Ocular Purine Receptors as Drug Targets in the Eye

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Abstract

Agonists and antagonists of various subtypes of G protein coupled adenosine receptors (ARs), P2Y receptors (P2YRs), and ATP-gated P2X receptor ion channels (P2XRs) are under consideration as agents for the treatment of ocular diseases, including glaucoma and dry eye. Numerous nucleoside and nonnucleoside modulators of the receptors are available as research tools and potential therapeutic molecules. Three of the 4 subtypes of ARs have been exploited with clinical candidate molecules for treatment of the eye: A₁, A_{2A}, and A₃. An A₁AR agonist is in clinical trials for glaucoma, A_{2A}AR reduces neuroinflammation, A₃AR protects retinal ganglion cells from apoptosis, and both A₃AR agonists and antagonists had been reported to lower intraocular pressure (IOP). Extracellular concentrations of endogenous nucleotides, including dinucleoside polyphosphates, are increased in pathological states, activating P2Y and P2XRs throughout the eye. P2YR agonists, including P2Y₂ and P2Y₆, lower IOP. Antagonists of the P2X₇R prevent the ATP-induced neuronal apoptosis in the retina. Thus, modulators of the purinome in the eye might be a source of new therapies for ocular diseases.

Keywords: adenosine receptor, P2Y receptor, P2X receptor, ATP

Introduction

ADENOSINE AND VARIOUS adenine and uracil nucleotides act as extracellular autocrine or paracrine signals by activating cell surface receptors.^{1,2} The adenosine receptors (ARs) represent 4 subtypes of G protein-coupled receptors (GPCRs), and nucleotides also activate distinct GPCRs (8 subtypes of P2Y receptors, P2YRs) and/or ligand-gated ion channels (7 subunits forming trimeric P2X receptors, P2XRs).

Numerous synthetic agonists and antagonists for exogenous application are available as tools to study these receptors (Figs. 1–5), and some of these molecules have progressed into clinical trials.^{3–5} These ligands are largely orthosteric, that is, they bind to the same site on the receptor as the native agonist, but some allosteric modulators of the ARs, P2YRs, and P2XRs have been reported.⁶ The allosteric modulators can either enhance the action of the native agonist, that is, act as a positive allosteric modulator, or antagonize it as a negative allosteric modulator. Recently, monoclonal antibodies were also reported to modulate P2XR activity.⁷

Table 1 lists receptors associated with the biological actions of extracellular purines and pyrimidines, which collectively could be considered a purinome and representative ligands. All ARs, P2XRs, and P2YRs are well expressed in all ocular tissues (Fig. 6), as indicated by the PLIER values generated by the Affymetrix algorithm.⁸ There is a generally

higher expression level for A_{2B}, P2Y₁₄, and P2X₂R in various ocular tissues. However, the level of expression of these receptors can vary greatly in pathological states; thus, use of purine receptor agonists and antagonists in disease models must take receptor regulation into account.⁴

Furthermore, a set of enzymes and transporters control the levels of extracellular purines and pyrimidines and, therefore, indirectly modulate the degree of activation of the various receptors.⁹ The numerous members of the ectonucleotidase families (such as CD39 and CD73) catalyze the hydrolysis of phosphate groups of the nucleotide ligands of P2Y and P2XRs. Thus, inhibition of these enzymes would reduce the amount of adenosine 1 available to activate ARs. There are both equilibrative (ENTs) and concentrative (CNTs) transporters for adenosine. For example, the approved vasodilator dipyridamole (Persantine, structure not shown) inhibits the equilibrative transporter ENT1 and other ENTs to increase the amount of extracellular adenosine.¹⁰ The therapeutic modulation of enzymes and transporters associated with the extracellular concentrations of purines and pyrimidines is also being considered for clinical development.¹¹

Adenosine receptor modulators as drug targets in the eye

The ARs are coupled to G proteins that cause either increases (A_{2A} and A_{2B}) or decreases (A₁ and A₃) of intracellular cyclic

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FIG. 1. Structures of AR agonist ligands. AR, adenosine receptor.

Nonselective antagonists

A₁ antagonists

A_{2A} antagonists

A_{2B} antagonists

A₃ antagonists

FIG. 2. Structures of AR antagonist ligands.

FIG. 3. Structures of P2YR and P2XR agonists.

