Pharmacodynamic Evaluation: Ocular Pharmacology

7

Najam A. Sharif

Contents

N. A. Sharif (✉)
Global Alliances and External Research, Global Ophthalmology Research and Development, Santen Incorporated, Emeryville, CA, USA
e-mail: najam.sharif@santen.com

F. J. Hock, M. R. Gralinski (eds.), *Drug Discovery and Evaluation: Methods in Clinical Pharmacology*,
https://doi.org/10.1007/978-3-319-68864-0_54

Abstract

The eye is a specialized organ that provides a relatively easy access for direct visualization of the different anatomical structures and assessment of the diseases associated with them. Despite this, however, the diagnosis and treatment of eye diseases has proven difficult over the years. Ocular surface diseases include allergic conjunctivitis, infection (viral, bacterial, and fungal), inflammation, and dry eye. Major anterior chamber and lens-associated disorders include cataracts, presbyopia, iritis/ureitis, elevated intraocular pressure-associated glaucoma and pseudo-exfoliation glaucoma. Diseases that primarily effect the retina in the posterior segment of the eye include wet and dry age-related macular degeneration, diabetic macular edema and retinopathy, and glaucomatous optic neuropathy that involves the retinal sensory neurons (retinal ganglion cells) and their axons that form the optic nerve that connects the retina to the brain. Many decades of basic and applied research have resulted in the discovery and development of different types of pharmacological agents (small molecules), peptides, and antibodies that help clinically manage the various ocular disorders mentioned above. Recent advances in gene- and cellular-therapeutics, and production of suitable miniature devices, have also revolutionized ocular disease management. The pharmacotherapeutic and pharmacodynamic aspects of these modalities will be discussed here. This will include target protein localization, assessment of drug engagement with the target, and mechanism of action of the drug entities in cellular and whole-eye efficacy systems using normal and disease-based assays and animal models. Such in vitro screening and in vivo evaluation and the types of results obtained from such studies will be also described and discussed.

General Introduction

Due to the fact that this chapter aims to cover numerous types of eye diseases that have a diverse set of etiologies and disease pathways, it is the author's opinion that a standardized format is unsuitable. As such, the chapter will cover the key elements of the requisite headings and subheadings but without strict adherence. It is hoped that the format followed is acceptable.

The World Health Organization (WHO 2018), National Eye Institute (NEI 2014), and American Academy of Ophthalmology have estimated that ~250 million individuals, including 36 million blind people, have some form of eyesight impairment. Furthermore, it is believed that the number of blind people will increase to 38.5 million by 2020, and to 115 million by 2050. In the USA alone, the total economic burden related to vision loss is expected to reach ~USD 715 billion by 2050. In fact, chronic eye diseases are one of the main causes of vision loss globally, and an ~90% cases of visual impairment are due to such conditions, and indeed a large portion of these chronic ophthalmological disorders affect back of the eye (WHO 2018; NEI 2014; American Academy of Ophthalmology).

Eyesight is an extremely vital sense in animals and humans and is critical for survival in most species that sense and interact with the environment through ocular cues. In fact, most humans place the highest value, among the five senses, on eyesight. While not life-threatening, visual

impairment due to eye disorders has serious implications for quality of life for the patient, caregivers, and the society at large. As some of the eye diseases are age-related in terms of onset and severity, the incidence of ocular diseases continues to increase as the aging population on our planet increases. For example, cataracts were reported to impact >20 million people in 2010, and glaucoma afflicts >65 million people worldwide (Tham et al. 2014; Weinreb et al. 2014; Jonas et al. 2017) with similar numbers for age-related macular degeneration and related visual acuity disorders. Similarly, dry eye (15 million patients in the USA alone), diabetic retinopathy (>5 million patients), refractive errors (>4.1 million patients), and ocular allergies (>3 million patients/year in the USA) also have a high prevalence. These eye diseases, vascular eye disorders, and myopia, continue to cause a sustained undue suffering, medical burden and expense to the patients and the society (WHO 2018; NEI 2014; American Academy of Ophthalmology). Accordingly, discovery and development of therapeutic agents and devices to treat ocular disorders has gained prominence and importance, eliciting an appropriate heightened sense of urgency to find better treatment modalities and ultimately cures for these maladies.

Introduction to Eye Anatomy and Basic Physiology

Being a uniquely specialized sensory organ, it is important to briefly outline the key anatomical elements of the eye and how the eye encodes the light it receives into well-defined color-coded images for us to see (Fig. 1). Most of the eyeball is encased in a white relatively thick, tough, yet flexible fibrous tissue called the sclera, which provides protection from sharp objects and noxious chemicals and shapes the eyeball. The sclera at the

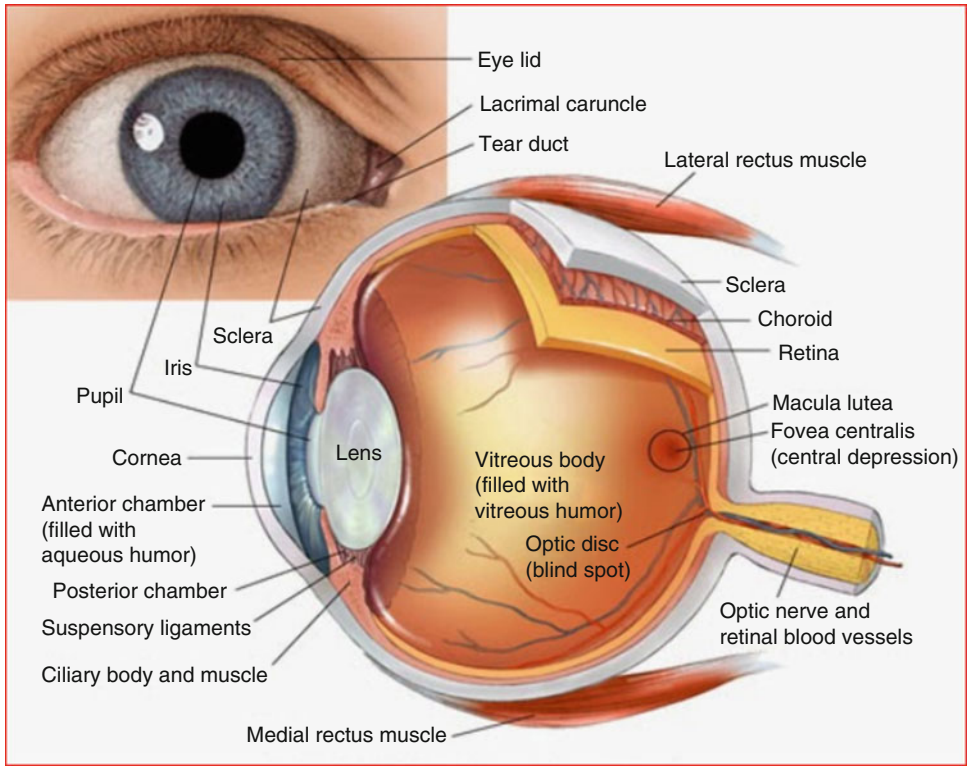

Fig. 1 Depiction of the anatomy and key structures of the human eye pertinent for the discussion of various eye diseases

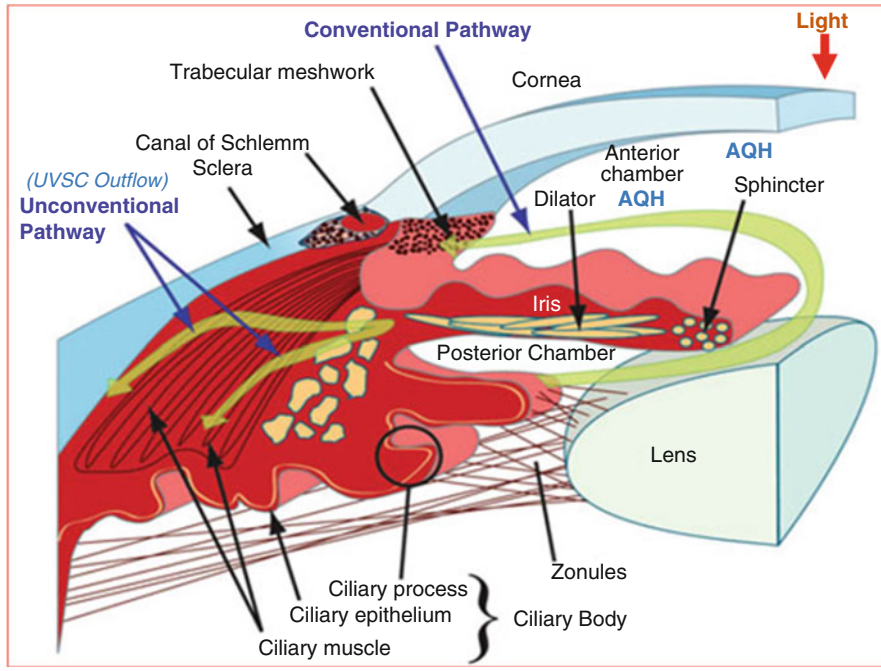

Fig. 2 Key elements of the anterior segment of the human eye are shown. Formation of the AQH by the ciliary processes, its flow from the latter in front of the lens, followed by its drainage from the anterior chamber via TM/SC and via the UVSC pathways is also depicted

front of the eyeball is further specialized and forms the transparent cornea that permits light to enter the eye and that also provides a barrier to airborne chemicals, noxious agents, and pathogens.

The eye is divided into anterior and posterior segments that are separated by the lens. The anterior chamber (ANC) contains a clear fluid (aqueous humor [AQH]), that contains various nutrients and oxygen, is generated by the ciliary epithelium within the ciliary body. As the AQH flows through the ANC, it nourishes the cells of the lens epithelium, corneal endothelium, and trabecular meshwork (TM) (Fig. 2). AQH also removes metabolites, dead cells, and other toxic waste as it drains through the TM/Schlemm's canal (SC) and into the venous circulation.

The posterior segment is filled with vitreous humor (VH), a gelatinous material, that does not turnover as much as the AQH. The AQH and VH, coupled with the sclera, provide the eyeball its unique shape and overall rigidity. In direct contact with the VH is a thin inner limiting membrane that isolates the retina from the VH but one that is fairly permeable to chemicals and gases. The retina is a highly specialized tissue being composed of multiple layers of cells that have unique functions in light perception and neuronal communication to the brain (Fig. 3). For the sake of brevity and focus, the most important cell types related to pathological aspects and treatment of retinal diseases include the retinal ganglion cells (RGCs) and their axons (connected with various forms of glaucoma; glaucomatous optic neuropathy (GON)), retinal pigment epithelial cells (RPE cells; connected with wet age-related macular degeneration (AMD) and diabetic retinopathy (DR), and photoreceptor cells (connected with retinitis pigmentosa (RP) and dry AMD) (Fig. 3). However, the interplay between many of these and other retinal cell types, for example, Muller glia and retinal interneurons, is also very important for visual perception. Thus, in humans a million or so RGC axons are bundled together to form the optic nerve that projects to the brain centers that are involved in visual perception.

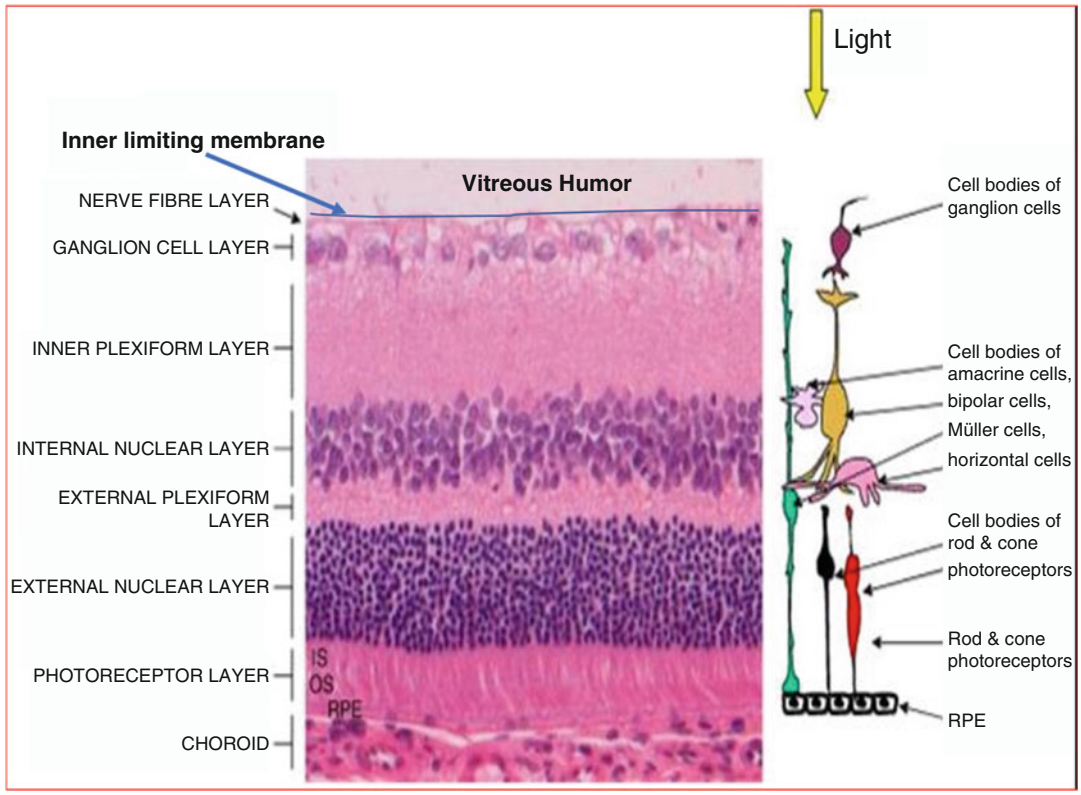

Fig. 3 Various layers of human retinal cells are shown indicating their relative positions and interconnections between them

Wrapped within the optic nerve are the central retinal artery and the central retinal vein that provide and remove, respectively, some of the blood to the retina. Another major artery supplying oxygenated blood to the retina is the posterior ciliary artery.

In terms of visual perception, light from the environment is first focused by the cornea and then passes through the pupil and is focused further by the lens onto the retina. Here, photoreceptors (rods and cones) then convert the light photons into membrane potential changes by closing of Na^+-channels and with resultant cellular hyperpolarization. This electrical change within the rods and cones decreases the release of the excitatory neurotransmitter glutamate (GLU) from their synaptic terminals onto bipolar cells. This changes the level of tonic electrical activity of bipolar cells that then release less GLU onto RGCs. In turn, since GLU activates many types of ionotropic and metabotropic receptor subtypes that modulate various Ca^{2+}-channels and a whole host of transient receptor potential channels, the electrical activity of the RGCs is well regulated. Also, since neighboring horizontal and amacrine cells modulate the activity of bipolar cells through release of inhibitory neurotransmitters such as gamma-butyric acid and glycine, the bipolar cells' ability to encode and transmit signals to the RGCs is extremely well coordinated and finely controlled. Finally, the RGCs integrate all the information received from bipolar cells and relay this as electrical impulses down their axons, within the optic nerve, to the lateral geniculate nucleus/superior colliculus within the thalamus of the mid-brain. From there, visual information is relayed to the visual cerebral cortex for final processing and generation of the exquisite images that we see. In this manner, the eyes represent windows for the brain.

Receptors, Ion-Channels Transporters, and Pharmacodynamics

In short, pharmacology is the study of drug action at a tissue and cellular level, while pharmacodynamics pertains to the study of mechanism of action of drugs at molecular, cellular, and tissue levels that reflects drug affinity and efficacy. In principle, ocular pharmacology is no different to pharmacology of other organs such as the heart, lungs, and brain. However, since the repertoire of receptors/ion-channels/transporters (target proteins; Alexander et al. 2017a, b, c, d) do not necessarily exist or function in exactly the same manner in each tissue/cell of these organs, it is worthwhile considering some of the basic aspects of pharmacology and their application to the eye. This necessitates the understanding of how neurotransmitters, hormones, cytokines, and other mediators and drugs exert their actions by interacting with receptors, enzymes, transporters, ion-channels, and nucleic acids that the cells possess (Alexander et al. 2017a, b, c, d). An important characteristic of most biologically and pharmacologically relevant molecules (whether they be small molecules, peptides, hormones, cytokines, or antibodies) is their affinity ("relative attraction") for the target protein. This affinity parameter is the dissociation constant (K_d or K_i) as determined by the ratio of (rate of dissociation/rate of association) of the ligand from the target protein. The K_d or K_i values are inversely proportional to the affinity of the ligand, thus a low K_d or K_i reflects a high affinity, usually represented as a concentration needed to occupy [or dissociate from half of the ligand binding sites of the target protein. Implied within the K_d and K_i values is a certain amount of specificity of the ligand for the receptor binding site such that mere adsorption onto the target protein can be discounted. Some examples of these pharmacological/pharmacodynamic parameters for ligands binding to various prostaglandin receptor subtypes can be seen in Tables 1 and 2.

Of the overall many druggable target proteins expressed by cells, receptors (39–41% of total), transporters (4–5%), and ion-channels (8–11%) (Alexander et al. 2017a, b, c, d) are primarily embedded in cellular membranes, while most kinase enzymes (22–24%) and proteases (8–11%) are located within the cytoplasm, and of course nucleic acids and nuclear receptors (2%) are located within the cell nucleus. The interplay among these targets and their signal transduction mechanisms, including positive or negative cooperativity, feed-forward or feedback pathways, allows the cellular machinery to dampen, amplify, or subtly modulate the response mechanisms of the cells and tissues to the pharmacological agent, thereby providing extra fine control and specificity. Added to this complexity is the ability of endogenous ligands and exogenous agents to behave either as full-agonists (producing a maximal biological response), or partial-agonists (only producing a submaximal response; e.g., Fig. 4, left panel), or inverse-agonists (inducing a response opposite to an agonist), or antagonists (blocking the actions of an agonist) (e.g., Fig. 4, right panel). Furthermore, there are instances when partial agonists at certain concentrations and under certain conditions can behave as antagonists (Griffin et al. 1999; Sharif and Klimko 2019), and this property can be exploited in the realm of disease management when truly bona fide high potency and high affinity antagonists are not available or are unsuitable as therapeutic drugs.

In general terms, receptors are functional transmembrane proteins that are coupled to signal transduction components such as G-proteins (or kinase enzymes) coupled to certain catabolizing enzymes on the inner leaflet of the cell membrane. Activation of the receptor by an agonist ligand (such as norepinephrine or prostaglandin $F_{2\alpha}$) changes the conformation of the receptor to an active state that triggers specific G-protein(s) and the associated enzyme(s) to generate intracellular second messengers such as cAMP (by activation of G_s-linked to adenylyl cyclase [AC] to increase cAMP and via G_i to inhibit AC and Ca^{2+-}channels, and open K^+-channels), cGMP, inositol phosphates, and diacylglycerol (by activation of G_q-linked to phospholipase C) (Alexander et al. 2017a, b, c, d) (Fig. 5). These second messengers amplify the signal transduction by activating protein kinases, opening/closing

Table 1 Receptor binding inhibition constants and relative receptor selectivities for some key natural PGs

Natural PG	PG binding inhibition constants (K_i, nM) and receptor selectivity (x)							
	DP	EP_1	EP_2	EP_3	EP_4	FP	IP	TP
PGD_2	**81 ± 5**	>19,000 (x 234)	2,973 ± 100	1,115 ± 118 (x 14)	2,139 ± 180 (x 26)	2,500 ± 760 (x 31)	>140,000 (x 1,728)	>35,000 (x 432)
PGE_2	>10,000 (x 667 *vs* EP_1)	**26 ± 10**	**4.9 ± 0.5**	**3 ± 0.2**	**0.9 ± 0.03**	3,400 ± 710 (x 227 *vs* EP_1)	53,708 ± 2,136 (x 3,581 *vs* EP_1)	>10,000 (x 667 *vs* EP_1)
$PGF_{2\alpha}$	18,000 ± 6,460 (x 138)	594 ± 12 (x 5)	964 ± 64	24 ± 8 (x 0.2)	433 ± 25 (x 3)	**130 ± 6**	≥ 50,000 (x 385)	≥ 190,000 (x 1,462)
PGI_2	3,537 (x 3)	>15,000 (x 11)	Nd	5,375 ± 1,394 (x 4)	8,074 ± 254 (x 6)	>86,000 (x 62)	**1,398 ± 724**	>65,000 (x 46)

Data are means ± SEM from 3–141 experiments. The bolded values represent the drug inhibition constant (K_i) of the PG for its primary preferred receptor. In the case of PGE_2 it is clear that it lacks selectivity between the subtypes of the EP receptors. The K_i value is inversely related to affinity of the PG for the receptor, i.e., the smaller the K_i value the greater the affinity. The values in parentheses denote the relative selectivity of the PG for its preferred (cognate) receptor compared to its affinity for other PG receptors
Nd not determined (Sharif et al. 2003)

Table 2 PG receptor inhibition constants and relative selectivities of synthetic PG receptor analogs of the FP-class

PG Analog	PG receptor binding inhibition constants (K_i, nM) and FP receptor selectivity (x)							
	DP Receptors	EP_1 Receptors	EP_2 Receptors	EP_3 Receptors	EP_4 Receptors	FP Receptors	IP Receptors	TP Receptors
Travoprost acid ((+)-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)
(±)-Fluprostenol	>50,000 (x 510)	12,300 ± 1,240 (x 126)	>100,000#	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 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 acid (PHXA85)	≥ 20,000 (x 204)	2,060 ± 688 (x 21)	39,667 ± 5,589#	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 (UF-021; 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)
S-1033 (Na^+-salt)	90,000 (x 4)	13,500 ± 1,670 (x −2)	Nd	≥ 77,000 (x 4)	6,650 ± 610 (x −3)	22,000 ± 2,600	>30,000 (x 1)	>30,000 (x 1)

