Title: USE OF DNUNUCLEOSIDE PHOSPHATE TO STIMULATE REMOVAL OF FLUID IN RETINAL DISORDERS

Abstract: The present invention provides a method of treating edematous retinal disorders. The method comprises administration of a pharmaceutical composition comprising a P2Y receptor agonist to stimulate the removal of pathological extraneous fluid from the subretinal and retinal spaces and thereby reduce the accumulation of said fluid associated with retinal detachment and retinal edema. The P2Y receptor agonist may be administered with therapeutic and adjuvant agents commonly used to treat edematous retinal disorders. The pharmaceutical composition useful in this invention comprises a P2Y receptor agonist with enhanced resistance to extracellular hydrolysis, such as dinucleoside polyphosphate compounds.
USE OF DINUCLEOSIDE POLYPHOSPHATE TO STIMULATE REMOVAL
OF FLUID IN RETINAL DISORDERS

TECHNICAL FIELD

This invention relates to a method of treating eye disease. Specifically, this invention relates to a method of removing pathological fluid accumulation in subretinal and intra-retinal spaces.

BACKGROUND OF THE INVENTION

The retinal pigment epithelium (RPE) lies in the back of the vertebrate eye and forms a barrier that separates the retina from the choroidal blood supply. A critical function of the RPE is to maintain and regulate the hydration of the subretinal space, the extracellular volume that exists between the retina and the RPE. (Marmor, pp. 3-12, in The Retinal Pigment Epithelium, Eds. M. F. Marmor and T. J. Wolfensberger, Oxford University Press, New York, (1998)). This function is achieved by the regulated transport of fluid, ions, and metabolites between the subretinal space and the choroidal blood supply. (Marmor, pp. 420-438, in The Retinal Pigment Epithelium, Eds. M. F. Marmor and T. J. Wolfensberger, Oxford University Press, New York, (1998); Pederson, pp. 1955-1968, in Retina, Ed. S. J. Ryan, Mosby, St. Louis, (1994)). Like all epithelia, the RPE contains two functionally and anatomically distinct membranes: an apical membrane that faces the retina, and a basolateral membrane that faces the choroidal blood supply. In the normal retina, fluid is absorbed across the RPE in the direction of the subretinal space to the choroid. This active absorption of fluid by the RPE, often referred to as the "RPE pump," plays a critical role in maintaining proper attachment of photoreceptors to the apical membrane of the RPE by pumping fluid out of the retinal spaces. (Marmor, pp. 1931-1954, in Retina, Ed. S. J. Ryan, Mosby, St. Louis, (1994); Hughes, et al., pp. xvii, 745, in The Retinal Pigment Epithelium, Eds. M. F. Marmor and T. J. Wolfensberger, Oxford University Press, New York, (1998)).

Retinal detachment is characterized by abnormal accumulation of fluid in the subretinal space leading to detachment of the retina from the underlying retinal pigment epithelium. Retinal edema refers to abnormal accumulation of fluid within the retina itself. Retinal detachment or edema in the central part of the retina (macula) produces significant loss of vision, and can ultimately lead to irreversible blindness. (Yanoff and Duker, Ophthalmology, Mosby, Philadelphia, (1999); Wilkinson, et al., Michels’ Retinal Detachment, 2nd ed. Mosby, St. Louis, (1997)) A wide variety of ocular pathologies can result in retinal
detachment or retinal edema. The most common type of retinal detachment is rhegmatogenous retinal detachment, which occurs as a result of single or multiple tears or holes in the retina that permit liquefied vitreous to enter into the subretinal space and create a retinal detachment.

There are no pharmacological approaches employed in the treatment of rhegmatogenous retinal detachment (RRD). The only current treatments for RRD are surgical (scleral buckling, pneumatic retinopexy, or vitrectomy). (Wilkinson, Michels’ Retinal Detachment, 2nd ed., Mosby, St. Louis, (1997)). There are two vital components for successful RRD surgery: reattachment of the retina and repair of the retinal break. The principal difference among the three surgical techniques for treating RRD is in the method employed to facilitate retinal reattachment.

Scleral buckle uses an extraocular buckle (usually a silicone sponge or solid silicone) that is sewn to the sclera towards the detached retina (Wilkinson, et al., Michels’ Retinal Detachment, 2nd ed., Mosby, St. Louis (1997)) The retina usually reattaches over a period of a few days, but may take up to a few weeks. The surgeon may elect to drain the subretinal fluid at the time of operation by inserting a needle through the sclera, choroid, and RPE. In general, the buckle remains permanently sewn to the sclera. In pneumatic retinopexy, a gas bubble is injected directly into the vitreous, and the head is positioned so that the gas bubble acts as a tamponade and covers the retinal break. (Tornambe and Hilton, Ophthalmology 96(6):772-83 (1989)). The subretinal fluid usually resolves within 1-2 days, but precise head positioning is required to insure that the bubble covers the retinal break. (Tomambe, 10 et al., Am. J. Ophthalmol. 127(6):741-3 (1999)). Vitrectomy is usually used for complex RRD associated with vitreous traction or hemorrhage, but is occasionally used for simple RRD (Chang, pp. 8.34.1-8.34.6, in Ophthalmology, Eds. M. Yanoff and J. S. Duker, Mosby, Philadelphia, (1999)). The procedure involves making three small incisions through the sclera to allow the introduction of instruments in the vitreous cavity. The vitreous is removed and replaced with a special saline solution. Depending on the type and cause of the detachment, a variety of instruments and techniques are then used to reattach the retina. For simple detachments, the retina is flattened via anterior drainage of the subretinal space by insertion of needle through the retinal tear.