AMP. However, a range of other G protein-dependent and independent signaling mechanisms are associated with activation of ARs.¹ Nucleoside derivatives that activate ARs (1–20) are shown in Fig. 1, and AR antagonists (both nucleosides and nonnucleosides, 25–49) are shown in Fig. 2. Compounds 21–24 indirectly modulate the activity at ARs as inhibitors of the removal of adenosine by phosphorylation (21 and 22, by adenosine kinase) or through allosteric enhancement of the A₁ 23 or A₃AR 24. There are potent agonists and antagonists associated with each of the 4 AR subtypes, but caution must be used when applying them to pharmacological experiments due to selectivities that are not completely definitive for the AR subtypes. For example, the “A₁AR agonist” R-PIA 3 causes

reductions in heart rate, activity, and body temperature in mice that are dependent on the presence of the A₃AR; genetic knockout of the A₃AR prevents some of these effects.¹² Various agonists were defined as A₁AR selective before the discovery of the A₃AR. For example, CPA 4 and CHA 8 are more potent at the A₃AR than at the A_{2A}AR.³ The A₁AR agonist CCPA 5 is more selective than CPA, but it has lower than full efficacy at the A₃AR, that is, it can act as a partial agonist or an antagonist.¹³ However, among the more selective agonists are: 5'-Cl-5'-d-ENBA (Cl-ENBA) 10 at the A₁AR and MRS5698 18 at the A₃AR,^{14,15} which have been shown to be selective *in vivo* using A₁AR and A₃AR knockout mice.¹⁶ Agonists of the A₁AR 9 (moderately selective) and the A₃AR 19 (highly

FIG. 4. Structures of P2YR antagonists.

FIG. 5. Structures of P2XR antagonists.

TABLE 1. RECEPTOR SUBTYPES INVOLVED IN THE ACTION OF EXTRACELLULAR PURINES AND PYRIMIDINES (pK_A VALUES AT HUMAN RECEPTORS FOR REPRESENTATIVE AGONIST AND ANTAGONIST LIGANDS ARE GIVEN IN PARENTHESES).^{1,3,5,6,106,121}

Subtype	Gene name	Chromosome	Native and synthetic agonist(s)	Representative antagonist(s)
Adenosine receptors (GPCRs)				
A ₁	<i>ADORA1</i>	1q32.1	Adenosine 1	PSB36 28 , SLV320 29
A _{2A}	<i>ADORA2A</i>	22q11.23	Adenosine 1	SCH442416 34
A _{2B}	<i>ADORA2B</i>	17p12	Adenosine 1	MRS1754 36
A ₃	<i>ADORA3</i>	1p13.2	Adenosine 1 , inosine 2	MRS1191 39 , MRS1523 42
P2Y receptors for nucleotides (GPCRs)				
P2Y ₁	<i>P2RY1</i>	3q25.2	ADP 47 (5.1), 2-MeSADP 48 (8.2), MRS2365 51 (9.4)	MRS2500 73 (9.0)
P2Y ₂	<i>P2RY2</i>	11q13.4	UTP 53 (8.1), ATP 49 (7.1), PSB1114 61 (6.9)	AR-C1189251XX 74 (7.4)
P2Y ₄	<i>P2RY4</i>	Xq13	UTP 53 (6.3)	-
P2Y ₆	<i>P2RY6</i>	11q13.4	UDP 64 (7.9), MR2693 65 (7.8)	MRS2578 75 (7.4)
P2Y ₁₁	<i>P2RY11</i>	19p13.2	ATP 49 (4.8), NF546 69 (6.3)	NF340 76 (7.1)
P2Y ₁₂	<i>P2RY12</i>	3q25.1	ADP 47 (7.2), 2-MeSADP 48 (8.3)	Cangrelor 77 (9.4), Ticagrelor 78 (7.9), AZD1283 79 (8.0)
P2Y ₁₃	<i>P2RY13</i>	3q24	ADP 47 (7.9)	MRS2211 82 (6.0),
P2Y ₁₄	<i>P2RY14</i>	3q21-q25	UDP 64 (6.8), UDP-glucose 70 (6.5)	PPTN 83 (8.7)
P2X receptors for ATP (in channels)				
P2X ₁	<i>P2RX1</i>	17p13.3	ATP 49 (7.3)	NF449 85 (10.7), MRS2159 86 (8.0)
P2X ₂	<i>P2RX2</i>	12q24.33	ATP 49 (6.0), Bz-ATP 52 (6.4)	PSB-1011 87 (7.1)
P2X ₃	<i>P2RX3</i>	11q12	ATP 49 (6.5)	RO-3 88 (7.0)
P2X ₄	<i>P2RX4</i>	12q24.32	ATP 49 (6.3)	5-BDBD 89 (6.3), PSB-12054 90 (6.7)
P2X ₅	<i>P2RX5</i>	17p13.3	ATP 49 (5.4)	BBG (6.3)
P2X ₆	<i>P2RX6</i>	22q11.21	ATP 49 (6.3)	TNP-ATP (6.1)
P2X ₇	<i>P2RX7</i>	12q24	ATP 49 (2.2), Bz-ATP 52 (4.3)	A438079 92 (6.9), JNJ 47965567 96 (8.3)

