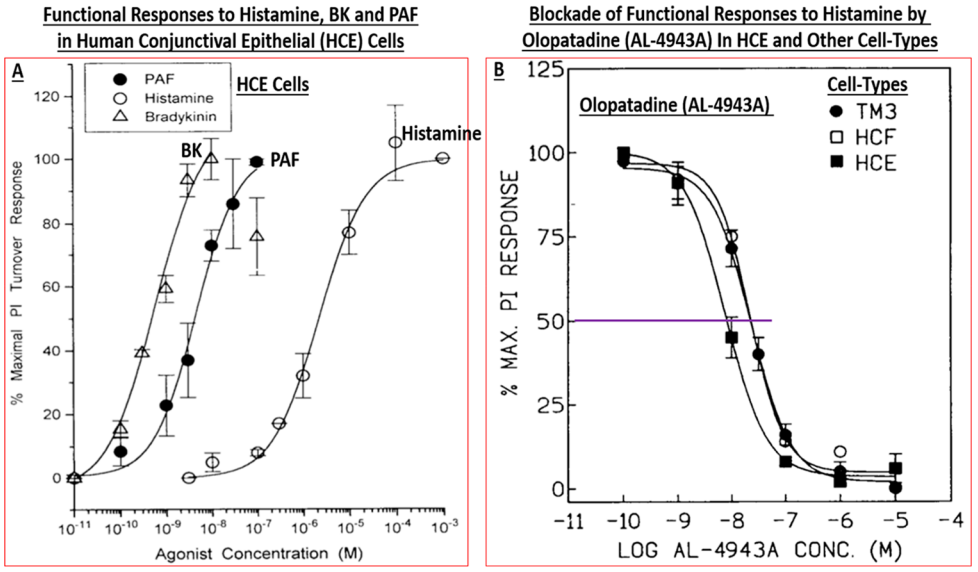

Figure 2. Panel A shows the concentration-dependent stimulation of PI turnover by three key mast cell mediators in HCE cells. The antagonism of histamine-induced responses in three different human cell-types by olopatadine (AL-4943A) are displayed in panel B. Reproduced and updated with permission from refs 29 and 32. Copyright 1996 and 1997 Mary Ann Liebert Publishing Inc.

which is induced under pathological conditions), and for PAF, synergistic activation of these cell-types could potentiate the allergic response in the cornea and conjunctiva due to the release of various cytokines by the latter epithelial cells,[39−43] perhaps in a yet to be defined coordinated or uncoordinated manner. In fact, HCEPI and HCF cells would be expected to respond immediately to the mast cells mediators such as histamine, BK, and PAF,[22] and promote an early phase of cytokine release upon the ocular surface since these receptors react very quickly to their cognate ligands to generate intracellular inositol phosphates (IPs) and mobilize intracellular Ca^{2+} $[Ca^{2+}]_i$ over a few seconds that leads to the final biological response.[28,33,36]

Clearly, the multiplicity of mediators involved in the onset and progression of ocular allergic disease is overwhelming and the interconnecting pathways and mechanisms very complex and complicated. For these reasons it was difficult to decide which mediator(s) and which cell-type(s) to target with respect to finding suitable new drugs to treat SAC/PAC around the 1992/1993 time-period. Historical data concerning the skin had shown histamine to be a potent itch-causing (pruritic) agent with BK inducing a milder and transient response but causing pain.[44] Similarly, serotonin was significantly weaker than histamine in provoking dermal itching, and since various prostaglandins while not very pruritogenic by themselves appeared to synergize with serotonin and histamine (PGE_2 actually promoted histamine release),[45] various neuropeptides, including BK, apparently produced itching by releasing histamine.[46] On the basis of these findings and the fact that only peptide antagonists for BK receptors were available at the time, and the complexity of trying to unravel and identify the specific type(s) of receptors involved in AC within each class of potential target(s), we ruled out BK (at least two receptor subtypes), serotonin (at least seven major receptors with several subtypes within each class) and PG (at least five major receptors with many subtypes) receptor antagonists as targets for our drug discovery program for AC. Added to this complexity was the uncertainty of translating results obtained from one organ to the next, and the known significant heterogeneity among mast cells and epithelial cells,[22,47] and of the many species differences in disease pathology and known differences in compound affinities and potencies across mammalian species.[1−3,47] Since PAF exhibited potent chemotactic and chemokinetic activity for eosinophils and PAF receptor antagonists were showing promise as antiasthmatic drugs,[48] and a dual PAF/histamine receptor antagonist (SCH-37370) had recently been reported,[49] our interest in PAF began to grow. However, since SCH-37370 exhibited a relatively low antagonist affinity/potency at PAF and H_1-histamine receptors (IC_{50} = 0.6 and 1.2 μM, respectively), we decided not to pursue PAF antagonists for AC.[38]

Importantly, Berdy et al.[50] had reported that H_1-receptor antagonists were effective at significantly reducing histamine-induced ocular itch, ocular congestion, and redness of the eye in healthy human volunteers.[50] The major problem seemed to be that existing antihistamines and other antiallergic agents at that time were not fast-acting and their efficacy was not durable, therefore requiring multiple topical ocular (t.o.) dosing regimens.[1−3] Collectively, it was decided that we would endeavor to find the next generation of histamine receptor antagonists with a high affinity, a greater receptor-selectivity, higher potency, perhaps having multiple mechanisms of actions, and having a superior *in vivo* efficacy and a longer duration of action than the current medications in the early 1990s in order to mitigate the signs and symptoms associated with AC. This necessitated a better understanding of the human ocular cells and tissues involved in, or implicated in, the pathology of AC using a fundamental pharmacological approach. Accordingly, the team established appropriate radioligand-based receptor binding assays, second messenger-based functional assays, and rendered them into a high throughput screening (HTS) platform. Likewise, other team members started a program to isolate, cultivate, and propagate various resident cells in post-mortem human conjunctiva and cornea that were deemed important for compound profiling. The use of human ocular cells was critical in order to remain focused on target tissues and the target population. Such primary cells included human conjunctival mast cells (HCMCs),[39,51−58] human conjunctival epithelial cells (HCE) cells,[26−32,58−60] human conjunctival fibroblasts (HCF),[26,27,29,30] human corneal epithelial (HCEPI)

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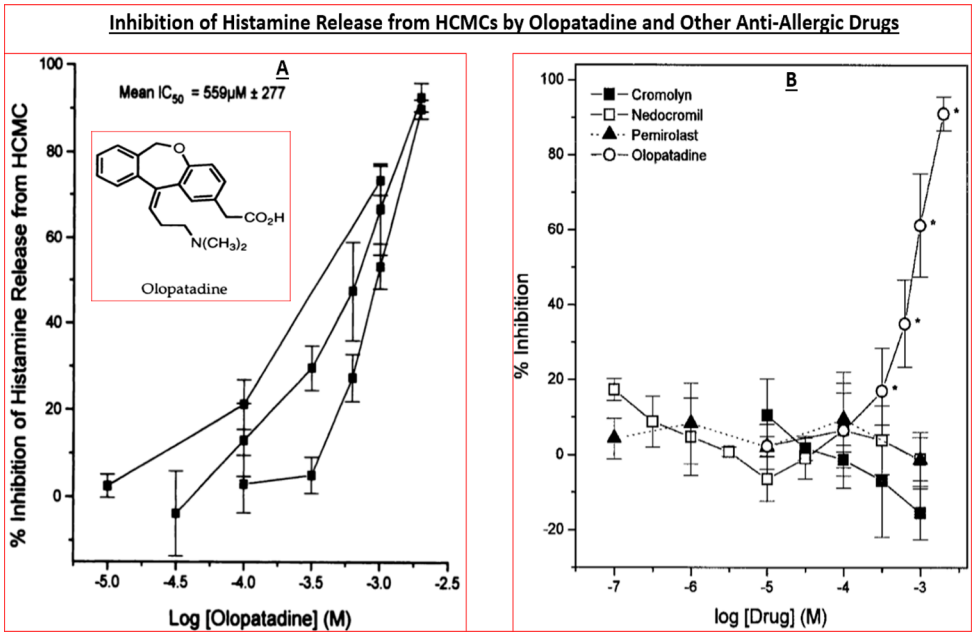

Figure 3. Histamine release from isolated human conjunctival mast cells (HCMCs) exposed to an immunological challenge and the ability of olopatadine and other antiallergic drugs to inhibit the release is depicted. Figure reproduced with permission from ref 30. Copyright 1996 American Society for Pharmacology and Experimental Therapeutics.

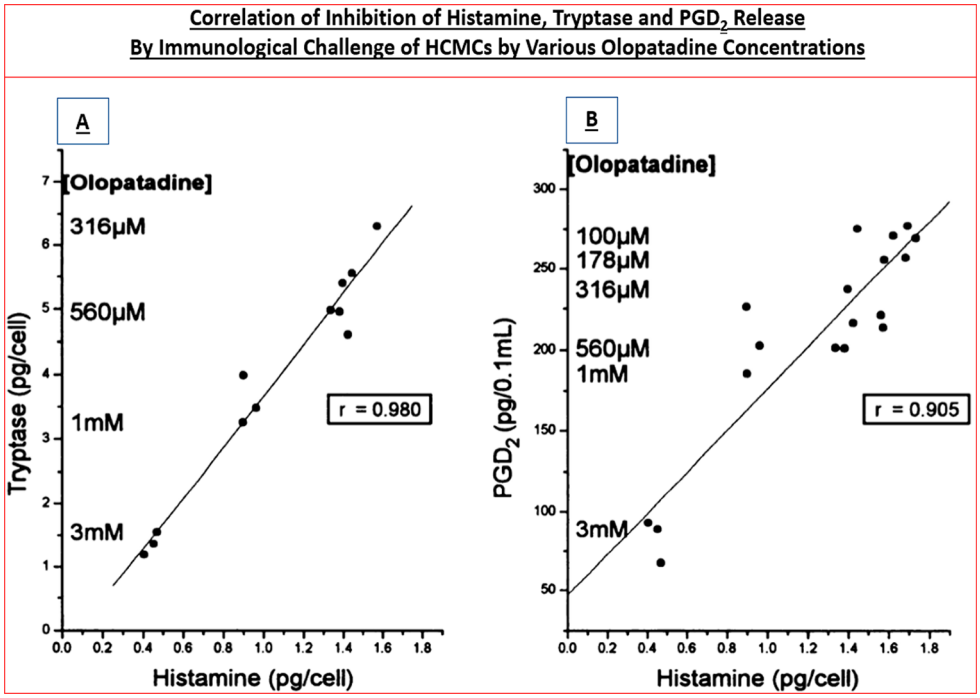

Figure 4. Correlations of immunologic-challenge-induced secretion of histamine, tryptase (A) and PGD_2 (B), and their inhibition by different concentrations of olopatadine, from human conjunctival mast cells is shown. Figure reproduced with permission from ref 30. Copyright 1996 American Society for Pharmacology and Experimental Therapeutics.

cells,[33−38] and also other cells obtained from human ocular tissues such as trabecular meshwork (TM) cells. The ready availability of sufficiently large quantities of these early passage primary cells allowed us to first perform a small survey of guanine protein-coupled receptors (GPCRs) present on these cell-types. It was discovered that primary target HCE cells expressed functionally active major mast cell mediator and neurotransmitter receptors including β_2-adrenergic, prostaglandin EP_4, vasoactive intestinal peptide, and 5-HT receptors positively coupled to adenylate cyclase, the activation of which resulted in cAMP generation.[32] Of the phospholipase C-coupled receptors present on HCE cells, BK, PAF, and histamine (and leukotrienes) robustly stimulated phosphoinositide (PI) turnover by generating [^{3}H]-IPs (e.g., Figure 2A)[31,32] and rapidly enhanced $[Ca^{2+}]_i$ mobilization.[31,32]

Interestingly, primary (and immortalized) HCEPI cells also expressed BK, PAF, and histamine-1 (H_1) receptors that were functionally responsive to various agonists and antagonists of

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Table 1. Competition by Selected Histamine Antagonists for Specific Radioligand Binding to H_1−H_3 Receptors[a]

test drugs	equilibrium inhibition constants of drugs at guinea pig brain H_1−H_3 receptors sub-types (K_i, nM ± SEM)		
	H_1 receptors	H_2 receptors	H_3 receptors
clemastine	0.23 ± 0.1	143 ± 33	4 015 ± 1 617
pyrilamine[b]	0.7 ± 0.1	8 612 ± 1 275	9 820 ± 1 098
emedastine	1.2 ± 0.1	39 860 ± 7 453	14 498 ± 2 257
ketotifen[b]	1.2 ± 0.1	1 122 ± 127	2 458 ± 203
chlorpheniramine	1.4 ± 0.3	7 980 ± 649	3 103 ± 198
brompheniramine	9.9 ± 0.9	5 350 ± 247	5 750 ± 1994
diphenhydramine	11.9 ± 2.9	1 595 ± 141	31 480 ± 12 020
pheniramine[b]	32.3 ± 2.8	14 475 ± 939	10 190 ± 1 190
olopatadine	36.0 ± 5.7	153 983 ± 94 313	137 980 ± 28 603
antazoline[b]	39.3 ± 3.4	40 850 ± 3 794	35 295 ± 8 380
levocabastine[b]	52.6 ± 9.9	27 075 ± 4 996	9 506 ± 5 825

[a]Selected antihistamines were evaluated for their ability to displace [^{3}H]-pyrilamine, [^{3}H]-tiotidine, and [^{3}H]-*N*-methyl-histamine from guinea pig brain H_1-, H_2-, and H_3-receptors, respectively. [b]Drugs marketed for treatment of AC around 1992/1994.[27,29]

these receptor classes.[33−38] Furthermore, stimulation of the latter receptors initiated release of various pro-inflammatory cytokines such as interleukin-6 (IL-6) and IL-8 from HCE cells[58−60] and also from HCEPI cells[33−38] *in vitro*, indicating that the machinery for creating, propagating, and sustaining the cellular signaling mechanisms involved in the allergic inflammation on the ocular surface all existed within these key ocular cell-types. We deemed it necessary to examine the effects of mast cell mediators, especially histamine, on HCEPI cells since they are also directly exposed to the allergens on the ocular surface and are also recipients and potential responders to HCMCs/HCE cell-secreted mediators, and because clusters of corneal epithelial cells apparently co-reside in the conjunctival epithelium.[43] HCMCs were isolated and interrogated for mediator release characteristics since they are the major and primary cells involved in the AC pathology. In the absence of the multiplexed screening tools of today where potentially several dozen mediators can be detected and quantified simultaneously, it was encouraging to observe that exposure of isolated HCMCs to human IgE and other provocative stimuli resulted in degranulation and release of histamine, tryptase, leukotrienes, and PGD_2 (e.g., Figures 3 and 4)[51,57] and numerous cytokines and adhesion molecules.[40,53,54,61]