Data are means ± SEM from 3–8 experiments. The bolded values represent the drug inhibition constant (K_i) of the PG for its primary preferred receptor, except for S-1033 which is left unbolded since it appears to possess a higher affinity for the EP_4 receptor than the FP receptor. The K_i value is inversely related to affinity of the PG for the receptor, i.e., the smaller the K_i value, the greater the affinity. The values in parentheses denotes the relative FP-receptor-selectivity of the PG analog compared to its affinity for the other PG receptors

Nd not determined (Sharif et al. 2003)

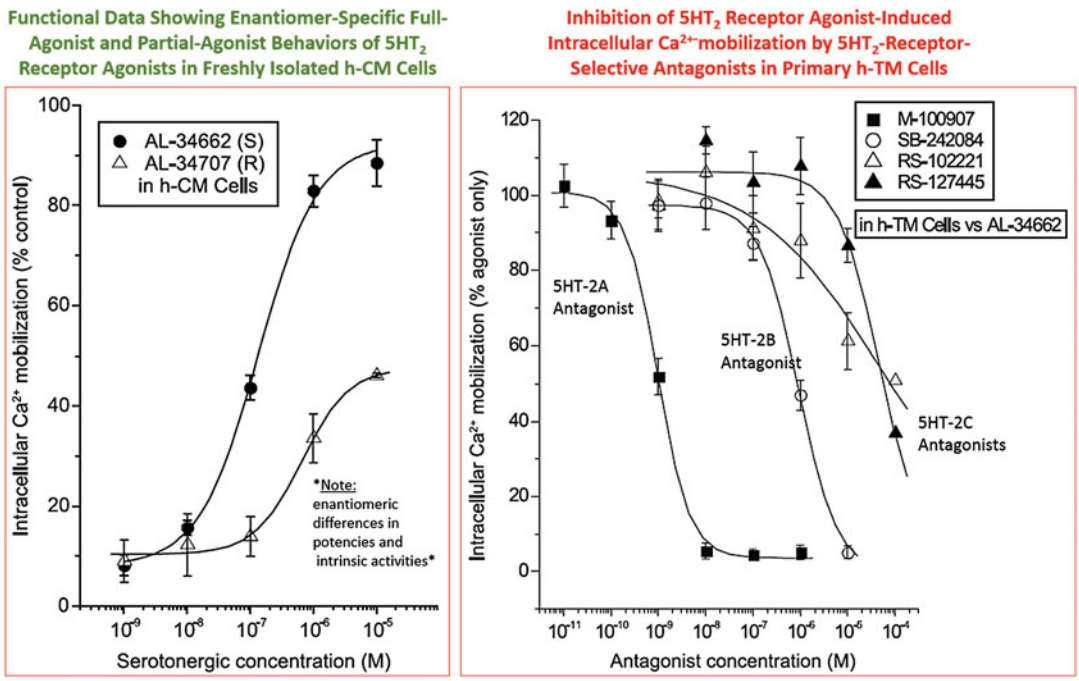

Fig. 4 Left-side panel of this figure shows the increase in intracellular Ca^{2+}-induced by AL-34662 and AL-34707, two enantiomers of a $5HT_2$-receptor agonist. Note that while AL-34662 is a full-agonist, AL-34707 is a much weaker agonist and is a partial-agonist based on its relative potency and intrinsic efficacy compared with AL-34662.The right-side panel depicts the ability of various $5HT_2$ receptor subtype-selective antagonists concentration dependently blocking the actions of AL-34662. (Modified from Sharif et al. (2007))

ion-channels or releasing intracellular Ca^{2+}, which ultimately results in a biological response such as hormone/cytokine release, muscle contraction, induction of inflammation and/or pain. On the other hand, kinase-linked receptors represent another large family of proteins that respond to certain growth factors and cytokines, and they trigger the phosphorylation of intracellular proteins that are involved in other types of signal transduction linked to cell growth, differentiation, and gene activation or inhibition. Furthermore, certain lipophilic ligands such as steroids enter the cell and have to move to the nucleus to activate gene transcription and thus influence protein synthesis (Fig. 5). Thus, rapid transmission of information (milliseconds to minutes) to modulate cellular activity and achieve communication among cells is undertaken by ion-channels/second messengers and intermediary mediators, while slow transfer of information (occurring over hours to days) is accomplished via gene expression changes (Alexander et al. 2017a, b, c, d). When the actions of the endogenous ligand or exogenously delivered drug are completed, these molecules are either degraded by specific enzymes or are taken up by cells via transporter systems and then metabolized and ultimately recycled. Transporters, as the name implies, shuttle chemicals, drugs, metabolites, and/or nutrients from one side of the cell membrane to the cytoplasm or vice versa, and this process occurs over a seconds-minutes time-scale. Thus, by effective and timely use of different receptors, ion-channels, transporters, and diverse signaling mechanisms, the body conserves energy, enhances efficiency and tries to maintain homeostasis and thus good health.

The degree to which the agonist ligand fits the receptor active-site and converts the inactive conformation of the receptor protein to a fully active or partially active state governs the relative

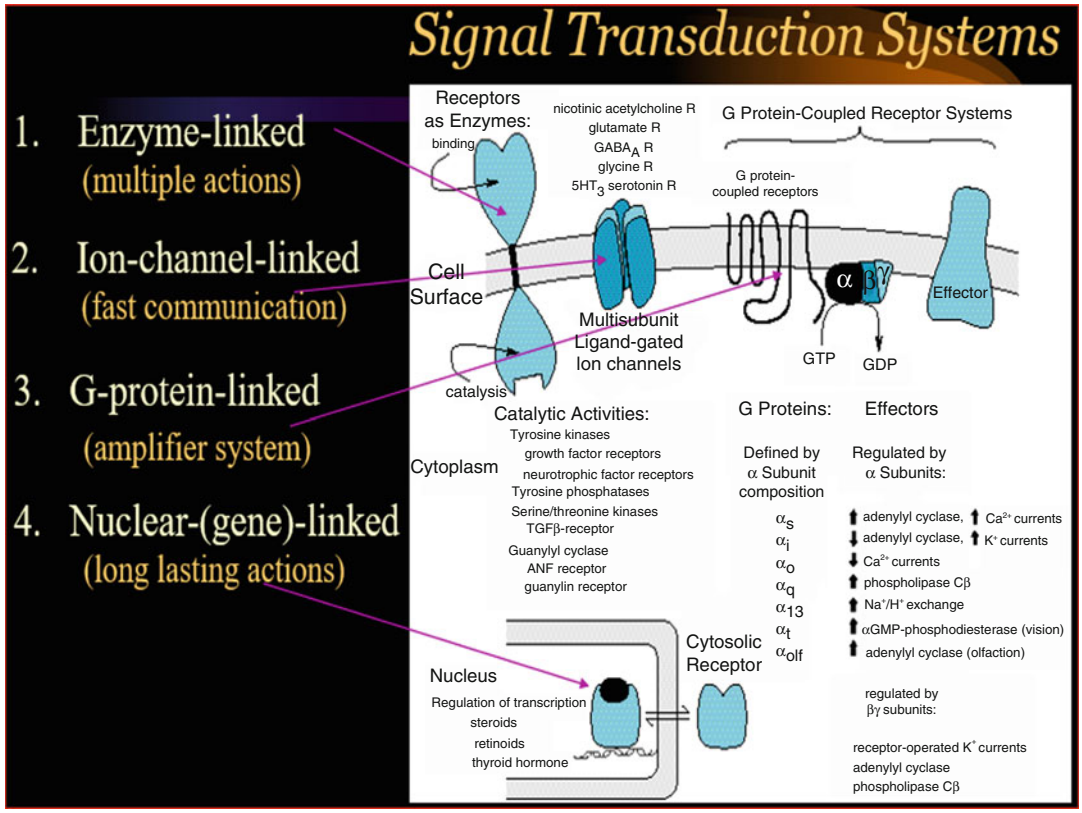

Fig. 5 The signal transduction systems associated with different kinds of receptor proteins present in most mammalian cells are shown

efficacy of the agonist (full- or partial agonist) to elicit the final cell or tissue response. The relative attraction of the ligand for the receptor to begin with is determined by the relative binding affinity of the ligand to fit the receptor-protein-pocket ("key and lock engagement") and the ability of the ligand-receptor complex to be formed and thus to cause a biological effect ("opening the lock phenomenon") (Fig. 5). For an agent to be classified as a pharmacologically relevant entity, its action at the receptor/ion-channel/transporter must follow concentration-response or dose-response relationship characteristics, and other compounds in the same class must demonstrate various degrees of affinity/potency/efficacy (intrinsic activity) with parallel concentration-response curves. This means that an agonist must elicit an increasingly larger response as its concentration or dose is increased until all the receptors/ion-channels/transporter ligand-binding sites are fully occupied and the induced response plateaus. In most cases, overstimulation of the receptor leads to dissociation of the receptor-G-protein complex, and a diminished response is observed. This desensitization phenomenon is well known and is responsible for the development of tolerance to a drug.

The potency of the agonist ligand is defined as the relative concentration needed to induce a given response via receptor activation or by engagement with its recipient protein. The relative potencies of agonist compounds (the concentration needed to induce 50% of the maximal response; EC_{50} or ED_{50}) is used to rank order such compounds (e.g., Tables 3 and 4.). Likewise, the relative potencies of antagonist or inhibitor

Table 3 Functional agonist potencies of selected PGs at various PG receptors

Test PG Compound	Agonist potency (EC_{50}; nM) at various prostaglandin receptors						
	DP-receptor (↑ cAMP)	EP_1-receptor (PI turnover; or other response)	EP_2-receptor (↑ cAMP; or other response)	EP_3-receptor (various functional responses)	EP_4-receptor (↑ cAMP)	IP-receptor (↑ cAMP or other response)	TP-receptor (PI turnover; or other response)
PGD_2	**74**	3,190	58,000	Nd	>10,000	>10,000	>10,000
PGI_2	>10,000	319	>10,000	3,019	>10,000	**7**	>10,000
PGE_2	>1,000	**2.9**	**67**	**19.9; 45; 4.5**	**40**	3,310	>10,000
$PGF_{2\alpha}$	>10,000	**29**	>10,000	691; >10,000; 2,000	>10,000	3,000	>10,000
Bimatoprost acid	>10,000	2.6	>10,000	Nd	>10,000	>10,000	>10,000
Travoprost acid	>10,000	Nd	>10,000	>10,000	>10,000	>10,000	>10,000
Latanoprost acid (PHXA85)	>10,000	119	20,000	12,000	>10,000	>10,000	>10,000
Cloprostenol	>10,000	93	>10,000	228	>10,000	>10,000	>10,000
S-1033	>10,000	>30,000	>10,000	>10,000	>10,000	>10,000	>10,000
Unoprostone (UF-021)	>10,000	>30,000	>10,000	>10,000	>10,000	>10,000	>10,000

Data are average values from up to three experiments.
Nd not determined (Sharif et al. 2003)

compounds (the concentration needed to block/inhibit 50% of the maximal response or event; IC_{50} or K_i) can be used to rank order such compounds in order to choose which compound(s) to pursue in animal studies, for instance. Furthermore, antagonists can be classified as competitive or noncompetitive. Competitive antagonists shift the concentration response of an agonist to the right (dextral shift) without diminishing the maximal effect of the agonist thereby reducing the agonist affinity for the receptor (Griffin et al. 1999). Noncompetitive antagonists invariably produce rightward shifts of the agonist concentration-response curves but prevent the agonist compound achieving its maximum effect (Sharif and Klimko 2019). Just as agonists cause receptor desensitization when the latter are exposed to excessively high a concentration (and/or exposed too often) of the agonist ligand, cells/tissue/animals challenged with very high levels of competitive antagonists (and/or on a high frequency) actually induce generation of more receptors (receptor upregulation) as a compensatory mechanism. This is often associated with a so-called "rebound effect" when the agonist actually produces a greater response than it induced before exposure to the high levels of the antagonist.

Ligand-gated ion-channels are important targets for neurotransmitter and drug interaction and thus for drug discovery/development. There are several different types of ion-channels that are present primarily on cell membranes of neurons and excitable cells where fast communication is needed. The most well-known ion-channels, that are made up of 3–5 protein subunits, are those permeable to Na^+, K^+, Cl^-, and Ca^{2+} ions and are responsible for depolarization or hyperpolarization of cells. Binding of specific ligands (e.g., serotonin, glutamate, zinc, acetylcholine, ATP) to certain subtypes of receptors activates these types of ion-channels (Alexander et al. 2017a, b, c, d). Certain cation-channels of the transient receptor potential (TRP) protein superfamily

Table 4 Functional agonist potencies of various FP-class PG analogs at native or cloned FP receptors in five different cell types of different species

Compound	Agonist potency for stimulating inositol phosphate production in different cell types (EC_{50}; nM)				
	Human ciliary muscle cells (h-CM cells)	Human trabecular meshwork cells (h-TM cells)	Human cells (HEK-293) expressing cloned human ocular FP receptor	Mouse Swiss 3T3 fibroblasts	Rat A7r5 vascular smooth muscle cells
Travoprost acid ((+)-fluprostenol	1.4 ± 0.2	3.6 ± 1.3	2.4 ± 0.3	2.6 ± 0.2	2.6 ± 0.5
(±)-fluprostenol	4.3 ± 1.3	11 ± 2	4.6 ± 0.4	3.7 ± 0.4	4.4 ± 0.2
Bimatoprost acid (17-phenyl-$PGF_{2\alpha}$)	3.8 ± 0.9	28 ± 18	3.3 ± 0.7	2.8 ± 0.2	2.8 ± 0.6
Latanoprost acid (PHXA85)	124 ± 47	35 ± 2	45.7 ± 8.4	32 ± 4	35 ± 8
Travoprost (Isopropyl ester)	123 ± 65	103 ± 27	40.2 ± 8.3	81 ± 18	46 ± 6
Latanoprost (Isopropyl ester)	313 ± 90	564 ± 168	173 ± 58	142 ± 24	110 ± 19
Bimatoprost (amide)	9,600 ± 1,100	3,245 ± 980	681 ± 165	12,100 ± 1,200	6,850 ± 1,590
Unoprostone (UF-021)	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
S-1033	4,701 ± 2,031	7,000 ± 2,600	2,610 ± 463	670 ± 320	767 ± 93
$PGF_{2\alpha}$	104 ± 19	62 ± 16	29 ± 2	26 ± 3	31 ± 3

Data are mean ± SEM from 3–23 experiments. *nd* not determined. $PGF_{2\alpha}$ (K_i = 122 ± 40 nM) and latanoprost acid (K_i = 149 ± 9 nM) exhibited relatively high affinity for the prostaglandin transporter transfected in host cells. The functional PI turnover activities of various PGs were blocked by the FP-receptor selective antagonist, AL-8810. The pooled antagonist potencies of AL-8810 were: cloned human FP receptor $K_i = 1.9 \pm 0.3$ μM; h-TM cell $K_i = 2.6 \pm 0.5$ μM; h-CM cell $K_i = 5.7$ μM; rat A7r5 cell $K_i = 0.4 \pm 0.1$ μM; and mouse 3 T3 cell $K_i = 0.2 \pm 0.06$ μM using a variety of FP agonists including fluprostenol, travoprost acid, unoprostone, bimatoprost, and bimatoprost acid (Sharif et al. 2003)

exist on nonexcitable and excitable cells and act as sensors of heat/cold, changes in osmolarity, odorants, and mechanical stimuli. The transient receptor potential vanilloid-1 (TRPV1) channel, for example, responds to capsaicin and detects "hot taste" associated with chili-peppers but is also activated by noxious heat (>43 °C), low pH, voltage, and various lipids.

All the pharmacodynamic aspects of drug-receptor (or drug-ion-channel) interactions mentioned above apply to in vitro and in vivo situations. Obviously, all drugs have side effects, some toxicological in nature, and thus the risk-benefit ratio must be determined for each in order to ensure that the therapeutic index is high and that the side effects are minimized for the subject. The drug safety elements also have to account for the dose of the drug administered, routes of administration, the speed with which the active drug reaches its intended site of action, the duration of action of the drug, and its rate of and safe elimination from the body. Such data are obtained

from pharmacokinetic (PK) and absorption/distribution/metabolism/elimination (ADME) studies conducted in suitable normal healthy animals or human subjects during the drug development processes. Direct efficacy of the drug can then be assessed at appropriate dosage(s) in animal models of disease and of course in humans in clinical trials in due course.

Due to the diversity of cell types involved in the anatomy and physiology of the eye and the mediation of pathogenesis of various ocular diseases, the eye offers a great opportunity to apply the principles of pharmacology. There is thus a rich history of delineation of physiological and pharmacological actions of endogenous ligands and exogenous experimental chemical agents. A few examples include the role of endogenous epinephrine and norepinephrine (NE) in promoting the synthesis of AQH by the nonpigmented ciliary epithelial cells of the ciliary body in the anterior chamber (ANC) of the eye, and the role of vascular endothelial growth factor (VEGF) to stimulate the growth of new blood vessels into the vitreous humor in wet AMD and diabetic retinopathy. The NE-induced AQH production is mediated through action at alpha- and beta-adrenoceptors and by raising the intracellular levels of cAMP, while the VEGF-induced neovascularization of the choroidal blood vessels is mediated through receptor-tyrosine kinase (RTK)-coupling. Hence, β-blockers and anti-VEGF antibodies reduce AQH production and neovascularization, respectively. Similarly, allergen-induced conjunctival mast cell degranulation results in the release of histamine and other mediators into the tear film with subsequent activation of histamine receptor-1 in conjunctival and corneal epithelial cells (Sharif et al. 1994, 1996). This raises intracellular Ca^{2+} and then results in secretion of various proinflammatory cytokines, that together with histamine, cause increased vascular permeability of both tissues causing ocular itching and redness. And thus, treatment of the ocular surface with H_1-receptor antagonists such as emedastine and olopatadine blocks the effects of histamine and curtails the itching and redness (Sharif et al. 1994, 1996; Yanni et al. 1999). Other examples of ocularly relevant receptors/ion-channels/transporters, and drugs that interact with them, will be discussed below in more detail.

Application of Pharmacodynamic Principles in Ocular Drug Discovery and Development

The first step in any drug discovery program is to identify the target protein whose activity needs to be modulated to achieve the therapeutic benefit. Next, it is important to verify the localization and relative distribution of the target protein in the specific tissue/cells connected with the disease process and in other areas where potential side effects may occur. Such target localization and visualization can be accomplished using techniques such as autoradiography (Fig. 6, left panel), immunohistochemistry (Fig. 6, right panel), and *in situ* hybridization. From an ocular perspective, the relative density and distribution and cellular localization of the target protein in animal and human eye sections is paramount. Examples of use of such techniques and results obtained are shown in Fig. 6 and Table 5.