ARs, adenosine receptors; BBG, Brilliant Blue G; GPCR, G protein-coupled receptor.

selective) do not cross biological membranes because of a charged sulfonate group that has been introduced.

The generally suppressant role of the A_{2A}AR and A_{2B}AR in the immune system has been explored.¹⁷ Potent A_{2A}AR agonist UK432,097 **12** failed clinical trials for COPD, but displays extended lung retention.¹⁸ Lexiscan **13a** is a short acting A_{2A}AR agonist that is approved for dilating coronary vessels during stress testing and was recently demonstrated to open the blood-brain barrier transiently, with a decreased P-glycoprotein expression, and to ameliorate sickle cell disease.^{19–21} Its A_{2A}AR affinity and selectivity are only moderate (K_i ~ 1.3 μM, >10-fold selective versus A₁AR). More potent and selective A_{2A}AR agonist ATL-313 **13b** and other A_{2A}AR agonists are being considered for treatment of inflammatory pain and sepsis.^{3,22,23} The potential of using A_{2A}AR antagonists, for example, **30–35**, to boost the immunotherapy of cancer is based on blocking the effects of elevated adenosine levels in the tumor microenvironment that suppress the immune response, and many pharma companies are actively pursuing this concept.²⁴ Nonnucleoside A_{2B}AR agonist BAY 60-6583 **14** has been noted to act as partial agonist or even antagonist at that receptor in different models.²⁵

Suggested antagonists for pharmacological experiments are provided in Table 1. A relatively nonselective but potent pan-AR antagonist is xanthine amine congener (XAC, structure not shown). One should also be cognizant of species differences in the affinity of a given ligand.²⁶ For example, the A₃AR antagonist MRS1191 **39** has a K_i value of 31 nM at the human A₃AR, 1.4 μM at the rat A₃AR, and >10 μM at the mouse A₃AR.^{27,28} A₃AR antagonist OT-7999 **46** has a K_i value of 0.61 nM at the human A₃AR and

>10 μM at the rat A₃AR.²⁷ For that reason, its preclinical validation for glaucoma had to be performed in primate species.²⁹ In some cases, such as human A₃AR antagonist MRS1220 **41**, the selectivity can be inverted, depending on species. The A₃AR affinities of nucleoside-derived antagonists, such as MRS1292 **43**, LJ1251 **44**, and LJ979 **45** tend to be more constant across species than the nonnucleotide antagonists. Thus, the ligand tools for the ARs must be used with all appropriate controls and blocking with antagonists or, preferably, genetic knockout. There are also species differences in the biology of a given purine receptor subtype, for example, the A₃AR in mast cells.¹⁵

A rise in the concentrations of adenosine **1** and inosine **2** in aqueous humor accompanies intraocular hypertension and correlates with the magnitude of the pressure.³⁰ Inosine is formed from adenosine by adenosine deaminase, a ubiquitous enzyme, and at high concentrations can activate various ARs.³¹ The increase in adenosine is thought to arise from release of ATP from both the nonpigmented ciliary epithelial cells responsible for aqueous humor inflow and from trabecular meshwork cells of the outflow tract. The ATP is subsequently converted to adenosine by ectoenzymes of those cells.^{32–36} It might be expected that solvent drag would preclude a contribution of adenosine by the trabecular meshwork cells in a direction against the flow of aqueous humor from the anterior chamber. However, even proteins, having a higher frictional interaction with water can proceed against net flow. As an example, net diffusion of proteins from posterior to anterior chambers occurs across the iris root in rabbits,³⁷ monkeys,³⁸ and human.³⁹ This net diffusion proceeds against the net flow.^{40,41} Adenosine concentration in the rabbit eye