Scleral buckle and vitrectomy often require general anesthesia and can involve hospitalization. Pneumatic retinopexy is usually done in the physician's office, but requires patient compliance for success. (Hilton and Tornambe, Retina 11(3):285-94 (1991); Hilton and

The conditions that are commonly associated with the more severe forms of intra-retina edema are diabetic macular edema, exudative age-related macular degeneration (AMD) and clinically significant cystoid macular edema. (Jampol and Po, pp. 999-1008, in *Retina*, Ed. S. J. Ryan, Mosby, St. Louis, (1994)). Other Pathological conditions associated abnormal fluid accumulation in intra-retinal or subretinal spaces include uveitis, central and branch vein occlusion, retinitis pigmentosa, central serous retinopathy, CMV retinitis, and choroidal melanoma. Physical trauma associated with ocular injury following certain surgical procedures (such as cataract surgery) can also produce retinal detachment or edema. (Ahmed and Ai, pp. 8.34.1-8.34.6 , in *Ophthalmology*, Eds. M. Yanoff and J. Duker, Mosby, Philadelphia, (1999)).

and topical administrations of corticosteroid, acetazolamide, and non-steroidal anti-inflammatory drugs, as well as surgical options such as vitrectomy, grid, and focal laser photoocoagulation. These therapies show limited utility in patients.

Although modern day RRD surgery has a relatively high success rate (60-90%), it is thought that a pharmaceutical composition that can reattach the retina in cases where surgery failed would be of enormous patient benefit. In addition, if the pharmaceutical composition can reattach the retina in the absence of surgical intervention, it would be most therapeutically useful, particularly in the treatment of rhegmatogenous retinal detachment.


Previous work has shown that the apical (retinal-facing) membrane of the RPE contains P2Y receptors that can be activated to stimulate fluid transport across the RPE in the direction of the subretinal space to the choroidal blood supply, and this mechanism of action was proposed to facilitate the removal of subretinal fluid in retinal detachment. (Peterson, et al, *J. Neurosci.* 17(7):2324-37 (1997)). However, the natural ligands for the P2Y2 receptor are
ATP and UTP, both of which are rapidly degraded by ubiquitous extracellular nucleotidases. Therefore, in order for ATP and UTP to be efficacious in the treatment of retinal detachment, these compounds need to be delivered directly into the subretinal space. However, drug delivery into the subretinal space is widely regarded to be unacceptably risky for patients because it involves the insertion of a needle between the retina and RPE, which can result in complications and blindness. Therefore, in order for ATP or UTP to be therapeutically useful, it must be delivered into the intravitreal cavity, which is a much less invasive procedure. However, in order for ATP or UTP to reach the RPE apical membrane, it must diffuse across the retina. It is unknown if intravitreal ATP or UTP is degraded by the time it reaches the RPE apical membrane and therefore effective in stimulating retinal reattachment. The present examples show that intravitreal UTP is ineffective in stimulating retinal reattachment and that novel hydrolysis resistant agonists are effective in stimulating retinal reattachment.

**SUMMARY OF THE INVENTION**

Pharmaceutical compositions and methods of use thereof for stimulating removal of extraneous fluid in the retina or subretinal space in a subject in need of such treatment are disclosed. The methods and compositions disclosed in the present invention are used to stimulate removal of extraneous intra-retinal or subretinal fluid for any reason, including, but not limited to, primary and adjunctive treatments of rhegmatogenous retinal detachment, serous retinal detachment, all forms of cystoid macular edema (uveitis, post-surgical, central and branch vein occlusion, and inherited retinal diseases such as retinitis pigmentosa), and all forms of retinal and macular edema (proliferative and non-proliferative, exudative age-related macular degeneration, and retinopathy of prematurity.)

The present invention discloses methods of treating a subject with retinal disorders such as retinal detachment or retinal edema by administering a pharmaceutical composition according to Formula I via intravitreal injection, intravitreal sustained release or delivery, ocular surface instillation, transcleral injection or infusion, or systemic injection or infusion.

The pharmaceutical compositions useful in this invention comprise P2Y receptor agonists, including certain adenine-, uridine-, and cytidine-containing dinucleoside polyphosphates, which are selective agonists of the P2Y receptor on epithelial cells of the retinal pigment epithelium. Activation of P2Y receptors by such agonists is associated with elevated intracellular calcium levels and increased fluid transport across the RPE.

The present invention also provides a novel composition of compounds of Formula I,
wherein the furanosyl sugar moieties of Formula I are selected from the group consisting of 3'-deoxyribofuranosyl, 2',3'-dideoxyribofuranosyl, arabinofuranosyl, 3'-deoxyarabinofuranosyl, xylofuranosyl, 2'-deoxyxylofuranosyl, and lyxofuranosyl.

**DESCRIPTION OF THE FIGURES**

FIGURE 1 represents cellular localization of P2Y2 receptor mRNA in fresh frozen cross sections of albino rabbit retina/RPE/choroid tissue using nonisotopic in situ hybridization techniques. Specifically, a representative in situ hybridization result from antisense and sense digoxigenin (DIG)-labeled riboprobes engineered based on the P2Y2 receptor mRNA sequence is shown. GCL: ganglion cell layer. IPL: inner plexiform layer. INL: inner nuclear layer. ONL: outer nuclear layer. IS: inner segments. OS: outer segments.

FIGURE 2 represents the effects of INS37217 (UP4dC) versus UTP on cytosolic calcium mobilization in 1321N1 cells overexpressing P2Y2 receptor.

FIGURE 3 represents the effect of INS37217 (UP4dC) versus UTP on inositol phosphate generation in 1321N1 cells overexpressing P2Y2 receptor.

FIGURE 4 represents the effects of INS372 17 (UP4dC) on fluid absorption in human fetal RPE.

FIGURE 5 represents the effects of INS37217 (UP4dC) on the magnitude and direction of fluid transport in bovine RPE.

FIGURE 6 represents the metabolism rates of INS37217 (UP4dC) and UTP from freshly isolated pig retinal tissue.

FIGURE 7 shows the effects of intravitreal INS37217 on size of subretinal blebs, as evaluated using indirect ophthalmoscopy, in large retinal detachments made in young pigs.

FIGURE 8 shows the effects of subretinal INS37217 on reabsorption of subretinal blebs. Subretinal blebs were created by injecting MPBS solution into the subretinal space with or without INS37217 (1 mM). Summarized results (mean ± SEM) show that INS37217 increased the rate of clearance of subretinal blebs when compared with vehicle control.