Testing funnel schemes are drawn up next that place the target protein into ligand binding assays (and/or directly into functional assays) using cell/tissue homogenates enriched in the target protein (Fig. 7). If the target receptor/transporter/ion-channel can be found at high levels in animal/human cells/tissues, then the naturally occurring protein can be used for screening purposes, and this is actually preferred (Sharif 2018a). The alternative strategy is to avail recombinant molecular biological techniques to express the desired protein at high levels in host cells to make the assay sensitive and reproducible. Using known high-affinity (usually nanomolar K_d) tritiated or iodinated radiolabeled ligands to tag the target protein, the relative affinity of unlabeled test compounds to compete for, and thus displace, the radioligand from its binding sites is determined using rapid vacuum filtration techniques to separate the free from bound radioligand followed by liquid

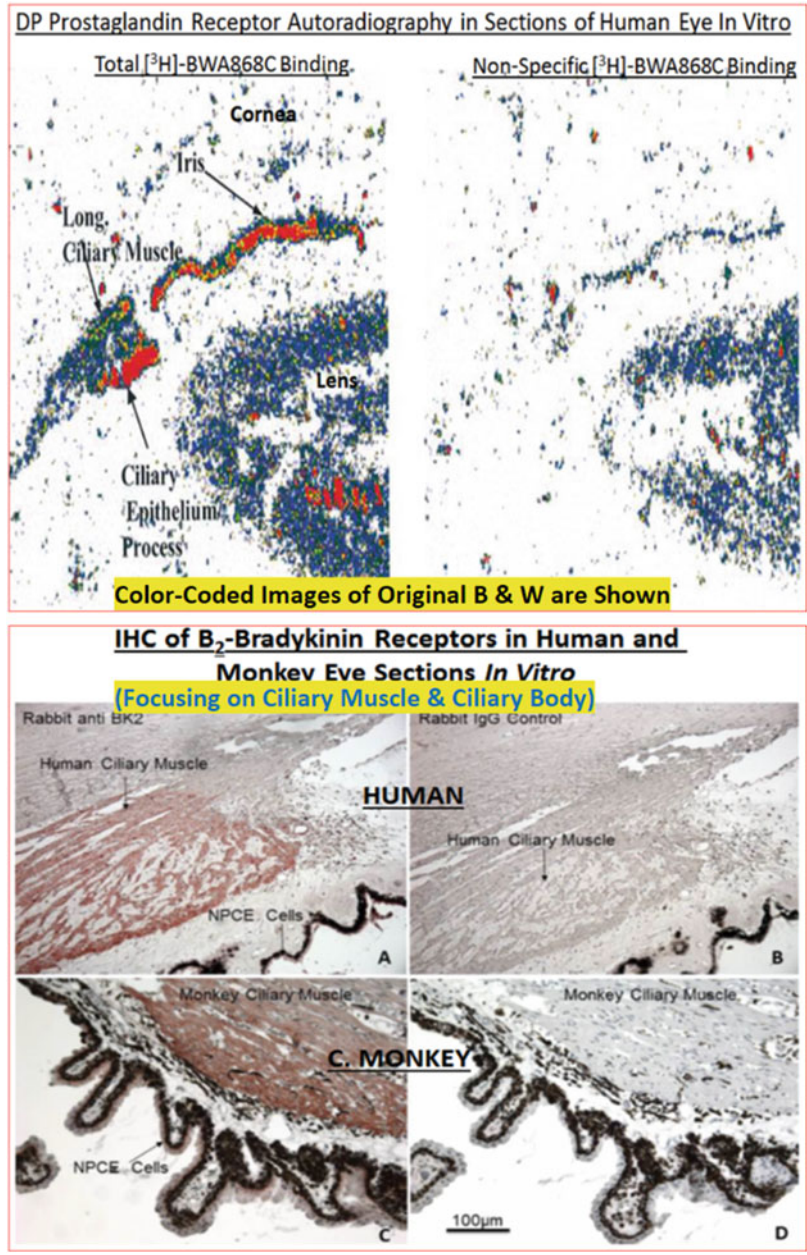

Fig. 6 Two different techniques of localizing and visualizing membrane-bound receptors in human and monkey eye sections are illustrated. The top panel shows the autoradiographic distribution of DP PG receptor binding sites in the anterior segment of the human eye using radio-labeled DP-receptor antagonist, [^{3}H]-BWA868C. Top-left panel shows the total binding, while the top-right panel shows the nonspecific binding of the radioligand. (Modified from Sharif et al. 2000). The bottom panel depicts localization of bradykinin B_2-receptors in human and monkey eye sections using the IHC technique– specific binding of the B_2-receptor anti-body is shown in the left-side panels (russet color), while the control (nonspecific binding) is shown in the right-side panels for each species.(Modified from Sharif et al. (2014).

scintillation spectrometry (Fig. 8, left panel; Sharif 2018a). Alternatively, homogeneous proximity-scintillation ligand binding assays can be utilized. The ligand binding affinity data obtained from such experiments are used to rank-order compounds and only those meeting specified pass criteria (e.g., $K_i \leq 50$ nM) are then tested in functional assays. Here, isolated primary cells from target tissue are used to determine if the compounds are agonists or antagonists (Sharif et al. 2007) (e.g., Fig. 8, right panel) or activators or inhibitors of enzymes (Chen et al. 2014) or channels (Patil et al. 2016). Compounds can then be ranked according to their relative functional potencies for instance for their ability to stimulate the production of intracellular cAMP (Crider and Sharif 2001) or cGMP (Katoli

Table 5 Quantitative autoradiographic distribution of various drug receptors in postmortem human eye sections

	All 5-HT Receptors	5-HT_2 Receptors	β-Adrenoceptors	DP PG Receptors	FP PG Receptors
	[^{3}H]-5-HT binding (5-HT receptors)	*[^{3}H]-ketanserin binding (5-HT_2 receptors)*	*[^{3}H]-levobetaxolol binding (β-receptors)*[a]	*[^{3}H]-BWA868C binding (DP prostaglandin)*[b]	*[^{3}H]-$PGF_{2\alpha}$ binding (FP prostaglandin)*[c]
Tissue	*Specific binding; DLU/mm^2 and (% specific binding)*	*Specific binding; DLU/mm^2 and (% specific binding)*	*Specific binding; DLU/mm^2 and (% specific binding)*	*Specific binding; DLU/mm^2 and (% specific binding)*	*Specific binding; DLU/mm^2 and (% specific binding)*
Ciliary epithelium (process)	71,780 ± 2,725 (70%)	13,683 ± 5,870 (40%)	51,459 (76%)	67,000 (82%)	2,554 (35%)
Longitudinal ciliary muscle	14,232 ± 7,937 (48%)	14,459 ± 3,683 (47%)	27,543 (83%)	37,900 (79%)	12,741 (68%)
Iris	174,943 ± 20,092 (74%)	20,026 ± 11,276 (20%)	78,140 (62%)	55,500 (54%)	3,776 (40%)
Lens	2,225 ± 582 (14%)	4,151 ± 2,762 (20%)	1,507 (18%)	23,000 (5%)	1,886 (12%)
Choroid	33,238 ± 5,950 (60%)	16,304 ± 3,792 (40%)	19,244 (72%)	39,700 (54%)	1,671 (28%)

DLU digital light units. These units represent an index of the relative density of the receptor population found in the tissues studied

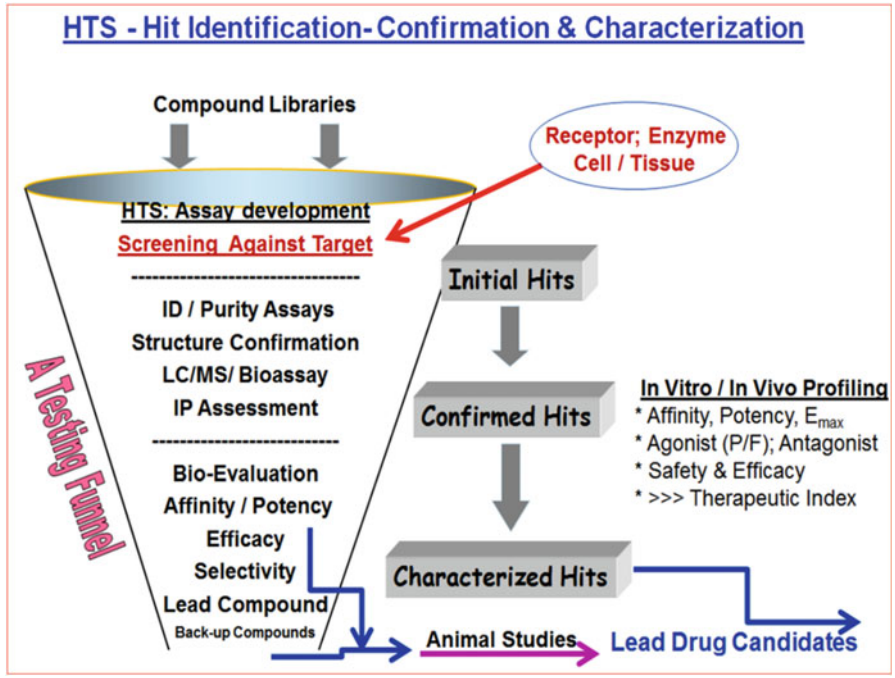

Fig. 7 A schematic illustration of testing funnels for profiling and selecting compounds as "Hits" and fully characterized compounds is shown. Note that the target can be a receptor, enzyme, transporter, or ion-channel expressed in isolated normal primary cells or genetically engineered cells or in isolated animal/human tissue samples

et al. 2010; Cavet and DeCory 2018; Fig. 9, left panel) or to mobilize intracellular Ca^{2+} (Kelly et al. 2003; Sharif et al. 2007; Fig. 8, right panel) or to induce cell/tissue contraction (Ohia et al. 2018).

Sometimes the radioligand binding assays are omitted from the testing funnels and compounds are directly screened in functional activity assay systems using multiple compound concentrations to construct full concentration-response curves (e.g., Fig. 4, left panel). Once again relative potency data are used to triage and select most potent and efficacious agonists (e.g., $EC_{50} \leq 10$ nM), or most potent antagonists ($K_i \leq 10$ nM), to advance into *in vivo* testing in animal models of ocular safety (e.g., guinea pigs or rabbits for ocular irritation, redness [hyperemia], or inflammation [mucus-containing discharge]), followed by testing in animal models of eye disease (e.g., ability of compounds to lower intraocular pressure (IOP) in rodent, rabbit, and monkey eyes, either normotensive or ocular hypertensive) (e.g., Fig. 9, right panel, Cavet and DeCory 2018; Fig. 10, Sharif et al. 2014).

In other cases where compound supply is limited, only one or two concentrations are tested to generate receptor binding and/or functional-response data to help make decisions. If none of the tested compounds meet the prespecified criteria of affinity/potency/in vitro efficacy, or in order to improve the overall profile of the compounds, the medicinal chemists would need to modify the chemical structure of the compounds and the biologists would need to retest the new compounds. This iterative process can be short or long depending on the complexity of the synthetic process for the compounds/peptides/antibodies and the degree of difficulty and complexity of the assay systems. Based on the physiochemical properties and structure-activity relationship (SAR) profile of the compounds, a suitable lead compound and possible backup compounds are selected for advancement to full development that requires gathering much more detailed information on the ocular/systemic safety, PK, ADME, and toxicology (genotoxicity; central and systemic toxicology in multiple species after repeated

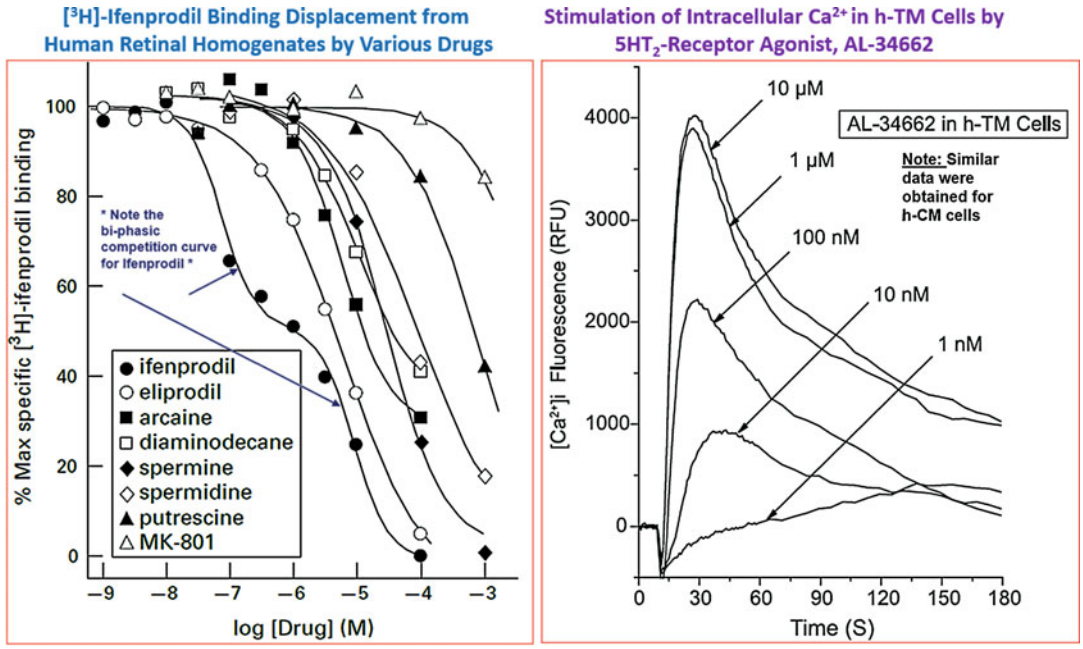

Fig. 8 Two different types of assays are shown. The left-side panel depicts a typical receptor-binding experiment where various compounds of interest are screened for their ability to displace a receptor-specific radioligand, in case from human retinal homogenates. Note how a compound can be shown to interact with the bind site in a high- and a low-affinity state(ifenprodil displacing [^{3}H]-ifenprodil), or to only bind to a low-affinity state of the receptor. The relative affinities of the different compounds are easily observed and determined in this manner. (Modified from Sharif and Xu, 1999). The right-side panel shows a typical profile of intracellular Ca^{2+} increase induced by different concentrations of the $5HT_2$-receptor agonist, AL-34662. The peak induction of responses from such traces are then used to construct concentration–response curves depicted previously in Fig. 4. (Modified from Sharif et al. (2007))

dosing at multiple doses) information in suitable animal species and test systems. Additional work requiring building a data package covering optimized formulation, route(s) of administration, duration, and mechanism of action of the lead compound (and a backup compound) would be undertaken next. These are lengthy, laborious, expensive, but necessary experiments to discover suitable drugs.

Ultimately, the lead compound data package would have met all the necessary requirements to be considered for clinical trials in humans. At this stage, a formal request to conduct Phase-1 (primarily for ocular safety at multiple doses) clinical trials is made to the regulatory agency of the country whence the complete data package on the Investigational New Drug is submitted for approval. If the lead compound is pronounced safe in a limited number of healthy humans (e.g., 20–30) at multiple doses administered once daily or multiple times/day via the best route of administration (e.g., topical ocular; intracameral; intravitreal; Hartman and Kompella 2018), Phase-II studies can be conducted. About 70% of drugs that enter Phase-I will be successful enough to proceed to Phase-II. Here, clinical studies are conducted in a small number of age-matched control patients lacking the ocular disease, and in those patients having the ocular disease. A control vehicle (placebo), multiple doses of the test drug candidate and a single dose of a suitable comparator drug (previously approved by the regulatory authority) are tested in patients who have the ocular disease (e.g., 30–50 per each treatment arm). Such Phase-II studies are conducted over several months and allow the selection of the most optimum dose of the lead compound. In Phase-III studies, the optimum dose of the lead compound is compared against specified marketed comparator drug (in

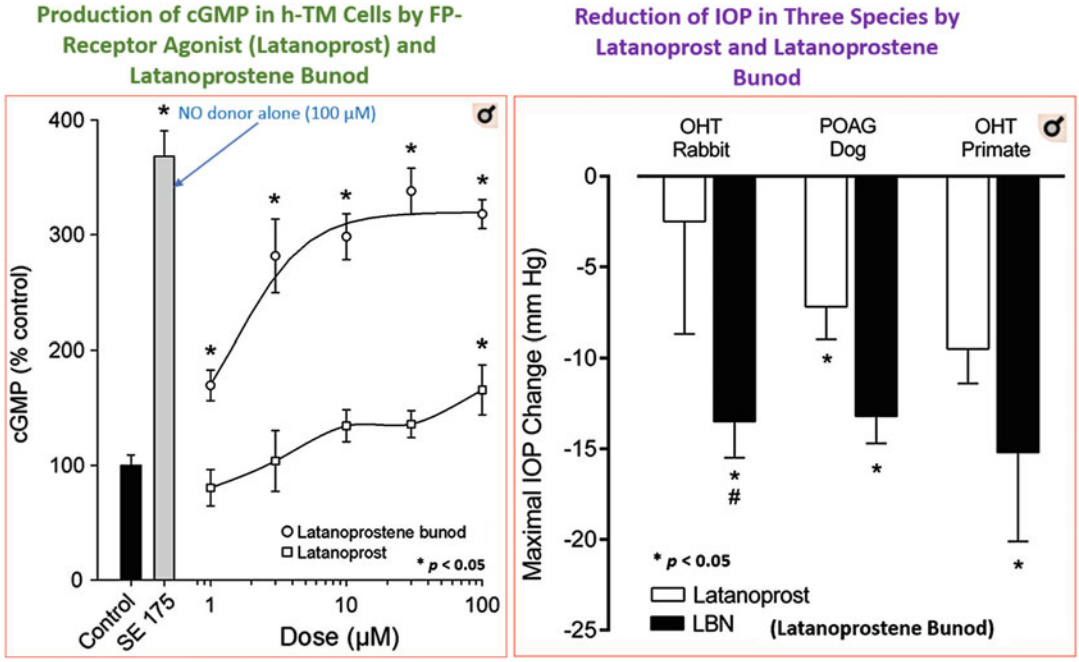

Fig. 9 The left-side panel illustrates the production of a second messenger, cGMP, in h-TM cells by various ligands, while the right-side panel shows how the different ligands lower IOP in rabbits, dogs, and monkeys. The cell-based and animal model-based data correlate well with each other. (Modified from Cavet and DeCory (2018).

the same drug class) in a much larger human population (e.g., 100–800 patients/treatment arm) of patients with the ocular disease and the trials conducted for several months. It is important to "statistically power" the clinical trials (with sufficient number of patients per treatment arm) in order to show statistically and clinically relevant efficacy and benefit to the patient suffering from the ocular disease.

Having established the necessary safety and efficacy of the drug candidate molecule, the regulatory agency can be approached for approval to market the drug by submitting a New Drug Application. This dossier contains all the necessary guidance on drug manufacturing procedures, stability and formulation data, PK, ADME, and other necessary data, and of course all the preclinical and clinical safety and efficacy data, as required by the specific health authority. After meeting all the regulatory authority's criteria and requirements, the agency may grant an approval to market the drug and thus make it available for use by clinicians to treat the patients with the ocular disease with appropriate guidance on dose/frequency of dosing/route of administration and of course side effects, etc. Based on the above, it is not surprising that drug discovery, drug development and approval by a health authority is a very long (taking 10–12 years from discovery to marketing and before being introduced into clinical medicine) and very expensive process (typically costing $50–100 million depending on the type of drug/its cost of goods/frequency of dosing, etc.). Nevertheless, such due diligence, time, and cost is worthwhile in order to reduce and/or prevent visual impairment and preserve eyesight for a global population of humans afflicted with the eye disorders for which the treatment was sought.

Eye Diseases and Their Pharmacological Treatments

As the earlier discourse above illustrates, the eye is a very complex organ being composed of a heterogeneous population of specialized tissues/

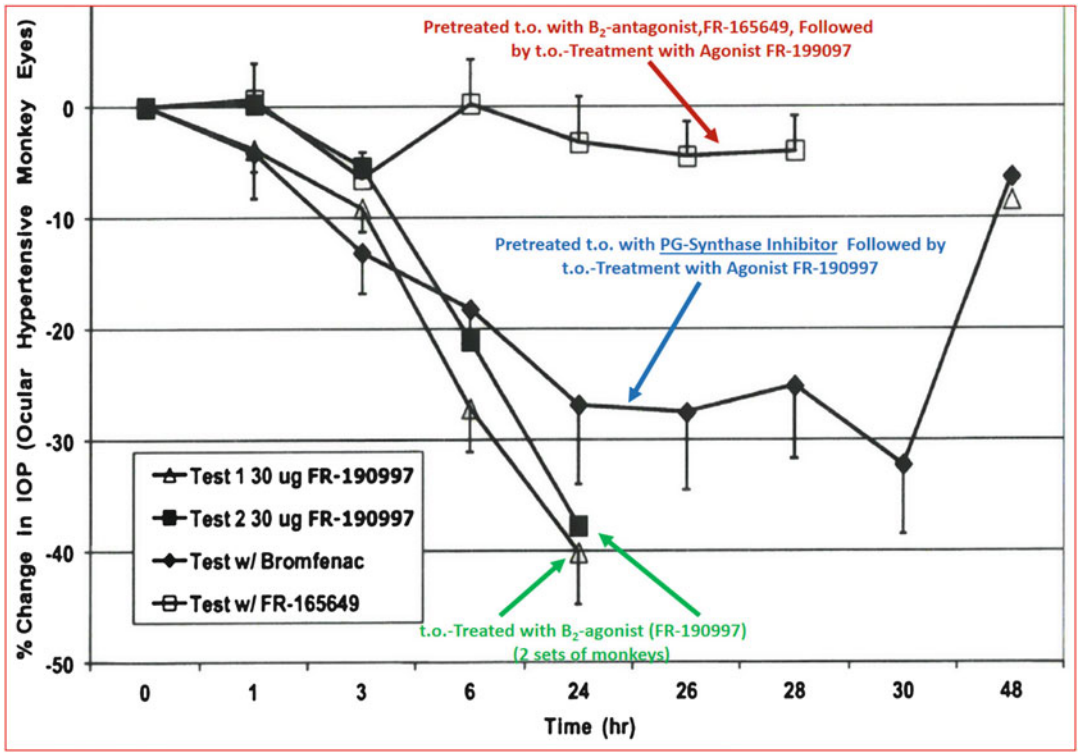

Fig. 10 IOP reduction in conscious cynomolgus monkeys. This figure shows how the nonpeptide B_2-bradykinin receptor agonist FR-190997 lowered IOP of ocular hypertensive eyes of the monkeys when delivered topical ocularly (t.o.). The effect of FR-190997 was reproduced in two different colonies of cynomolgus monkeys. Additionally, it was important to demonstrate that predosing t.o. with a B_2-receptor antagonist FR-165649 could block the IOP-lowering effects of the agonist. Likewise, in order to show that endogenous PGs were mediating some (or all) effects of activating the B_2-receptor, a PG synthase inhibitor partially reduced the agonistic effects of FR-190997. Such studies illustrate the role of different pharmacological treatments to better understand the mechanism of action of drugs in vivo. (Modified from Sharif et al. (2014))

cells which have diverse functions ranging from providing structural support, limiting pathogenic infiltration, keeping corneal transparency, focusing light, transducing light into electrical impulses, absorbing excess light, phagocytosing toxins and cellular debris, production and drainage of AQH, etc. It is therefore not surprising that dysfunction of these cell types results in various ocular disorders such as cataracts (51% of total eye diseases), glaucoma (8%), AMD (5%), corneal opacity/scarring (4%), pediatric eye diseases (4%), trachoma (3%), uncorrected refractive errors (e.g., myopia; presbyopia; 3%), diabetic retinopathy (1%), and infections, ocular allergies, inflammation, retinoblastoma, etc. (21%) (WHO 2018; NEI 2014).

The other aspects worthy of note include the fact that while somewhat isolated from the rest of the body, medications administered to the eye topically still drain into the naso-pharynx area and eventually into the stomach and then into the blood stream. As the blood is supplied to each organ, the latter are then exposed to the drug that was administered to the eye, albeit at lower concentrations. Depending on the concentration/affinity/potency of the drug or its metabolite(s), off-target side effects are always possible and thus the physician and the patient need to be aware of such issues. Likewise, drugs that are delivered to the anterior or posterior chambers of the eye via different routes of administration also are routed via the venous circulation and eventually

metabolized by the liver and then eliminated via the kidneys and/or the alimentary canal. Due to these reasons, local ocular and systemic and central side effects of drug treatments to the eye have serious consequences and need to be considered in the treatment regimens.

In order to emphasize the pharmacodynamic and translational aspects of ocular pathologies and their treatments, eye disorders that are more prevalent and which are debilitating from a visual impairment perspective will be covered first followed by others that are lesser common and where pharmacological elements are less well characterized from the structure activity and pharmacological viewpoint. Due to space limitations, only certain eye diseases will be discussed in detail where the pharmacology is quite well defined and robust. It is hoped that readers will follow-up on the cited references, especially review articles, to help advance their understanding of the eye disease pathogenesis and the treatment modalities available.