FIG. 6. Occurrence of purinergic receptors (A, ARs; B, P2YRs; C, P2XRs;) determined in exon-level expression profiling of ocular tissues.⁸

can be increased by application of dipyridamole.⁴² Modulation of ARs has been explored for several decades for the treatment of glaucoma. Three of the 4 subtypes of ARs have been exploited with clinical candidate molecules for treatment of the eye: A₁, A_{2A}, and A₃.^{43–45} Clinical trials for glaucoma have ensued.⁴⁶

The effects of AR modulation on specific tissues in the eye and on intraocular pressure (IOP) have been extensively probed by multiple techniques. For example, activation of A₁AR or A_{2A}AR in juxtacanalicular tissue cells increased the concentrations of Cl[−] and K⁺ and the cell volume measured by electron probe X-ray microanalysis.⁴⁷ The intracellular ionic composition of cells in the inner wall of Schlemm's canal was similarly affected by activating the

A_{2A}AR, but not the A₁AR. The differential effects of AR agonists to alter the resistance to outflow of aqueous humor have also been studied. Topically applied A₁AR agonists lower resistance, thereby enhancing aqueous humor outflow and, consequently, reducing IOP.^{48,49} The reduction in resistance appears mediated by release of matrix metalloproteinases (MMP-2 and MMP-9) from trabecular meshwork cells of the aqueous outflow tract.^{32,48} However, the time course of the effects of MMPs in the perfused bovine anterior segments⁴⁸ was much faster than the effects of MMPs on human eyes in organ culture.⁵⁰ Whether the different time courses reflected species or experimental differences remains unknown. Topically applied ATP also stimulated release of MMPs, but this release is dependent on conversion of the

nucleotide to adenosine.⁴⁵ The finding that A₁AR agonists can lower IOP has led to clinical trials of INO-8875 (Trabedonon) **6** for glaucoma.⁴⁹ In the anesthetized rat, INO-8875 displayed a longer duration of action in lowering atrioventricular (AV)-nodal conduction, reflective of activation of the A₁AR compared to another A₁AR agonist CVT-510 (Tecadenoson) **7**.⁵¹ A single topical dose of INO-8875 **6** reduced IOP in glaucoma patients in a Phase1/2 clinical trial.⁵² A Phase 3 study of topically applied **6** in adults with ocular hypertension or primary open-angle glaucoma (MATrX-1) is underway (NCT02565173).⁴⁶

A_{2A}AR agonists and antagonists have also been undergoing study to treat glaucoma.⁵³ The A_{2A}AR agonist, OPA-6566 (structure not disclosed), underwent Phase1/2 clinical trials (NCT01410188) by Acucela, Inc. and Otsuka Pharmaceutical Co., Ltd. for the management of open-angle glaucoma or ocular hypertension.⁴⁶ The trials have been completed, but no study results have yet been posted. Santen Pharmaceuticals has also been exploring the potential use of A_{2A}AR agonist ATL-313 **13b** for lowering IOP.⁵⁴ The promise of A_{2A}AR agonists is uncertain since they have exerted both unfavorable and favorable effects in preclinical studies. Activation of the A_{2A}AR was found to be neuroprotective against traumatic optic neuropathy by attenuating the inflammatory response to optic nerve trauma.⁵⁵ Low levels of traumatic optic neuropathy that would otherwise induce only a minimal neuroinflammatory response in wild-type mice induced severe inflammation in A_{2A}AR^{-/-} mice. In addition, A_{2A}AR had an anti-inflammatory effect on retinal ganglion cells subjected to elevated pressure.⁵⁶ Given that inflammation plays a role in the pathogenesis of glaucoma,^{57–59} A_{2A}AR agonists might be helpful. However, A_{2A}AR agonists also act to reduce vascular resistance and increase blood flow to the retina and optic nerve head.⁴³ Antagonists, but not agonists, of the A_{2A}AR can improve recovery of retinal function after ischemia reperfusion.⁴³ Furthermore, a selective A_{2A}AR antagonist (SCH58261, **33**) prevented ischemia-reperfusion death of retinal ganglion cells arising from transient elevation of IOP.⁶⁰ The neuroprotection was thought to have been mediated by attenuating the microglial-mediated neuroinflammatory response.^{55,60} Another caveat is that the effect of A_{2A}AR agonists on IOP is expected to be unfavorable since the A_{2A}AR antagonist ZM241385 **31** lowered pressure in the mouse,³³ while activation of A_{2A}ARs transiently increased pressure in rabbit and cynomolgus monkey eyes.⁶¹ In short, A_{2A}AR agonists and antagonists have multiple effects, and current published evidence that A_{2A}AR agonists would be helpful in glaucoma is not yet compelling.