Using these encouraging data, we embarked on our drug discovery campaign to find high affinity, high potency, and highly selective, more efficacious, and fast-acting antihistamines for the topical treatment of AC. At the time the commercially available H_1-antagonists included antazoline, brompheniramine, chlorpheniramine, clemastine, diphenhydramine, ketotifen, pheniramine, and pyrilamine.[50] Additional compounds in this class were obtained as generous gifts from other companies and tested in parallel (e.g., Emedastine from Kanebo Ltd., Osaka, Japan; Levocabastine from Janssen Pharmaceuticals, Beerse, Belgium). All these drugs were quickly profiled for their relative affinities and selectivities at the guinea pig brain histamine receptor subtypes (Tables 1 and 2), tested for their ability to prevent histamine-induced [^{3}H]-inositol phosphates ([^{3}H]-IPs) production and $[Ca^{2+}]_i$ mobilization in HCE cells, and to reduce guinea pig conjunctival vascular permeability *in vivo*. To our delight emedastine was found to be a high affinity (K_i = 1.2 nM; Table 1) and a high potency H_1-receptor antagonist preventing PI turnover in HCE cells (K_B = 0.88 nM)[26,27] and at blocking histamine-induced IL-6, IL-8, and granulocyte macrophage-colony stimulating factor (GM-CSF) secretion from HCE cells (IC_{50} = 1.5−3.4 nM);[60] Weimer et al., 1998), and the most H_1-receptor selective (>12 000−33 000 fold) among all the antihistamines tested (Tables 1 and 2).[26,27] Emedastine was also found to be the most potent/efficacious drug at inhibiting histamine-induced conjunctival vascular leakage in guinea pig eyes,[62] being 3−17-times more potent than ketotifen, pheniramine, and antazoline, and equipotent with pyrilamine. Moreover, emedastine was 7, 10, 10, 100, 3333, 357, and 5813 times more potent than brompheniramine, chlorpheniramine, clemastine, levocabastine, diphenhydramine, pheniramine, and antazoline, respectively, in this animal model.[62]

Table 2. Relative Selectivities of Key Compounds for Histamine Receptor Sub-Types (H_1−H_3)[a]

test drug	relative selectivities for guinea pig brain histamine receptor sub-types		
	H_1 relative to H_2	H_1 relative to H_3	H_3 relative to H_2
emedastine	33 217	12 082	3
pyrilamine[b]	12 303	14 028	<1
chlorpheniramine	5 700	2 216	3
olopatadine	4 277	3 833	<1
antazoline[b]	1 039	898	1
ketotifen[b]	935	2 048	<1
clemastine	621	17 456	<1
brompheniramine	540	580	<1
levocabastine[b]	515	181	3
pheniramine[b]	448	315	1
diphenhydramine	134	2 645	<1

[a]The Table has been arranged to reflect high to low relative selectivity with focus on the H_1-receptor since that was predominantly involved in mediating the majority of the proinflammatory effects of histamine in AC by enhancing conjunctival vascular permeability and causing itching. [b]Drugs marketed for treatment of AC at the time of our studies, around 1992/1994.[27,29]

In clinical studies, emedastine (0.05%; twice daily topically applied) was compared with levocabastine (0.05%; twice daily topically applied) in one main study involving 222 patients with SAC aged four years and older.[63] The main end point for effectiveness was the reduction in itching and redness, measured on a nine-point scale over and up to 6 weeks. Emedastine was as effective as levocabastine in reducing symptoms of seasonal conjunctivitis. In both groups of patients, itching scores fell from around 5.1 at the start of the study, to around 3.8 after five minutes and around 2.7 after two hours. Similar reductions in redness scores were seen, falling from 4.5 to 3.7 after five

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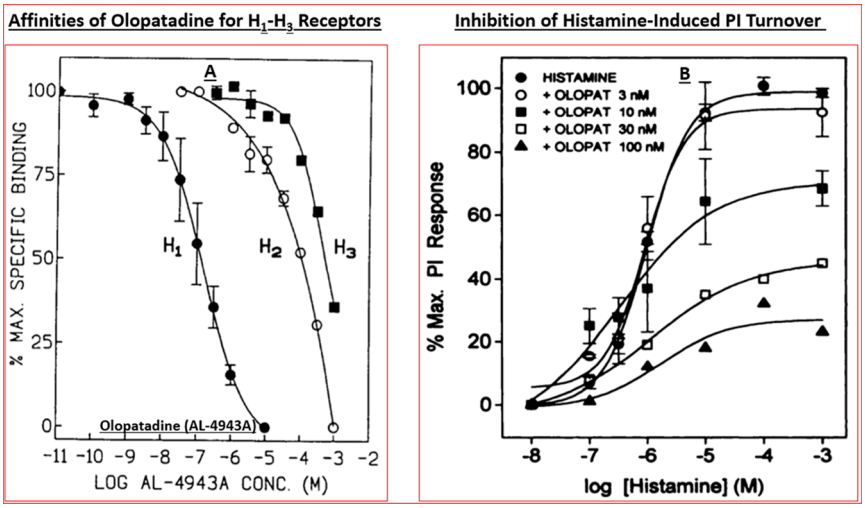

Figure 5. This montage depicts the relative affinity and selectivity of olopatadine for H_1−H_3 receptors subtypes (A), and the ability of histamine to increase production of [^{3}H]-IPs in isolated h-TM cells and the noncompetitive antagonism of these responses by different concentrations of olopatadine (B). Panel A reproduced with permission from ref 29. Copyright 1996 Mary Ann Liebert Publishing Inc. Panel B reproduced with permission from ref 30. Copyright 1996 American Society for Pharmacology and Experimental Therapeutics.

minutes and 2.7 after two hours.[63] In the long term, the itching scores fell from an average of around 3.9 on the first day, falling to 0.8 for emedastine and 2.0 for levocabastine after 6 weeks. For redness, the scores fell from around 2.7 to 0.5 for emedastine and to 1.1 for levocabastine.[63] Similar results were obtained for emedastine in earlier studies in which a conjunctival allergen-challenge (CAC) model was utilized to compare the efficacy of 0.05% emedastine with 0.5% ketorolac[64] and with 0.05% levocabastine.[65] On the basis of all these collective data, the FDA approved emedastine 0.05% (Emadine) in December 1997, and the European Medicines Agency (EMA) approved emedastine 0.05% (Emadine) in January 1999 for use in treating SAC. While emedastine exhibited superior pharmacological properties to both levocabastine and ketotifen in terms of a higher or equivalent H_1-receptor affinity, greater *in vitro* potency, greater H_1-receptor selectivity[26,27] and efficacy in the animal models of AC,[62] the 5−8 h duration of action in the CAC model[66] and twice-daily topical ocular dosing requirement to control the signs and symptoms of SAC were considered less than ideal.[62−66] Therefore, Emadine was strategically only marketed in the EU while we continued our search for a better ocularly suited antihistamine with superior characteristics to Emadine.

During the ongoing research described above, we had also profiled an antiallergic drug, olopatadine, from another Japanese company (Kyowa Hakko Kogyo, Tokyo, Japan). Olopatadine was originally synthesized and reported by the team of Ohshima et al.[67] and it was shown to be effective at inhibiting histamine-induced skin weal. The Alcon team had obtained olopatadine and profiled it in several *in vitro* assays and *in vivo* models of AC. Although olopatadine exhibited a lower H_1-receptor affinity (K_i = 36 nM) as compared with emedastine (K_i = 1.2 nM) (Tables 1,2), it possessed a greater H_1-receptor selectivity than antazoline, ketotifen, and levocabastine vs H_2- and H_3-receptors of the guinea pig brain preparation (Table 2; Figure 5A).[29] It was interesting to find later on that olopatadine had a higher affinity (K_i = 2.5 nM) for the human H_1-receptor[68] than for the guinea pig H_1-receptor.[29] Olopatadine potently antagonized histamine-induced PI turnover in isolated HCE, HCF, and HTM cells (IC_{50}s = 10−40 nM; Figure 2B) and potently inhibited cytokine secretion from HCE cells.[60,69] In isolated HCMCs, olopatadine concentration-dependently inhibited anti-IgG-stimulated histamine secretion (IC_{50} = 559 ± 277 μM; Figure 3), but unlike ketotifen which promoted histamine release (also PGD_2 and tryptase release) from HCMCs at high concentrations, olopatadine did not exhibit such toxicity effects even up to 2 mM.[57−59]

The specific way olopatadine and epinastine interact with cell membranes appears to stabilize and perhaps strengthen the latter[70] thereby preventing HMC degranulation in response to the pollen-induced immune reaction in the conjunctiva. Such characteristics and additional specific binding of olopatadine may also explain why this drug was 10-fold more potent at inhibiting histamine-stimulated cytokine release from HCE cells[59,60,69] than its H_1-receptor binding affinity using guinea pig brain cell membranes.[26,27] Olopatadine, levocabastine, and emedastine were significantly more potent antagonists than antazoline and pheniramine in the histamine-mediated cytokine secretion assays.[58−60] These mast cell stabilizing and anti-histaminergic activities of olopatadine translated well to the *in vivo* models of AC. Thus, topical ocular application of olopatadine effectively blocked antigen- and histamine-stimulated conjunctivitis in guinea pigs.[56,58] Passive anaphylaxis in guinea pig conjunctiva was also attenuated by olopatadine applied 30 min prior to intravenous or topical ocular antigen challenge (ED_{50} values 0.0067% and 0.017%, w/v, respectively).[30,56] Likewise, olopatadine applied topical ocularly (t.o.) from 5 min to 24 h prior to a histamine challenge effectively and concentration-dependently attenuated the vascular permeability response.[30,56] These data strongly indicated that olopatadine had an acceptable onset of action, and a durable therapeutic effect. Such preclinical results helped elevate olopatadine for clinical testing in the CAC and SAC models of AC after suitable Investigation New Drug (IND)-enabling studies were conducted to ensure requisite safety of the drug, and eventual effectiveness in human subjects. Results from an environmental study demonstrated that Patanol was effective in the treatment of the signs and symptoms of allergic conjunctivitis when dosed twice daily for up to 6 weeks. Results from conjunctival antigen challenge studies demonstrated that Patanol, when subjects

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Table 3. Reduction of Itching Scores by Pataday and Pazeo in Human Subjects[a]

		Pazeo (olopatadine, 0.7%)	Pataday (olopatadine, 0.2%)		Vehicle	
	time point	mean	mean	difference (95% CI)	mean	difference (95% CI)
Study 1		$N = 66$		($N = 68$)		($N = 68$)
onset	3 min	0.36	0.39	−0.02 (−0.31 0.26)	1.90	−1.54 (−1.82 −1.25)
	5 min	0.53	0.61	−0.08 (−0.39 0.22)	2.06	−1.53 (−1.84 −1.22)
	7 min	0.48	0.61	−0.13 (−0.44 0.17)	1.97	−1.49 (−1.80 −1.18)
16 h	3 min	0.70	0.87	−0.17 (−0.44 0−11)	2.20	−1.50 (−1.77 −1.23)
	5 min	0.79	1.04	−0.24 (−0.55 0.07)	2.27	−1.4S (−1.79 −1.16)
	7 min	0.75	0.98	−0.23 (−0.54 0.08)	2.13	−1.38 (−1.69 −1.07)
24 h	3 min	0.93	1.41	−0.48 (−0.76 −0.20)	2.54	−1.61 (−1.88 −1.33)
	5 min	1.10	1.52	−0.42 (−0.72 −0.12)	2.62	−1.51 (−1.81 −1.21)
	7 min	1.09	1.50	−0.41 (−0.72 −0.10)	2.50	−1.41 (−1.72 −1.11)
Study 2		($N = 98$)		($N = 99$)		($N = 49$)
onset	3 min	0.38	0.47	−0.09 (−0.28 0.09)	1.91	−1.53 (−1.76 −1.30)
	5 min	0.53	0.61	−0.08 (−0.29 0.12)	1.99	−1.46 (−1.71 −1.22)
	7 min	0.65	0.61	0.04 (−0.18 0.26)	1.82	−1.17 (−1.45 −0.90)
24 h	3 min	1.01	1.33	−0.31 (−0.57 −0.06)	2.30	−1.29 (−1.60 −0.97)
	5 min	1.22	1.48	−0.26 (−0.51 -0.01)	2.37	−1.15 (−1.46 -0.84)
	7 min	1.25	1.41	−0.16 (−0.42 0.11)	2.14	−0.89 (−1.22 −0.57)

[a]Mean score estimates, treatment differences, and corresponding 95% confidence intervals (CIs) were based on analysis of repeated measures using a mined model with itching scores frm each eye (left or right) as the dependent variable and fixed effect terms for investigator, treatment, eye-type (left or right), time, and treatment-by-time interaction. The ocular itching score range is 0−4, where 0 is none and 4 is incapacitating itch. The comparative clinical data shown above are from the package insert of Pazeo available from the FDA Web site.

were challenged with antigen both initially and up to 8 h after dosing, was significantly more effective than its vehicle in preventing ocular itching associated with allergic conjunctivitis. Such clinical evaluations of olopatadine (0.01−0.15%) in the CAC model of AC demonstrated optimal efficacy at 0.1% with a duration of action up to 8 h using a twice-daily dosing paradigm relative to placebo.[71−76] Olopatadine 0.1% (Patanol), in a relatively simple formulation, was approved by the FDA and marketed in 1996 for treating SAC-related ocular itching. Subsequently, Patanol was shown to be more efficacious than oral loratadine (Claritin),[73,74] and more effective than topical ocular azelastine[75] and nedocromil.[76] Hence, the use of a dual pharmacophoric compound (antihistaminic and mast cell stabilizer) for the treatment of SAC and PAC became the standard of care during the mid-1990s.[25,54,58]

Even though the team and the company were delighted to make this ground-breaking contribution, the relatively short duration of action and twice-daily dosing regimen remained a concern. These challenges were overcome by finding a solubilization formulation (that contained povidone and edentate disodium) that permitted generation of olopatadine 0.2% that possessed a greater efficacy and was compatible with a once-daily dosing with up to 16 h of effectiveness. Thus, results from clinical studies of up to 12 weeks duration demonstrated that olopatadine 0.2% solution when dosed once a day is effective in the treatment of ocular itching associated with allergic conjunctivitis. This became Pataday and was FDA-approved and marketed in 2004 to treat the itching due to SAC (Table 3),[77−81] and which is now available over the counter. With further refinement of the formulation for olopatadine, a higher concentration became possible a few years later when the formulation was augmented with viscosity enhancing excipients such as hydroxypropyl-gamma-cyclodextrin, polyethylene glycol 400, and hypromellose, and with a slightly higher concentration of the preservative benzalkonium chloride (0.015% vs 0.01%). Patients were evaluated with an ocular itching severity score ranging from 0 (no itching) to 4 (incapacitating itch) at several time points after CAC administration. Table 3 displays the mean ocular itching severity scores after ocular administration of a specific antigen using the CAC model in Studies 1 and 2, respectively. A one-unit difference compared to vehicle is considered a clinically meaningful change in the ocular itching severity score. Olopatadine 0.77% demonstrated statistically significantly improved relief of ocular itching compared to vehicle at 30−34 min, 16 h, and 24 h after study treatment. Olopatadine 0.77% provided statistically significantly improved relief of ocular itching compared to Pataday at 24 h after study treatment, but not at 30−34 min after study treatment. Olopatadine 0.77% demonstrated once-daily dosing efficacy and a 24-h duration of action to reduce ocular pruritis in pollen-sensitive patients in the CAC model of AC (e.g., Table 3).[82−84] Olopatadine 0.77% became Pazeo and was FDA-approved and marketed for clinical introduction for SAC and PAC in 2015 (Table 3).[82−84] While all the marketed antihistamines and mast cell stabilizer drugs for AC treatment are safe and effective (to varying degrees with differences in their onset and duration of action), all have side-effects as can be found in the package inserts of these drugs. Thus, for Pazeo the most commonly reported adverse reactions occurred in 2−5% of patients and included blurred vision, dry eye, superficial punctate keratitis, dysgeusia (bad taste) and abnormal sensation in eye.[84−86]