Primary Open-Angle Glaucoma (POAG) and Ocular Hypertension (OHT)

Glaucoma is a heterogeneous group of ocular diseases that have their origin due to pathogenic events occurring in at least three distinct parts of the eye, namely, ANC, retina, and optic nerve. Over >65 million people around the world suffer from the most common form of glaucoma (primary open-angle glaucoma (POAG)). It is a slowly developing ocular disease that the patient only notices once some of the peripheral vision has already been lost and when only 60% of the original RGCs remain functional and capable of transmitting retinal signals to the brain. Eyesight is lost due to POAG and other types of glaucoma when the RGCs die, their axons atrophy and the RGCs are disconnected from the brain where neurons also die (Yucel et al. 2000). Other characteristic features of POAG are thinning of the retinal nerve fiber layer (RNFL) due to RGC axon atrophy, excavation of the optic nerve head (OHN), and subsequent cupping of the optic nerve disc (Tham et al. 2014; Weinreb et al. 2014; Jonas et al. 2017).

Behind cataracts, POAG is the next most prevalent preventable blinding eye disease. Despite linkage of numerous risk factors to POAG, abnormally high IOP is the most high IOP accepted modifiable element in the disease process. This ocular hypertension (OHT) is caused by an imbalance between the amount of AQH being produced and the amount draining from the ANC. The blockage of the TM and SC with aberrant or age-related accumulation of extracellular matrix (ECM) and cell debris is primarily the culprit responsible for OHT (Xu et al. 2014). The OHT has been shown to be directly responsible for demise of RGCs and their axons in animal models of POAG and in humans such that it is calculated that every 1 mmHg reduction of IOP results in up to 13% lowering of the progression of POAG (Weinreb et al. 2014; Jonas et al. 2017). Therefore, reducing IOP has been an effective way to treat OHT/POAG for many decades. This has resulted from the fundamental understanding that either the production of AQH can be reduced and/or the efflux of AQH can be accelerated in order to lower IOP. Even though inhibiting AQH generation by blocking the Na^+-K^+-ATPase in the CEP cells of the ciliary processes is not usually recommended, since the AQH provides nutrients to the ANC cells and removes their waste products, early treatment options were quite limited and clinicians had no choice but to inhibit the inflow process. The relatively recent discovery and development of drugs, surgical procedures and devices that can effectively reduce IOP by stimulating the drainage of AQH via the trabecular meshwork and/or uveoscleral pathways and newly created pathways have revolutionized clinical management of OHT/POAG.

Several decades ago, pharmacological management of IOP was achieved using pilocarpine, a plant-derived alkaloid muscarinic receptor agonist. However, while it reduced IOP it also caused miosis and brow ache by contracting the iris sphincter and ciliary muscle (CM). Additionally, its IOP-lowering effect was rather short-lived (4–6 h depending on the concentration of the drug and dosing frequency). Since that time, an enormous amount of progress has been made in discovering, developing, and marketing a variety of pharmacological agents that have

diverse mechanisms of action to lower IOP. Today, prostaglandin FP-receptor agonist analogs (PGAs; e.g., latanoprost; travoprost; tafluprost; bimatoprost) (Hellberg et al. 2002) represent the drugs of choice for treating ocular hypertension and POAG since they exhibit excellent efficacy, long duration of action (at least 24 h) with relatively low incidence and severity of side effects after a single topical ocularly administered drop of the drug. These PGAs promote egress of AQH from the ANC of the eye via the uveoscleral pathway and to some extent via the conventional TM-SC outflow pathway by releasing matrix metalloproteinases that digest ECM and other cellular debris and thus enlarging existing spaces between CM bundles and the sclera and/or creating new drainage channels in the latter tissues. The notable side effects of PGAs, however, are hyperemia (eye redness), darkening of the iris and orbital skin, lengthening and thickening of eyelashes, deepening of the orbital sulcus, and to a lesser extent, cystoid macular edema.

While alpha-2 adrenergic agonists like brimonidine and apraclonidine lower IOP by inhibiting inflow (generating cAMP that suppress Na^+-K^+-ATPase in ciliary epithelium) and by stimulating some outflow, their ocular (e.g., ocular allergy) and systemic and central (lowering CNS activity and causing lethargy) side effects limit their utility. Other agents that inhibit production of AQH, inflow inhibitors, also include carbonic anhydrase inhibitors (e.g., dorzolamide and brinzolamide), and beta-blockers (e.g., timolol and betaxolol) that do lower IOP but exhibit a number of ocular side effects (burning, stinging, foreign-body sensation) and systemic side effects (drop in blood pressure, bradycardia, palpitations, arrythmias, and bronchospasms). The latter inflow and outflow drugs have been combined in suitable formulations to generate so-called "combination products" with certain degree of enhanced efficacy (Holló et al. 2014), that have expanded the treatment options for treating elevated IOP and glaucoma. The exceptional value of the inflow suppressors and outflow stimulators is highlighted by the latter combination products but also their utility in glaucoma and OHT patients who become refractory to or are nonresponders to PGAs, especially to latanoprost.

In order to overcome some of the deficiencies and side effects of the aforementioned drugs for the treatment of OHT and glallcoma, two recent FDA-approved medications have been marketed: netarsudil (Rhopressa; Lin et al. 2018) and latanoprostene bunod (Vyzulta; Cavet and DeCory 2018). While netarsudil is a rho-kinase inhibitor that lowers IOP by relaxing the ciliary muscle and TM (Lin et al. 2018) thereby stimulating AQH to flow out of the ANC, latanoprostene bunod is a conjugate drug made up of latanoprost and a nitric oxide donating agent (Cavet and DeCory 2018), that reduces IOP by relaxing CM and TM tissues (engaging the outflow pathway) and by activating the UVS pathway. How these new drugs will fare after being introduced into clinical management of OHT/glaucoma remains to be seen, but in the meantime, the search for even better pharmacological agents with unique characteristics and better side effect profiles continues. It is encouraging that continued research in this area is poised to deliver additional drugs as judged by the multitude of reports published in recent years. The most succinct way to show this progress is via a tabular listing of such pharmacological agents (Table 6). Pharmacodynamic aspects, including mechanism of action, of each class of these agents is shown in this table and also described in detail in the relevant citations. Another exciting recent development is the ability of implanted microdevices, after Minimally Invasive Glaucoma Surgeries (MIGS), to literally drain the excess AQH from the ANC of OHT/POAG patients (e.g., Batlle et al. 2016; Fig. 11) without causing collapse of the ANC.

The MIGS-related devices have revolutionized AQH drainage from the ANC and have added another means to lower IOP, which was previously dominated by tubes and trabeculectomies (Batlle et al. 2016).

Receptor Binding and Functional Assays to Discover New IOP-Lowering Agents

Testing of potential ocular hypotensive agents is simplified since in most cases the target protein and its signal transduction mechanism is known.

Table 6 Pharmacological agents that lower IOP in various mammals and the mode of action of the compounds

Compound classes	Pharmacological agent	Reported or potential mode(s) of action
Conventional outflow (TM) stimulators		
Muscarinic receptor agonists (mostly M_1 receptor agonists)	Pilocarpine; Acecledine; Carbachol	Contract ciliary muscle/TM to promote outflow of AQH via the TM-SC pathway
Inhibitors of chloride transport	Ethacrynic acid; Ticrynafen; Indacrinone	Inhibition of Na^+-K^+-Cl^--transporter activity in the TM changes cell shape and volume and thus AQH efflux is increased
Kinase inhibitors	*Rho kinase (ROCK) inhibitors*: AR-12286 (Netarsudil); Ripasudil (K115); Y-27632; Y-39983; AMA-0076; H-7; ML-9; Chelerythrine; Staurosporin	Modification of actomyosin contractility that leads to changes in actin cytoskeleton of TM (relaxation) and this leads to AQH efflux
	LIM-K inhibitor	
	Myosin-II ATPase inhibitor: Blebbistatin	
	Src kinase inhibitor	
Marine macrolids	Latrunculins A and B; Bumetanide; Swinholide	Promote sequestration of actin monomers and dimers in TM; cause cell TM shape change and thus AH efflux
Guanylate cyclase activators	*Natriuretic peptides*: ANP; CNP; SHP-639	Type-A and type-B receptor activation leads to cGMP production, TM relaxation and AQH efflux via TM
NO donors	Sodium nitroprusside; Hydralazine; 3-morpholinosyndnonimine; (S)-nitroso-acetyl-penicillamine; NCX-125	
Soluble guanylate cyclase activators	YC-1; BAY-58-2667; IWP-953	
κ-opioid receptor agonists	Bremazocine; dynorphin	Release natriuretic peptides and thus raise cGMP in TM leading to its relaxation and thus AQH efflux
Cannabinoid receptor agonists	WIN55212-2; CP55940; SR141716A	Receptor stimulation opens BKC-channels and relaxes TM which then causes AQH efflux via TM and SC
FP-class PG-receptor agonists	Latanoprost; Travoprost; Tafluprost; Bimatoprost; Unoprostone isopropyl ester	Some clinical evidence of promoting conventional outflow in addition to UVS outflow
Serotonin-2 receptor antagonists	BVT-28949; ketanserin and its analogs	Unknown and unverifiable mechanism(s) of action (may block beta-adrenergic receptors indirectly?)
Releasers of MMP and AP-1	FP-class PGs (see above); and *t*-butylhydroquinone (t-BHQ); β-naphthoflavone;	Local production of MMPs; ECM degradation; stimulation of AQH efflux via TM
Uveoslceral (UVSC) outflow stimulators (via gaps in CM fiber bundles and scleral tissue)		
FP-class PG-receptor agonists	Latanoprost; Travoprost; Tafluprost; Bimatoprost; Unoprostone isopropyl ester	FP receptor activation in CM causes release of MMPs that breakdown ECM ("clog") around CM bundles and within sclera thus causing UVS outflow of AQH
EP_2- and EP_4-PG-receptor agonists	Omidenepag Isopropyl (DE-117); Butaprost; AL-6598; ONO-AE1-259-01; PF-04217329; PF-04475270	Receptor activation increases cAMP that relaxes CM and TM; EP_2 agonists also cause release of MMPs that breakdown ECM ("clog") around CM bundles and within sclera thus causing UVSC outflow of AQH
Serotonin-2 ($5HT_2$) receptor agonists	(R)-DOI; α-methyl-5HT; AL-34662	Contraction/relaxation of CM and TM by activation of $5HT_2$ receptors. May also release MMPs and/or PGs or other local

(continued)

Table 6 (continued)

Compound classes	Pharmacological agent	Reported or potential mode(s) of action
		mediators that promote CM remodeling and thus promote UVS outflow
Bradykinin B_2-receptor agonists	Bradykinin; FR-190997; BKA278	B_2-receptor activation causes PI hydrolysis production of IPs and DAG; cause PG release and release of MMPs that digest ECM and this promote UVS outflow in cynomolgus monkey; conventional outflow also stimulated in isolated bovine/porcine anterior eye segments
Dual activity PGs, and conjugated compounds	FP/EP_3 receptor agonist (ONO-954)	Promotes UVSC outflow
	AL-6598 (DP/EP_2 receptor agonist)	Inhibits inflow and stimulates outflow (TM and UVSC)
	Latanoprostene bunod (latanoprost-NO donor conjugate)	Promotes UVSC and TM outflow
Inflow inhibitors (reduce AQH production)		
β-adrenergic antagonists	Timolol; Betaxolol; Levobetaxolol; Levobunolol; Metipranolol	Block β-adrenergic receptors in the ciliary process, decrease cAMP generation and thus decrease AQH formation
β-adrenergic receptor silencer	SYL-040012; siRNA (Bamosiran)	Downregulates endogenous β-adrenergic receptors and their signaling
α2-adrenergic agonists	Brimonidine; Apraclonidine; Clonidine	Intracellular cAMP reduced in CP that decreases AQH generation; may also prevent NE release Brimonidine also promotes TM outflow
Carbonic anhydrase inhibitors (CAIs)	Dorzolamide; Brinzolamide	Inhibit ciliary process CA-II and CA-IV and thus reduce bicarbonate production that in turn reduces AQH generation
Chloride channels inhibitors	5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB)	Ion flux of CP NPE cells causes reduction of AQH formation
Na^+-K^+-ATPase inhibitors	Ouabain; Digoxin analogs	Ciliary process Na^+-K^+-ATPase inhibited leading to inhibition of AQH production
Dopamine receptor agonists	PD-128907; CHF-1035; CHF-1024; SDZ GLC-756; (S)-(-)-3-hydroxyphenyl)-N-n-propylpiperidine (3-PPP)	Inhibit release of NE and prevent AQH production; may also release natriuretic peptides
Na^+-K^+-ATPase inhibitors	Ouabain; Digoxin analogs	Ciliary process Na^+-K^+-ATPase inhibited leading to inhibition of AQH production
Aquaporin inhibitors	Various aromatic sulfonamides and dihydrobenzofurans	Inhibit release of NE and prevent AQH production
Additional IOP-lowering agents		
Mas receptor stimulator	DIZE via ACE-2 activation	Prevent ECM (including TGFβ) accumulation (outflow stimulation?)
Angiotensin-II receptor antagonists	CS-088	Various mechanisms of action; not robust IOP-lowering
Ca^{2+}-channel inhibitors	Lomerazine; Nivaldipine; Nifedipine; Nimodipine; Verapamil; Brovincamine; Iganidipine	Enhance retinal blood flow; some may lower IOP; work well in normal tension glaucoma patients
Alpha-adrenergic receptor antagonists	Oxymetazoline; 5-methylurapidil; Ketanserin	Work mostly via outflow mechanism but this needs to be defined
Other prostaglandin receptor agonists	AL-6598 (DP/EP_2 receptor agonist); AGN192093 (TP receptor agonist); BW245C (DP receptor agonist); Sulprostone (EP_3 receptor agonist)	These work through multiple mechanisms of action involving cAMP production, Ca^{2+} mobilization leading to relaxation/ contraction of ciliary muscles/TM

(continued)

Table 6 (continued)

Compound classes	Pharmacological agent	Reported or potential mode(s) of action
PG-conjugates	Latanoprostene Bunod (NO donor coupled to latanoprost)	Combination of NO-cGMP production and FP-receptor activation
Combination products	Brinzolamide-brimonidine; Brinzolamide-brimonidine; Acetozolamide-Timolol-Brimonidine; Travoprost-brimonidine; Bimatoprost-brimonidine; Tafluprost-Timolol	Complementary mechanisms of action encompassing inflow-outflow inhibition, and inflow-uveoscleral outflow inhibition

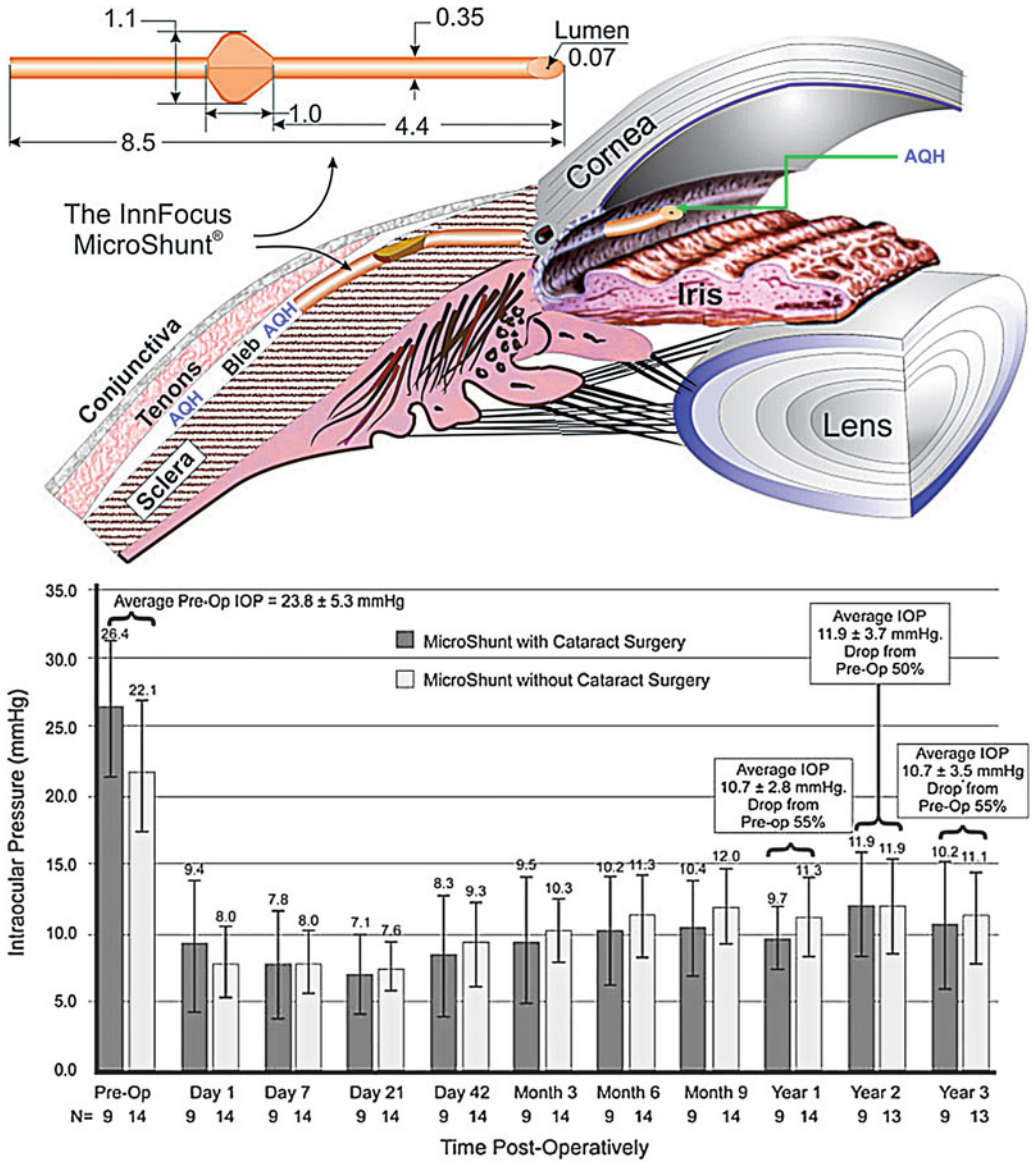

Fig. 11 Top panel shows the dimensions of and the placement of the InnFocus Microshunt in the anterior chamber of the eye to drain the AQH in order to lower IOP. The efficacy of this MIGS device is clearly demonstrated by the data in the lower panel in longitudinal studies performed in human OHT/POAG patients. (Modified from Batlle et al. (2016))

Thus, for instance, if a new small molecule needs to be discovered for an EP_2 or FP prostaglandin receptor, then ligand binding assays for all the known PG receptors and their subtypes, preferably human cloned or using fresh target tissues/cells, and using selective radiolabeled ligand, are established and validated. Then test compounds are used to compete for the receptor-radioligand complex in cell/tissue homogenates suspended in suitable buffers at equilibrium using rapid filtration techniques and thus evaluated for their relative affinities, relative selectivities, and then ranked ordered accordingly. Examples of such receptor-ligand binding assays and the data obtained are shown in Tables 1 and 2 (Sharif et al. 2003; Kirihara et al. 2018).

Since agonist compounds are being sought as novel ocular hypotensives, the compounds selected from the above assays are then tested for their ability to stimulate the desired receptor in freshly isolated target cells (preferably human CM or TM cells known to be involved in AQH dynamics and IOP regulation) (e.g., 5HT2-receptor-mediated intracellular Ca^{2+}-mobilization, Fig. 4, left panel), or in host cells transfected with the human cloned receptors of interest, and appropriate second messengers detected and quantitated. The data are graphed to determine the relative potencies of the test compounds relative to a positive control drug. Thus, for instance, cAMP (Crider and Sharif 2001; Kirihara et al. 2018) or cGMP (Katoli et al. 2010) or inositol phosphates (Sharif et al. 1994, 1996) or intracellular Ca^{2+} (Sharif et al. 2007) are used as receptor-induced signal readouts (Fig. 4, left panel; Fig. 8, right panel) and the receptor-specific blockade of these responses (e.g., Fig. 4, right panel). Other assays to examine functional activity in vitro of potential IOP-reducing agents involve use of cell- (Ramachandran et al. 2011) and/or tissue-based relaxation/contraction assays (Ohia et al. 2018) using cells/tissues known to be mediators of AQH dynamics and thus ocular hypotensive activity in vivo (e.g., CM and TM [Sharif et al. 2007, 2014] or SC [Dismuke et al. 2010] cells or CM/TM tissue strips [Wiederholt et al. 2000; Ohia et al. 2018]), coupled with cellular impedance changes that reflect cell relaxation/contraction (Wang et al. 2013) or cell-volume changes (Dismuke et al. 2009, 2010). Examples of some of these data for different classes of ocular hypotensive drugs are shown in Figs. 4, 8, and 9; Tables 3 and 4.

Testing of Compounds for AQH/Fluid Extrusion in Ex-Vivo Systems

The results from cell/tissue-based experiments are useful to define the agonist/antagonist nature of test compounds but they only provide a glimpse into the pharmacological activity of the latter. Therefore, researchers have utilized partially intact ANC segments of the eye from a number of species (human, bovine, and porcine) (e.g., Sharif et al. 2014) in culture to assess the ability of drugs to stimulate outflow of the fluid from such models. The perfusion buffer can also contain suitable inhibitors of receptors or enzymes in order to dissect potential molecular mechanisms of action of the perfused test agents.