FIGURE 9 (A-C) shows the effects of intravitreal INS37217 on reabsorption of subretinal blebs. MPBS solution was injected into the subretinal to create subretinal blebs, followed immediately by an intravitreal injection of MPBS solution with or without INS37217 (12 mM, 1.4 mM, and 0.15 mM). Summarized results show that INS37217 administered at 12 and 1.4 mM, but not 0.15 mM, increased the rate of clearance of subretinal blebs when compared with vehicle control.
FIGURE 10 shows a significant difference (p < 0.05) between INS37217 (open bars) and vehicle control (placebo, solid bars) on the scoring of subretinal blebs.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention provides a method of increasing fluid absorption across the retinal pigment epithelium (RPE) to facilitate the removal of extraneous intraretinal or subretinal fluid from the posterior portion of the eye to treat diseases that lead to retinal detachment and retinal edema. The method comprises administering to a subject a P2Y receptor agonist in an amount effective to stimulate the removal of pathological fluid accumulation in intra-retinal and subretinal spaces associated with edematous retinal disorders.

The P2Y receptor agonist is administered with or without other therapeutic and adjuvant agents commonly used to treat or manage retinal detachment and retinal edema. An effective dose will be the amount of such agonist necessary to activate P2Y receptors at the retinal-facing (apical) membrane of retinal pigment epithelial cells and to enhance fluid absorption (retinal-to-choroidal direction) across the RPE. The present invention provides a method for stimulating the removal of extraneous fluid from the retina and subretinal spaces, and thus can be useful in the prevention, management and treatment of edematous retinal disorders such as retinal detachment and retinal edema.

The method of the present invention is useful for the management and/or treatment of all disorders associated with retinal detachment and retinal edema, including but not limited to rhegmatogenous retinal detachment, serous retinal detachment, all forms of cystoid macular edema (uveitis, post-surgical, central and branch vein occlusion, and inherited retinal diseases such as retinitis pigmentosa), and all forms of retinal and macular edema (proliferative and non-proliferative diabetic macular edema, exudative age-related macular degeneration, and retinopathy of prematurity).

This invention provides a method of administering to a subject a pharmaceutical composition comprising P2Y receptor agonists for removing pathological fluid accumulation in subretinal and intra-retinal spaces. P2Y receptor agonists include dinucleoside polyphosphate and their analogues, which activate P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11 and preferably P2Y2 receptors.
Description of Compounds

Dinucleoside polyphosphate useful for this invention are compounds of general Formula I or the pharmaceutically acceptable non-toxic salts thereof:

wherein:

X is oxygen, methylene, dihalomethylene (with difluoromethylene and dichloromethylene preferred), or imido;

n = 0, 1 or 2;

m = 0, 1 or 2;

n + m = 0, 1, 2, 3, or 4;

Z = OH or H;

Z' = OH or H;

Y = OH or H;

Y' = OH or H; and

B and B' are each independently a purine residue or a pyrimidine residue, as defined in Formula Ia or Ib, linked through the 9- or 1-position, respectively;
R₁ is hydrogen, chlorine, amino, monosubstituted amino, disubstituted amino, alkylthio, arylthio, or aralkylthio, wherein the substituent on sulfur contains up to a maximum of 20 carbon atoms, with or without unsaturation;

R₂ is hydroxy, alkenyl, o xo, amino, mercapto, thione, alkylthio, arylthio, aralkylthio, acylthio, alkyloxy, aryloxy, aralkyloxy, acyloxy, monosubstituted alkylamino, heterocyclic, monosubstituted cycloalkylamino, monosubstituted aralkylamino, monosubstituted arylamino, diaralkylamino, diarylamino, dialkylamino, acylamino, or diacylamino,

R₅ is O, H or is absent;

R₂ and R₅ are optionally taken together to form a 5-membered fused imidazole ring of 1,N₆-etheno adenine derivatives, optionally substituted on the 4- or 5-positions of the etheno moiety with alkyl, aryl or aralkyl moieties as defined below;

R₃ is hydrogen, azido, alkoxy, aryloxy, aralkyloxy, alkylthio, arylthio, or aralkylthio as defined below; or T(C₁₆alkyl)OCONH(C₁₆alkyl)W, wherein T and W are independently amino, mercapto, hydroxy or carboxyl; or pharmaceutically acceptable esters, amides or salts thereof; or absent;

Thus, the substituted derivatives of adenine include adenine 1-oxide; 1,N₆-(4- or 5-substituted etheno) adenine; N₆-substituted adenine; or N-substituted 8-aminoadenine, where R' of the 6- or 8-HNR' groups are chosen from among: arylalkyl (C₁₋₆) groups with the aryl moiety optionally functionalized as described below; alkyl; and alkyl groups with functional groups therein, such as: ([6-aminohexyl]carbamoylmethyl)-, and ω-acylated- amino(hydroxy, thiol and carboxy)alkyl(C₂₋₁₀)- and their ω-acylated-amino (hydroxy, thiol and carboxy) derivatives where the acyl group is chosen from among, but not limited to, acetyl, trifluoroacetyl, benzoyl, substituted-benzoyl, etc., or the carboxylic moiety is present as its

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ester or amide derivative, for example, the ethyl or methyl ester or its methyl, ethyl or benzamido derivative. The $\omega$-amino(hydroxy, thiol) moiety can be alkylated with a C$_{1-4}$ alkyl group;

    J is carbon or nitrogen, with the provision that when J is nitrogen, R$_3$ is not present;

wherein the alkyls are straight-chain, branched or cyclic;

wherein the aryl groups are optionally substituted with lower alkyl, aryl, amino, mono- or dialkylamino, NO$_2$, N$_3$, cyano, carboxylic, amido, sulfonamido, sulphonic acid, phosphate, or halo group;

\[ \text{Formula Ib} \]

wherein:

R$_4$ is hydroxy, oxo, mercapto, thione, amino, cyano, C$_{7-12}$aryloxy, C$_{1-6}$ alkythio, C$_{1-6}$ alkoxy, C$_{1-6}$ alkylamino or diC$_{1-4}$alkylamino, wherein the alkyl groups are optionally linked to form a heterocycle;

R$_5$ is hydrogen, acetyl, benzoyl, C$_{1-6}$ alkyl, C$_{1-5}$ alkanoyl, aroyl, or absent;