In closing out this section, it is worth mentioning that since the FDA approvals of Patanol and Pataday and since our research began on emedastine and olopatadine, there has been progress made in identifying additional mast cell mediators/mast cell chemoattractants including a host of chemokine ligands (e.g., CCL2, CCL3, CCL5-CCL11) and adhesion molecules (ICAM-1 and VCAM-1). CCL7, for example, is a potent chemoattractant for monocytes, memory T-lymphocytes, eosinophils, basophils, dendritic cells, and natural killer cells, all of which are heavily implicated in the secondary phase of AC following the increased vascular permeability induced by histamine and other inflammatory mediators during the early/acute phase of AC discussed above. The cloning of a fourth

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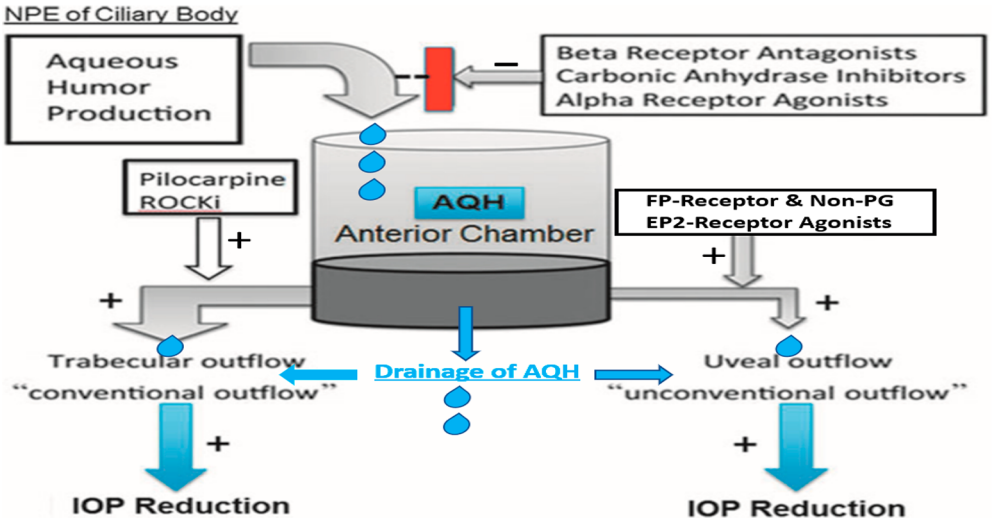

Figure 6. Diagram showing the generation of AQH and its inhibition by certain drugs, and drainage of AQH from the anterior chamber of the eye via two different outflow pathways as promoted by different drug classes. Reproduced and updated with permission from ref 7. Copyright 2018 Mary Ann Liebert Publishing Inc.

histamine receptor and its localization on animal and human conjunctiva and eosinophils[87] led to the finding that only H_1-and H_4-receptors are involved in mediating the itching sensation.[88] Furthermore, the involvement of serotonin-1 and 2-receptors along with protease-activated receptor-2 in propagating the itch response involves transient receptor potential-ion-channel mediated signaling pathways,[18] thereby laying the foundation for potential future therapeutic intervention for AC using these targets. Alcaftadine appears to be the only ocularly utilized antihistamine to-date that possesses a somewhat weak micromolar affinity (IC_{50} = 4.4 μM) and micromolar antagonist potency at the H_4-receptor.[85,89] However, since a bona fide H_4-receptor antagonist of nanomolar affinity and high potency/selectivity, JNJ7777120, induced histamine release on the rat conjunctiva,[90] and H_4-receptors may not be fully operational on human eosinophils,[91] more research is warranted to clarify the relative contribution of H_4-receptors in mediating the ocular inflammatory actions of mast cell-derived histamine in AC. Because H_4-receptors are expressed by mast cells, leukocytes, and CD4+ cells, there is the potential for drugs with H_4-antagonist activity to inhibit recruitment of eosinophils and thus reduce the severity of the late-phase of allergic phenomenon, in particular the ocular itching.[18,88,92]

Lastly, since the advent of the first mast cell stabilizer with potent H_1-receptor antagonists activity, olopatadine, and the approval of Patanol (1996), Pataday (2004), and Pazeo (2015), some other dual action drugs approved for SAC treatment have surfaced. These include: ketotifen (Zaditor, approved 1999), azelastine (Optivar, approved 2000), epinastine (Elastat, approved 2003), bepotastine (Bepreve, approved 2009), alcaftadine (Lastacaft, approved 2010) and cetirizine (Zerviate, approved 2017). In the early clinical trials for suppressing ocular itching (and hyperemia) in the CAC model of AC, ketotifen, azelastine, and epinastine performed poorly against olopatadine 0.1−0.2%. Only alcaftadine (0.25%) exhibited a greater efficacy than olopatadine 0.2% in preventing ocular itching at 3 min and up to 16 h postchallenge/instillation.[92] However, it would be interesting to see how alcaftadine (0.25%) would compare with olopatadine 0.77% (Pazeo) in a future clinical trial for AC treatment. Regardless, it would appear that olopatadine and alcaftadine may remain the gold standards for treating SAC and PAC until more superior drugs are discovered, developed, and approved by health authorities. It is hoped and anticipated that novel medicines for treatment of SAC may come from the many areas of active research involving synthetic organic drugs, immunomodulators, and antibodies directed to integrins, adhesion molecules, leukotriene, and Toll-like receptors, among other modalities.[12,85,92−96]

Taken together, this three-generational product-line featuring olopatadine for the treatment of SAC/PAC earned the major contributors involved in the olopatadine research and development for AC research at Alcon (Dr. Najam Sharif, Dr. John Yanni, and Mr. Steve Miller, and Mr. Shouxi Xu) the "Sir James Black Award for contributions to drug discovery" from the British Pharmacological Society in December 2017.

Discovery, Development, and Approval of Travatan for Treatment of Ocular Hypertension (OHT) and Primary Open-Angle Glaucoma (POAG). The neurodegenerative eye disease "glaucoma" comprises several different multifactorial optic neuropathies, the cardinal features of which encompass slow but progressive destruction of the optic nerve that connects the retinal ganglion cells via their axons in the anterior retina to the brain. The loss of such connectivity can result in vision deterioration and ultimately blindness in the absence of suitable treatment(s). Around 80 million people worldwide are currently suffering from Primary Open-Angle Glaucoma (POAG), the predominant form of glaucoma, which is the second leading cause of blindness around the world. Epidemiological surveys project this number to increase to over 112 million victims of POAG by 2040,[10] with resultant poor quality of life and high economic and social burdens. Risk factor analyses have indicated that elevated intraocular pressure (IOP) is highly correlated with the onset and progression of POAG,[5,6,10] but increasing age, comorbidities such as diabetes, retinal vascular abnormalities,[97] and lower than normal intracranial fluid pressure (ICFP)[98] all exacerbate the condition and may under certain circumstances be more responsible causative factors than high IOP.[6,14,97] Thus, some people with fairly normal IOPs (~16−21 mmHg) still experience progressive visual impairment and blindness, suggesting that factors other than IOP are involved in the pathology and progression of "normotensive glaucoma" (NTG).[99,100] Research over the years

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Table 4. Classes of Clinically Utilized Drugs for Treating Ocular Hypertension/POAG/NTG[a]

pharmacological class of drug	general name of approved drugs (brand name)	mode(s) of action	pertinent comments
		Conventional AQH Outflow Promoting Drugs	
cholinergic muscarinic receptor agonists	pilocarpine (Isopto Carpine); carbachol (Miostat)	enhancement of AQH via conventional outflow pathway	oldest drug therapy known for glaucoma; use limited by 4× daily topical ocular [t.o.] dosing and brow ache and meiosis
rho kinase (ROCK) inhibitors	ripasudil (Galanatec); netarsudil (Rhopressa)	increase conventional outflow of AQH (perhaps also enhancing episcleral venous outflow)	relatively efficacious IOP-lowering; increased propensity for hyperemia induction
		AQH Production (Inflow) Inhibitor Drugs	
carbonic anhydrase inhibitors	dorzolamide (Trusopt); brinzolamide (Azopt)	AQH Inflow inhibition at ciliary processes	oral acetazolamide and methazolamide were used in the past; currently used for acute IOP control instead of chronic therapy; 2x-t.o. daily dosing
beta-adrenergic receptor antagonists ("beta blockers")	timolol (Timoptic); betaxolol (Betoptic); levobunolol (Betagan)	AQH Inflow inhibition at ciliary processes	widely utilized; 2×-t.o. daily dosing; can induce bradycardia; asthmatics treated very cautiously.
alpha$_2$-adrenergic receptor agonists	brimonidine (Alphagan); apraclonidine (Iopidine)	AQH Inflow suppression at ciliary processes and enhancement of uveoscleral outflow of AQH	epinephrine and dipivefrin used historically; brimonidine widely used nowadays; 2×- daily t.o. dosing but propensity to cause ocular allergic reaction
		Uveoscleral Outflow Promoting Drugs	
prostaglandin analogs (FP-receptor agonists), a novel non-PG EP2-receptor agonist (OMDI))	latanoprost (Xalatan); travoprost (Travatan); bimatoprost (Lumigan); tafluprost (Zioptan). omidenepag isopropyl (OMDI) (Eybelis)	enhancement of uveoscleral and also conventional outflow of AQH En-hancement of uveoscleral outflow of AQH	FP-receptor agonists are the most widely used most potent and most efficacious drug class enabling 1x-t.o. dosing; cosmetic side-effects in and around eyes (iridial color change; deepening of eyelid sulcus). OMDI approved in Japan does not have the aforementioned side-effects.
		Multiple Modes of Action Drugs	
prostaglandin conjugates	latanoprostene bunod (latanoprost conjugated to an nitric oxide [NO] donor) (Vyzulta)	increase uveoscleral and also conventional outflow of AQH	efficacious IOP-lowering using dual mechanisms of action; 1×-t.o. dosing; propensity for greater hyperemia induction due to NO
combination products	examples include: dorzolamide + timolol (Cosopt); brimonidine + brinzolamide (Simbrinza); travoprost + timolol (DuoTrav); latanoprost + netarsudil (Roclatan)	enhancement of outflow and suppression of inflow of AQH	efficacious IOP-lowering using dual modes of action; 1×-t.o. dosing; Patients who are refractory or poor responders to standards of care usually require combination products.

[a]While t.o. drugs are the mainstay treatment for OHT/POAG/NTG, some patients are recalcitrant to pharmaceutical agents. Thus, use of the above-mentioned drugs is often secondarily supplemented with implantation of AQH microshunts or surgeries to reduce the IOP down to or below the normal range in order to help preserve vision in these patients.[5−7,13,99,116]

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has yielded some clues including the concept that RGCs and their axons in NTG patients have a lower threshold for damage due to even relative low IOPs, perhaps they are more sensitive and susceptible to IOP fluctuations, ischemia/hypoxia, and to metabolic and oxidative stress than POAG patients.[99,100]

Homeostatic control of IOP is maintained due to a balance between aqueous humor (AQH) production by the ciliary processes (nonpigmented ciliary epithelium [NPCE] cells) and its drainage from the anterior chamber (ANC) of the eye through two different pathways, the major conventional trabecular meshwork (TM) outflow and the minor uveoscleral outflow (UVSC) pathway (Figure 1A/B; Figure 6). In most POAG patients, AQH does not egress or the drainage is extremely slow due to severe blockage of the trabecular meshwork (TM) and Schlemm's canal (SC) (Figure 1A/B) resulting from accumulation of cellular debris and excessive extracellular matrix (ECM).[5,6] The IOP rises and is propagated throughout the eyeball with a major impact on the rear of the globe. This process starts damaging the delicate fenestrated tissue at the back of the eye in the optic nerve head (ONH) region, the lamina cribosa (LC),[100−104] which supports the million RGC axons as they pass through to form the optic nerve. The stress and strain of the high IOP initiates local release of inflammatory substances and matrix metalloproteinases (MMPs) that degrade the ECM of the LC, and its structural integrity declines and the optic nerve and associated blood vessels bend and constrict,[101−104] causing ischemia.[105,106] This aberrant tissue remodeling[103,107] adversely affects the RGC axons, and their tensile strength decreases. The ensuing ischemia/hypoxia causes further inflammatory factors to be released, and the vicious cycle continues. During this time, the axonal transport of mitochondria and neurotrophic factors from the brain to the RGC somas and dendrites via the axons in the optic nerve is retarded,[108−111] and the axonal injury is increased to the point where their terminals in the brain thalamic nuclei begin to atrophy.[112] RGC axons, followed by the RGCs themselves, are depleted of energy[109,110,113,114] and growth factors,[111,112] and apoptotic death of the RGCs follows. While these are slow processes and their detrimental effects take several years to manifest as visual disturbances, the cascade of deleterious events and factors continues to spiral out of control unless there is therapeutic intervention. Also, because POAG is a "silent thief of sight" the patient is usually unaware of their disease until quite late into the progression phase. It appears that in the early phase of glaucoma development the brain compensates for the loss of contrast sensitivity,[115] and due to the asymptomatic nature of this insidious disease, the patient finally notices visual impairment when ∼40% of the RGCs have been destroyed and peripheral vision has significantly deteriorated. It is now critical to quickly diagnose and begin treatment to lower the elevated IOP,[4−6] the only modifiable end point that has thus far shown to alleviate the damage caused to the RGCs and their axons during the pathogenesis and progression of POAG and NTG.[4−6,97,99] AQH production can be slowed and/or its drainage stimulated using pharmaceutical or surgical means to lower the IOP and to save the sight of these patients (Figure 6; Table 4).[5−7,116]

In the early 1990s, at the time our research into drug discovery for treating OHT/POAG started, only some old drugs such as pilocarpine, timolol, brimonidine, dorzolamide, brinzolamide, and trabeculoplasty were available to the clinicians (see Table 4). Even though these drugs offered IOP-lowering efficacy, their low potencies necessitated 2−4-times daily ocular dosing, and their side-effect profiles were not ideal.[5−7,116] Early stage academic research (and later, work at Pharmacia Inc.) had begun to show that various classes of prostaglandins (PGs) possessed ocular hypotensive activity in various animals.[117−123] However, the natural PGs in their free acid forms (and later as esters) caused corneal/conjunctival vasodilation and thus hyperemia.[117−123] In some cases, inflammation, foreign body sensation, and localized hemorrhages on the ocular surface were also observed when t.o. dosed. The FP-receptor agonist class became the preferred target when researchers demonstrated that upon esterification of the free acid and modification of the lower chain of $PGF_{2\alpha}$, the IOP-lowering efficacy could be enhanced and the side-effects significantly reduced due to improved receptor selectivity.[13,119,121,124−126] Pharmacia Inc., which later became part of Pfizer, had numerous compounds they were trying to optimize for their clinical trials. Our internal research review of such a program revealed that Alcon could compete in this area of ocular discovery research and bolster the portfolio beyond betaxolol (beta-blocker) and brinzolamide (carbonic anhydrase inhibitor) (Table 4).