Animal Models Used to Discover Novel Ocular Hypotensive Drugs

Testing of potential IOP-lowering agents is obviously best performed in animal models where the initial baseline IOP is naturally high so that the change in IOP induced by the test compound is more easily identified. To this end, there are only a few animal species that express a naturally high IOP and that are readily accessible. These include DBA/2J strain of mice, Dutch-Belt rabbits, and Beagle dogs (see McNally and O'Brien 2014; Sharif 2018a, b, for reviews). In the absence of the latter, researchers have had to artificially elevate IOPs in rodents and monkeys by a number of different techniques. For rodents, the TM can be partially or completely destroyed using injection of hypertonic saline via the episcleral vein, by injecting latex or magnetic microbeads into the ANC of the eye, or by lasering the TM or episcleral veins (reviewed in McNally and O'Brien 2014; Sharif 2018a, b). In the monkeys, the preferred reproducible method of raising IOP is by lasering the TM where the IOP can be elevated and maintained around 30–40 mmHg

for many months and in some cases for years. To obtain optimal results from testing new compounds for ocular hypotensive activity, it has been reported that the animals need to be unrestrained, well trained, comfortable, and conscious to have their IOPs measured on a frequent basis accompanied by suitable rewards. Some investigators anesthetize animals before they measure IOPs, but the data from such methods are somewhat suspect since unconscious animals have a different set of baseline IOPs and also respond differentially from fully awake animals. The age of the test animals also needs careful attention and requires standardization to older animals to ensure that OHT/POAG condition in humans is being well represented.

Ocular normotensive and ocular hypertensive (naturally or induced) animals are used to directly assess IOP-lowering potential of new test compounds. Due to cost and genetic considerations, rodent models of OHT have been preferred for primary screening using t.o. dosing of known and new compounds dissolved or suspended in suitable ocularly compatible and previously approved vehicle solvents/buffers. First, test compounds are freshly prepared in the vehicle of choice and evaluated using either single installation (20–30 μl drop) or after multiple administrations for ocular safety using rabbits and/or guinea pigs. Albino New Zealand White rabbits are preferred since guinea pigs are overtly too sensitive to most t.o. drugs. When rodents are used for IOP-lowering efficacy studies, they can also be used for ocular safety assessments as well. Compounds are considered safe when they do not induce excessive irritation/redness, excessive blinking, and discharge, and when no vocalization is recorded.

For the actual ocular hypotensive efficacy experiments, animals are grouped according to the treatment they will receive. The eye is first mildly numbed with proparacaine and IOP measured 3–4 times in a quiet and dimly lit environment using various pneumotonometers (e.g., Tono-Pen, Tono-Lab, Goldman appellation pneumotonometer) in order to establish the baseline IOP. Future IOPs are determined at the same time every day in the same room. Usually, a 5–10 μl drop of the vehicle or positive control drug (for rodents; or 20–30 μl drop for larger species) or the new test agent is instilled in one eye of the mice/rats and the IOPs recorded over time in each eye of each group of animals. A number of IOP readings are taken at each time point to ensure accuracy and reproducibility of the data. Those compounds that meet acceptable criteria of ocular safety (see above) and sufficient IOP reduction (e.g., 20% from baseline) are then tested in secondary models (e.g., ocular normotensive New Zealand White rabbits or naturally ocular hypertensive Dutch-Belted rabbits), and then ultimately in tertiary screening models (Beagle dogs; nonhuman primates such as Cynomolgus or Rhesus monkeys, either ocular normotensive and/or ocular hypertensive) (Fig. 12; Fig. 13, top panel) (Sharif et al. 2014; Kirihara et al. 2018; Fuwa et al. 2018). Typically, compounds that reduce IOP by >20% from baseline are considered worthy of further pursuit. Now, more detailed dose-response and mechanistic studies, involving testing of selective enzyme inhibitors and/or receptor antagonists can be undertaken (e.g., Sharif et al. 2014; Fig. 10). Additionally, AQH dynamic studies (Sharif et al. 2014) can be conducted in order to define whether the test compound(s) promote outflow of AQH from TM and/or UVSC pathways or inhibit the production of AQH (e.g., Fig. 13, bottom panel; Fuwa et al. 2018). In order for these results to be biologically and perhaps clinically relevant, the latter AQH modulation studies are usually performed in anesthetized ocular hypertensive nonhuman primates before being tested in human subjects in clinical trials.

Neuroprotective Therapeutics for Treating Glaucomatous Optic Neuropathy

It is unfortunate that despite use of many different classes of ocular hypotensive agents for multiple decades to lower and control IOP, patients with OHT/POAG (and even those with normal IOPs),

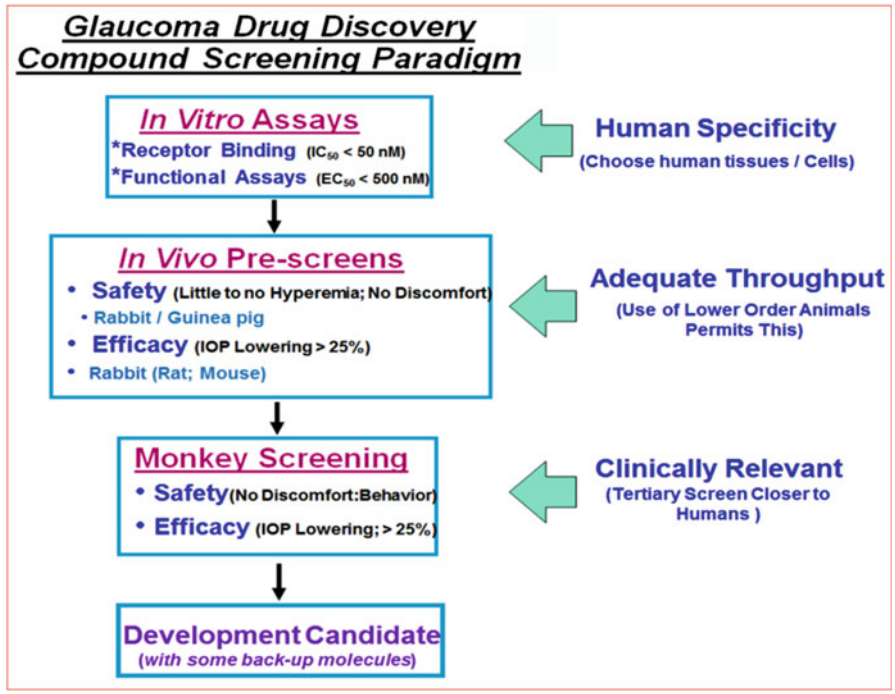

Fig. 12 A typical testing funnel for screening compounds directed at ocular hypertension and POAG is shown. Once a target protein is identified and incorporated into the in vitro screening paradigm, radioligand binding assays and/or functional cell-based assays are conducted in order to rank-order the test compounds in terms of their affinity and functional potency/intrinsic activity (agonist or antagonist). This information is used to select compounds to be tested for ocular/systemic toxicity/irritability and efficacy in rodents and/or rabbits. Those compounds meeting selection criteria are then tested in ocular normotensive and OHT monkey eyes for safety and efficacy. All this information is ultimately used to improve the structure–activity relationship of compounds by medicinal chemists. This iterative process is expected to yield clinical candidate drug molecules

continue to lose vision, and some may ultimately succumb to blindness. Thus, it is now accepted that direct protection of the retinal neurons (especially RGCs), their axons and terminals within the brain need to be strongly considered in addition to IOP reduction. Due to the multiplicity of damaging factors and insults impacting the RGC-brain axis that results in glaucomatous optic neuropathy (GON) (Fig. 14), including constriction of optic nerve axons at ONH (Hollander et al. 1995), complement activation (Tezel et al. 2010), locally released inflammatory/toxic substances (excess neurotoxic amino acids, cytokines, endothelins, NO, etc.) and tissue/cell remodeling enzymes (MMPs; calpains; caspases), blockage of neurotrophin (Quigley et al. 2000) and mitochondrial transport up and down the RGC axons (hence loss of energy [Thomas et al. 2000], etc., a multipronged neuroprotective strategy is necessary to preserve RGCs and their axons. As far as optic neuropathy (ON; encompassing Leber's hereditary ON, nonarteritic ischemic ON) is concerned, there has already been a number of a drug launched/approved such as the powerful antioxidant and Ca^{2+}-channel blocker idebenone, with others in late-stage clinical trials (e.g., lenadogene/nolparvovec (mitochondrially encoded NADH dehydrogenease-4 expression enhancer), QPI-1007 (caspase-2 expression inhibitor), and elamipretide (apoptosis inhibitor). Other novel strategies for protecting RGCs and optic nerve components are discussed in more detail in recent publications (Smedowski et al. 2016; Hines-Beard et al. 2016; Venugopalan et al. 2016; Williams et al. 2017; Mead et al. 2018; Sharif 2018b).

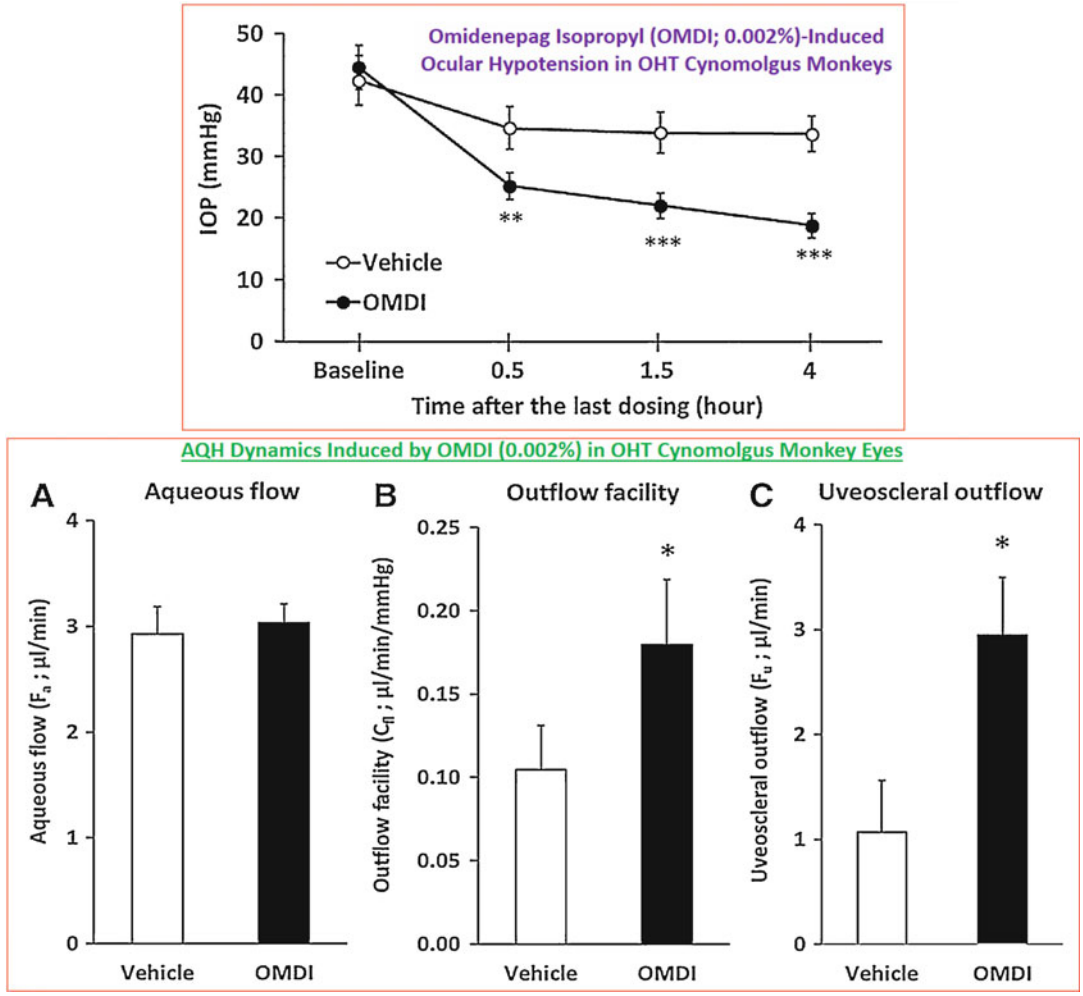

Fig. 13 A new-generation non-PG EP_2-receptor agonist omidenepag isopropyl (OMDI) and its IOP-lowering effects in monkeys (top panel), and its stimulation of both conventional outflow and uveoscleral outflow in OHT eyes of monkeys (lower panel) is shown.(Modified from Fuwa et al. (2018))

Cell-Based Assays and Animal Models for Discovering Neuroprotective Drugs

A number of cell/tissue-based assay systems have been developed to assess the potential protective activity of selected compounds of interest. These have included isolated primary rat RGCs, co-cultures of RGCs and other retinal cells, ONH or retinal astrocytes, surrogate CNS neurons, retinal explants, whole retinas, and whole retina-optic nerve explants (see He et al. 2018; Sharif 2018b for recent reviews). Despite the fact that GON is caused by a plethora of chemical, biological, mechanical, and local environmental factors simultaneously or in a close time frame at the level of the RGCs, their axons and terminals (Fig. 14), the majority of the reported test systems investigating neuroprotection in vitro have only introduced a single insult to the retinal/axonal cells/tissues. The types of challenges used have included hypoxia (to mimic retinal ischemia), elevated hydrostatic pressure (to mimic high IOP), glutamate- or NMDA- or TNFα- or IL-1-β-induced cell death (to recapitulate neuronal toxicity), glucose or neurotrophic factor withdrawal (to mimic hypoglycemia as a result of ischemia

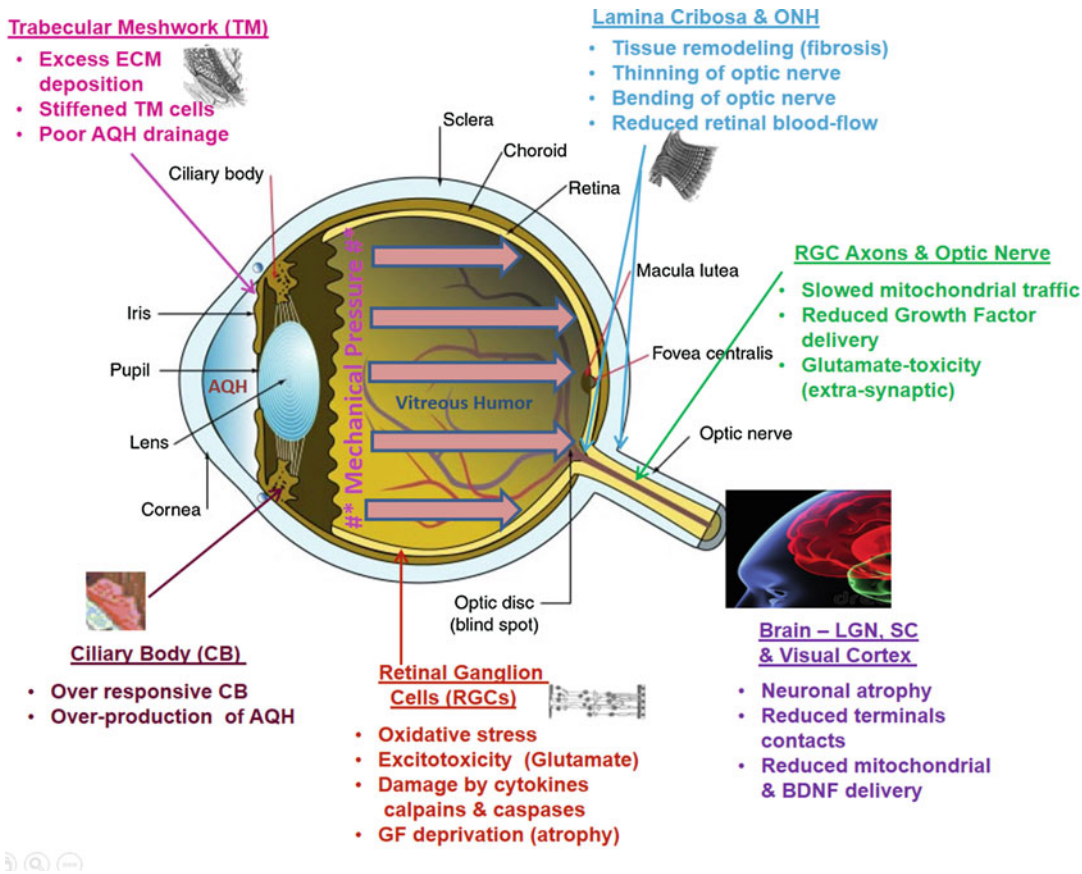

Fig. 14 Illustrationof the numerous sites within the eye-brain axis where various pathological events (e.g., mechanical, chemical, and environmental) occur to damage the anterior chamber cells (e.g., TM cells), retinal cells (e.g., RGCs), RGC-axons, and the optic nerve connections to the brain. The net result of such damage is progressive visual impairment that can ultimately lead to blindness as in various forms of glaucoma. (Modified from Sharif (2018b))

(Osborne et al. 2014), and axonal/optic nerve constriction/blockage, respectively). Techniques and types of signal readouts that have provided means to gauge neuroprotective activity of test compounds in these in vitro systems included the following: cell viability (cellular toxicity) assays using measurement of extracellular lactate dehydrogenase and/or cytochrome c oxidase (released when cell membranes become compromised due to cell ill health), quantifying membrane potential using JC-1 dye, detecting mitochondrial destruction by fission, quantitating mitochondrial viability by cyan fluorescent protein labeling, monitoring cell apoptosis using the TdT-mediated dUTP nick-end labeling (TUNEL assay), Brn3A-staining, propidium iodide labeling to assess cell death, measuring caspase-1/3 activity, multiple electrode array recording of cellular activity, measuring cellular levels of ATP using nuclear magnetic resonance, calcein AM staining, gelatin zymography for MMPs and other proteases, inhibition of [^{3}H]-D-aspartate release from retinas as an index of neurotoxicity prevention, neurite elongation using high content screening, etc. (see references in: He et al. 2018; Sharif 2018b). If compounds show cytoprotective activity in a number of these assay systems, then chances are high that they will exhibit some level of neuroprotective efficacy in vivo.

Animal models of GON are somewhat limited and generally tend to be labor-intensive, yielding data of variable value. The major reason for this is again the singularity approach of chemical, mechanical, and other insults/challenges used to evaluate the in vivo neuroprotective efficacy of compounds. This necessitates the utility of multiple animal models to robustly assess the neurotherapeutic activity of any compound that may ultimately be used in a clinical setting.

Despite the above issues, much progress has been made in finding novel compounds and new pathways that represent useful intervention points with therapeutic end points for testing in animal models of retinal/optic nerve damage as encountered in GON/POAG. Consequently, numerous classes of compounds have been qualified as neuroprotective based on their ability to reduce the loss of RGCs and/or their axons. The following represent some of the most commonly used animal models to study GON and to screen compounds for their neuroprotective efficacy: rodent models of acute/chronically elevated IOP that results in retinal ischemia; rodent models of partial transection or crush of the optic nerve at the level of the ONH; ivt injections of neurotoxins such as NMDA, endothelin, amyloid-beta peptides, or staurosporin, or phorbol ester to capitulate endogenous inflammatory reactions common in GON; uveitic glaucoma model and inflammatory demyelination models (see references in Sharif 2018b). In the majority of the cases, the number of RGCs and their axons are quantified postmortem to assess the degree of damage/protection in control *vs.* the treated animals. This is achieved by retrograde-labeling of RGCs using fluorogold- or Brn3A-labeling after injection of the latter markers into the superior colliculus of the animals, and by axonal counts in transverse sections of the optic nerve, respectively.

In some cases, intravenously injected fluorescently labeled annexin-5 has been used to monitor and quantify retinal cells undergoing apoptosis in living animals subjected to various experimental challenges pertinent to GON as described above. Interestingly, this technique has now been used in patients with POAG/OHT and certain neurodegenerative diseases and useful baseline data gathered (Cordeiro et al. 2017). Perhaps such diagnostic/prognostic biomarkers can be utilized to evaluate neuroprotective drugs in nonhuman primates and human subjects in the near future. Likewise, the recent use of flavoprotein fluorescence to monitor mitochondrial health of retinal cells in vivo in POAG and control patients appears a promising tool for assessing cytoprotective actions of compounds believed to possess neuroprotective efficacy (see references in Sharif 2018b). Furthermore, the use of novel technologies such as high-resolution adaptive optics and visible-light-OCT coupled with standard functional readouts like visual-evoked potential measurements will enhance our understanding of the GON and lead to better therapies in the future.

Age-Related Macular Degeneration (AMD)

Another ocular disease that has a relatively rich history of drug discovery and pharmacodynamics associated with it is age-related macular degeneration (AMD) (Lambert et al. 2016). Unlike POAG where peripheral vision is lost, in AMD the loss of macular photoreceptors impacts central vision. AMD is believed to be responsible for nearly half of all severe vision loss in the US adults over the age of 40. AMD has been divided into two forms: nonexudative or "dry" AMD (dAMD; 90% of total) and exudative or "wet" AMD (wAMD; 10% of the total). While dAMD is characterized by the loss of photoreceptor cells in the macula following the death of supporting RPE cells, wAMD's hallmarks are retinal edema and rampant neovascularization of choroidal capillaries (choroidal neovascularization or CNV) (Al-Zamil and Yassin 2017; Hernandez-Zimbron et al. 2018). Such aberrant angiogenesis causes retinal fibrosis, scar formation (and perhaps retinal detachment), culminating in loss of central visual acuity.

While dAMD develops over months and years, wAMD is highly progressive and rapidly develops to rob vision of the patient unless treatment is sought. Advancing age is the strongest demographic risk factor associated with AMD

although Caucasian heritage predisposes patients to this ocular disease. However, chronic excessive oxidative stress from cigarette smoke and other sources, autoimmune disease involving complement activation, and chronic local inflammation are also causative factors in the development of dAMD (Rickman et al. 2013). An increased blood plasma concentration of the proinflammatory proteins C-reactive protein, IL-6, cholesterol/triglycerides, and a family history of AMD also been positively correlated to some degree with AMD development and progression. Recent angiography-coupled-OCT evidence suggests that poor choriocapillary blood flow leads to poor clearance of cellular and other debris and may be the source of deposited drusen and related materials that ultimately lead to dAMD/GA (Qin et al. 2018).