R$_6$ is hydroxy, oxo, mercapto, thione, C$_{1-4}$alkoxy, C$_{7-12}$aryloxy, C$_{1-4}$alkythio, S-phenyl, arlythio, arylalkythio, triazolyl, amino, C$_{1-6}$alkylamino, C$_{1-5}$ disubstituted amino, or di-C$_{1-4}$alkylamino, wherein said dialkyl groups are optionally linked to form a heterocycle or linked to form a substituted ring such as morpholino, pyrrolo, etc.; or

R$_5$ and R$_6$ taken together form a 5-membered fused imidazole ring between positions 3 and 4 of the pyrimidine ring and form a 3,N$^4$-ethenocytosine derivative, wherein said etheno moiety is optionally substituted on the 4- or 5-positions with C$_{1-4}$ alkyl, phenyl or phenolxy;

wherein at least one hydrogen of said C$_{1-4}$ alkyl, phenyl or phenolxy is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy, C$_{1-4}$ alkoxy, C$_{1-4}$ alkyl, C$_{6-10}$ aryl, C$_{7-12}$ arylalkyl, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino, C$_{1-4}$ alkylamino, and di- C$_{1-4}$ alkylamino, wherein said dialkyl groups are optionally linked to form a heterocycle;
R₇ is selected from the group consisting of hydrogen, hydroxy, cyano, nitro, C₁₋₆ alkyl or phenyl; substituted C₂₋₄ alkynyl, halogen, substituted C₁₋₄ alkyl, CF₃, C₂₋₃ alkenyl, C₂₋₃ alkynyl, allylamino, bromovinyl, ethyl propenoate, or propenoic acid and C₂₋₄ alkenyl; or

R₆ and R₇ together form a 5 or 6-membered saturated or unsaturated ring bonded through N or O or S at R₆, such ring optionally contains substituents that themselves contain functionalities; and

R₈ is selected from the group consisting of hydrogen, amino, di-C₁₋₄ alkylamino, C₁₋₄ alkoxy, C₇₋₁₂ arylalkoxy, C₁₋₄ alkylthio, C₇₋₁₂ arylalkylthio, carboxamidomethyl, carboxymethyl, methoxy, methylthio, phenoxy, and phenylthio; provided that when R₈ is amino or substituted amino, R₇ is hydrogen.

The ribosyl moieties are in the D-configuration, as shown, but can be L-, or D-and L-. The D-configuration is preferred. The nucleoside residues include the sugar moieties ribofuranosyl, arabinofuranosyl, 2′-deoxyribofuranosyl, 3′-deoxyribofuranosyl, 2′,3′-dideoxyribofuranosyl, xylofuranosyl, 2′-deoxyxylofuranosyl and lyxofuranosyl; and can be in the alpha-or beta-and D-or L-configurations, but most preferably the beta-D-configuration.

In the general structure of Formulae I, Ia and Ib above, the dotted lines in the 2-to 6-positions are intended to indicate the presence of single or double bonds in these positions; the relative positions of the double or single bonds being determined by whether the R₄, R₅ and R₆ substituents are capable of keto-enol tautomerism.

In the general structures of Formula I above, the acyl groups comprise alkanoyl or aroyl groups. The alkyl groups contain 1 to 8 carbon atoms, particularly 1 to 4 carbon atoms optionally substituted by one or more appropriate substituents, as described below. The aryl groups including the aryl moieties of such groups as arylxy are preferably phenyl groups optionally substituted by one or more appropriate substituents, as described below. The above-mentioned alkenyl and alkynyl groups contain 2 to 8 carbon atoms, particularly 2 to 6 carbon atoms, e.g., ethenyl or ethynyl, optionally substituted by one or more appropriate substituents as described below.