With senior management's support, a number of existing biologists were reassigned to my newly created Molecular Pharmacology Unit, and I rapidly hired several new scientist biologists and began establishing and validating numerous specific PG receptor binding and functional assays and rendered them into the HTS platform. Simultaneously, our expert medicinal chemists had begun synthesizing key reference and novel PG molecules. Together, we launched a multidimensional drug discovery program, initially focusing on FP-receptor agonists but then also spreading the net wider to capture novel compounds that may have IOP-lowering potential by engaging other PG receptor types and/or subtypes. At first, progress was slow due to the novelty of the new drug discovery paradigm being implemented. However, as the team members and other associates sharpened their focus, gelled together scientifically and personality-wise, and some good reproducible data began to emerge, the team got the necessary motivational boost, and productivity and innovation accelerated. The team established compound screening funnels with stringent criteria for Go/No Go decisions to be made. A pharmacological mindset was also a huge catalyst that yielded dividends! Full concentration−inhibition and concentration−response *in vitro* studies allowed the team to rank order compounds and select leads for animal safety and efficacy studies based on receptor affinity and agonist potency (+ relative intrinsic activity). Thankfully, the full range of PG receptor binding and functional assays had been established and validated with suitable agonists and antagonists, and thus we began to also generate relative PG-receptor selectivity data for our key compounds of interest. This was critical since the published literature on various PGs was incomplete or somewhat inaccurate. In certain cases, the literature data could not be reproduced, and thus our internal database became our guide that gave everyone much more confidence on results from our internal screening efforts.

The HTS paradigm and system permitted automatic transfer of raw data from receptor binding and second messenger assay readout machines, automatic curve-fitting, and data archival.[7,29,31] Thus, data sharing became routinely automated and the biologists and medicinal chemists in various departments utilized the information to render further design modifications to the compounds, and the triaged *in vitro*-active compounds meeting defined criteria were flagged and prioritized for *in vivo* testing for ocular safety (topical ocular testing at defined

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Table 5. Relative Affinities and Selectivities of Synthetic Prostaglandins for PG Receptor Sub-Types[a]

PG analogue	PG receptor binding inhibition constants (K_i, nM) and FP receptor selectivity (x)							
	DP	EP_1	EP_2	EP_3	EP_4	FP	IP	TP
travoprost free acid ((*S*)-fluprostenol)	52 000 ± 7 200 (*x 1 486*)	9 540 ± 1 240 (*x 273*)	nd	3 501 ± 461 (*x 100*)	41 000 ± 2 590 (*x 1 171*)	35 ± 5	≥90 000 (*x 2 571*)	≥121 000 (*x 3 457*)
(*R*/*S*)-fluprostenol free acid	>50 000 (*x 510*)	12 300 ± 1 240 (*x 126*)	>100 000 (*x 1020*)	4 533 ± 597 (*x 46*)	14 400 ± 1 550 (*x 147*)	98 ± 9	>60 500 (*x 617*)	121 063 ± 20 714 (*x 1 235*)
bimatoprost free acid (17-phenyl-$PGF_{2\alpha}$)	>90 000 (*x 1 084*)	95 ± 27 (*x 1*)	nd	387 ± 126 (*x 5*)	25 700 ± 2 060 (*x 310*)	83 ± 2	>100 000 (*x 1 205*)	>77 000 (*x 928*)
latanoprost free acid (PHXA85)	≥20 000 (*x 204*)	2 060 ± 688 (*x 21*)	39 667 ± 5 589 (*x 405*)	7 519 ± 879 (*x 77*)	75 000 ± 2 830 (*x 765*)	98 ± 11	≥90 000 (*x 918*)	≥60 000 (*x 612*)
bimatoprost (Amide)	>90 000 (x 14)	19 100 ± 1 450 (*x 3*)	nd	>100 000 (*x 16*)	>100 000 (*x 16*)	6 310 ± 1 650	>100 000 (*x 16*)	>100 000 (*x 16*)
unoprostone (free acid)	>43 000 (x 7)	11 700 ± 2 710 (*x 2*)	nd	≥22 000 (*x 4*)	15 200 ± 3 500 (*x 3*)	5 900 ± 710	>30 000 (*x 5*)	>30 000 (*x 5*)
natural endogenous PG ligand $PGF_{2\alpha}$	18 000 ± 6 460 (*x 138*)	±12 (*x 5*)	964 ± 64 #	24 ± 8 (*x 0.2*)	±25 (*x 3*)	130 ± 6	≥50 000 (*x 385*)	≥190 000 (*x 1 462*)

[a]Data are mean ± SEMs from >3 experiments for each compound in each assay. The values in parentheses are the relative FP-receptor selectivities of the compounds. Note that the naturally occurring $PGF_{2\alpha}$ lacks selectivity but the synthetic compounds such as travoprost free acid and latanoprost free acid exhibit significant FP-receptor selectivity.[158]

concentration(s) in preagreed standardized formulation in rabbits).[7] Those compounds that met the "Go-Criteria" were scheduled and tested for effectiveness in the cat pupil diameter measurement model and ocular hypertensive cynomolgus monkey eyes model for IOP-lowering activity.[7,127] Typically, efficacy results from a single standardized t.o.-dose study and any side-effects were reviewed by the team before a dose−response study was conducted. This stage-gate screening paradigm ensured speed without impacting data quality, ensured data integrity due to internal data access restrictions, reduced burden, ensured animal safety and health and minimized animal usage and associated cost, especially those connected with tertiary animal models such as the OHT monkeys.[7] The iterative molecular design of new compounds helped develop the structure−activity-relationship (SAR), and novelty and patentability was thereby assured. The team built various correlation plots of *in vitro* receptor binding and functional assay data, and the latter compared with *in vivo* data from different animals and models. It was gratifying to find good correlations between these various parameters,[7] and the discovery program continued to experience growth and continued internal funding support.

With sufficient novel data we also began laying the foundation for quality publications that pharmacologically validated various *in vitro* techniques such as RT-PCR[128] and receptor autoradiography[129] and assays involving receptor binding,[130−136] cell-based functional assays,[135−158] and *in vivo* animal models,[127,155−157] and which permitted intellectual property protection via strategic patent filings before public presentations and/or publication of data. The screening and profiling of natural PGs along with new synthetic compounds revealed that indeed the endogenous PGs were on the whole not that selective for their cognate receptor types. Thus, for instance $PGF_{2\alpha}$ exhibited appreciable affinity for EP_3, FP, EP_4, and EP_1 receptors (Table 5), while R/S-fluprostenol and its S-enantiomer (travoprost acid; AL-5858) were significantly more FP-receptor-selective than other synthetic PGs tested (Figure 7; Table 5).[158]

Furthermore, second messenger-based functional assays confirmed that the natural PGs were quite promiscuous and nonselective with respect to which PG receptors they activated

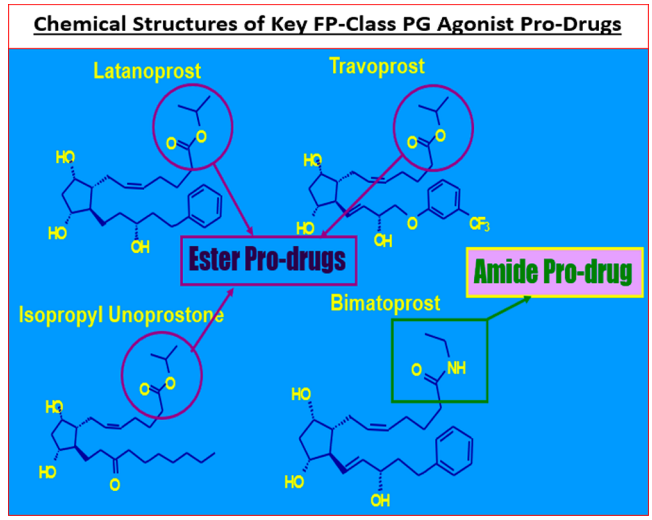

Figure 7. Structures of the key PG pro-drugs discussed in this article are shown in this figure.

(Table 6). These findings supported the ocular side-effect observed with the natural PGs when they were instilled to the animal eyes,[117,120,122] and hence the need for more potent and PG-receptor-selective agents.[158] Even though some of the synthetic PGs such as bimatoprost acid, latanoprost acid, and cloprostenol exhibited relatively high potency at the their cognate FP-receptor, they also stimulated additional PG receptors at fairly low concentrations thereby rendering them to be also not so FP-selective (Table 6). Fortuitously, the most FP-receptor-selective and potent new PG turned out to be travoprost acid as shown by the bolded potency values in Table 6 below.[155,158] These types of receptor affinity, potency, and receptor-selectivity data helped the team to frame S-fluprostenol as a viable drug candidate, and this compound progressed to detailed side-effect profiling, rabbit/guinea pig hyperemia, cat eye meiosis, and conscious OHT monkey eyes IOP studies (see ahead).

Along the way, target localization studies were conducted to verify the presence of the key PG receptors of interest in post-

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Table 6. Relative Agonist Potencies of Natural and Synthetic Prostaglandins for PG Receptor Subtypes[a]

compound	agonist potency (EC_{50}\; nM) at various prostaglandin receptors and subtypes							
	DP-receptor (↑ cAMP)	EP_1-receptor (↑ PI turnover; or other response)	EP_2-receptor (↑ cAMP; or other response	EP_3-receptor (↓ cAMP various functional responses)	EP_4-receptor (↑ cAMP)	FP-receptor (↑ PI turnover at human cloned FP-receptor; or other responses)	IP-receptor (↑ cAMP or other response)	TP-receptor (↑ PI turnover; or other response)
PGD_2	74	3190	58 000	nd	>10 000	>100; 222	>10 000	>10 000
PGI_2	>10 000	319	>10 000	3 019	>10 000	>5 000	7	>10 000
PGE_2	>1 000	2.9	67	19.9; 45; 4.5	40	>2 500	3 310	>10 000
$PGF_{2\alpha}$	>10 000	29	>10 000	691; 2 000	>10 000	29 ± 2	3 000	>10 000
bimatoprost free acid	>10 000	2.7	>10 000	nd	>10 000	3.3 ± 0.7	>10 000	>10 000
travoprost free acid	>10 000	nd	>10 000	>10 000	>10 000	2.4 ± 0.3	>10 000	>10 000
latanoprost free acid (PHXA85)	>10 000	119	20 000	12 000	>10 000	45.7 ± 8.4	>10 000	>10 000
cloprostenol	>10 000	93	>10 000	228	>10 000	0.73 ± 0.1	>10 000	>10 000
unoprostone (free acid; UF-021)	>10 000	>30 000	>10 000	>10 000	>10 000	3 220 ± 358	>10 000	>10 000

[a]Data are from various sources using different methodologies and functional readouts. Note that the endogenously produced PGs exhibit poor receptor selectivity in isolated cell/tissue preparations. Receptor selectivity by the natural PGs may be achieved at the site of action *in vivo* depending on the local PG concentration. nd = not determined.[158]

mortem human eye sections using quantitative autoradiography technology.[129,136,159−161] Indeed, FP-receptors visualized with [^{3}H]-$PGF_{2\alpha}$[129,153] and then later with [^{3}H]-AL-5848 (S-fluprostenol acid)[129,136] demonstrated a relatively high density of receptors in the longitudinal and circular ciliary muscle (CM) (e.g., Figure 8A) whose functional activity was confirmed by measuring PI turnover, $[Ca^{2+}]_i$ mobilization, MAP-kinase studies in isolated human CM (h-CM) cells (Figure 8B,C).[158,162] These results were supported by similar findings using isolated and propagated human TM (h-TM) cells,[163] and human ciliary body cloned FP-receptor expressed in host HEK-293 cells.[164,165]

Since CM and TM cells are responsible for mediating the AQH outflow enhancing ability of FP-receptor agonists to lower IOP (Figure 1B), the presence of functionally responsive FP-receptors in these key cells helped explain the relatively high potency and efficacy of FP-agonists, in particular travoprost acid (AL-5848; S-fluprostenol) in efficaciously lowering IOP in the OHT monkey eyes.[155,156] These data helped further support our research discovery program. Additional data were obtained for certain compounds such as extensive receptor profiling (on- and off-target) and using Alcon sponsored research with academic collaborators[162,164,166] and contract research facilities[155,167] to round-off the SAR development. Compounds of interest were tested at multiple doses for their ability to lower and control IOPs in the relevant animal models including the OHT cynomolgus monkeys with 8−12 animals per group (e.g., Figure 9).[155,156] Such IOP data were also confirmed in different colonies of OHT monkeys in-house and also at external collaborator facilities to confirm the efficacy of lead compounds, thereby confirming internal data and enhancing the overall confidence in the testing paradigms.

Additionally, the latter collaborations permitted studies to delineate the mechanisms of action of the lead compounds in terms of their effects on Ca^{2+}-mediated (Figure 10A)[162−165] secretion of matrix metalloproteinases (MMPs) from h-CM cells (e.g., Figure 10B), and AQH outflow via the conventional TM[164] pathway and via the uveoscleral pathway (which involves egress of AQH through expanded spaces in the CM and sclera)[162] to lower IOP (Figure 9). It became apparent that the new FP-receptor agonist class of PGs (Figure 7) primarily activated phospholipase C to generate inositol phosphates (IPs; Figure 8)[162] and mobilized $[Ca^{2+}]_i$ (Figures 10A),[162−165] with similar potencies in a variety of human ocular and animal cells (Table 7),[158] that induced MMPs secretions (e.g., Figure 10B) which then digested the ECM in CM and TM to create/expand UVSC outflow pathway (and to some degree the TM-pathway) to lower IOP (Figure 9) following a single drop t.o. instillation of the FP-receptor agonist drug.

Such multiyear *in vitro* and *in vivo* research resulted in the identification and nomination of clinically viable lead compounds. Following IND-enabling studies and having met all stage-gate "Go Criteria", some of the leads entered clinical trials for human ocular safety, efficacy, and durability of the IOP-lowering effect based on the classic Phase 1−3 studies paradigm. These proof-of-concept and formal clinical investigations culminated in the approval of Travatan (0.004% travoprost isopropyl ester) by the US FDA in 2001 and by EMA for the treatment of OHT associated with POAG (Table 8). Specifically, Travatan (travoprost 0.004%) and a slightly lower concentration FDA-approved drug (Izba; travoprost 0.003%) lowered IOP by 7.1−8.4 mmHg from baseline and maintained this reduced IOP at various time points during the day after a single topical ocularly administered drop of either drug at night in up to 442 OHT/POAG patients (Table 8).