RPE cell dysfunction likely begins with intralysosomal accumulation of a fluorescent material called lipofuscin, a complex mixture rich in polyunsaturated lipids and probably is derived from phagocytosed photoreceptor outer segments that cannot be broken down. Over time, this material renders lysosomal enzymes inactive and raises the pH level causing lysosomal membrane dysfunction and RPE cell death. A likely functionally important component of lipofuscin is the amphiphilic pyridinium ion N-retinyldene-N-retinylethanolamine (A2E) which is generated from the condensation of phosphatidylethanolamine with 11-*trans* retinaldehyde, followed by phospholipase D-catalyzed dephosphorylation. It has been postulated that A2E might be the major toxic chemical in lipofuscin producing reactive oxygen species and oxiranes in the presence of light and O_2 (Fig. 15). Additionally, A2E likely acts as a detergent causing leakage of toxic reagents into the RPE cell, thereby killing the latter cells.

Once RPE cells are injured, their ability to phagocytose photoreceptor cell outer segments is hindered and this causes the incomplete recycling of the components of the latter. This is responsible for accumulation of cellular debris, and the production and release of additional inflammatory

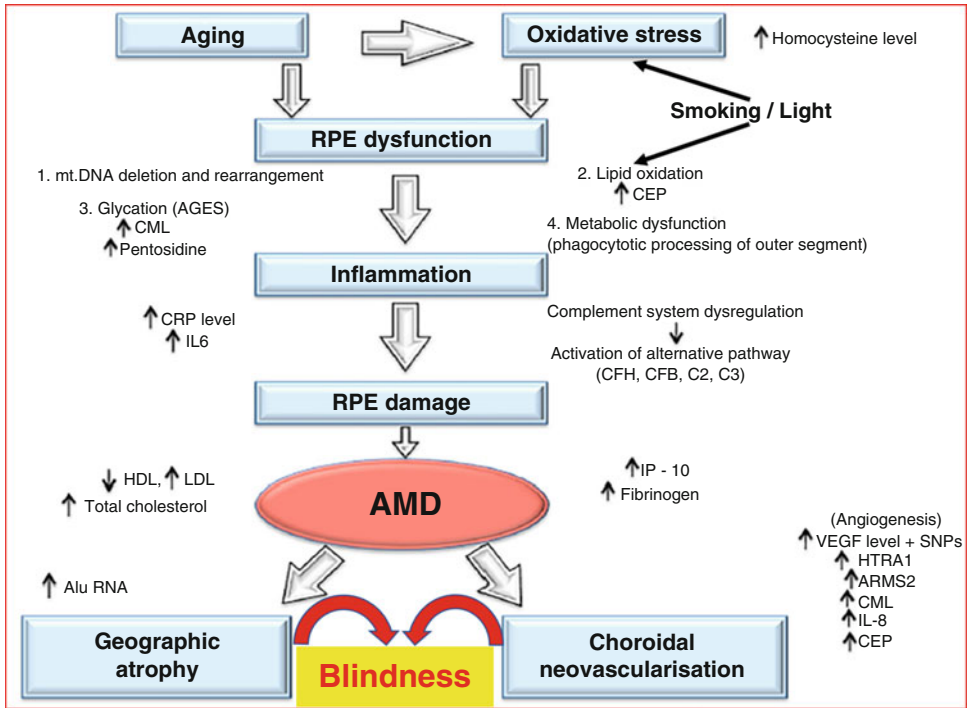

Fig. 15 Pathogenesis of dAMD/GA. (Modified from Ambati et al. (2003)).

agents from the dying RPEs such as oxidized lipids, proinflammatory cytokines (e.g., IL-1β and IL-17A [Zhang et al. 2016]), and acute-phase inflammatory proteins, that accumulate between the RPE cell layer and the Bruch's membrane that separates the RPEs from the choroidal capillaries. All this deposited toxic material (drusen) then kills more RPE cells and the homeostasis is disrupted further (Rickman et al. 2013) (Fig. 15). The drusen and other cellular debris also act as a barrier and less O_2 and nutrients are made available to the rest of the retina, and this results in the development of a hypoxic environment at the back of the eye. In order to overcome this situation, hypoxia-inducible transcription factors like HIF-1α are upregulated and vascular endothelial growth actor (VEGF) and other angiogenic factors are locally released that cause the generation of new blood vessels from the existing choroidal capillaries. This aberrant neovascularization temporarily helps remove some of the metabolic and cellular waste and provides nutrients and O_2 to the remaining RPE and photoreceptor cells. However, the new blood vessels breach the Bruch's membrane and start to grow into the retina and eventually into the vitreous and start to interfere with light transmission to the retina and the communication among the inner retinal cells. Additionally, since the new blood vessels are leaky, blood starts to accumulate in the vitreous and local hemorrhages develop at the rear of the globe that begins to detach the retina. The patient loses more and more visual acuity and requires urgent care to curb the loss of all vision in that eye. This is how dAMD can cause wAMD. However, there are patients who do not develop wAMD, and their dAMD keeps progressing till their retina has widespread drusen deposited that is characterized as geographic atrophy (GA) (advanced dAMD) that robs the patient's central and eventually peripheral vision and thus causes irreversible blindness (Fig. 16).

As to the possible treatment options for combating dAMD/GA, there is currently no accepted pharmacological treatment in use in the clinical setting. While dietary supplementation with a combination of high-dose vitamins E and C, beta-carotene, and zinc afforded a 25% risk reduction for progression of high risk dAMD patients to wAMD form, the interpretation of the results of this study has been controversial. It is thought, however, that based on the involvement of oxidative stress and inflammation in the etiology of dAMD that drugs that can reduce the severity of the latter conditions may have beneficial effects in dAMD patients. Therefore, additional research and clinical trials are in progress using a number of agents and treatment options for dAMD/GA which are at various stages of development (Ishikawa et al. 2015; Waugh et al. 2018) (e.g., Table 7).

Several wAMD treatments centered around reducing levels of VEGF have been successfully developed and introduced into the clinical management of the disease including use of bevacizumab and ranibizumab (both truncated antibodies bind VEGF and remove it), and aflibercept (VEGF-receptor as a VEGF trap) (Ishikawa et al. 2015; Al-Zamil and Yassin 2017; Hernandez-Zimbron et al. 2018). Despite the undeniable success of the anti-VEGF treatment modalities, there is increasing concern about the reported development of tolerance/resistance to these medications after the 2nd-year of treatment (Maguire et al. 2016; Yang et al. 2016). Thus, it is imperative that additional therapeutic agents be discovered and developed to mitigate such issues. Consequently, several therapeutic strategies have been proposed to reduce and/or prevent the development and progression of wAMD that appears to be driven by the angiogenic factors such as HIF-1α and VEGF (Table 8), along with other emerging growth factor culprits such as angiopoietins that trigger angiogenesis via the Tie-1/2 receptors. It may be necessary to also begin combinatorial therapy for wAMD (e.g., endogolin + anti-VEGF combination [Shen et al. 2018]), as was the case for OHT/POAG when it was realized that conventional single-agent therapies were unsuitable for recalcitrant patients and also those who were not maximally controlled by monotherapeutic agents (see above). Likewise, the novel cell-replacement therapies involving growth of polarized monolayer of human embryonic stem cell-derived RPE (hESC-RPE) cells on an ultrathin parlene substrate and placing them into human subjects' retinas with severe vision loss due to dAMD/GA as a cell-patch (Kashani et al. 2018), or placing similarly engineered hESC-RPE patches

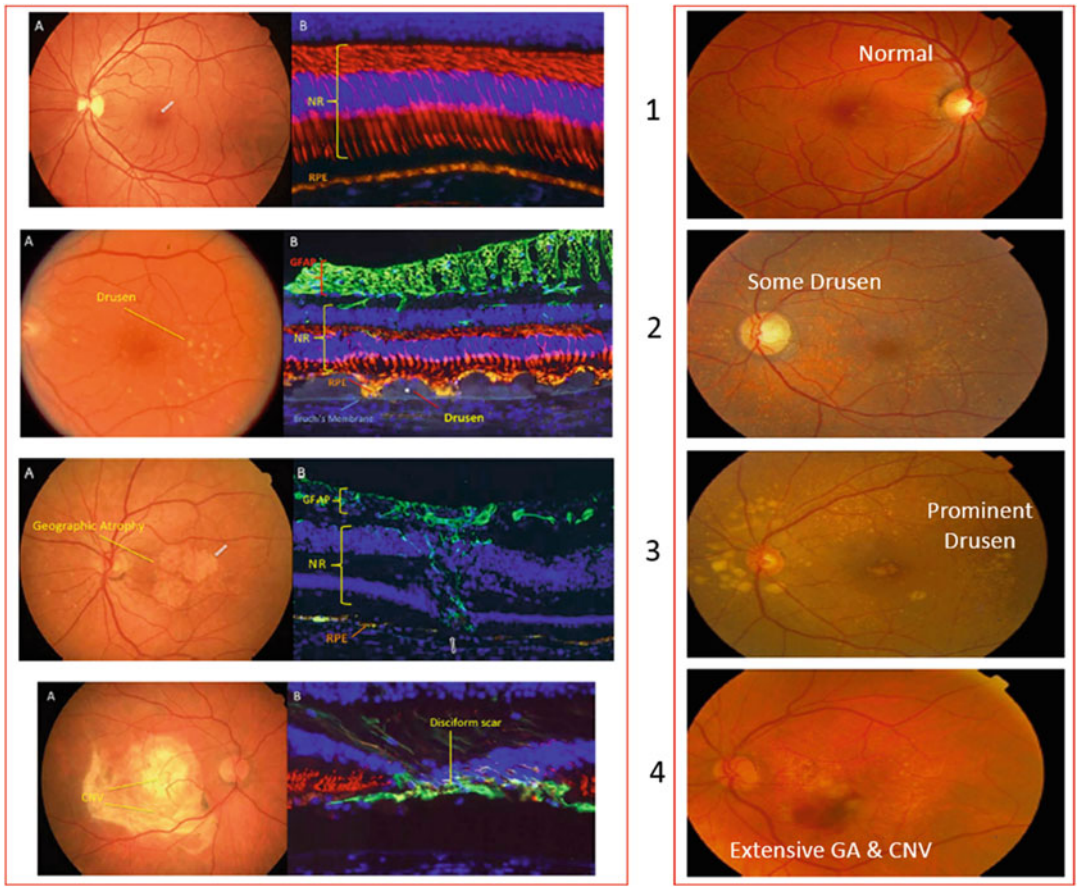

Fig. 16 Pictorial depiction, via fundus photos, of human dAMD progressing to widespread GA due to increasing drusen deposition in the retina.(Modified from Ambati et al. (2003))

into subretinal space of patients with wAMD (da Cruz et al. 2018) to improve visual acuity holds tremendous promise.

Assay Systems Deployed for Anti-dAMD/Anti-GA Drug Discovery

As with OHT/POAG and GON, cell-based assays utilize anatomically relevant retinal cells known to be involved in the etiology of or as comprised cell type(s) in the disease process of dAMD/GA for screening purposes. Since the cascade of events linked to the disease involve inflammation, deposition of ECM in Bruch's membrane, and drusen accumulation in the RPE cells, the latter cells have been used as target cells to study ways to prevent the former and latter phenomena. Anderson et al. (2013) used ARP-19 cells and tested various components of drusen (e.g., carboxyethypyrrole (CEP)-modified proteins, amyloid-β (1-42), Nε-(carboxymethyl) lysine (CML)-modified proteins, and aggregated vitronectin) and the key component of lipofucin (A2E) and monitored production of major inflammatory cytokines, chemokines, and VEGF-A as biomarkers. They concluded that A2E was the most active proinflammatory substance studied, and that it promoted the release the aforementioned biomarkers from RPE-19 cells by activating the inflammasome (NLRP3/caspase-1) pathway activation. Such a screening tools can thus be used to find suitable blockers of the NLRP3/caspase-1 system, and these may prove useful in ameliorating the dAMD/GA.

Table 7 Therapeutic agents, devices and other treatments under development for dAMD/GA

Mode of action	Therapeutic agent/ product	Sponsoring institution	Disease indication	Development phase	Route of delivery
Small molecules					
Visual cycle modulator	Emixustat HCl	Acucela	GA/dry AMD	Phase-2b/3	Oral daily tablet
Modulating choroidal blood flow	MC-1101	Macuclear	Dry AMD	Phase-2/3	Topical, twice daily
Tetracycline antibiotic 40 mg doxycycline	Oracea	University of Virginia	GA/dry AMD	Phase-2/3	Daily oral capsules
Antioxidant; slows DNA damage, reduces ROS levels	Metformin	University of California San Francisco	Nondiabetic GA/dry AMD	Phase-2	Daily oral tablets
Aptamers/peptides/antibodies					
mAb fragment for complement factor D	Lampalizumab	Genentech	GA/ advanced AMD	Phase-3	Intravitreal injection
Inhibition of complement C3	APL-2	Apellis Pharma	GA/AMD	Phase-2	Intravitreal injection
Antibody for C5 complement	LFG 316	Novartis	GA/AMD	Phase-2	Intravitreal injection
Aptamer; inhibits complement Factor C5	Zimura	Ophthotech	GA/dry AMD	Phase-2/3	Monthly intravitreal injection
Hu-mAb for Aβ peptide	GSK933776	GlaxoSmith-Kline	Retinal amyloidosis/ GA/dry AMD	Phase-2a	Intravenous infusion
Cellular therapeutics					
Stem cell transplantation	Hu-CNS SC	StemCells Inc.	GA/ Advanced dry AMD	Phase-1/2	Subretinal transplantation
hESC-derived RPE cells	MA09-hRPE	Ocata Therapeutics – Astellas Pharma	Advanced dry AMD	Phase-1/2	Subretinal transplantation
Human umbilical tissue-derived cells	CNTO 2476	Janssen Research and Development	GA/AMD	Phase-1/2a	Subretinal administration
hESC-derived RPE cells seeded on polymeric substrate	CPCB-RPE1	Regenerative Patch Technologies (RPT)	Advanced dry AMD	Phase-1/2	Subretinal implantation
hESC-derived RPE cells	OpRegen	Cell Cure Neurosciences	Advanced AMD	Phase-1/2	Subretinal transplantation
Patient-derived iPSC transplantation	iPSC-derived RPE cells	Moorefields Eye Hospital NHS Foundation Trust	Wet and dry AMD	Phase-1/2	Transplantation
Autologous bone marrow–derived stem cells	BMSC- SCOTS study	Retina Associates of South Florida and MD Stem Cells	AMD	Early stage interventional study	Sub-Tenon injection

(continued)

Table 7 (continued)

Mode of action	Therapeutic agent/ product	Sponsoring institution	Disease indication	Development phase	Route of delivery
Devices and other treatments					
Photobiomodula-tion or low light level therapy	LumiThera LT 300 light delivery system	LumiThera	Vision loss associated with AMD	Early stage interventional study	Light exposure
Electrical stimulation	Argus-II System	Second Sight Medical Products	Late-stage AMD	Phase-1	Implantable visual retinal prosthesis
Transpalpebral microcurrent electrical stimulation	Nova Oculus	The Eye Machine Canada	Vision loss associated with dry AMD	Early stage interventional study	Externally applied microcurrent electrical stimulation
Electrophysiologic methods (double plasma filtration or cascade filtration)	Rheohemapheresis	University Hospital Hradec Kralove	High-risk dry AMD	Phase-4	Cascade filtration

Abbreviations: *AMD* age-related macular degeneration, *GA* geographic atrophy, *hESC* human embryonic stem cell, *iPSC* induced pluripotent stem cell, *mAb* monoclonal antibody, *MOA* mode of action, *ROS* reactive oxygen species, *RPE* retinal pigment epithelium

Primary human RPE (ph-RPE) cells represent a better cellular system than the cell line ARPE-19. Zhang et al. (2016) demonstrated that ph-RPE cells expressed all three IL-17 receptors and that addition of IL-7A to these cells upregulated the production of IL-1β secretion via the NLRP3 inflammasome activation mechanism. Importantly, these authors found that inhibiting caspase-1 activity and silencing NLRP3 significantly reduced IL-1 β release from RPE cells. Thus, this assay system can be used to find new potent and efficacious blockers of caspase-1 and NLRP3 to help prevent dAMD/GA. Additional work in this area using human patient-derived iPSC-RPE cells is also very encouraging (Galloway et al. 2017, 2018).

Animal Models to Find Anti-dAMD/ Anti-GA Drugs

While not truly reflecting the human dAMD/GA disease, a number of animal models have been developed to study the condition and use for potential drug discovery efforts. The light damage models utilize rodents and expose them to very bright light (white or blue) for a number of days/ weeks and the retinal damaged assessed by electroretinograms and histology (Chader 2002). The genetic rodent models include the Royal College of Surgeons (RCS) rats (a recessive genetic defect that prevents phagocytosis of rod-outer-segments by RPE cells), P23H rhodopsin defect rat, and an ABCR-1- rat (which has the transporter of 11-trans-retinaldehyde (ABCR-protein) knocked-out). The most appealing of the animal models created thus far is the chemokine receptor-2 knockout mouse which exhibits many of the hallmark features of human dAMD/GA (drusen accumulation under RPE, photoreceptor loss followed by CNV; Ambati et al. 2003). How these animal models are exploited to discovery novel therapeutics for treating dAMD/GA remains to be seen.

Cell-Based Assays for Finding New Anti-wAMD/Anti-CNV Drugs

As described above, wAMD involves poor retinal circulation leading to retinal hypoxia, release of angiogenic factors (e.g., HIF-1/2α; VEGF; ANG-2), and abnormal growth and development of leaky new blood vessels from the choroidal

Table 8 Recently approved and emerging treatment options being pursued for GA and neovascular AMD

Drug agent	Drug class	Developer/ sponsor	Targeted pathology	Mechanism of action	Route of administration
Ranibizumab (Lucentis)	Anti-VEGF antibody	Novartis	wAMD/ Neovascular AMD	Removes VEGF	Intravitreal
Aflibercept (Eylea)	VEGF-Receptor (VEGF-trap)	Regeneron	wAMD/ Neovascular AMD	Removes VEGF	Intravitreal
Conbercept	VEGF-Receptor (KH-902; Biosimilar)	Chengdu Kanghong	wAMD/ Neovascular AMD	Removes VEGF	Intravitreal
Brolucizumab	Anti-VEGF antibody (RTH-258)	Novartis	wAMD/ Neovascular AMD	Removes VEGF	Intravitreal
Lampalizumab	Anti-factor D Fab	Genentech	GA	Anti-factor D Fab	Intravitreal
MA09-hRPE	Cell therapy	Astellis Pharma	GA	Human umbilical tissue-derived cells	Subretinal injection
Brimonidine tartrate implant	α2-adrenoceptor agonist	Allergan	GA	Alpha-2-agonist	Intravitreal implant
Eculizumab	Anti-C5 mAb	Alexion	GA	mAb against complement factor C5	Intravitreal
Fovista (E10030)	Anti-PDGF aptamer	Ophthotech	Neovascular AMD	Anti-PDGF PEGylated aptamer	Intravitreal
Abicipar pegol	Anti-VEGF Aptamer	Allergan/ Molecular Partners	Neovascular AMD	Anti-VEGF	Intravitreal injection
RBM-007	Anti-FGF aptamer	Ribomic	Neovascular AMD		
PAN-90806	RTK Inhibitor	PanOptica	Neovascular AMD	Kinase inhibitor	Topical ocular
RXI-109	rX-RNA	RXi Pharma	Neovascular AMD	CTGF expression inhibitor	Intravitreal
Sunitinib	RTK Inhibitor	Graybug	Neovascular AMD	Multikinase inhibitor	Topical
APL-2	C3 Inhibitor	Apellis	Neovascular AMD	Complement-C3 Inhibitor	Intravitreal
Faricimab	Bispecific Ab	Chugai	Neovascular AMD	Anti-VEGF-A/ Ang-2 Ab	Intravitreal
RGX-314	Gene therapy vector (VEGF neutralizer)	RegenxBio	Neovascular AMD	Anti-VEGF AAV	Intravitreal
Retinostat	Gene therapy vector (Angiostatic stimulator)	Oxford Biomedica	Neovascular AMD	Lentivirus vector	Intravitreal

Abbreviations: *Ab* antibody, *Ang-2* angiopoeitin-2, *AMD* age-related macular degeneration, *CTGF* connective tissue growth factor, *GA* geographic atrophy, *mAb* monoclonal antibody, *PDGF* platelet-derived growth factor, *VEGF* vascular endothelial growth factor

system. Such CNV compromises Bruch's membrane (due to release of MMPs from RPE and Muller cell that digest the latter), retinal interneurons and eventually RGCs as the new blood vessels branch out into the vitreous and cause local hemorrhages and loss of vision, especially at the macula. Therefore, pathologically relevant cell types (e.g., human micro- and macrovascular

retinal endothelial cells [REC]; RPE [including primary cells and ARPE-19 cells, and Muller glial cells), surrogate cells (e.g., human umbilical vein endothelial cells (HUVEC)), and co-cultures (Chen et al. 2017), have been utilized to evaluate new drug modalities directed at the neovascular component of wAMD. Functional readouts relevant to the CNV using in vitro assays have involved RPE-stretch-induced release of VEGF (Farjood and Vargis 2018), growth factor-induced REC proliferation/migration/tube formation using a matrigel assay (Chen et al. 2016), cellular permeability, and expression and secretion of various MMPs (Di and Chen 2018).