Appropriate substituents on the above-mentioned alkyl, alkenyl, alkynyl, and aryl groups are selected from halogen, hydroxy, C₁₋₄ alkoxy, C₁₋₄ alkyl, C₆₋₁₂ aryl, C₆₋₁₂ arylalkoxy, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic, amino and substituted amino wherein the amino is singly or doubly substituted by a C₁₋₄ alkyl, and when doubly substituted, the alkyl groups optionally being linked to form a heterocycle.
Dinucleoside polyphosphates of general Formula I include dinucleoside tetraphosphates selected from the group consisting of P\(^{1}\)-P\(^{4}\)-di (uridine 5\(^{-}\)-tetraphosphate (UP\(_{4}\)U); P\(^{1}\)-(cytosine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-tetraphosphate; P\(^{1}\),P\(^{4}\)-di(adenosine 5\(^{-}\)-tetraphosphate; P\(^{1}\)-(adenosine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-tetraphosphate; P\(^{1}\)-P\(^{4}\)-di(ethenoadenosine)tetraphosphate; P\(^{1}\)-(uridine 5\(^{-}\))-P\(^{4}\)-(cytosine 5\(^{-}\)-tetraphosphate; P\(^{1}\),P\(^{4}\)-di(ethenoadenosine)tetraphosphate; P\(^{1}\)-(uridine 5\(^{-}\))-P\(^{4}\)-(thymidine 5\(^{-}\))-tetraphosphate; P\(^{1}\)-(adenosine 5\(^{-}\))-P\(^{4}\)-(inosine 5\(^{-}\)-tetraphosphate; P\(^{1}\),P\(^{4}\)-di(adenosine 5\(^{-}\))-P\(^{4}\)-methylene tetraphosphate; P\(^{1}\),P\(^{4}\)-di(adenosine 5\(^{-}\))-P\(^{4}\)-difluoromethylenetetraphosphate; P\(^{1}\),P\(^{4}\)-di(adenosine 5\(^{-}\))-P\(^{4}\)-imidotetraphosphate; P\(^{1}\),P\(^{4}\)-di(4-thiouridine 5\(^{-}\)-tetraphosphate; P\(^{1}\),P\(^{4}\)-di(3\(^{\prime}\),N\(^{4}\)-ethenocytidine 5\(^{-}\))-tetraphosphate; P\(^{1}\),P\(^{4}\)-di(imidazo[1,2-c]pyrimidine-5(6H)-one-2-(3-nitro)-phenyl-6-β-D-ribofuranoside 5\(^{-}\)-tetraphosphate, tetraammonium salt; P\(^{1}\)-(adenosine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-tetraphosphate; P\(^{1}\)-(4-thiouridine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-tetraphosphate; P\(^{1}\)-(cytosine β-D-arabinofuranoside 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-tetraphosphate; P\(^{1}\)-(uridine 5\(^{-}\))-P\(^{4}\)-(xanthosine 5\(^{-}\)-tetraphosphate; P\(^{1}\)-(2\(^{-}\)-deoxyuridine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-tetraphosphate; P\(^{1}\),P\(^{4}\)-di(3\(^{\prime}\)-azido-3\(^{-}\)-deoxythymidine 5\(^{-}\)-)tetraphosphate; P\(^{1}\),P\(^{4}\)-di(3\(^{\prime}\)-azido-3\(^{-}\)-deoxythymidine 5\(^{-}\)-)tetraphosphate; P\(^{1}\),P\(^{4}\)-di(3\(^{\prime}\)-azido-3\(^{-}\)-deoxythymidine 5\(^{-}\)-)tetraphosphate; 2\(^{\prime}\)(3\(^{-}\))-benzoyl-P\(^{1}\),P\(^{4}\)-di(adenosine 5\(^{-}\)-)tetraphosphate; P\(^{1}\),P\(^{4}\)-di(2\(^{-}\),3\(^{-}\))-benzoyl uridine 5\(^{-}\)- tetraphosphate; P\(^{1}\)-(2\(^{-}\)-deoxyguanosine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(2\(^{-}\)-deoxyadenosine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(2\(^{-}\)-deoxyadenosine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(2\(^{-}\)-deoxyadenosine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(2\(^{-}\)-deoxyadenosine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(4-thiouridine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(4-thiouridine 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(6-mercaptopurine riboside 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(6-mercaptopurine riboside 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(6-mercaptopurine riboside 5\(^{-}\))-P\(^{4}\)-(2\(^{-}\)-deoxyuridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(4-thiouridine 5\(^{-}\))-P\(^{4}\)-(arabinocytidine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(adenosine 5\(^{-}\))-P\(^{4}\)-(4-thiomethyluridine 5\(^{-}\)-)tetraphosphate; P\(^{1}\)-(2\(^{-}\)-deoxyadenosine 5\(^{-}\))-P\(^{4}\)-(6-thiohexyluridine riboside 5\(^{-}\)-)tetraphosphate, and P\(^{1}\)-(6-eicosanoyloxypurine riboside 5\(^{-}\))-P\(^{4}\)-(uridine 5\(^{-}\)-)tetraphosphate. UP\(_{4}\)U and dCP\(_{4}\)U are preferred compounds.

Dinucleoside polyphosphates of general Formula I include dinucleoside triphosphates selected from a group consisting of: P\(^{3}\)-di (uridine 5\(^{-}\)-)triphosphate; P\(^{1}\)-(cytosine 5\(^{-}\))-P\(^{3}\)-(uridine 5\(^{-}\)-)triphosphate; P\(^{1}\),P\(^{3}\)-di(adenosine 5\(^{-}\)-)triphosphate; P\(^{1}\)-(adenosine 5\(^{-}\))-P\(^{3}\)-(cytosine 5\(^{-}\)-)triphosphate; P\(^{1}\),P\(^{3}\)-di(ethenoadenosine)triphosphate; P\(^{1}\)-(uridine 5\(^{-}\))-P\(^{3}\)-(thymidine 5\(^{-}\)-)triphosphate; P\(^{1}\)-(adenosine 5\(^{-}\))-P\(^{3}\)-(inosine 5\(^{-}\)-)triphosphate; P\(^{1}\),P\(^{3}\)-di(2-thiouridine 5\(^{-}\)-)P\(^{3}\)-methylene tetraphosphate;
P1, P3-di(adenosine 5'-P2, P3-di(fluoromethylenetriphosphate); P1, P3-di(adenosine 5'-P2, P3-imidotriphosphate); P1, P3-di(thiouridine 5'-triphosphate); P1, P3-di(3, N1-ethenocytidine 5'-triphosphate; P1, P3-di(imidazol[1,2-c]pyrimidine-5(6H)-1-one-2(3-nitro)-phenyl-6-β-D-ribofuranoside 5'-triphosphate; tetrammonium salt; P1-(inosine 5')-P3-(uridine 5')-triphosphate; P1-(4-thiouridine 5')-P3-(uridine 5')-triphosphate; P1-(cytosine β-D-arabinofuranoside 5')-P3-(uridine 5')-triphosphate; P1-(uridine 5')-P3-(xanthosine 5'-triphosphate; P1-(2'-deoxyuridine 5')-P3-(uridine 5')-triphosphate; P1-(3'-azido-3'-deoxythymidine 5')-P3-(uridine 5')-triphosphate; P1, P3-di(3'-azido-3'-deoxythymidine 5'-triphosphate; P1, P2-di(3'-azido-3'-deoxythymidine 5'-triphosphate; 2'-O(benzoyl)-P1, P3-di(uridine 5')-triphosphate; P1, P3-di(2'-(3')-benzoyl uridine 5')-triphosphate; P1-(2'-deoxyguanosine 5')-P3-(uridine 5')-triphosphate; P1-(2'-deoxyadenosine 5')-P3-(uridine 5')-triphosphate; P1-(2'-deoxyinosine 5')-P3-(uridine 5')-triphosphate; P1-(2'-deoxythymidine 5')-P3-(uridine 5')-triphosphate; P1-(4-thiouridine 5')-P3-(uridine 5')-triphosphate; P1-(8-azaadenosine 5')-P3-(uridine 5')-triphosphate; P1-(6-mercaptopurine riboside 5')-P3-(uridine 5')-triphosphate; P1-(6-mercaptopurine riboside 5')-P3-(2'-deoxyuridine 5')-triphosphate; P1-(4-thiouridine 5')-P3-(arabinocytidine 5')-triphosphate; P1-(adenosine 5')-P3-(4-thiomethyluridine 5')-triphosphate; P1-(2'-deoxyadenosine 5')-P2-(6-thiohexylypurine riboside 5')-tetraphosphate; and P1-(6-mercaptopurine riboside 5')-P3-(uridine 5')-triphosphate.