These and additional IOP-lowering clinical data for Travatan compared well with those published for latanoprost (0.005%; Xalatan) in terms of efficacy and duration of action over a 24-h period studied during several months (recently reviewed, ref 168.) Travoprost isopropyl ester's active moiety, travoprost free acid, was found to be a potent and efficacious FP-receptor full agonist (e.g., Figure 8B, 8C),[155,158,162−166] and the parent drug suitable for once-daily t.o. dosing at night. Such FP-class-PG-directed research also helped identify and characterize other useful FP-receptor class PG analogues that met *in vitro* and *in vivo* potency/efficacy parameters and were qualified as clinical candidate ocular hypotensive drugs. These included compounds such as AL-12128[170,171] and several other novel FP-receptor

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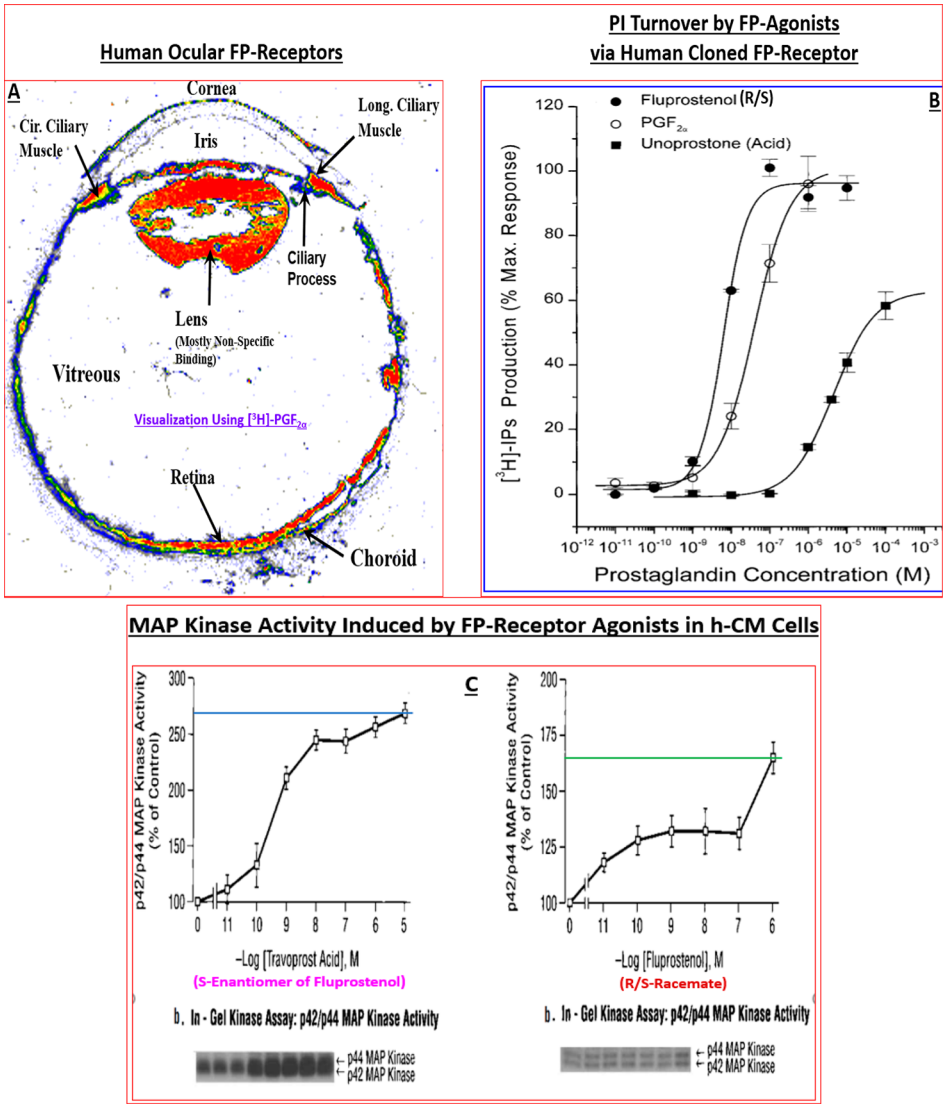

Figure 8. (A) Autoradiographically visualized FP-receptors in a section of human eye *in vitro* are shown (total binding of [^{3}H]-PGF$_{2\alpha}$). The black/white radioautograph was pseudocolor coded to illustrate the relative density of the FP-receptors. Red indicates the highest density, followed by orange, yellow, green, and blue. (B) PI turnover and accumulation of intracellular [^{3}H]-IPs following stimulation by fluprostenol (R/S; racemate), PGF$_{2\alpha}$, and unoprostone free acid in HEK-943 cells expressing human cloned ciliary body FP-receptor. Reproduced with permission from ref 164. Copyright 2002 Mary Ann Liebert Publishing Inc. (C) MAP kinase activity stimulated by travoprost acid (*S*-enantiomer of fluprostenol) and fluprostenol (*R*/*S*; racemate) in isolated and cultivated hCM cells. Reproduced with permission from ref 162. Copyright 2003 Mary Ann Liebert Publishing Inc.

agonists that were potent and efficacious ocular hypotensive agents as demonstrated in the OHT eyes of conscious cynomolgus monkeys in multiple studies.[126,172,173]

Even though FP-class PGs (latanoprost 0.005, travoprost 0.004, bimatoprost 0.03%, tafluprost 0.0015%) became first-line therapeutic drugs for treating OHT/POAG in the late 1990s and early 2000s, it is important to balance their excellent IOP-lowering activities with several ocular side-effects which are well documented.[119,121,156,168,169,174] By example, the most common ocular adverse event observed in controlled clinical studies with Travatan 0.004% was ocular hyperemia which was reported in 35 to 50% of patients. Approximately 3% of patients discontinued therapy due to conjunctival hyperemia. Ocular adverse events reported at an incidence of 5 to 10% included decreased visual acuity, eye discomfort, foreign body sensation, pain, and pruritus. Ocular adverse events reported at an incidence of 1 to 4% included, abnormal vision, blepharitis, blurred vision, cataract, cells, conjunctivitis, dry eye, eye disorder, flare, iris discoloration, keratitis, lid margin crusting, photophobia, subconjunctival hemorrhage, and tearing. Non-ocular adverse events reported at a rate of of 1 to 5% were accidental injury, angina pectoris, anxiety, arthritis, back pain, bradycardia, bronchitis, chest pain, cold syndrome, depression, dyspepsia, gastrointestinal disorder, headache, hypercholesterolemia, hypertension, hypotension, infection, pain, prostate disorder, sinusitis, urinary incontinence, and urinary tract infection. The latter are reported in the package insert for this ocular hypotensive drug. It is evident from all the reported studies that all FP-class PG analogues, including bimatoprost and its free acid, share the same common side-effects described above.[174]

During the aforementioned research, the Alcon medicinal chemistry team also synthesized and tested many analogues of PGD$_2$, both free acids and various esters, and successfully

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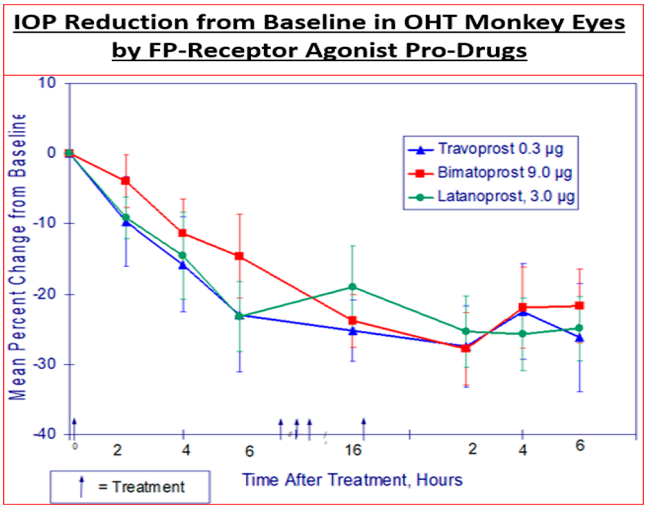

Figure 9. IOP reduction by three different PG pro-drug compounds tested t.o. at different doses in the OHT eyes of conscious cynomolgus monkeys.

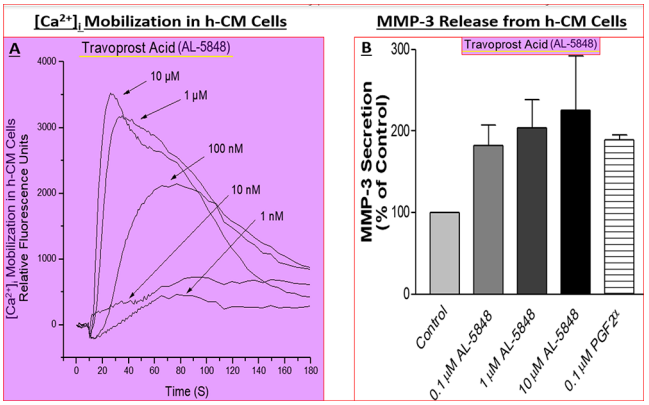

Figure 10. (A,B) Concentration-dependent mobilization of $[Ca^{2+}]_i$ (A) and MMP-3 secretion from h-CM cells (B) in response to travoprost acid (AL-5848). Reproduced and updated with permission from ref 162. Coopyright 2003 Mary Ann Liebert Publishing Inc.

identified safe and efficacious drugs such as AL-6598 that entered clinical trials.[156,161] Although initial studies identified the free acid of AL-6598 as a DP-receptor agonist,[156] more detailed investigations of AL-6556 led to the discovery that it possessed dual pharmacophoric activity being a full agonist at the DP receptor but a partial agonist at the EP_2 receptor.[161] AL-6598 was a potent and efficacious ocular hypotensive in OHT monkey and human eyes.[156] However, the relatively high degree of hyperemia associated with t.o. dosing of AL-6598 precluded its future development, even though we tried to lower the risk and intensity of this side-effect by utilizing a low dose of a vasoconstrictor α2-agonist, brimonidine, that did not interfere with the IOP-lowering efficacy of AL-6598.[156]

Some years later, the antiglaucoma team also successfully characterized[147,157,175] and obtained effective lowering of IOP by the S-enantiomer of betaxolol (Betaxon) in animal models of OHT[157] and in OHT/POAG patients.[176] However, for strategic marketing purposes, even though Betaxon was approved by the FDA, Betaxon was not marketed in the US. Instead, the antiglaucoma team focused on generating sufficient data to secure FDA approval and then successfully launched a few combination products such as DuoTrav (travoprost + timolol; see ref 13 for review), and Simbrinza (brinzolamide + brimonidine) (Table 9), the latter being the first combination product devoid of a β-blocker, to help those patients who were low-responders to individual ocular hypotensive drugs or those who were refractory to other pharmaceutical treatments to lower their IOPs.

Similarly, for expansion of the Alcon antiglaucoma drug treatment portfolio and franchise, additional drug targets were investigated, and several drug discovery projects were launched in the ensuing years after global approval and marketing of Travatan. Thus, we also found some relatively potent and efficacious ocular hypotensive rho kinase (ROCK) inhibitors[176−180] that matched the *in vitro* and *in vivo* properties of ripasudil and netarsudil, and that showed the classic actomyosin-relaxing activity observed with other well-known literature reference ROCK inhibitors such as Y-27632 and Y-39983 (e.g., Figure 11).[180,181]

Table 7. Agonist Potencies of Synthetic Prostaglandins for FP-Receptors Expressed in Various Cell-Types[a]

	stimulation of PI turnover and production of IPs (functional response) in different cell types (agonist potency, EC_{50} [nM])				
compound	human ciliary muscle (h-CM) cells	human trabecular meshwork (h-TM) cells	human cells (HEK-293) expressing cloned human ocular FP receptor	mouse Swiss 3T3 fibroblast cells	rat A7r5 vascular smooth muscle cells
travoprost free acid ((*S*)-fluprostenol)	1.4 ± 0.2	3.6 ± 1.3	2.4 ± 0.3	2.6 ± 0.2	2.6 ± 0.5
bimatoprost free acid (17-phenyl-$PGF_{2\alpha}$)	3.8 ± 0.9	28 ± 18	3.3 ± 0.7	2.8 ± 0.2	2.8 ± 0.6
(R/S)-fluprostenol	4.3 ± 1.3	11 ± 2	4.6 ± 0.4	3.7 ± 0.4	4.4 ± 0.2
$PGF_{2\alpha}$	104 ± 19	62 ± 16	29 ± 2	26 ± 3	31 ± 3
travoprost (isopropyl ester)	123 ± 65	103 ± 27	40.2 ± 8.3	81 ± 18	46 ± 6
latanoprost free acid (PHXA85)	124 ± 47	35 ± 2	45.7 ± 8.4	32 ± 4	35 ± 8
latanoprost (isopropyl ester)	313 ± 90	564 ± 168	173 ± 58	142 ± 24	110 ± 19
unoprostone (UF-021; free acid)	3 503 ± 1 107	3 306 ± 1 700	3 220 ± 358	617 ± 99	878 ± 473
unoprostone isopropyl ester	8 420 ± 912	2 310 ± 1 240	9 100 ± 2 870	560 ± 200	458 ± 85
bimatoprost (amide)	9 600 ± 1 100	3 245 ± 980	681 ± 165	12 100 ± 1 200	6 850 ± 1 590

[a]Data taken from ref 158.

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Table 8. Mean IOP (mmHg) by Treatment Group and Treatment Difference in Mean IOP in Response to Travatan and Izba[a]

		IOP change from baseline (mmHg)							
		IZBA				TRAVATAN			
visit		*N*	8 AM	10 AM	4 PM	*N*	8 AM	10 AM	4 PM
week 2	mean	442	−8.0	−7.3	−7.1	416	−8.1	−7.5	−7.1
	95% CI		(−8.3, −7.7)	(−7.6, −7.0)	(−7.4, −6.8)		(−8.4, −7.8)	(−7.8, −7.2)	(−7.4, −6.8)
week 6	mean	440*	−8.1	−7.4	−7.2	413	−8.3	−7.5	−7.2
	95% CI		(−8.4, −7.9)	(−7.6, −7.1)	(−7.5, −6.9)		(−8.7, −8.0)	(−7.9, −7.2)	(−7.5, −6.9)
month 3	mean	432*	−8.2	−7.5	−7.1	408	−8 4	−7.6	−7.3
	95% CI		(−8.6, −7.9)	(−7.9, −7.2)	(−7.4, −6.8)		(−8.7, −8.1)	(−7.9, −7 2)	(−7.7, −7.0)

	IZBA (Travoprost 0.003%)	TRAVATAN (Travaprost 0.004%)	Difference
visit/time point	mean (SE)	mean (SE)	mean (95% CI) *
baseline	(*N* = 442)	(*N* = 418)	
8 AM	26.9 (0.12)	27.1 (0.14)	−0.2 (−0.5 0.2)
10 AM	25.4 (0.13)	25.6 (0.15)	−0.2 (−0.6 0.2)
4 PM	24.6 (0.14)	24.8 (0.16)	−0.2 (−0.6 0.2)
week 2	(*N* = 442)	(*N* = 416)	
8 AM	19.4 (0.16)	19.5 (0.17)	−0.1 (−0.5 0.3)
10 AM	18.6 (0.16)	18.6 (0.16)	−0.0 (−0.4 0.4)
4 PM	18.0 (0.16)	18.3 (0.16)	−0.3 (−0.7 0.1)
week 6	(*N* = 440**)	(*N* = 413)	
8 AM	19.3 (0.16)	19.3 (0.17)	−0.0 (−0.4 0.4)
10 AM	18.5 (0.16)	18.6 (0.17)	−0.1 (−0.5 0.3)
4 PM	18.0 (0.16)	18.1 (0.17)	−0.2 (−0.6 0.2)
month 3	(*N* = 432**)	(*N* = 408)	
8 AM	19.2 (0.17)	19.3 (0.18)	−0.1 (−0.5 0.3)
10 AM	18.3 (0.17)	18.6 (0.18)	−0.3 (−0.7 0.1)
4 PM	18.0 (0.16)	18.0 (0.17)	0.0 (−0.4 0.4)

[a]Data are from the package insert of Izba that compares the IOP-lowering efficacy with Travatan. The asterisks (∗) indicate statistical significance.