As an alternative to the use of side effect-prone AR agonists, adenosine kinase inhibitor ABT-702 **21** was administered to mice to increase the level of endogenous adenosine and, thereby, protect against traumatic optic neuropathy-induced retinal injury and reduce pro-inflammatory cytokines.⁶² Nevertheless, adenosine kinase inhibitors have their own side effects, led to the discontinuation of past clinical trials of centrally-acting inhibitors.⁶³

At the A₃AR, there is evidence that topically applied selective antagonists reduce IOP, and topically applied selective agonists increase IOP.^{33,64–66} The mechanistic basis for this action is that the A₃AR is coupled to a chloride channel in the nonpigmented ciliary epithelial layer, such that its activation causes inflow leading to a rise in IOP.⁶⁷

This concept was supported by studies of A₃AR knockout mice, in which the absence of the A₃AR lowered IOP.⁶⁸ In addition, the A₃AR is more highly expressed in the non-pigmented ciliary epithelium in glaucoma associated with pseudoexfoliation syndrome.⁶⁹ The finding of A₃AR as a regulator of chloride transport in the nonpigmented ciliary epithelium led to the development of selective A₃AR antagonists for the treatment of glaucoma. A nucleoside-based antagonist of the A₃AR, LJ1251 **44**, was found to lower IOP.⁶⁶ Acorn Biomedical has proposed using ACN-1052 (structure not disclosed) for glaucoma treatment.⁷⁰ In addition, a “first-in-human” Phase I trial (NCT02639975) of Palobiofarma’s orally administered A₃AR antagonist PBF-677 (structure not disclosed) intended for treatment of glaucoma is expected to begin enrolling healthy volunteers to test safety and tolerability, as it has received the approval of the Spanish Regulatory Agency.^{46,71}

Interestingly, agonists of the A₃AR improved aqueous humor outflow in an animal model and in preliminary human clinical data.^{72,73} OphthaliX is a daughter company of Can-Fite Biopharma, which is already sponsoring clinical trials of A₃AR agonists, originally reported by the NIDDK laboratory,^{74,75} for autoimmune inflammatory diseases (rheumatoid arthritis and psoriasis) and hepatocellular carcinoma. During a previous unsuccessful trial of the A₃AR agonist CF101 (IB-MECA, Piclodenoson **15**) for dry eye disease, a modest reduction of IOP was noted in patients receiving the drug.⁷² The reported reduction of IOP was 0.92 mm Hg at a dose of 1 mg CF101 twice daily. This prompted a separate clinical path sponsored by OphthaliX for the same A₃AR agonist in glaucoma treatment. However, in a phase 2 trial of systemic **15** (1 or 2 mg oral dose, twice daily) for 16 weeks in patients with elevated IOP, no statistically significant differences were found between the drug-treated group and placebo group (NCT01033422).^{45,76}

The apparent contradiction that both agonists and antagonists of the A₃AR might be useful in treating the same conditions, other than glaucoma, still lacks a mechanistic explanation. The agonist-stimulated downregulation of the A₃AR in cell systems has been reported to occur in ~20 min,^{77,78} but in animals a sustained action of A₃AR activation in chronic pain has been noted.⁷⁹ The limitation of using adenosine agonists in glaucoma due to the desensitization of ARs has been noted.⁸⁰ Nucleoside prodrugs of A₃AR agonists and antagonists have been explored for their action on IOP.^{65,66,81,82}