Animal Models to Find Anti-wAMD/Anti-CNV Drugs

Aberrant ocular angiogenesis is the hallmark of CNV/wAMD. Animal models that have been used to study the pathological and drug discovery aspects of these diseases have involved laser-induced and surgically induced CNV in rodents, rabbits, and monkeys (reviewed by Liu et al. 2017). Retinopathy of prematurity (ROI) as induced by high levels of oxygen soon after birth of rodents (OIR) is another useful model of CNV and wAMD (Liu et al. 2017). These models are self-explanatory and have been deployed to study effects of disease-prevention/reduction using vascular permeability, measurement of VEGF release, and angiogenesis as biomarkers and readouts. In view of the importance of these retinal disorders, a number of transgenic mouse models with spontaneous sub- and intraretinal angiogenesis have also been established for possible screening purposes where other angiogenic signaling molecules may be involved other than or in addition to VEGF (Liu et al. 2017). Due to the aberrant involvement of the complement system in the etiology of wAMD/CNV, an important interaction between the oxidative stress and the latter systems is also being studied (Du et al. 2016), as is the measurement of numerous cytokines and chemokines using multiplex and microarray technologies to find additional targets for wAMD/CNV conditions that may lead to discovery and development of new medicines to treat these blinding disorders (Liu et al. 2016; Lambert et al. 2016).

Diabetic Macular Edema and Diabetic Retinopathy

Diabetes-induced retinopathy is also a major ocular disease that causes preventable blindness around the world, currently estimated at 93 million worldwide in total. Two forms of diabetic retinopathy (DR) have been described: non-proliferative (NPDR) and proliferative (PDR). Abnormal neovascularization induced by prolonged hyperglycemia and retinal hypoxia causes PDR which results in vision loss (Wang and Lo 2018). Diabetic macular edema (DME) is a hallmark of NPDR and corticosteroids and biologics are used in its treatment (Wang and Lo 2018). Since many of the signs and symptoms of PDR resemble those of wAMD, anti-VEGFs biologies (antibodies (ABs) and small AB fragments [Fabs]) have paved the therapeutic pathways for treatment of PDR in addition to traditional laser photocoagulation of leaky retinal blood vessels. Similarly, the side effects associated with the latter, and the tachyphylactic responses to anti-VEGFs observed in wAMD patients will also necessitate requirement of alternative treatment options for DR and DME. Some novel approaches encompass the explorative use of bispecific antibodies (RO-6867461 [Anti-Ang-2 + Anti-VEGF]), AKB-9778 (Tie-2 activator), EBI-031 and Tocilizumab (IL-6 inhibitors), Luminate (Integrin inhibitor), MTP-131 (cardiolipin inhibitor), Lutein, and ALA (mitochondrion-specific antioxidant) (Wang and Lo 2018). Recently, it has been proposed that not only are the components of aberrant neovascularization and inflammation important in the etiology and ultimate resolution of PDR and NPDR but that degeneration of the neural retinal cells is also a key element leading to blindness resulting from diabetes (Simo et al. 2018). Therefore, interventional and perhaps even prophylactic neuroprotective therapies should be considered for both wAMD and DR.

Assay Systems and Animal Models for Discovering New Treatments for DR and DME

In an effort to explore possible means to ameliorate the effects of DR and DME, researchers have focused attention on identifying possible interventions point of these retinal disorders. Since hyperglycemia is the core of the problem, the effects of high glucose on RPE, Muller glia, vascular endothelial cells, and pericytes have been studied and used for screening compounds for reducing various biomarkers of disease (e.g., secretion of cytokines and angiogenic factors, cell permeability, mitochondrial dysfunction; microglial activation, RPE, and REC tube formation) (e.g., Tien et al. 2017; also see above in section "Cell-Based Assays for Finding New Anti-wAMD/Anti-CNV Drugs"). While none of these in vitro systems truly capitulates the DR/DME, direct effect of potential drugs of benefit in treating the latter have been examined using a variety of animal models (chemically [alloxan/streptozotocin/diet]/surgically [pancreatomy]-induced and genetic) ranging from zebrafish to rodents, to cats, dogs, pigs, and monkey; Olivares et al. 2017). It is worth mentioning that alloxan/streptozotocin/diet-induced retinal disease outcomes resemble human DR/DME, where rodents generally exhibit the major defects in terms of hyperglycemia, damaged pancreatic beta-cells, damaged/reduced pericytes, increased acellular capillaries, microglial changes, basement membrane thickening, microaneurysms, RGC, and inner retinal cell loss (Olivares et al. 2017). Retinal neovascularization was observed in rodents after systemic hyperglycemia and was most profound under hypoxic conditions in zebrafish, rodents, and monkey (Olivares et al. 2017). Of the genetically induced DR/DME, rodents were the most susceptible showing many of the aforementioned phenotypic changes in their retinal anatomy and pathology. These results emphasize the need to use multiple assays and animal models to assess the therapeutic efficacy of any treatment modalities for DR/DME.

Ocular Surface Diseases

Despite the protection afforded by the blink response, the placement of the eyeball within the orbital socket, and protection provided by the sclera, the eye still remains a target for airborne allergens, pollutants, bacteria, and viruses that fall onto the ocular surface. The tear film covering the cornea and conjunctiva also acts as a barrier but can harbor some of the agents mentioned above.

Allergic Conjunctivitis

Seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC) are allergic reaction of the cornea and conjunctiva to airborne allergens such as pollen, mold, pet dander, and air pollution (Yanni et al. 1999; O'Brien 2013). SAC afflicts millions of patients of all ages every few months and causes debilitating and extremely bothersome excessive tearing, intense itching, grittiness, burning, photophobia, redness and swelling of the eyelids (O'Brien 2013; Gomes 2014). These symptoms are caused by release of histamine, prostaglandins, cytokines, and chemokines from resident mast cells in conjunctiva of the eyelids (Sharif et al. 1996; Yanni et al. 1999). SAC leads to decreased work productivity, increased absenteeism from work and school, limitation of everyday activities, significantly reduced quality of life, including decreased sleep quality. These SAC symptoms combined with seasonal rhinitis cause further ill health and detrimental psychological ill effects leading to impaired social interaction on top of the physical morbidity. Overall, SAC and rhinitis due to their perennial occurrence requires potent and efficacious treatment options. Accordingly, there are now several approved/launched histamine-1 (H_1) antagonists that are used in clinical management of SAC. These include emedastine (Sharif et al. 1994), olopatadine (Sharif et al. 1996), epinastine, alcaftadine, and cetirizine. However, by far the most effective agent, with a dual mechanism of action, that provides 24-h relief from SAC upon a single t.o. dose is the H_1-antagonist/mast cell

stabilizer, olopatadine (Patanol (o.1% olopatadine); Pataday (0.2% olopatadine); Pazeo (0.7% olopatadine). Elesion/Elestat [0.05% epinastine) is also quite and effective drug for the treatment of signs and symptoms of SAC. A number of other treatment options for SAC at various stages of development are allergy vaccines (MK-3641; MK-8237; SQ tree SLIT-tablet), an aldehyde scavenger (ADX-102), Syk tyrosine kinase inhibitor (PRT-2761), and an anti-IgE monoclonal antibody (xmab7195) (Gomes 2014).

Assays and Animals Models for Discovering Drugs to Treat Allergic Conjunctivitis

Once again, exploiting the knowledge of the possible disease-causing elements, relevant cell types, and animal models have been deployed in the screening for new drugs to treat seasonal and perennial allergic conjunctivitis. Thus, isolated human primary conjunctival epithelial and mast cell (Sharif et al. 1996; Yanni et al. 1997), and corneal epithelial cells (Offord et al. 1999) has been at the forefront of the cell-based assays systems. These cells have been challenged with various allergens and the release of cytokines and other mediators quantified in the presence or absence of test drugs of interest. These assays proved effective in yielding compelling data for advancing H_1-antagonists like emedastine (Sharif et al. 1994) and dual pharmacophoric drugs like olopatadine (H_1-antagonist and mast cell stabilizer) (Sharif et al. 1996; Yanni et al. 1997) into animal models of allergic conjunctivitis (Yanni et al. 1997).

Several different mammalian species have been deployed to test drugs for inflammatory and allergic conjunctivitis including guinea pigs and rodents (Groneberg et al. 2003). The most frequently and preferred models use active immunization sensitization protocols. In the guinea pig model, for instance (Yanni et al. 1997), animals are sensitized with anti-ovalbumin (OA) serum injected subconjunctivally in one eye. Twenty-four hours after passive sensitization, ovalbumin (OA) was administered either intravenously (i.v.) or topically onto the eye. The anti-allergic effect of compounds following i.v. antigen administration was determined as follows: 30 min prior to i.v. antigen challenge, the animals received 20 μl of the drug solution or saline applied topically to the eye. The animals are then challenged i.v. via the marginal ear vein or lateral tail vein with 1.0 ml of an OA:Evans Blue solution (100 pg: l mg, guinea pigs; 1 mg:2.5 mg, rats). For assessment of the allergic response following topical ocular antigen challenge, 20 μl of ovalbumin (1.0%, w/v) was administered to the sensitized eye 5 min after topical ocular application of the test drug (20 μl). During dose-response studies, the order of compound administration is randomized. Thirty minutes later, the reaction is quantitated, using a scoring of ocular allergic reactions accounting for swelling, discharge, and congestion of the conjunctiva/eye lids.

In the histamine-induced vascular permeability model using guinea pigs, animals are injected i.v. via the marginal ear vein with 1.0 ml of Evans Blue dye (1.0 mg/ml). Forty-five minutes post-dye injection, 20 μl of test compound or saline vehicle is applied topically onto one eye of each experimental animal. Thirty minutes following topical drug application, the guinea pigs are anesthetized and challenged subconjunctivally with histamine (300 ng/10 μl). The appearance of blue color on the ocular surface is then quantitated (Yanni et al., 1996).

Dry Eye Disease (DED)

Dry eye disease (DED), keratoconjunctivitis sicca, is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the cornea and conjunctiva (Messmer 2015; Marshall and Roach 2016; Baudouin et al. 2018; Dogru et al. 2018). It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface. Sjogren's syndrome is present in ~10% of the total DED patients. Other patients tend to be women who are postmenopausal, pregnant, or who are on hormone replacement therapy or are

taking oral contraceptives. Defects of the meibomian gland, that normally produces the oily/lipid components of the tear film which limit evaporation of the aqueous fluid of the tears, are also now recognized as key contributors to the development of DED (Baudouin et al. 2018). The signs and symptoms of DED have been difficult to address over the years apart from the use of artificial tears to provide brief and temporary relief. However, there are now four drugs available to the physician to treat DED including various formulations of cyclosporine (Restasis; Ikervis; Cyclokat; all are anti-inflammatories that are calcineurin inhibitors), diquafosol tetrasodium (a P_2Y_2 receptor agonist that stimulates tear production), rebamipide (Mucosta; mucin liberator that works on conjunctival goblet cells), and lifitigrast (Xiidra; an LFA-1 antagonist) (Messmer 2015; Marshall and Roach 2016; Baudouin et al. 2018; Dogru et al. 2018).

Several other classes of drugs are currently in early-late-stage research and development that cover diverse mechanisms of action such as thymosin-beta-4 ligand (RGN-259), TRPV1 expression inhibitor (siRNA; SYL-1001), NGF receptor agonist (tavilermide; MIM-D3), ICAM1 expression inhibitor/antioxidant (visomitin; SkQ1), nicotinic receptor agonist (cytisine; OC-02), alpha-4 integrin antagonist (AXR-159), JAK3/Syk Kinase inhibitor (R932348), aldehyde scavenger (reproxalap; ADX-102), multikinase inhibitor (TOP-1630), and RAR-gamma receptor agonist (palovarotene; RG-667) (Messmer 2015; Marshall and Roach 2016). It is hoped that some of these agents will prove to be safe and effective alternative treatment options for the DED patients in the near future.

In Vitro Assays and Animal Models of Dry Eye Disease

There are a number of therapeutic intervention points in DED including the ocular surface itself (corneal and conjunctival epithelia and goblet cells), and tear and meibomian glands. Accordingly, in vitro assays have been established that incorporate cells derived from these tissues. Cultured epithelial cells and cell lines of human cornea, conjunctiva (epithelial and goblet cells), and meibomian gland have been exposed to various treatments to simulate dry eye conditions including hyperosmolarity (400–500 mOsM) (Clouzeau et al. 2012), desiccation Hovakimyan et al. 2012), and inflammation (e.g., exposure to formaldehyde; Vitoux et al. 2018) and the effects on cell viability (lysosomal integrity), cell apoptosis/death (cell membrane permeability and chromatin condensation), secreted cytokines (e.g., IL-1, IL-8), oxidative stress (reactive oxygen species and superoxide anion), and cellular hyperpolarization measured. These indices were then used to determine the potential therapeutic effects of test compounds (e.g., Hagan et al. 2018).

Animal models for assessing the impact of test compounds in dry eye conditions have been difficult to establish and correlate with the human disease. Nevertheless, there has been progress made in understanding the DED processes and some level of screening performed using mice subjected to a dry, drafty environment (20% humidity) for 5–10 days after they receive daily subcutaneous injections of scopolamine that inhibits tear secretion. The animals exhibited a desiccating-stress-activated innate immune response resulting in release of cytokines, chemokines and MMPs on the ocular surface, increased intercellular adhesion molecule-1, increased loss of conjunctival epithelial and goblet cells, and CD4 T-cell infiltration (Stern and Pflugfelder 2017). Rabbits have also been employed for dry eye models using desiccation (Gamache et al. 2002) and lacrimal gland inflammation (Negelhout et al. 2005) with limited success.

Bacterial Infection/Ocular Inflammation

Even though bacterial infection of the cornea is fairly rare due to increased public awareness and enhanced ocular hygiene, bacterial keratitis is often caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Willcox 2011). Infections caused by these bacteria are best treated with the fluroquinolones levofloxacin, moxifloxacin and gatifloxicin, and/or with the aminoglycoside tobramycin. Inflammation of the eyelid hair follicles often caused by bacteria (blepharitis) is a

bothersome disorder that causes itching, redness, and irritation. Common treatments include t.o. antibiotic alone, or antibiotic + a corticosteroid. However, due to development of bacterial resistance, other drugs like ciprofloxacin, ofloxacin, and levofloxacin are less prescribed but may still be useful and the only drugs available in less developed world. New broad spectrum antibiotics are eagerly awaited in order that the bacterial resistance can be overcome.

Postoperative inflammation and pain are common when patients undergo elective Lasik, cataract or photorefractive keratectomy (PRK) eye surgery. PRK is used to correct mild to moderate nearsightedness, farsightedness, and/or astigmatism and/or mild myopia. Patients often receive nonsteroidal anti-inflammatory drugs such as the prostaglandin synthase inhibitors bromfenac, nepafenac, ketorolac, and/or low-dose steroids such as loteprednol to reduce the pain and inflammation associated with the eye surgeries (Waterbury et al. 2011).

Uveitis is caused by inflammatory responses inside the eye as a result of tissue damage, bacterial/viral infection, or due to toxins (Tsirouki et al. 2018). The disease will cause various symptoms, such as decreased vision, pain, light sensitivity, and increased "floaters" in the vitreous, and it causes 10–15% of blindness in the USA. In many cases, the cause is unknown and thus is idiopathic. Anterior uveitis occurs in the front of the eye and is the most common form of uveitis, predominantly occurring in young and middle-aged people. Many cases occur in healthy people and may only affect one eye but some are associated with rheumatologic, skin, gastrointestinal, lung, and infectious diseases. Intermediate uveitis is commonly seen in young adults and is observed in the vitreous. It has been linked to several disorders including, sarcoidosis and multiple sclerosis. Posterior uveitis is the least common form of uveitis, and it occurs in the back of the eye, often involving both the retina and the choroid. It is often called choroditis or chorioretinitis (Tsirouki et al. 2018). There are many infectious and noninfectious causes to posterior uveitis. Lastly, panuveitis is a term used when all three major parts of the eye are affected by inflammation. Behcet's disease is one of the most well-known forms of panuveitis, and it greatly damages the retina. Intermediate, posterior, and panuveitis are the most severe and highly recurrent forms of uveitis. They often cause blindness if left untreated. Treatment modalities for uveitis primarily try to eliminate inflammation, alleviate pain, prevent further tissue damage, and restore any loss of vision. Treatments depend on the type of uveitis a patient displays. Some, such as using corticosteroid eye drops and injections around the eye or inside the eye, may exclusively target the eye whereas other treatments, such immunosuppressive agents taken by mouth, may be used when the disease is occurring in both eyes, particularly in the back of both eyes (Tsirouki et al. 2018). However, these steroid-based treatments adversely affect the body and can cause glaucoma. A recent development for treating posterior uveitis that overcomes the latter side effect issues associated with corticosteroids centers around ivt injection of the immunosuppressant inhibitor of mammalian target of rapamycin, sirolimus (Nguyen et al. 2018). This drug has shown significant efficacy in a number of patients suffering from noninfectious posterior uveitis (Nguyen et al. 2018).

Screening Assays and Animal Models for Ocular Infectious Diseases and Uveitis

Infectious keratitis can be caused by various bacterial strains and fungi like *fusarium solani*. Obviously bacterial strains and fungi can be grown in vitro and effects of potential antibiotics and antifungal agents determined by direct application to these infectious agents (Jett et al. 1997). Researchers have also developed ex-vivo models using rabbit and human anterior eye segments (Pinnock et al. 2017) and animal models of infectious keratitis using mice and rabbits (Zhang et al. 2017b; Zhu et al. 2017).

The study of uveitis and drugs to treat this condition are best studied using animal models of the disease involving rats (Pepple et al. 2018) and mice (Chen et al. 2015). The different methods of inducing experimental autoimmune uveitis, panuveitis, and posterior uveitis are well described and reviewed by Bansal et al. (2015).

There is also an AIRE knockout mouse model of posterior uveitis and another Tg-knockout mouse model that reflects Birdshot retinochoroidopathy (Bansal et al. 2015).

Refractive Disorders/Errors

The most common types of refractive disorders, that result from misalignment of the light focusing on the retina, include nearsightedness (myopia), farsightedness (hyperopia), astigmatism, and presbyopia. While astigmatism results from uneven surface of the cornea, presbyopia develops due to stiffness of the lens. The Latter can lead to formation of cataracts of the lens. While in general refractive disorders can be corrected with eyeglasses, contact lenses, Lasik or PRK, a recent advancement includes the ability of EV-06 (a lipoic acid synthase modulator/S-adenosylmethionine decarboxylase stimulator) to temporarily change the fluidity of the lens to overcome presbyopia. On the other hand, myopia has been far more difficult to address since it primarily affects children, although due to the increasing use of computers/tablets and handheld wireless phones and a reduction in time spent outdoors the incidence and prevalence of myopia is steadily rising. Asian children experience myopia disproportionately greater than Caucasian children. Recent projections indicate that >50% of the world's population will have myopia by 2050, and more children and young adults will be affected. Since high myopia causes vision loss due to myopic macular degeneration, myopia and it comorbidities (cataracts, retinal detachment, and glaucoma) may become the leading cause of irreversible blindness worldwide. While t.o. dosing of atropine solution/ointment appears promising for treating myopia, additional modalities are urgently needed to stem the tide of myopia development around the globe.

Animal Models of Myopia

For sake of brevity, only myopia will be dealt with here. Several animal models using chicks, rats, mice, guinea pigs, and monkeys have been developed over the years (reviewed by Schaeffel and Feldkaemper 2015). Form deprivation (suturing of eyelids or patching the eye) and lens-induced methods are the most common and effective ways to induce myopia, and several classes of drugs have been tested for their efficacy in slowing down and/or preventing myopia. By far the most effective treatment observed in numerous animal species is the use of t.o. antimuscarinic agents (Schaeffel and Feldkaemper 2015; Jiang et al. 2018) that are able to reduce axial length within the globe. A recent study demonstrated that PG FP-receptor agonist, latanoprost (30 μg peribulbarly injected daily for 4 weeks) reduced form-deprivation-induced myopia in guinea pigs by 41% (Yang et al. 2018). Interestingly, peribulbarly injected FP-receptor antagonist, AL-8810 0.5 μg/day for 4 weeks, actually induced myopia in naïve animals (Yang et al. 2018).

Conclusions

Of the many ocular disorders, glaucoma, AMD, DR, and myopia represent the major sight-threatening diseases that afflict millions of people on the planet. Even irritating ocular disorders that do not necessarily directly cause blindness such as dry eye and seasonal allergic conjunctivitis affects millions on an annual basis. Since sight is such a precious sense, much effort has been expended to find suitable treatment modalities for the various eye diseases discussed above. It will be evident from the above discourse that while majority of the drugs discovered, developed, and launched for ocular utility represented agonists or antagonist of several GPCRs, that the newer drug classes are antibodies, gene therapeutics, growth factor proteins, cellular therapies, and miniature devices. This agnostic approach to combating ocular diseases is very encouraging and it is hoped that combination products using these foundational elements will be even more productive in helping the patients who suffer from the diseases of the eye. It is also hoped that diagnostics encompassing various biomarkers and devices, and new technologies such as adaptive optics and OCT-coupled with angiography will prove helpful in our deeper understanding of the ocular disease processes and thus lead to superior preventative measures in the future.