In the intervening months we decided to launch a totally diverse pioneering drug discovery program that involved finding suitable ocular hypotensive drugs from the serotonergic (5-hydroxy tryptamine; 5-HT) receptor field, even though this was an extremely difficult task. The literature and our early studies in this field had shown that 5-HT was an important transmitter in the eye.[182−185] What confounded the issue was the diversity of conflicting reports connected with IOP-lowering actions of various 5-HT receptor agonists and antagonists (see refs 186 and 187 for reviews). Initial studies in-house centered around routinely and methodically testing commercially available serotonergic compounds in the rabbit and monkey models of OHT in order to delineate involvement of specific 5-HT receptors in the process of IOP-lowering and to generate a database upon which to build the discovery program. The team successfully ruled out several receptor classes and zoned in on the 5-HT_2 receptor family.[185] We patented certain novel serotonergic compounds that were potent and efficacious ocular hypotensives with IOP-lowering up to 30% or greater in the OHT eyes of conscious Cynomolgus monkeys[188−191] and published several patents and papers[182−205] describing our efforts to lead the field and inspire other researchers to join the hunt for novel and efficacious drugs to treat OHT/POAG. Lessons learnt from the PG drug discovery programs helped us stay focused and we successfully established in-house receptor binding, cell-based functional assays and deployed the *in vivo* models to rapidly screen compounds that medicinal chemists synthesized. We mapped 5-HT receptor mRNAs in human ocular tissues,[194] and localized the 5-HT_2 receptors in human eye sections by autoradiography[199] to ensure our target receptors were indeed accessible and engageable with compounds of interest. Again, as with PG receptor studies, we demonstrated that functional 5-HT_2 receptors were present on isolated and propagated h-CM[199] and h-TM,[200] and in NPCE,[194] the key cells/tissues that modulate AQH dynamics in the ANC of the eye. This research project finally yielded a novel class of 5-HT_2 receptor agonists with high affinity and selectivity for the 5-HT_{2A} receptor subtype and which potently and effectively reduced IOP in OHT monkey eyes,[188,190,191,193,196,201,202] and some of which (AL-34662; AL-37807)[189,190,201] exhibited efficacy in OHT/POAG patients. Agents with dual receptor activity such as cabergoline[202] were found to activate 5-HT_2 and dopamine receptors and to enhance outflow facility via the TM pathway in porcine eyes *in vitro* (Figure 12A) and 5-HT_{2A}-receptor-selective compounds such as 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane (DOI) to lower IOP in the OHT eyes of monkeys (e.g., Figure 13).[202]

Serendipity (or creative luck?) played a big part in the final ocular hypotensive drug discovery program that I was involved in as a project leader. Because of my early interest in bradykinin (BK) as an edema-causing algesic endogenous inflammatory peptide that also has numerous other functions in the body,[206−209] I was intrigued by the potential role that this peptide may play in ocular physiology and pathology. The discovery and characterization of excitable B_2-subtype of BK receptors on immortalized h-TM cells[207] was also a catalyst that inspired me to patent certain nonpeptide B_2-receptor antagonists (e.g., WIN-64338) as potential IOP-lowering drugs.[208] At that time, I reasoned that compounds such as WIN-64338, being organic stable structure molecules would likely penetrate the cornea upon t.o. dosing, whereas the natural nonapeptide would

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Table 9. IOP Lowering Data for Simbrinza in OHT/POAG Patients[a] (Data Are from the Package Insert for Simbrinza)

	Simbrinza	brinzolamide		brimonidine	
	mean	mean	difference (95% CI)[b]	mean	difference (95% CI)[b]
Study 1	(*N* = 209)	(*N* = 224)		(*N* = 216)	
week 2					
8 AM	20.4	22.0	−1.6 (−2.3, −0.9)	22.4	−2.0 (−2.7, −1.3)
10 AM	17.1	20.5	−3.4 (−4.1, −2.7)	19.4	−2.3 (−3.0, −1.6)
3 PM	18.4	20.4	−1.9 (−2.6, −1.3)	20.6	−2.2 (−2.9, −1.5)
5 PM	16.6	19.7	−3.2 (−3.9, −2.5)	18.4	−1.9 (−2.6, −1.2)
week 6					
8 AM	20.4	21.9	−1.5 (−2.2, −0.8)	22.6	−2.3 (−3.0, −1.6)
10 AM	17.5	20.2	−2.7 (−3.4, −2.0)	19.5	−2.0 {-2.7, −1.3)
3 PM	18.9	20.2	−1.2 (−1.9, −0.5)	21.1	−2.1 (−2.8, −1.4)
5 PM	17.0	19.7	−2.6 (−3.3, −1.9)	18.6	−1.5 (−2.2, −0.8)
month 3					
8 AM	20.5	21.6	−1.1 (−1.8, −0.4)	23.3	−2.8 (−3.5 −2.1)
10 AM	17.2	20.4	−3.2 (−3.9, −2.5)	19.7	−2.5 (−3.2, −l.8)
3 PM	18.7	20.4	−1.8 (−2.5, −1.1)	21.3	−2.6 (−3.3, −1.9)
5 PM	17.0	20.0	−3.0 (−3.7, −2.3)	18.8	−1.8 (−2.5, −1.1)
Study 2	(*N* = 218)	(*N* = 229)		(*N* = 232)	
week 2					
8 AM	20.5	22.2	-1.7 (−2.4, −1.0)	22.8	−2.4 (−3.1, −1.7)
10 AM	17.4	20.7	−3.3 (−4.0, −2.6)	19.2	-1.8 (−2.5, −1.2)
3 PM	18.7	20.5	-1.7 (−2.4, −1.1)	21.1	−2.3 (−3.0, −1.6)
5 PM	16.5	20.1	−3.6 (−4.3, −2.9)	18.3	-1.8 (−2.4, −1.1)
week 6					
8 AM	20.7	21.9	-1.2 (−1.9, −0.5)	23.2	−2.5 (−3.2, −l.8)
10 AM	17.4	20.5	−3.1 (−3.8, −2.4)	19.7	−2.3 (−3.0, −1.6)
3 PM	19.3	20.2	−0.8 (−1.5, −0.2)	21.2	−1.9 (−2.6, −1.2)
5 PM	16.9	19.9	−3.0 (−3.7, −2.3)	18.5	−1.7 (−2.4, −1.0)
month 3					
8 AM	21.1	22.0	−1.0 (−1.7, −0.3)	23.2	−2.2 (−2.9, −1.5)
10 AM	18.0	20.8	−2.8 (−3.5, −2.1)	19.9	−1.9 (−2.6, −1.2)
3 PM	19.5	20.7	−1.2 (−1.9, −0.5)	21.5	−2.0 (−2.7, −1.3)
5 PM	17.2	20.4	−3.2 (−3.9, −2.5)	18.9	−1.7 (−2.4, −1.0)

[a]Based on the Intent-to-Treat Population defined as all patients who received study drug and completed at least 1 on-therapy study visit. [b]The estimates are based on least-squares means derived from a linear mixed model that accounts for correlated IOP measurements within patient; Treatment difference is Simbrinza minus individual component. CI = 95% confidence interval.

not cross the barriers on the ocular surface and may actually cause too much inflammation, a lesson from the AC story relayed above.[33−35] What happened next was a total but a delightful surprise. Since we had a very successful program of material transfer agreements with other pharma companies with subsequent in-licensing and marketing of drugs such as for AC treatment (see above discourse), I requested a sample of FR-199097, a recently reported nonpeptide B_2-receptor BK agonist[209] from Fugisawa Pharmaceutical Co Ltd. (Tokyo, Japan) because I wished to compare its activity with the B_2-Antagonist (WIN-64338) and to see whether an agonist or an antagonist may be better suited for lowering IOP. After months of waiting, the compound arrived and was tested for ocular safety followed by ocular hypotensive activity as per our standard protocol. To my amazement, FR-190997 exhibited very little ocular side-effects but potently and efficaciously reduced OHT monkey IOP at low microgram doses of the compounds, akin to the PGs our team had tested in the past! These data were subsequently reproduced in the same original monkey colony and the compound was also effective in another colony of OHT cynomolgus monkeys. Dose−response studies revealed that the effect was specific and not a random unphysiological response. Indeed, later we showed that FR-190997's IOP-lowering effect could be totally blocked by t.o. dosing with a B_2-receptor-selective BK antagonist (FR-165649), making the response totally pharmacologically relevant and validated. These compelling data, and with management's full support, helped me launch and lead this discovery program in an effort to find better and more effective ocular hypotensive nonpeptide B_2-receptor agonist drugs.

A robust array of receptor binding and functional assays using prior knowledge of the kininergic system[210−212] were setup to begin the screening process for analogues of FR-190997 that our medicinal chemists designed and synthesized and that we patented.[213−215] B_2-receptors were mapped using immunohistochemical techniques,[216,217] and functional responses were observed in isolated h-CM,[216] h-TM,[218] and h-NPCE[217] cells that involved BK- and FR-190997-induced PI hydrolysis, IPs accumulation, $[Ca^{2+}]_i$ mobilization, PGE_2 release, MMP secretion, h-TM cell volume reduction, stimulation of TM-outflow in isolated/perfused porcine eyes (Figure 12B), and of course IOP reduction *in vivo* in numerous OHT cynomolgus monkeys that involved mostly enhancement of UVSC outflow of AQH (Table 10).[219−221] These studies involved much in-house and external collaborative effort with multiple academic colleagues[219,220] who were coauthors on the publications who

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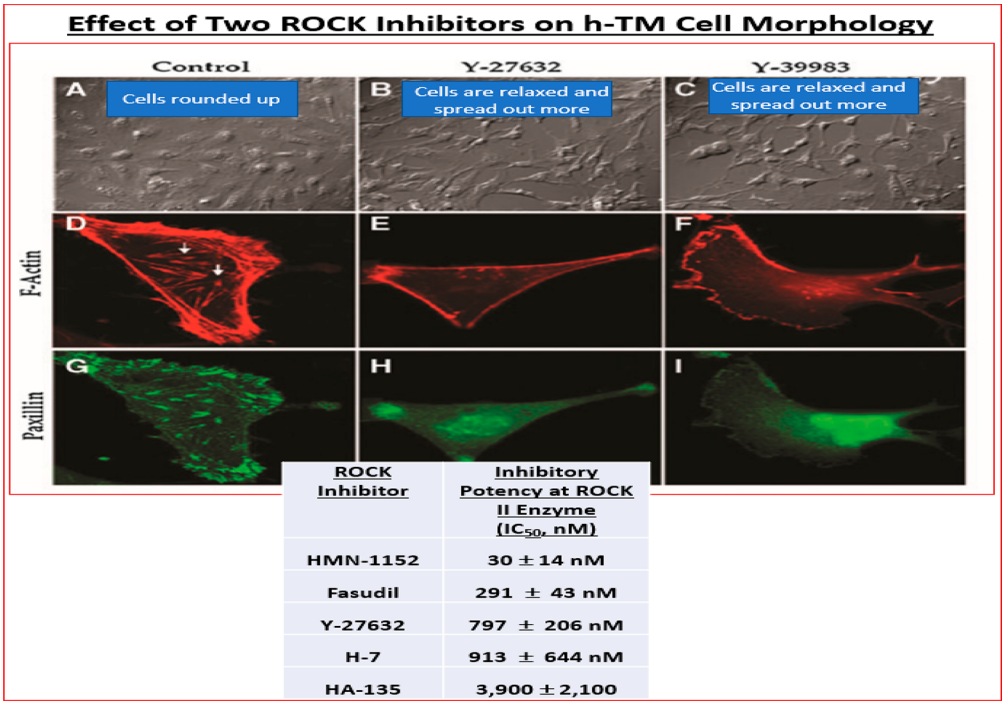

ROCK Inhibitor	Inhibitory Potency at ROCK II Enzyme (IC_{50}, nM)
HMN-1152	30 ± 14 nM
Fasudil	291 ± 43 nM
Y-27632	797 ± 206 nM
H-7	913 ± 644 nM
HA-135	3,900 ± 2,100

Figure 11. Morphological changes induced by ROCK inhibitors in h-TM cells. Reproduced with permission from ref 7. Copyright 2018 Mary Ann Liebert Publishing Inc.

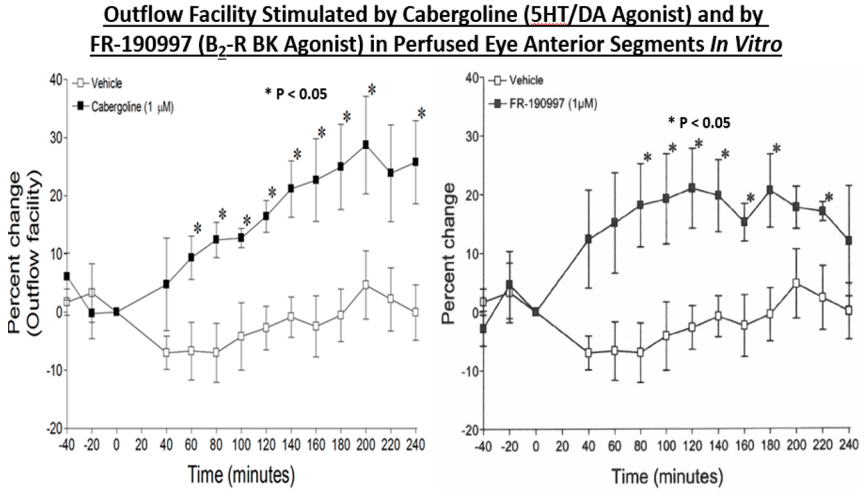

Figure 12. Ability of two different classes of compounds to stimulate conventional outflow facility in isolated anterior chambers of porcine eyes. Reproduced with permission from ref 7. Copyright 2018 Mary Ann Liebert Publishing Inc.

had tested our coded compounds in a masked manner. As the project moved forward, another important nonpeptide B_2-receptor agonist (BK2A78)[222] was identified and fully characterized in biochemical, pharmacological assays and in the *in vivo* models and which also demonstrated potent and effective ocular hypotensive efficacy and minimal ocular and systemic side-effects. Last but not least, the apparent contradictory results of the first generation B_2-receptor antagonist, WIN-64338, causing pronounced IOP reduction in rabbits after ivt injection of the compound[223] was due to the multiplicity of receptors of different classes being activated by this molecule (a good example of polypharmacology in action).[223] This conclusion was supported by the fact that other more selective and potent nonpeptidic B_1- (LF23-1591) and B_2-receptor antagonists (FR-165649; FR-173657) failed to lower IOP when injected into the rabbit eye vitreous.[223] Taken together, as with the "so-called" inflammatory PGs (see above), which were chemically modified to render them into suitable non-inflammatory drugs to lower and control IOP in OHT/POAG/NTG patients,[119,121,155,156,161] the possibility of using non-peptide B_2-kinin receptor partial agonists such as FR-190997 as future ocular hypotensive drugs provides an intriguing novel target to pursue for future drug discovery to help glaucoma patients.[206,219,220,222]

However, during all the above-mentioned antiglaucoma ocular research, the team had also considered generating dual pharmacophoric drug conjugate drugs, as opposed to using fixed-dose combination products, for example Simbrinza and Duotrav. Conceptually it was thought that travoprost could be linked to other ocular hypotensive drugs to maximally lower IOP