Furthermore, dinucleoside polyphosphates of general Formula III include compounds selected from a group consisting of: P1-(uridine 5')-P2-(4-thiouridine 5')-diphosphate; P1, P5-di(uridine 5')-pentaphosphate; and P1, P6-di(uridine 5')-hexaphosphate.

A preferred nucleotide agonist is a hydrolysis-resistant agonist. A hydrolysis-resistant agonist is a nucleotide with a modified phosphate ester backbone, e.g. a methylene, imido or other group that protects the phosphate ester bonds from being readily hydrolyzed.

Dinucleotides are also resistant to hydrolysis due to a lack of a terminal phosphate group. Certain dinucleotides are especially resistant to hydrolysis. For example, P1-(cytosine 5')-P4-(uridine 5')-tetraphosphate is more resistant in comparison with P1, P4-di(uridine 5')-tetraphosphate. Furthermore, groups placed on the end of the phosphate chain of a mononucleotide impart some stability against hydrolysis, e.g. simple alkyl phosphate esters (methyl, ethyl, benzyl, etc.) or a thio group (e.g. UTPgammaS).

The present invention also provides a novel composition of compounds of Formula I, wherein the furanosyl sugar moieties of Formula I are selected from the group consisting of 3'-deoxyribofuranosyl, 2',3'-dideoxyribofuranosyl, arabinofuranosyl, 3'-
deoxyarabinofuranosyl, xylofuranosyl, 2'-deoxyxylofuranosyl, and lyxofuranosyl.

The follow specific compounds are also novel: P\(^1\)-(6-mercaptopurine riboside 5'-)P\(^4\)-
(uridine 5'-)tetraphosphate, P\(^1\)-(6-mercaptouridine riboside 5'-)P\(^4\)-(2'-deoxyuridine 5'-)
tetraphosphate, P\(^1\)-(4-thiouridine 5'-)P\(^4\)-(arabinocytidine 5'-)tetraphosphate, P\(^1\)-(2'-
deoxycytidine 5'-)P\(^4\)-(6-thiohexylpurine riboside 5'-) tetraphosphate, P\(^1\)-(6-
eicosanyloxypurine riboside 5'-)P\(^4\)-(uridine 5'-)tetraphosphate, P\(^1\)-(arabinoadenosine-5')P\(^4\)-
(uridine-5')tetraphosphate, P\(^1\)-(lyxofuranosylthymine-5')P\(^4\)-(uridine-5')tetraphosphate, and
P\(^1\)-(xylofuranosyluracil-5')P\(^4\)-(uridine-5')tetraphosphate.

Compounds encompassed by the present invention can be prepared by condensation of
a nucleoside mono-, di-, or triphosphate, activated with a condensing agent such as, but not
limited to, carbonyldimidazole or dicyclohexylcarbodiimide, with a second molecule of the
same or a different mono-, di-, or triphosphate to form the desired dinucleotide polyphosphate.
Another method of preparation is the sequential condensation of a nucleoside phosphate,
activated as above, with a non-nucleoside mono-, di- or polyphosphate moiety, such as, but not
limited, to a monophosphate or pyrophosphate anion to yield the desired dinucleotide
polyphosphate, the non-isolated intermediate in such a case being a mononucleotide
polyphosphate. Yet another preparative approach is the sequential condensation of a mono-,
di- or polyphosphate moiety, activated as mentioned above, or in the form of an acid halide or
other derivative reactive toward nucleophilic displacement, with a nucleoside phosphate or
polyphosphate to yield the desired dinucleotide polyphosphate. The desired dinucleotide
polyphosphate can be formed by modification of a pre-formed dinucleotide polyphosphate by
substitution or derivatization of a moiety or moieties on the purine, pyrimidine or carbohydrate
ring. Nucleoside phosphates used as starting materials are commercially available, or can be
made from the corresponding nucleosides by methods well known to those skilled in the art.
Likewise, where nucleosides are not commercially available, they can be made by
modification of other readily available nucleosides, or by synthesis from heterocyclic and
carbohydrate precursors by methods well known to those skilled in the art (WO 96/40059,
(1995); Visscher, et al., Nucleic Acids Research 20, 5749-5752 (1992); Holler, et al.,
Biochemistry 22,4924-10 4933 (1983); Orr, et al., Biochem. Pharmacol. 673-677 (1988);
Plateau, et al., Biochemistry 24,914-922 (1985); Hagneier, et al., J. Chromatography 237,
174-177 (1982); Scheffzek, et al., Biochemistry 35,9716-9727 (1996); Stridh, et al., Antiviral

Those having skill in the art will recognize that the starting materials can be varied and additional steps employed to produce compounds encompassed by the present invention, as demonstrated by the following examples. In some cases, protection of certain reactive functionalities is useful to achieve some of the above transformations. In general, the need for such protecting groups will be apparent to those skilled in the art of organic synthesis as well as the conditions necessary to attach and remove such groups.

The compounds of the present invention also encompass their non-toxic pharmaceutically acceptable salts, such as, but not limited to, an alkali metal salt such as lithium, sodium or potassium; an alkaline earth metal salt such as manganese, magnesium or calcium; or an ammonium or tetraalkyl ammonium salt, i.e., NX₄⁺ (wherein X is C₁₋₄).

Pharmaceutically acceptable salts are salts that retain the desired biological activity of the parent compound and do not impart undesired toxicological effects. The present invention also encompasses the acylated prodrugs of the compounds disclosed herein. Those skilled in the art will recognize various synthetic methodologies, which can be employed to prepare non-toxic pharmaceutically acceptable salts and acylated prodrugs of the compounds.

Though the compounds of the present invention are primarily concerned with the treatment of human subjects, they can also be employed for the treatment of other mammalian subjects such as dogs and cats for veterinary purposes.

The pharmaceutical utility of compounds of this invention is indicated by the inositol phosphate assay for P2Y₂ and other P2Y receptor activity. This widely used assay, as described in Lazarowski, et al. (1995)(Brit. J. Pharm. 116, 1619-27), relies on the measurement of inositol phosphate formation as a measurement of activity of compounds activating receptors linked via G-proteins to phospholipase C.