References and Further Reading

Alexander SPH, Christopoulos A, Davenport AP et al (2017a) The concise guide to PHARMACOLOGY 2017/18: G protein-coupled receptors. Br J Pharmacol 174:S17–S129

Alexander SPH, Striessnig J, Kelly E et al (2017b) The concise guide to PHARMACOLOGY 2017/18: voltage-gated ion channels. Br J Pharmacol 174: S160–S194

Alexander SPH, Kelly E, Marrion NV et al (2017c) The concise guide to PHARMACOLOGY 2017/18: transporters. Br J Pharmacol 174:S360–S446

Alexander SPH, Fabbro D, Kelly E et al (2017d) The concise guide to PHARMACOLOGY 2017/18: enzymes. Br J Pharmacol 174:S272–S359

Al-Zamil WM, Yassin SA (2017) Recent developments in age-related macular degeneration: a review. Clin Interv Aging 12:1313–1330

Ambati J, Ambati BK, Yoo SH et al (2003) Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies. Surv Ophthalmol 48:257–293

American Academy Ophthalmology – Eye Health Statistics – American Academy of Ophthalmology. https://www.aao.org/newsroom/eye-health-statistics

Anderson OA, Finkelstein A, Shima DT (2013) A2E induces IL-1β production in retinal pigment epithelial cells via the NLRP3 inflammasome. PLoS One 8: e67263

Bansal S, Barathi VA, Iwata D et al (2015) Experimental autoimmune uveitis and other animal models of uveitis: an update. Indian J Ophthalmol 63:211–2118

Batlle JF, Fantes F, Riss I et al (2016) Three-year follow-up of a novel aqueous humor microshunt. J Glaucoma 25: e58–e65

Baudouin C, Rolando M, Benitez Del Castillo JM, et al (2018) Reconsidering the central role of mucins in dry eye and ocular surface diseases. Prog Retin Eye Res. pii: S1350-9462 (18) 30062-4

Cavet ME, DeCory HH (2018) The role of nitric oxide in the intraocular pressure lowering efficacy of latanoprostene bunod: review of nonclinical studies. J Ocul Pharmacol Ther 34:52–60

Chader GJ (2002) Animal models in research on retinal degenerations: past progress and future hope. Vis Res 42:393–399

Chen H-H, Namil A, Severns B et al (2014) In vivo optimization of 2,3-diaminopyrazine Rho Kinase inhibitors. Bioorg Med Chem Lett 24:1875–1879

Chen J, Qian H, Horai R et al (2015) Mouse models of experimental autoimmune uveitis: comparative analysis of adjuvant-induced vs spontaneous models of uveitis. Curr Mol Med 15:550–557

Chen L, Bai Y, Zhao M (2016) TLR4 inhibitor attenuates amyloid-b-induced angiogenic and inflammatory factors in ARPE-19 cells: implications for age-related macular degeneration. Mol Med Rep 13:3249–3256

Chen LJ, Ito S, Kai H et al (2017) Microfluidic co-cultures of retinal pigment epithelial cells and vascular endothelial cells to investigate choroidal angiogenesis. Sci Rep 7:3538

Clouzeau C, Godefrov D, Riancho L et al (2012) Hyperosmolarity potentiates toxic effects of benzalkonium chloride on conjunctival epithelial cells in vitro. Mol Vis 18:851–863

Cordeiro MF, Normando EM, Cardoso MJ et al (2017) Real-time imaging of single neuronal cell apoptosis in patients with glaucoma. Brain 140:1757–1767

Crider JY, Sharif NA (2001) Functional pharmacological evidence for EP_2 and EP_4 prostanoid receptors in immortalized human trabecular meshwork and N-PCE cells. J Ocul Pharmacol Ther 17:35–46

Da Cruz L, Fynes K, Georgiadis O et al (2018) Phase 1 clinical study of an embryonic stem cell-derived retinal pigment epithelium patch in age-related macular degeneration. Nat Biotechnol. https://doi.org/10.1038/nbt.4114

Di Y, Chen XL (2018) Effects of LY294002 on the function of retinal endothelial cells in vitro. Int J Ophthalmol 11:1447–1450

Dismuke WM, Sharif NA, Ellis DZ (2009) Human trabecular meshwork cell volume decrease by NO-independent soluble guanylate cyclase activators YC-1 and BAY-58-2667 involves the BK_{Ca} ion channel. Invest Ophthalmol Vis Sci 50:3353–3359

Dismuke WM, Sharif NA, Ellis DZ (2010) Endogenous regulation of human Schlemm's canal cell volume by nitric oxide signaling. Invest Ophthalmol Vis Sci 51:5817–5824

Dogru M, Kojima T, Simsek C et al (2018) Potential role of oxidative stress in ocular surface inflammation and dry eye disease. Invest Ophthalmol Vis Sci 59: DES163–DES168

Du H, Xiao X, Stiles T et al (2016) Novel mechanistic interplay between products of oxidative stress and components of the complement system in AMD pathogenesis. Open J Ophthalmol 6:43–50

Farjood F, Vargis E (2018) Novel devices for studying acute and chronic mechanical stress in retinal pigment epithelial cells. Lab Chip 18:3413–3424

Fuwa M, Toris CB, Fan S et al (2018) Effects of a novel selective EP2 receptor agonist, omidenepag isopropyl, on aqueous humor dynamics in laser-induced ocular hypertensive monkeys. J Ocul Pharmacol Ther 34:531–537

Galloway CA, Dalvi S, Hung SC et al (2017) Drusen in patient-derived hiPSC-RPE models of macular dystrophies. Proc Natl Acad Sci U S A 114:E8214–E8223

Galloway CA, Dalvi S, Shadforth AMA et al (2018) Characterization of human iPSC-RPE on a prosthetic Brusch's membrane manufactured from silk fibroin. Invest Ophthalmol Vis Sci 59:2792–2800

Gamache DA, Wei ZY, Weimer LK et al (2002) Corneal protection by the ocular mucin secretagogue 15(S)-HETE in a rabbit model of desiccation-induced corneal defect. J Ocular Pharmacol Ther. 18:349–361

Gomes PJ (2014) Trends in prevalence and treatment of ocular allergy. Curr Opin Allergy Clin Immunol 14:451–456

Griffin BW, Klimko P, Crider JY et al (1999) AL-8810: a novel $PGF_{2\alpha}$ analog with selective antagonist effects at the FP prostaglandin receptor. J Pharmacol Exp Ther 290:1278–1284

Groneberg DA, Bielory L, Fischer A et al (2003) Animal models of allergic and inflammatory conjunctivitis. Allergy 58:1101–1113

Hagan S, Fyfe MCT, Ofen-Frimpong B et al (2018) Narrow spectrum kinase inhibitors demonstrate promise for the treatment of dry eye disease and other ocular inflammatory disorders. Invest Ophthalmol Vis Sci 59:1443–1453

Hartman RR, Kompella UB (2018) Intravitreal, subretinal, and suprachoroidal injections: evolution of microneedles for drug delivery. J Ocular Pharmacol Ther 34:141–153

He S, Stankowska DL, Ellis DZ et al (2018) Targets of neuroprotection in glaucoma. J Ocul Pharmacol Ther 34:85–106

Hellberg MR, McLaughlin MA, Sharif NA et al (2002) Identification and characterization of the ocular hypotensive efficacy of travoprost, a potent and selective FP prostaglandin receptor agonist, and AL-6598, a DP prostaglandin receptor agonist. Surv Ophthalmol 47 (Suppl 1):S13–S33

Hernandez-Zimbron LF, Zamora-Alvarado R, Ochoa-Del la Paz L et al (2018) Age-related macular degeneration: new paradigms for treatment and management of AMD. Oxid Med Cell Longev: 2018:8374647

Hines-Beard J, Wesley S, Bond WS et al (2016) Virus-mediated EpoR76E gene therapy preserves vision in a glaucoma model by modulating neuroinflammation and decreasing oxidative stress. J Neuroinflammation 13:39

Hollander H, Makarov F, Stefani FH et al (1995) Evidence of constriction of optic axons at the lamina cribrosa in the normotensive eye in humans and other mammals. Ophthalmic Res 27:296–309

Holló G, Topouzis F, Fechtner RD (2014) Fixed-combination intraocular pressure-lowering therapy for glaucoma and ocular hypertension: advantages in clinical practice. Expert Opin Pharmacother 15:1737–1747

Hovakimyan M, Ramoth T, Lobler M et al (2012) Evaluation of protective effects of trehalose on desiccation of epithelial cells in three dimensional reconstructed human corneal epithelium. Curr Eye Res 37:082–089

Ishikawa M, Jin D, Sawada Y et al (2015) Future therapies of wet age-related macular degeneration. J Ophthalmol: 2015:138070

Jett BD, Hatter KL, Huycke MM et al (1997) Simplified agar plate method for quantifying viable bacteria. Biotechniques 23:648–650

Jiang L, Zhang S, Chen R et al (2018) Effects of the tyrosinase-dependent dopaminergic system on refractive error development in guinea pigs. Invest Ophthalmol Vis Sci 59:4631–4638

Jonas JB, Aung T, Bourne RR et al (2017) Glaucoma. Lancet 390:2183–2193

Kashani A, Lebkowski JS, Rahhal FM et al (2018) A bioengineered retinal pigment epithelial monolayer for advanced, dry age-related macular degeneration. Sci Transl Med 10:eaao4097, 10 pages

Katoli P, Sharif NA, Sule A et al (2010) NPR-B natriuretic peptide receptors in human corneal epithelium: mRNA, immunohistochemical, protein and biochemical pharmacology studies. Mol Vis 16:1241–1252

Kelly CR, Williams GW, Sharif NA (2003) Real-time intracellular Ca^{2+}-mobilization by travoprost acid, bimatoprost, unoprostone and other analogs via endogenous mouse, rat and cloned human FP prostaglandin receptors. J Pharmacol Exp Ther 304:238–245

Kirihara T, Taniguchi T, Yamamura K et al (2018) Pharmacologic characterization of omidenepag isopropyl, a novel selective EP2 receptor agonist, as an ocular hypotensive agent. Invest Ophthalmol Vis Sci 59:145–153

Lambert NG, Singh MK, ElShelmani H et al (2016) Risk factors and biomarkers of age-related macular degeneration. Prog Retin Eye Res 54:64–102

Liu F, Ding X, Li J et al (2016) Aqueous humor cytokine profiling in patients with wet AMD. Mol Vis 22:352–361

Liu CH, Wang Z, Ye S et al (2017) Animal models of ocular angiogenesis: from development to pathologies. FASEB J 31:4665–4681

Lin CW, Sherman B, Moore LA et al (2018) Discovery and preclinical development of netarsudil, a novel ocular hypotensive agent for the treatment of glaucoma. J Ocul Pharmacol Ther 34:40–51

Maguire MG, Martin DF, Ying G-S et al (2016) Five-year outcomes with anti-vascular endothelial growth factor treatment of neovascular age-related macular degeneration. The comparison of age-related macular degeneration treatments trials. Ophthalmology 123:1751–1761

Marquart ME (2011) Animal models of bacterial keratitis. J Biomed Biotechnol 2011:680642

Marshall LL, Roach JM (2016) Treatment of dry eye disease. Consult Pharm 31:96–106

McNally S, O'Brien CJ (2014) Metabolomics/proteomics strategies used to identify biomarkers for exfoliation glaucoma. J Glaucoma 23(8 Suppl 1):S51–S54

Mead B, Ahmed Z, Tomarev S (2018) Mesenchymal stem cell-derived small extracellular vesicles promote neuroprotection in a genetic DBA/2J mouse model of glaucoma. Invest Ophthalmol Vis Sci 59:5473–5480

Messmer EM (2015) The pathophysiology, diagnosis, and treatment of dry eye disease. Dtsch Arztebl Int 112:71–81

Negelhout TJ, Gamache DA, Roberts L et al (2005) Preservation of tear film integrity and inhibition of corneal injury by dexamethasone in a rabbit model of lacrimal gland inflammation-induced dry eye. J Ocular Pharmacol Ther 21:139–148

NEI (2014) Eye Disease Statistics Fact Sheet – National Eye Institute – NIH. https://nei.nih.gov/sites/default/.../NEI_Eye_Disease_Statistics_Factsheet_2014_V10.pdf

Nguyen QD, Merrill PT, Sepah YJ et al (2018) Intravitreal sirolimus for the treatment of noninfectious uveitis: evolution through preclinical and clinical studies. Ophthalmology 125:1984–1993

O'Brien TP (2013) Allergic conjunctivitis: an update on diagnosis and management. Curr Opin Allergy Clin Immunol 13:543–549

Ohia SE, Njie-Mbye YF, Robinson J et al (2018) Serotonin-2B/2C receptors-mediate bovine ciliary muscle contraction: role in IOP regulation. J Ocul Pharmacol Ther 34:70–75

Offord E, Sharif NA, Mace K et al (1999) Immortalized human corneal epithelial cells for ocular toxicity and inflammation studies. Invest Ophthalmol Vis Sci 40:1091–1101

Olivares AM, Althoff K, Chen GF et al (2017) Animal models of diabetic retinopathy. Curr Diab Rep 17:93. (17 pages)

Osborne NN, Álvarez CN, del Olmo Aguado S (2014) Targeting mitochondrial dysfunction as in aging and glaucoma. Drug Discov Today 19:1613–1622

Patil R, Xu S, Rusinko A, Feng Z et al (2016) Rapid identification of novel inhibitors of aquaporin-1 channel by high-throughput screening. Chem Biol Drug Des 87:794–805

Pepple KL, Wilson L, Van Gelder RN (2018) Comparison of aqueous and vitreous lymphocyte population from two rat models of experimental uveitis. Invest Ophthalmol Vis Sci 59:2504–2511

Pinnock A, Shivshetty N, Roy S et al (2017) Ex vivo rabbit and human corneas as models for bacterial and fungal keratitis. Graefes Arch Clin Exp Ophthalmol 255:333–342

Qin J, Rinella N, Zhang Q et al (2018) OCT angiography and cone photoreceptor imaging in geographic atrophy. Invest Ophthalmol Vis Sci 59:5985–5992

Quigley HA, McKinnon SJ, Zack DJ et al (2000) Retrograde axonal transport of BDNF in retinal ganglion cells is blocked by acute IOP elevation in rats. Invest Ophthalmol Vis Sci 41:3460–3466

Ramachandran C, Patil RV, Sharif NA et al (2011) Effect of elevated intracellular cAMP on actomyosin contraction in bovine trabecular meshwork cells. Invest Ophthalmol Vis Sci 52:1474–1485

Rickman CB, Farsiu S, Toth CA et al (2013) Dry age-related macular degeneration: mechanism, therapeutic targets, and imaging. Invest Ophthalmol Vis Sci 54:ORSF68–ORSF80

Schaeffel F, Feldkaemper M (2015) Animal models in myopia research. Clin Exp Optom 98:507–517

Sharif NA (2017) Ocular hypertension and glaucoma: a review and current perspectives. Int. J. ophthalmol Vis Sci. 2:22–36

Sharif NA (2018a) *i*Drugs and *i*Devices discovery and development- preclinical assays, techniques and animal model studies for ocular hypotensives and neuroprotectants. J Ocular Pharmacol Ther 34:7–39

Sharif NA (2018b) Glaucomatous optic neuropathy treatment options: the promise of novel therapeutics, techniques and tools to help preserve vision. Neural Regen Res 13:1145–1150

Sharif NA, Klimko P (2019) FP prostaglandin-receptor antagonists: discovery, pharmacological characterization and therapeutic utility. Br J Pharmacol. https://doi.org/10.1111/bph.14335

Sharif NA, May JA (2011) Potential for serotonergic agents to treat elevated intraocular pressure and glaucoma: focus on $5HT_2$ receptor agonists. Expert Rev Ophthalmol 6:105–120

Sharif NA, Xu SX (1999) Human retina contains polyamine-sensitive [^{3}H]-ifenprodil binding sites: implications for neuroprotection? Br J Pharmacol 83: 236–240.

Sharif NA, Xu S, Yanni JM (1994) Histamine receptor-subtype affinities, selectivities and potencies of emedastine, a novel H_1-selective antagonist, and other ocularly employed antihistamines. Drug Dev Res 33:448–453

Sharif NA, Xu SX, Miller ST et al (1996) Characterization of the ocular anti-allergic and anti-histaminic effects of olopatadine, a novel drug for treating ocular allergic diseases. J Pharmacol Exp Ther 278:1251–1260

Sharif NA, Kelly CR, Crider JY et al (2003) Ocular hypotensive FP prostaglandin (PG) analogs: PG receptor subtype binding affinities and selectivities, and agonist potencies at FP and other PG receptors in cultured cells. J Ocul Pharmacol Ther 19:501–515

Sharif NA, McLaughlin MA, Kelly CR (2007) AL-34662: a potent, selective, and efficacious ocular hypotensive serotonin-2 receptor agonist. J Ocular Pharmacol Ther 23:1–13

Sharif NA, Katoli P, Scott D et al (2014) FR-190997, a non-peptide bradykinin B_2-receptor partial agonist, is a potent and efficacious intraocular pressure lowering agent in ocular hypertensive cynomolgus monkeys. Drug Dev Res 5:211–223

Sharif NA, Williams GW, Davis TL (2000) Pharmacology and autoradiography of human DP prostanoid receptors using [^{3}H]-BWA868C, a DP receptor-selective antagonist radioligand. Br J Ophthalmol 131:1025–1038

Shen W, Lee S-R, Yam M et al (2018) A combination therapy targeting endoglin and VEGF-A prevents sub-retinal fibro-neovascularization caused by induced Muller cell disruption. Invest Ophthalmol Vis Sci 59:6075–6088

Simo R, Stitt AW, Gardner TW (2018) Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia 61:1902–1912

Smedowski A, Liu X, Pietrucha-Dutczak M et al (2016) Predegenerated Schwann cells–a novel prospect for cell therapy for glaucoma: neuroprotection, neuroregeneration and neuroplasticity. Sci Rep 6:23187

Stern ME, Pflugfelder SC (2017) What have we learned from animal models of dry eye. Int Ophthalmol Clin 57:109–118

Tezel G, Yang X, Luo C et al (2010) Oxidative stress and the regulation of complement activation in human glaucoma. Invest Ophthalmol Vis Sci 51:5071–5082

Tham Y-C, Li X, Wong TY et al (2014) Global prevalence of glaucoma and projections of glaucoma burden through 2040. Ophthalmology 121:2081–2090

Thomas D, Papadopoulo O, Doshi R et al (2000) Retinal ATP and phosphorus metabolites: reduction by hypoxia and recovery with MK-801 and diltiazem. Med Sci Res 28:87–91

Tien T, Zhang J, Muto T et al (2017) High glucose induces mitochondrial dysfunction in retinal Müller cells: implications for diabetic retinopathy. Invest Ophthalmol Vis Sci 58:2915–2921

Tsirouki T, Dastiridou A, Symeonidis C et al (2018) A focus on the epidemiology of uveitis. Ocul Immunol Inflamm 26:2–16

Venugopalan P, Wang Y, Nguyen T et al (2016) Transplanted neurons integrate into adult retinas and respond to light. Nat Commun 7:10472

Vitoux MA, Kessal K, Baudouin C et al (2018) Formaldehyde gas exposure increases inflammation in an in vitro model of dry eye. Toxicol Sci 1(160):108–117

Wang W, Lo ACY (2018) Diabetic retinopathy: pathophysiology and treatments. Int J Mol Sci 19:1816, 14 pages

Wang JW, Woodward DF, Stamer WD (2013) Differential effects of prostaglandin E2-sensitive receptors on contractility of human ocular cells that regulate conventional outflow. Invest Ophthalmol Vis Sci 54:4782–4790

Waterbury LD, Galindo D, Villanueva L et al (2011) Ocular penetration and anti-inflammatory activity of ketorolac 0.45% and bromfenac 0.09% against lipopolysaccharide-induced inflammation. J Ocul Pharmacol Ther 27:173–179

Waugh N, Loveman E, Colquitt J et al (2018) Treatments for dry age-related macular degeneration and Stargardt disease: a systematic review. Health Technol Assess 22:1–168

Weinreb RN, Aung T, Medeiros FA (2014) The pathophysiology and treatment of glaucoma: a review. JAMA 311:1901–1911

WHO (2018) Blindness and vision impairment. https://www.who.int/news-room/fact-sheets/detail/blindness-and-visual-impairment. WHO priority eye diseases. https://www.who.int/blindness/causes/priority/en/

Wiederholt M, Thieme H, Stumpff F (2000) The regulation of trabecular meshwork and ciliary muscle contractility. Prog Retin Eye Res 19:271–295

Willcox MDP (2011) Review of resistance of ocular isolates of Pseudomonas aeruginosa and staphylococci from keratitis to ciprofloxacin, gentamicin and cephalosporins. Clin Exp Optom 94:161–168

Williams PA, Harder JM, Foxworth NE et al (2017) Vitamin B_3 modulates mitochondrial vulnerability and prevents glaucoma in aged mice. Science 355:756–760

Xu G, Weinreb RN, Leung CK (2014) Optic nerve head deformation in glaucoma: the temporal relationship between optic nerve head surface depression and retinal nerve fiber layer thinning. Ophthalmology 121:2362–2370

Yang S, Zhao J, Sun X (2016) Resistance to anti-VEGF therapy in neovascular age-related macular degeneration: a comprehensive review. Drug Des Devel Ther 10:1857–1867

Yang J, Pan M, Reinech PS et al (2018) Prostaglandin F2α receptor modulation affects eye development in guinea pigs. Basic Clin Pharmacol Toxicol 123:263–270

Yanni JM, Miller ST, Gamache DA et al (1997) A comparison of topical ocular anti-allergy drugs: effects on human conjunctival mast cell mediator release. Ann Allergy Asthma Immunol 79:541–545

Yanni JM, Sharif NA, Gamache DA et al (1999) A current appreciation of sites for pharmacological intervention in allergic conjunctivitis: effects of new topical ocular drugs. Acta Ophthalmol Scand 77:33–37

Yucel YH, Zhang Q, Gupta N et al (2000) Loss of neurons in magnocellular and parvocellular layers of the lateral geniculate nucleus in glaucoma. Arch Ophthalmol 118:378–384

Zhang S, Yu N, Zhang R et al (2016) Interleukin-17A induces IL-1β secretion from RPE cells via NLRP3. Invest Ophthalmol Vis Sci 57:312–319

Zhang TT, Muto J, Kim D et al (2017a) High glucose induced mitochondrial dysfunction in retinal Muller cells: implications for diabetic retinopathy. Invest Ophthalmol Vis Sci 58:2915–2921

Zhang Z, Abdel-Razek O, Wang G (2017b) The mouse model for ocular surface staphylococcus aureus infection. Curr Protoc Mouse Biol 7:55–63

Zhu H, Kochevar IE, Behlau I et al (2017) Antimicrobial blue light therapy for infectious keratitis: ex vivo and in vivo studies. Invest Ophthalmol Vis Sci 58:586–593