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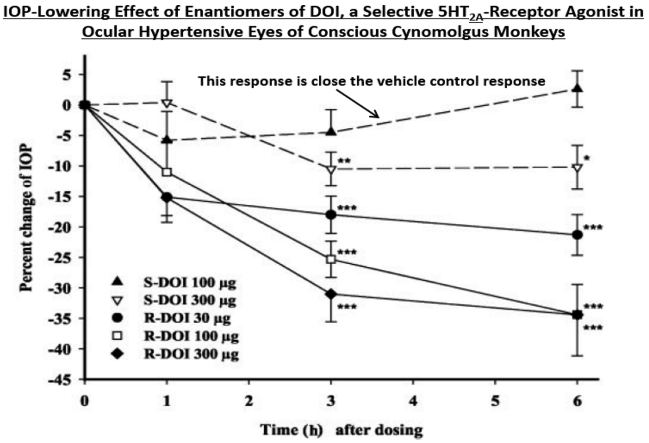

Figure 13. Ability of different t.o. doses of the two enantiomers of a potent and selective 5-HT_{2A} receptor agonist (DOI) to lower and control IOP in conscious OHT cynomolgus eyes. Reproduced with permission from ref 7. Copyright 2018 Mary Ann Liebert Publishing Inc.

by engaging different mechanisms to modulate AQH dynamics, and patented that idea.[224] Sticking with my keen interest in therapeutics for glaucoma treatment, lately I noticed the rather rapid onset of action and the extraordinary magnitude of IOP-reduction in OHT monkey eyes by a novel non-PG EP2-receptor agonist drug (omidenepag isopropyl [OMDI]; Eybelis).[225] The profound IOP-lowering by OMDI (−46% at 1.5−2 h, −54% at 3−4 h, and −56% at 6−8 h post t.o. dosing of OHT monkey eyes) was remarkably greater than other EP2-receptor agonists reported in the literature. Therefore, I postulated that OMDI may be of value in emergency treatment of rapidly rising IOP and/or for treating angle-closure and uveitic glaucoma and quickly filed appropriate patent applications in Japan and USA followed by discussions of the novel pharmacological attributes of OMDI in the public forum.[226−228] To support this hypothesis, the historic quantitative autoradiographic distribution of [^{3}H]-PGE_2-labeled receptor sites in human eye sections became useful.[161] The relatively high density of specific [^{3}H]-PGE_2-labeled receptor binding to both longitudinal and circular ciliary muscle[161] provides some basis of the action of OMDI lowering IOP in OHT/POAG patients by stimulating both uveoscleral and TM pathways.[149]

Discovery of Novel Pharmacological Tools for Ocular and Nonocular Research. The research conducted during the 1990s to find, characterize, develop, and finally launch Travatan (AL-6221, travoprost isopropyl ester; AL-5848 being the free acid, *S*-fluprostenol) to treat OHT/POAG as described above, yielded a number of other benefits. One such example worthy of mention is our unexpected discovery of a low intrinsic activity (E_{max} = 19−23% vs cloprostenol [100% E_{max}]) FP-receptor partial agonist (AL-8810). As described above, our team was focused on finding novel agonists to match the profile of travoprost acid. Thus, the team members were not so interested in AL-8810 since it exhibited a low potency (EC_{50} = 186−260 nM) for stimulating PI turnover in Swiss 3T3 fibroblasts and in A7r5 rat aortic smooth muscle cells, and its efficacy (*in vitro* intrinsic activity) was so low (Figure 14A).[152,229−232] However, remembering my pharmacological training, I immediately recognized the potential value of AL-8810—low intrinsic activity agonists behave as antagonists in cells/tissues where the receptor reserve is in the low-to-moderate range. AL-8810 was quickly profiled for its PG receptor activity and it indeed behaved as a fairly selective and competitive antagonist (Figure 14B,C)[152] at the FP-receptor in multiple assay systems. This was indeed fortuitous since no bona fide antagonist existed for the FP-receptor at that time. AL-8810 was further characterized,[152,233] and along with close analogues (e.g., AL-3138),[233] patented[229−233] and out-licensed to commercial companies for other researchers to use.

In the intervening years, AL-8810 has been successfully utilized by numerous researchers to probe the involvement of FP-receptors in normal and pathological conditions. AL-8810 has proven a valuable tool in ocular research *in vitro*[235−242,163−165,171] and *in vivo*.[242] Likewise, despite being a relatively low potency FP-antagonist (IC_{50}/K_i = 734 ± 228 nM in numerous systems), AL-8810 has shown robust efficacy in abrogating parasitic infections,[243] reducing structural and functional damage from experimental traumatic brain injury,[244] significantly decreasing demyelination and motor dysfunction,[245] reducing ischemic brain damage,[246,247] and attenuating bacterial-toxin-induced inflammation.[248] A full description of the discovery, characterization and possible uses of AL-8810 as a therapeutic agent has been recently reviewed.[234]

One significant element of AL-8810 pharmacology pertains to its use to address the possible mechanism of action at the receptor level of a new compound, Lumigan (bimatoprost; 17-phenyl-$PGF_{2\alpha}$-amide), launched in 2001 by a competitor company for the treatment of OHT/POAG. Authors of the controversial publication[249] claimed, without any tangible and reproducible proof, that bimatoprost lowered IOP by interacting

Table 10. FR-190997 (a Nonpeptide BK Agonist) Promotes UVSC Outflow of AQH from OHT Eyes of Ketamine-Sedated Cynomolgus Monkeys[a]

	baseline					T.O. treatment with FR-190997			
	normotensive eye OD	*n*	hypertensive eye OS	*n*	stats. *p*-values		hypertensive eye OS	*n*	.stats. *p*-values
F_a	1.63 ± 0.54	12	1.54 ± 0.80	12	0.50	F_a	1.48 ± 0.53	12	0.79
C_{fl}	0.42 ± 0.21	9	0.16 ± 0.17**	12	0.00**	C_{fl}	0.18 ± 0.16	9	0.47
C_{ton}	0.22 ± 0.14	12	0.15 ± 0.09	10	0.21	C_{ton}	0.17 ± 0.11	9	0.59
Fu_{fl}	0.16 ± 0.51	7	0.47 ± 0.57	11	0.14	Fu_{fl}	1.23 ± 0.91	9	0.00**
Fu_{ton}	0.48 ± 0.99	12	0.37 ± 1.04	9	0.46	Fu_{ton}	1.45 ± 0.45	10	0.03*
ACvol	76.0 ± 11.3	12	79.9 ± 9.12	12	0.16	ACvol	79.8 ± 9.2	12	0.74
CCT	0.48 ± 0.03	12	0.48 ± 0.03	12	0.36	CCT	0.48 ± 0.03	12	0.74

[a]Note that only UVSC outflow of AQH (determined by 2-methods) is enhanced by FR-190997 in this monkey model of OHT. However, this drug also promoted TM-mediated conventional outflow of AQH as demonstrated in *ex-vivo* perfused porcine eyes (ref 219). Data taken from ref 7. The asterisks (∗) indicate statistical significance.

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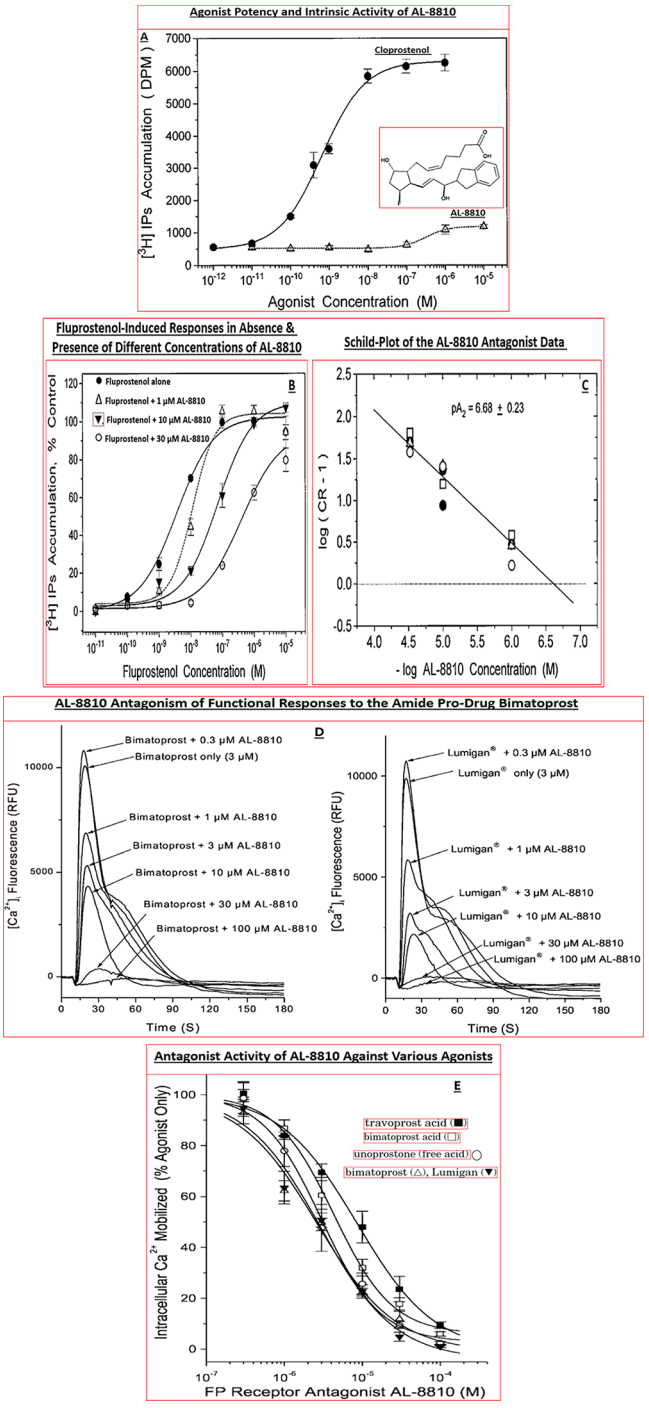

Figure 14. (A) Agonist potency and intrinsic activity of AL-8810 vs the full-agonist cloprostenol. (B,C) Schild analysis of the antagonist potency of AL-8810 vs fluprostenol-induced [^{3}H]-IPs accumulation in A7r5 rat aortic cells *in vitro*. (D) AL-8810 concentration-dependently antagonized the functional activity of bimatoprost within a few seconds. (E) AL-8810 antagonized the functional responses to various FP-receptor agonists in a concentration-dependent manner. Overall, the antagonist potency of AL-8810 against a range of FP-agonists in various assay systems was 734 ± 228 nM. While not highly potent, it has proven very useful in clarifying the role of FP-receptors and/or endogenous and exogenous FP-receptor agonist in numerous systems *in vitro* and *in vivo*. Panels A−E are reproduced/modified with permission from refs 152 and 234. Copyright 1999 American Society for Pharmacology and Experimental Therapeutics and 2019 Wiley.

with a postulated "prostamide receptor" rather than being hydrolyzed to its free acid form, 17-phenyl-PGF$_{2\alpha}$, which is a potent FP-receptor agonist with nanomolar potency in many cells and tissues.[158] Additionally, those authors claimed that the intact amide was the active moiety and that it directly activated the "prostamide receptor" without interacting with the FP-receptor, and thus was a novel lipid that was different from all the other PG antiglaucoma drugs. Many investigators voiced skepticism about this "labeling" of bimatoprost, and this was deemed an unacceptable way to promote Lumigan. The curiosity and controversy centered around the aforementioned proposed mechanism of action of bimatoprost prompted many arguments and studies. Several investigators independently showed that amidases present in animal and human cornea were able to convert bimatoprost into its free acid[250−256] as hypothesized by many researchers. Therefore, it was likely that *in vivo* this process would be expected to liberate bimatoprost free acid into the AQH and this would activate the FP-receptors in the CM and TM like the free acids of other FP-class PG pro-drugs (latanoprost, travoprost, and tafluprost) to lower IOP. Indeed, a number of studies in cataract patients demonstrated that t.o. dosing with Lumigan resulted in detection of 3.2 nM, 11 nM, 16 nM, and 10 nM of bimatoprost free acid (observed at 0.5, 1, 3, 5 h postdose).[252,253,256] These *in vivo* concentrations of bimatoprost free acid were at or several fold above the concentration required to achieve half-maximal activation of the FP-receptors in h-CM cells,[162] human TM cells,[163] at the human cloned ciliary body FP-receptor[164,165] in mouse 3T3 cells,[135,150,165] and in rat uterus[167] and cat iris[166] contraction assays. Hence, bimatoprost was no different from the other PG drugs approved for OHT/POAG treatment. Additionally, bimatoprost and its marketed version (Lumigan) were shown to directly interact with the FP-receptor since both "powder" and clinical solution forms of the compounds displaced [^{3}H]-PGF$_{2\alpha}$ binding, and since both compounds stimulated rapid $[Ca^{2+}]_i$ mobilization in numerous cell-types including mouse 3T3 fibroblasts, A7r5 cells, h-CM and h-TM cells, and via the human cloned FP-receptor expressed in HEK-293 cells (e.g., Figure 14D; see above), and which also contracted rat uterine and cat iris strips like many FP-receptor agonists (see above and ref 257 for review). Furthermore, these actions of bimatoprost were concentration-dependently blocked by AL-8810 (e.g., Figure 14D,E), the FP-receptor antagonist described earlier. The collective data reported by a multitude of researchers therefore cast doubt on the existence of the "prostamide receptor", and most scientists agreed that bimatoprost was indeed a pro-drug in the form of an amide, whereas the other FP-receptor agonist pro-drugs were isopropyl esters (Figure 7). Also, that all these drugs were metabolized to their respective free acids which were in fact the active moieties responsible for reducing IOP in animals and human subjects.[257] The aforementioned controversy, resulting in heated debates at numerous conferences about the proposed mechanism(s) of action of the marketed PG analogues for glaucoma treatment, in particular for bimatoprost, was captured in an article titled "*The Prostaglandin Wars*"[258] and was discussed in more detail in a review article.[257]

Another useful outcome of our detailed pharmacological studies in human ocular cells, in particular h-TM cells, relates to the mechanism of action of the FP-class PG agonists. The majority of the AQH dynamic studies conducted in OHT monkey eyes and OHT/POAG patients had concluded that drugs such as Xalatan, Travatan, Lumigan, Taflutan, and