The efficacy of these compounds is reflected in their ability to facilitate removal of pathological fluid accumulation in the sub-retinal and intra-retinal spaces, associated with edematous retinal disorders including retinal detachment and retinal edema. The effective dose will depend on characteristics of the individual patient, activity of the specific compound employed, mode of administration, and characteristics of the disease or disorder, and can be determined by those skilled in the art.
Dosage levels to remove extraneous fluid within intra-retinal or subretinal spaces are of the range of 10 μg/eye to 10 mg/eye, preferably in the range 50 μg/eye to 6 mg/eye, and most preferably 0.1 mg/eye to 4 mg/eye.

Administration of Compounds

The active compounds disclosed herein can be administered to the eyes of a patient by any suitable means, but are preferably administered by administering a liquid or gel suspension of the active compound in the form of drops, spray or gel. Alternatively, the active compounds can be applied to the eye via liposomes. Further, the active compounds can be infused into the tear film via a pump-catheter system. Another embodiment of the present invention involves the active compound contained within a continuous or selective-release device, for example, membranes such as, but not limited to, those employed in the Ocusert™ System (Alza Corp., Palo Alto, CA). As an additional embodiment, the active compounds can be contained within, carried by, or attached to contact lenses that are placed on the eye.

Another embodiment of the present invention involves the active compound contained within a swab or sponge that can be applied to the ocular surface. Another embodiment of the present invention involves the active compound contained within a liquid spray that can be applied to the ocular surface. Another embodiment of the present invention involves an injection of the active compound directly into the lachrymal tissues or onto the eye surface.

The active compounds disclosed herein are preferably administered by administering an aqueous suspension into the vitreous. Intravitreal administration comprising: single or multiple intravitreal injections; administration directly into the vitreal chamber during surgery separately or in conjunction with intraocular irrigation solutions, or other similar solutions or devices, routinely used during vitreoretinal surgery; administration via liposomes or other suitable pharmaceutical carriers; administration via continuous or selective-release intravitreal-implantable devices, including, but not limited to, Ocusert™ (Alza Corp., Palo, Alto, CA) and Vitrasert (Bausch and Lomb, Inc., Rochester, NY). The intravitreal solution containing the active compound optionally contains a physiologically compatible vehicle, as those skilled in the ophthalmic art can select using conventional criteria. The vehicles are selected from the known ophthalmic vehicles which include, but are not limited to, saline solution, water polyethers such as polyethylene glycol, polyvinyls such as polyvinyl alcohol and povidone, cellulose derivatives such as methylcellulose and hydroxypropyl methylcellulose, petroleum derivatives such as mineral oil and white petrolatum, animal fats
such as lanolin, polymers of acrylic acid such as carboxypolymethylene gel, vegetable fats such as peanut oil and polysaccharides such as dextrans, and glycosaminoglycans such as sodium hyaluronate and salts such as sodium chloride and potassium chloride. The preferred embodiment is an intravitreal solution comprising active compound and saline at neutral pH and physiological osmolarity.

The topical solution containing the active compound can also contain a physiologically compatible vehicle, as those skilled in the ophthalmic art can select using conventional criteria. The vehicles are selected from the known ophthalmic vehicles which include, but are not limited to, saline solution, water polyethers such as polyethylene glycol, polyvinyls such as polyvinyl alcohol and povidone, cellulose derivatives such as methylcellulose and hydroxypropyl methylcellulose, petroleum derivatives such as mineral oil and white petrolatsum, animal fats such as lanolin, polymers of acrylic acid such as carboxypolymethylene gel, vegetable fats such as peanut oil and polysaccharides such as dextrans, and glycosaminoglycans such as sodium hyaluronate and salts such as sodium chloride and potassium chloride.

In addition to the topical method of administration described above, there are various methods of administering the active compounds of the present invention systemically. One such means would involve an aerosol suspension of respirable particles comprised of the active compound, which the subject inhales. The active compound would be absorbed into the bloodstream via the lungs or contact the ocular tissues via the nasolacrimal ducts, and subsequently contact the retinal pigment epithelial cells in a pharmaceutically effective amount. The respirable particles can be liquid or solid, with a particle size sufficiently small to pass through the mouth and larynx upon inhalation; in general, particles ranging from about 1 to 10 microns, but more preferably 1-5 microns, in size are considered respirable.

Another means of systemically administering the active compounds to the eyes of the subject would involve administering a liquid/liquid suspension in the form of eye drops or eye wash or nasal drops of a liquid formulation, or a nasal spray of respirable particles that the subject inhales. Liquid pharmaceutical compositions of the active compound for producing a nasal spray or nasal or eye drops can be prepared by combining the active compound with a suitable vehicle, such as sterile pyrogen free water or sterile saline by techniques known to those skilled in the art.

Other means of systemic administration of the active compound would involve oral administration, in which pharmaceutical compositions containing compounds of Formula I are
in the form of tablets, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more agents selected from the group consisting of: of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with nontoxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients include, for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example, starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate can be employed. Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin or olive oil.

Additional means of systemic administration of the active compound to the eyes of the subject would involve a suppository form of the active compound, such that a therapeutically effective amount of the compound reaches the eyes via systemic absorption and circulation.

Further means of systemic administration of the active compound would involve direct intra-operative instillation of a gel, cream, or liquid suspension form of a therapeutically effective amount of the active compound.

The method of the present invention is useful to enhance the effects of surgery, pharmacotherapy, and adjunctive agents used to treat and manage disorders associated with retinal detachment and retinal edema. Surgical approaches include scleral buckle, pneumatic retinopexy, macular translocation and vitrectomy. Pharmacotherapeutic agents such as corticosteroids and acetazolamide have been used to manage macular edema.

High doses may be required for some therapeutic agents to achieve levels to effectuate the target response, but may often be associated with a greater frequency of dose-related adverse effects. Thus, combined use of the compounds of the present invention with agents
commonly used to treat retinal detachment and retinal edema permits relatively lower doses of such agents resulting in a lower frequency of adverse side effects associated with long-term administration of such therapeutic agents. Thus, another indication of the compounds in this invention is to reduce adverse side effects of drugs used to treat retinal detachment and retinal edema, such as the development of systemic effects with acetazolamide.