Elevated IOP causes degeneration and eventually apoptotic death of retinal ganglion cells (RGCs). ARs are known to be present in the retina^{8,83} and their activation provides protection against this apoptosis. Specifically, A₃AR agonists such as CI-IB-MECA **16** and MRS3558 **17** protect RGCs from apoptosis induced by ATP and other nucleotides that activate the P2X7R, such as Bz-ATP **52**. A mixed A₁/A₃ agonist MRS3630 **20** was also effective. A₃AR agonist IB-MECA **15** also induced an anti-inflammatory effect in experimental autoimmune uveitis induced by retinal antigen interphotoreceptor retinoid-binding protein.⁸⁴

The pathophysiology of retinal ARs and dysregulated levels of adenosine have been explored with the goal of correcting the excitotoxicity and inflammation associated with diabetic neuropathy.⁸⁵ Exposure of rat retinal cells to sustained (12 week) high glucose upregulated the A₁AR, and the retinal A_{2A}AR is upregulated in diabetic animal and

cellular models, while the A₃AR displayed only a transient increase. However, conflicting results do not provide a clear path forward to clinical approaches using AR ligands.

P2YR modulators as drug targets in the eye

Nucleotide derivatives that activate P2YRs and P2XRs (47–71) are shown in Fig. 3 and P2YR antagonists (both nucleotides and nonnucleotides, 72–84) are shown in Fig. 4. ATP is the common agonist used for all of the P2XRs, but several subtypes (e.g., P2X₁R and P2X₇R) are activated more potently by Bz-ATP 52. We still lack potent agonists and antagonists for various subtypes of P2YRs and P2XRs.³ Nevertheless, potent (~nM) antagonists for the P2Y₁R, P2Y₁₂R, and P2Y₁₄R and for the P2X₁R, P2X₃R, and P2X₇R are available (Fig. 5). P2Y₂R ligand AR-C1189251XX 74 has been used effectively as a selective antagonist for this subtype.⁸⁶ Four antagonists of the P2Y₁₂R receptor are approved as antithrombotic agents. Two of them (thienopyridines Clopidogrel 80 and Prasugrel 81) are prodrugs that must be first activated enzymatically *in vivo*; the other 2 are nucleotide Cangrelor 77 and nucleoside Ticagrelor 78, both of which are competitive at the receptor.⁸⁷ Applications of P2YR agonists in the eye and elsewhere are envisioned,³ and consequently, selective agonists for P2Y₁R, P2Y₂R, P2Y₄R, P2Y₆R, P2Y₁₁R, and P2Y₁₄R have been reported.

P2YRs are important in many physiological and pathophysiological ocular functions and have been immunolocalized in the eye.^{45,88,89} The P2Y₁R is localized in the cornea, ciliary processes, and trabecular meshwork. Activation of the P2Y₁R has contradictory effects in the retina. It affects volume control and worsens reactive gliosis in Müller cells (astrocytes) in the retina, but it reduces ischemia-induced apoptosis of cells in all retinal layers.^{65,90} The P2Y₂R, P2Y₄R, and P2Y₆R are expressed in the cornea and ciliary processes, while the retinal pigmented epithelium expresses P2Y₂R. The P2Y₄R and P2Y₆R are also found in the photoreceptors, outer plexiform layer, and ganglion cell layer. The retinal pigmented epithelium also expressed the P2Y₁₁R. P2Y₂R, P2Y₄R, P2Y₁₁R, and P2Y₁₃R are present on lachrymal glands, where UTP causes myoepithelial cell contraction.⁹¹

P2YRs have multiple actions in the eye: they control tear production, corneal wound healing, aqueous humor dynamics, and retinal physiology. Nucleotides can affect both the volume and composition of tears, while regulating processes necessary for corneal wound healing. In the lens, osmotic stress activates TRPV4 channels, which leads to ATP release through connexin and pannexin hemichannels.⁴⁵ The released ATP activates a P2YR to help maintain lens transparency by activating a Na/K ATPase pump on the epithelium. Both UTP 53 and Ap₄A 58 applied topically to the rabbit cornea stimulate a MAPK-dependent increase in epithelial cell migration to accelerate wound healing.⁹² Where P2Y₂R activation accelerates, P2Y₆R activation impedes the migration rate of corneal epithelial cells in rabbit primary cell cultures.⁹³ Nucleotides cause an increase in the content of secreted mucins, lysozyme, and other tear proteins. The complicated distribution of P2Y₂R, P2Y₄R, and P2Y₆R suggested some redundancy in the role of receptors for uracil nucleotides in the eye, for example, in tear production and IOP regulation. However, the use of P2YR knockout mice to clarify their role in the eye has been limited.⁹⁰ siRNA knockdown was used to show that the P2Y₂R promotes cell migration in epithelial scratch