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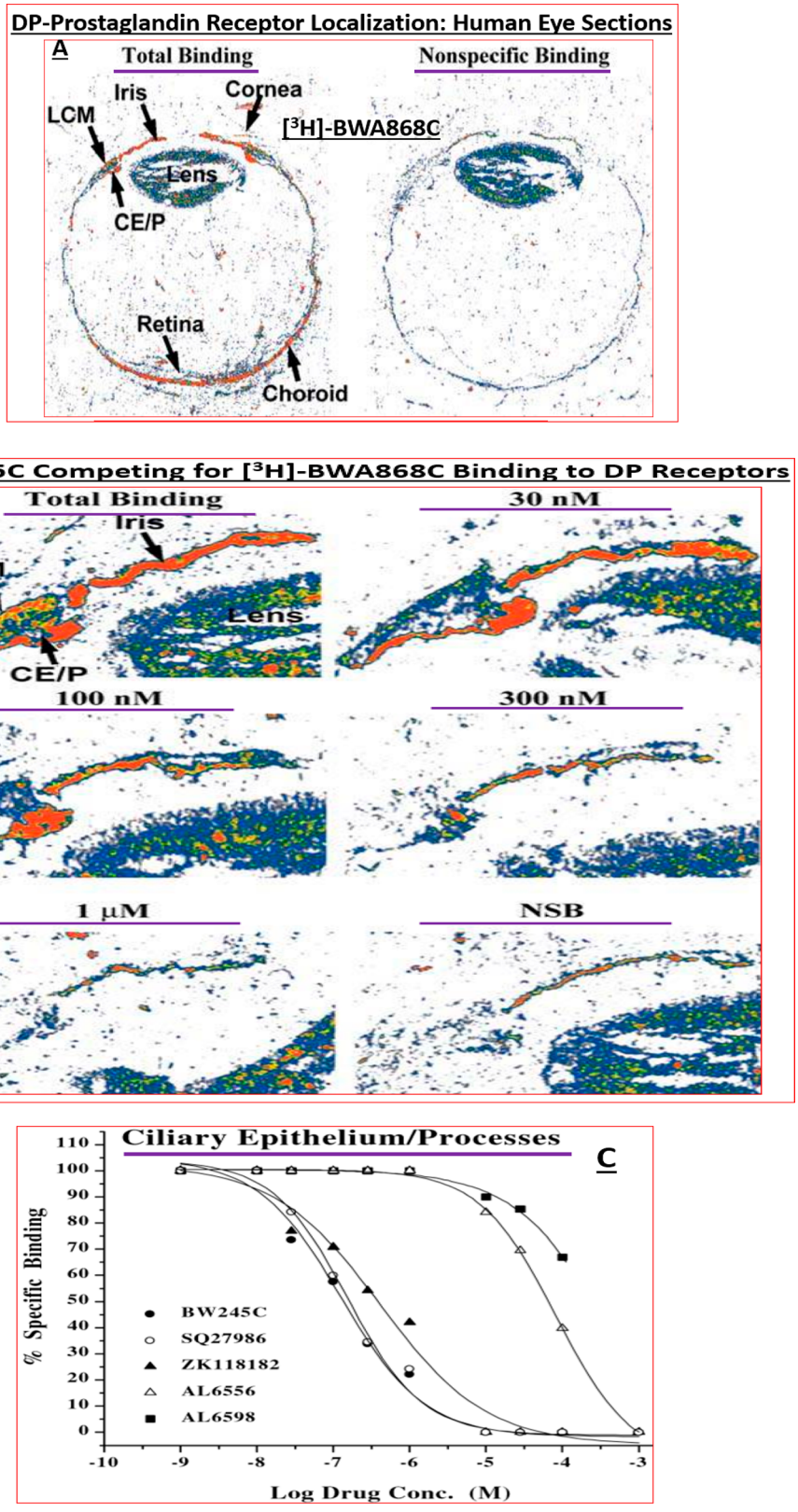

Figure 15. (A) Autoradiographic localization and quantification of DP-receptors in post-mortem human eye sections determined *in vitro* using the highly selective DP-receptor antagonist radioligand, [^{3}H]-BWA868C. (B) The concentration-dependent displacement of [^{3}H]-BWA868C from DP-receptors in human eye sections *in vitro* by a potent and selective DP-receptor agonist (BW245C), and (C) graphic representation of such data for a number of DP-receptor agonists. NSB = nonspecific binding. Reproduced with permission from ref 274. Copyright 2005 Mary Ann Liebert Publishing Inc.

unoprostone isopropyl (Rescula) lowered IOP[259−265] by releasing MMPs through activation of FP-receptors in the CM[235,266,267] (Figure 8C), and through CM and scleral tissue remodeling, enhanced UVSC egress of AQH.[268,269] However,

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when we demonstrated and fully characterized the presence of functionally active FP-receptors in h-TM cells[163] clinical investigators became more aware and hence noticed and reported a significant enhancement of trabecular outflow of AQH induced by bimatoprost,[270,271] latanoprost,[271,272] travoprost,[263,269,271] and by unoprostone isopropyl[262] in ocular normotensive and OHT/POAG patients, in addition to the elevated UVSC outflow induced by these drugs. The TM conventional outflow facility enhancement by bimatoprost, for instance, was 23% of total in ocular normotensive human subjects, and by latanoprost in perfused human anterior eye segments TM outflow facility was increased by up to 67% of total AQH outflow.[269] Interestingly, travoprost, tafluprost, and 15-keto-latanoprost did not appear to influence TM outflow in cynomolgus monkey eyes (see ref 269 for review). However, latanoprost was shown to increase murine outflow of AQH via the conventional TM pathway by 39% of total outflow after a single t.o. dose and within 2 h postdose.[273] This acute ocular hypotensive activity correlated with up-regulation of MMP-2[235] to enhance TM outflow facility, whereas the long-term protracted efficacy of the FP-PG agonists due to UVSC outflow was mediated through release of MMP-1, MMP-3, and MMP-9 which were synthesized and secreted over many hours postdosing (reviewed in ref 267). These collective studies helped identify important species differences in how these drugs mediate their biological effects and how the FP-receptors located in the CM and TM are coresponsible for increasing AQH drainage to lower IOP.

Other helpful tools that resulted from our drug discovery campaigns for treating OHT/POAG included generation of more potent and more receptor-selective radioligands to study the pharmacological properties and autoradiographic visualization of FP-receptors using [^{3}H]-travoprost acid ([^{3}H]-AL-5848),[136] and DP-receptors using [^{3}H]-BWA868C (Figure 15A–C).[138] The use of the latter radioligands coupled with quantitative phosphor-imaging technology[129,136,138,153] allowed us to determine equilibrium dissociation constants (K_is) for a range of FP- and DP-class drugs on thin sections of post-mortem human eyes, an unparalleled accomplishment thus far as far as we know (Figure 15C, Table 11).[153,274]

In a similar vein, even though we obtained useful functional data for FP-receptor directed compounds using Swiss 3T3 mouse fibroblasts[135,150] and rat aortic cells,[151,152] the latter findings being confirmed in isolated h-TM[163] and h-CM cells,[158,162] it was deemed imperative that we also demonstrate activity of our compounds at a human cloned FP-receptor directly without the issues of cross-activity through other receptor systems found on native cells and tissues. Accordingly, through an Alcon-funded collaboration, the human ciliary body FP-receptor was cloned and expressed in HEK-293 cells that were devoid of endogenous FP-receptors.[164] Using this cloned receptor system and AL-8810 as the tools, we then verified and cross-correlated our data previously obtained from the cells and tissues expressing natural FP-receptors (see above). We were of course delighted to find excellent correlations between radioligand binding, PI turnover, $[Ca^{2+}]_i$ mobilization, mitogen-activated protein kinase (MAPK) activity, tissue contractions and IOP-lowering for a range of FP-receptor agonists and the FP-receptor antagonist, AL-8810.[13,127,167,234]

Lastly, despite decades of research since the original discovery and clinical adoption of FP-class PG analogues as first-line therapeutics to treat OHT/POAG/NTG in the early 2000s, lowering and controlling IOP remains the single most validated

Table 11. Relative Affinities of DP-Receptor Agonists for Tissues in Human Eye Sections Determined by Quantitative Autoradiographic Techniques and Using [^{3}H]-BWA868C as the Radioligand[a]

	DP-Class prostaglandin inhibition constant (K_i, nM; mean ± SEMs) in human ocular tissues determined by quantitative autoradiography			
compound	ciliary epithelium/ processes	longitudinal ciliary muscle	circular ciliary muscle	iris
BW-245C (free acid)	8.0 ± 1.8	7.0 ± 1.0	5.7 ± 0.8	4.4 ± 0.7
SQ-27986 (free acid)	9.0 ± 1.9	6.1 ± 0.8	6.4 ± 1.3	8.5; 6.6
ZK-118182 (free acid)	32.9 ± 7.4	±2.9	±2.6	7.3; 15.4
AL-6556 (free acid of AL-6598)	±1310	±160	±413	nd
AL-6598 (isopropyl ester)	±3000	±665	±825	nd

[a]Note: the smaller is the K_i value, the higher is the affinity of the compound for the DP-receptors. nd = not determined. Data reproduced and updated from ref 274.

treatment for this collection of ocular diseases.[4−6] Therefore, new drugs and treatment options were still being sought to mitigate the damage caused to the optic nerve, RGCs, and their axons by elevated IOP. Only recently have new drugs to reduce IOP been added to the clinicians' toolbox encompassing a conjugate of latanoprost and a nitric oxide donor (Latanoprostene Bunod),[275] two ROCK inhibitors, one approved in Japan (Ripasudil)[276] and the other in the US (Netarsudil),[277] and a novel non-PG EP2-receptor agonist (Omidenepag Isopropyl).[225−228] Sadly POAG/OHT patients who are recalcitrant to pharmaceutical drugs, and in some cases NTG patients, often require invasive ocular surgeries to reduce their IOPs to preserve their sight.[4−7] The advent and introduction of microshunts to extrude AQH from the ANC of the eye are less invasive[278] but still require significant surgical procedures, and will often be given fixed-dose combination[279] ocular hypotensive drugs to maintain their lowered IOPs. Consequently, research has been directed toward finding ways to directly protect the RGCs and their axons from the ravages of IOP-induced visual impairment. Much effort has been expended in delineating the sequence of events that lead to optic nerve damage. It would appear that loss of energy at the level of mitochondrial ATP synthesis[109,110,113,114,280,281] is at the heart of the problem in causing GON, and this is now a well accepted theory with some compelling evidence from recent animal and human clinical studies.[282,283] We recognized this aspect during early years of our research in the late 1990s. Using nuclear magnetic resonance technology we showed that human and rat retinas subjected to hypoxic conditions, thereby simulating what may happen *in vivo* in GON, leads to a sharp decline in ATP and which could be recovered to a large extent using a Ca^{2+}-channel blocker (diltiazem) or using a blocker of the *N*-methyl-D-aspartate receptor-coupled-channel (MK-801) (Figure 16; Table 12).[113] Likewise we showed the presence of specific polyamine binding sites in human and rabbit retinas[130,131] that may endogenously serve a neuroprotective function,[284,285] and where reduction of glutamate-induced retinal toxicity can also protect the RGCs and their dendrites.[286] Nevertheless, it is also a fact that the best treatment paradigm for the good eyesight and preservation of

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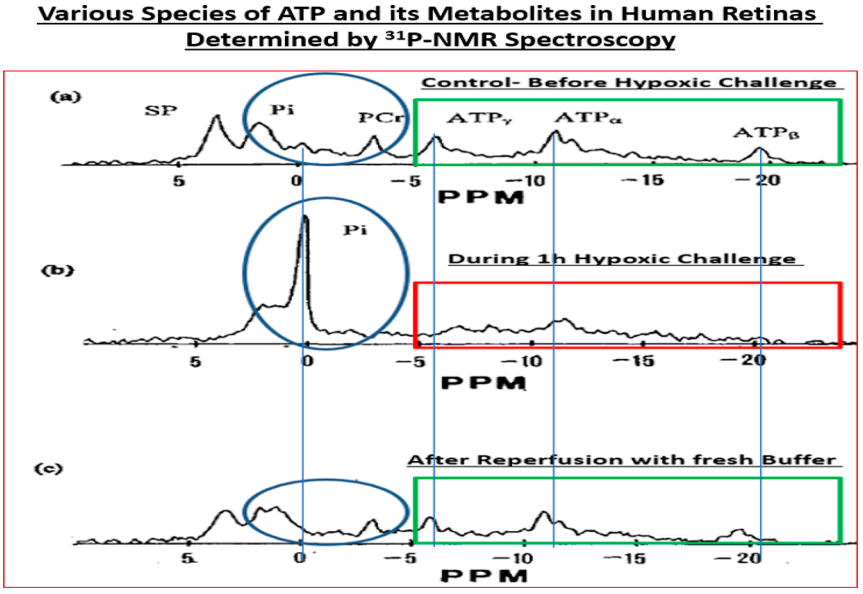

Figure 16. ATP energy depletion and restoration after hypoxia/reperfusion of human (panel A) and rat retinas (panel B) in the absence and presence of MK-801 NMDA-receptor-channel blocker as determined by ^{31}P NMR spectroscopy. Reproduced with permission from ref 7. Copyright 2018 Mary Ann Liebert Publishing Inc.

Table 12. Nuclear Magnetic Resonance (^{31}P-NMR)-Derived ATP Levels in Rat and Human Retinas under Normal and Hypoxic Conditions and Restoration of ATP with Two Different Drugs[a]

experiments/ treatments	ATP and its phosphorus metabolites (% of control levels)		
rat retinas	ATP	PCr	P_i
normal controls	98.4 ± 1.15	85.7 ± 2.3	95.0 ± 2.3
after 2.0 h hypoxia	48.1 ± 2.1***	nd	nd
after 1.0 h hypoxia	69.5 ± 3.46***	56.0 ± 4.6***	75.0 ± 8.6
after 1 h with MK-801 at 50 μM	97.4 ± 4.6***	102.0 ± 4.5***	89.3 ± 4.04
after 2 h with MK-801 at 50 μM	72.6 ± 1.4**	nd	nd
after 1 h with MK-801 at 5 nM	92.0 ± 1.4***	101.0 ± 5.7***	76.8 ± 3.57

human retinas	% of control ATP
normal controls	98 ± 12
after 2.0 h hypoxia	52 ± 8**
after 2 h with MK-801 at 50 μM	74 ± 6*
after 2 h with diltiazem at 50 μM	67 ± 4

[a]Effect on tissue ATP and its metabolites after hypoxia/reperfusion of rat and human retinas in the absence and presence of MK-801 (NMDA-receptor-channel blocker) and diltiazem (Ca^{2+}-channel blocker) as determined by ^{31}P NMR spectroscopy. PCr = phosphocreatinin; P_i = inorganic phosphate; nd = not determined; *P < 0.05; **P < 0.02; ***P < 0.01. Data from ref 7.

vision in glaucoma patients is to reduce and maintain the lowest possible IOP since that has repeatedly shown efficacy in reducing RGC injury and reversing their cellular dysfunctions.[287]

Clearly much more needs to be accomplished to help preserve the sight of glaucoma patients, and it is hoped that the research described above may contribute in some small way in this endeavor and lead to novel means to arrest vision loss. In the quest for such mitigation strategies, the early diagnosis of OHT/POAG is tantamount to early intervention. It is hoped that the near-term availability and therapeutic utility of novel diagnostic and prognostic reagents, and rapid clinical introduction of safe and effective neuroprotective drugs, gene-, and cell-therapies, and innovative devices (including prostheses) to combat GON will positively impact patients losing their sight.

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Notes

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Sincere thanks and gratitude are extended to team members, colleagues, mentors, and supervisors at Alcon. The intention of this article has been to describe the collective efforts of many talented and dedicated scientists, managers, and leaders to bring suitable products to the market place and to introduce them into medical treatments of certain ophthalmic diseases that ail millions worldwide. As such, I declare that there is no conflict of interest in providing this treatise to help future drug hunters and students gain an insight into the drug discovery research processes, to learn about the history of the discovery/characterization of some now well-known drugs, and to review some of the diverse kinds of data gathered during such studies. Views in this work are those of the author and do not express the views of anyone else.

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