wound repair.⁴⁰ P2YR agonists, including those of P2Y₂R and P2Y₆R, lower IOP. The potential role of P2Y₁₄R is particularly puzzling. Exon-level profiling has indicated that P2Y₁₄R is more highly expressed than any other purine receptor⁸ (Fig. 6). Furthermore, the gene is expressed far higher in the trabecular meshwork than in any of the 9 other ocular tissues studied (Fig. 6B). One might speculate that the P2Y₁₄R protein product could play a role in the regulation of outflow resistance, which is regulated by the trabecular meshwork. Indeed, Podos presented results in abstract form, suggesting that topical application of UDP-glucose to activate P2Y₁₄R did lower IOP in glaucomatous monkey eyes.⁹⁴ However, no peer-reviewed publication has since verified those early promising observations.

Naturally occurring diadenosine polyphosphates, such as Ap₄A 58, activate P2YRs to regulate tear secretion and other functions.^{45,95–97} These dinucleotides are typically found in higher concentrations in eye diseases, such as Sjögren and non-Sjögren dry eye disease and glaucoma. It was suggested that lowering IOP by Ap₄A is mediated by the P2X₂R, causing acetylcholine release from nerve terminals that regulate ciliary processes.⁴⁵ In addition to the adenine dinucleotides, the concentration of Ip₄I 60 was shown to be elevated in glaucoma. Ip₄I 60 reduced IOP by activating P2YRs, although the subtype(s) involved were not clearly delineated.

The role of the P2Y₂R in the eye has been reviewed.⁹⁸ The dinucleotide INS365 59b (Diquafosol, also known as Diquas[®]) is a mixed agonist of the P2Y₂R and P2Y₄R that has been shown to improve the corneal barrier function.⁹⁹ In a rat dry eye model, this agonist increased both tear fluid secretion and corneal epithelial resistance and induced the release of glycoprotein-containing moieties from goblet cells. These successful preclinical results led to the introduction of Diquafosol in Japan and South Korea for the treatment of dry eye syndrome.¹⁰⁰ As a topical ophthalmic solution (3%, 6-times daily), it stimulated secretion of tears and mucin in patients with dry eye disease, and its effectiveness was maintained for 12 months.¹⁰¹ In a large randomized, double-blind trial, it improved the fluorescein staining score in dry eye disease comparably to 0.1% sodium hyaluronate and improved the rose bengal subjective symptom scores significantly better than hyaluronate.¹⁰² The encouraging clinical results with Diquafosol in dry eye disease were recently reviewed.¹⁰³ It was shown to be effective in various types of dry eye disease, such as aqueous deficient, short tear film break-up time, and obstructive meibomian glandular. It is also useful in treating dry eye disease resulting from surgery (for *in situ* keratomileusis and cataracts) and from use of contact lenses and visual display terminals. Although this compound is a GPCR agonist, which is prone to inducing desensitization of the receptor, its effect was maintained over the long term (3% solution, 6-times daily, for 6 months or 52 weeks).¹⁰³ Eye irritation was reported as an adverse event in only 6.3% of the cases in a trial of Diquafosol and there were no serious adverse effects.¹⁰⁴ Diquafosol was applied topically because the P2Y₂R occurs on the ocular surface, and systemic absorption was not detectable and without effect. The enzyme mainly responsible for hydrolysis of the drug in airway epithelial cells was found to be alkaline phosphodiesterase 1 (PDE1), which is membrane bound and has a broad substrate specificity. Nucleotides are also rapidly metabolized when in contact with the corneal cells.¹⁰⁵

P2Y₆R agonists have also been explored for reducing IOP.^{106–108} Topically applied UDP **64** reduced rabbit IOP by a maximal 17% compared to control, corresponding to an EC₅₀ value of 27 nM. A potent agonist of the P2Y₆R, TG46 (**67**, not to be confused with the JAK2 inhibitor TG-46), was administered topically to reduce IOP in rabbits by 45%. P2Y₆R is also important in the retina. Müller cells in the retina release UTP, which is hydrolyzed rapidly to UDP, to act at the P2Y₆R in neighboring RGCs.¹⁰⁹ P2Y₆R activation induced neurite outgrowth, which would be beneficial in glaucoma. In glaucoma, both the expression level of the P2Y₆R and its endogenous agonists might be downregulated.¹⁰⁹ However, in glaucomatous mice, topical application of Ap₄A lowered the expression of mRNA for P2Y₂R and P2Y₆R that was elevated due to the disease state.¹¹⁰

P2XR modulators as drug targets in the eye

P2XRs also have important roles in the eye and their modulation has been explored for therapeutic purposes. The pharmacology of P2XRs is complicated by the presence of heterotrimers, which have distinct ligand profiles compared to the homotrimeric channels. In theory, there is a therapeutic opportunity due to unique heterotrimer pharmacology, but most of the known combinations of subunits are not yet associated with specific biological effects and do not yet have selective ligands.^{2,3,5} In addition, splice variants have been shown to display different pharmacological properties.¹¹¹ Neuronal P2XRs in the inner and outer retina contribute to visual processing, as well as cell death in retinal ganglia. P2X₂R is present in the trigeminal ganglion sensory neurons and ciliary body.¹¹² In chronic glaucoma, ATP is released through pannexin hemichannels in astrocytes of the optic nerve and activates P2X₇R that can increase cell death in the retina. The ATP release from astrocytes can result from chronic mechanical strain (stretch) as it occurs in glaucoma through the upregulation of pannexins.¹¹³ The P2X₇R in lacrimal glands interacts with M₃ muscarinic acetylcholine receptors and α_{1D} -adrenergic receptors to regulate tear secretion.⁴⁵ P2X₇R plays an essential role in corneal epithelial cell migration and stromal organization during healing from abrasion wounds.¹¹⁴ The P2X₁R mediates ATP release in trabecular meshwork and ciliary epithelial cells.^{45,115} P2X₂, P2X₃, and P2X₇R colocalize with GABA in amacrine cells, including neurons postsynaptic to cone bipolar cells (all 3 subtypes) and rod bipolar cells (only P2X₃ and P2X₇R).¹¹⁶ Although all P2XRs are present in the rat lacrimal gland, ATP acting at P2X₃R and P2X₇R increases protein secretion by raising intracellular [Ca²⁺].¹¹⁷ ATP acts at P2X₇R to modulate both M₃ muscarinic cholinergic and α_{1D} -adrenergic signaling in the lacrimal gland.⁴⁵

The retinal P2X₇R is overactivated in diabetes, leading to pathological gliosis.¹¹⁸ Furthermore, both degeneration of the retinal pigment epithelium and choroidal neovascularization associated with different types of age-related macular degeneration are dependent on the P2X₇R.¹¹⁸ Thus, P2X₇R antagonists might prove useful in treating these conditions.

Nonnucleotide P2X₃R antagonists are proceeding toward the clinic for treatment of cough,¹¹⁹ and P2X₄R antagonist (structure not disclosed) is scheduled to enter clinical trials for chronic neuropathic pain.¹²⁰ P2X₇R antagonists that have been in clinical trials for conditions other than in the eye include AZD9056 **93** and CE-224,535 **94** (rheumatoid

arthritis) and GSK1482160 **95** (inflammatory pain).^{4,121} The trials for rheumatoid arthritis failed to show efficacy, but the trial of GSK1482160 **95** showed a reduction of IL1 β following exposure to LPS.¹²² Antagonists of the P2X₇R prevent neuronal apoptosis in the retina induced by ATP or by hypoxia.^{118,123–126,127,128} Such antagonists include Brilliant Blue G (BBG, structure not shown) and MRS 2540 **91**. JNJ-47965567 **96** is a CNS penetrant, selective P2X₇R antagonist, while JNJ-54173717 **97** was recently reported as a PET ligand for imaging the P2X₇R.¹²⁹

Summary

In summary, the current review has focused broadly upon the gene and protein expression of the 19 purine receptors so far identified in human ocular tissues and their physiological role. The current puzzlements and apparent inconsistencies in the results thus far have also been highlighted, as well as promising leads for future probes. In many cases, there are beneficial effects of both the nucleotides that are released locally to activate P2XRs and P2YRs and the adenosine that is produced enzymatically to activate ARs. The enormous spectrum of functions and the early utilization of probes in clinical studies have been addressed. Synthetic ligands that are selective for various purine receptors have promise for ocular therapeutics.

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