The invention is illustrated further by the following examples, which are not to be construed as limiting the invention in scope or spirit to the specific procedures described in them.

EXAMPLES

Example 1. Localization of P2Y2-Receptor mRNA in Retina and RPE

Cellular localization of P2Y2-receptor mRNA in fresh frozen cross-sections of albino rabbit retina/RPE/choroid tissue was investigated by using nonisotopic in situ hybridization techniques. Figure 1 shows a representative in situ hybridization result from antisense and sense digoxigenin (DIG)-labeled riboprobes engineered based on the P2Y2 receptor mRNA sequence. Hybridization of antisense and sense riboprobes was visualized by immunohistochemistry using alkaline phosphatase-conjugated anti DIG antibody, and DIG-specific signal was detected using a chromophore reaction against the alkaline phosphatase, yielding purple/black staining. The tissues were also counterstained with nuclear fast red. The control sense probe (right) shows no specific labeling. Labeling with the anti-sense probe showed P2Y2 receptor mRNA localization in scattered nuclei in the ganglion cell and inner nuclear layers and through the inner segment layer of photoreceptors. Strong labeling throughout the RPE was also detected, and in endothelial cells of the choroidal blood vessels.

Example 2. Effects of Synthetic P2Y2 Agonist UP₄dC (INS37217) on Cloned Human P2Y2 Receptors

The dimucleotide [P1-(uridine 5’-)-P4-(2’-deoxycytidine 5’-)tetrathosphate tetrasodium salt](UP₄dC), also known as INS37217, was tested for its activity (potency, efficacy, and selectivity) at cloned human P2Y receptor subtypes, which were stably expressed in 132IN1 astrocytoma cells. Activity was assessed using two in vitro indices of cell activation: 1) mobilization of intracellular calcium stores, and 2) accumulation of [³H]-inositol phosphates ([³H]-IP). UP₄dC was evaluated for activity in both assays against cells expressing the P2Y₁, P2Y₂, P2Y₄, or P2Y₆ receptors.
UTP and UP₄dC induced mobilization of cytosolic calcium in 1321N1 astrocytoma cells expressing human P2Y₂ (Figure 2) receptors with EC₅₀ values of 0.22 μM and 0.8 μM, respectively. The calcium response to 100 PM UP₄dC was 100% of the maximal response to UTP at P2Y₂ receptors. In conclusion, UP₄dC is a full agonist for calcium mobilization at P2Y₂ receptors compared to UTP.

UTP and UP₄dC stimulated [³H]-IP accumulation in 1321N1 cells expressing human P2Y₁₀ P2Y₁, (Figure 3) receptors with an EC₅₀ values of 1.1 and 2.2 μM. The inositol phosphate response to 100 μM UP₄dC was approximately that of the maximal response to UTP. In conclusion, UP₄dC is a full agonist for inositol phosphate release at P2Y₂ receptors compared to UTP in the test system.

**Example 3. UP₄dC (INS37217) Stimulates Fluid Absorption in Freshly Isolated RPE Monolayers**

Fluid transport across freshly isolated, intact bovine and human fetal RPE monolayers was studied using a modified capacitance probe technique (Frambach, et al., Biophys. J. 47(4):547-52 (1985); Hughes, et al., J Gen. Physiol. 83(6):875-99 (1984)).

The RPE was mounted vertically in a modified Ussing chamber such that apical and basolateral membranes were separately exposed to Ringer's solutions held in bathing reservoirs. Stainless steel capacitative probes were lowered into the apical and basolateral bathing reservoirs to sense the capacitance of the air gap between the probe and fluid meniscus. Fluid transport rate Jᵥ (μL cm⁻² hr⁻¹) was determined by monitoring fluid movement-induced changes in the air gap capacitance at the apical and basolateral baths.

Representative effects of agonist on Jᵥ in human fetal RPE are shown in Figure 4. Positive Jᵥ values reflect fluid absorption (apical-to-basolateral) and negative Jᵥ values reflect fluid secretion (basolateral-to-apical). In the experiment shown in Figure 4, control fluid movement across the freshly isolated human fetal RPE monolayer is absorptive at a rate of ~5 μL cm⁻² hr⁻¹. The addition of 50 μM agonist to Ringer's solution bathing the apical membrane elicited a transient increase in fluid absorption to ~40 μL cm⁻² hr⁻¹ before returning back to pre-stimulated levels. During the 1-hour treatment period, UP₄dC (INS37217) increased total fluid absorption by approximately a factor of three.

Although the RPE is normally a fluid-absorbing epithelium, fluid secretion has occasionally been observed in freshly isolated RPE preparations. It has been postulated that fluid secretion *in vivo* may be a normal component of RPE physiology under certain
conditions, such as following a transition between dark and light, or under pathological conditions, such as in serous retinal detachments. Figure 5 shows that in a freshly isolated bovine RPE monolayer in which J_v secretion is observed under control conditions, the agonist can reverse the direction of fluid transport to absorption. The effects of agonist are reversible upon returning to control Ringer’s solution. Such an effect of agonist in vivo will offer therapeutic potential in the treatment of serous retinal detachments, such as central serous retinopathy, in which abnormal RPE-mediated fluid secretion is postulated to mediate the effects of transport of choroidal fluid into the subretinal space.

Example 4. Comparisons of Metabolic Stability of UP₄dC (INS37217) and UTP in Freshly Isolated Retinal Tissue

The metabolism rate of UP₄dC (INS37217) and UTP in freshly isolated pig retinal tissue was determined using a high performance liquid chromatography (HPLC) method with UV-coupled detection. Freshly isolated retinal tissues of uniform size were isolated from euthanized young pigs (2-3 months) and retinal tissues were individually placed in an incubation chamber at 37°C. After an equilibration period of 30 minutes, each tissue was spiked with 100 μM UP₄dC or UTP in physiological buffer, and incubated at for 0.5, 1, 2, and 4 hours. An aliquot of buffer from each chamber well was then processed for W-coupled HPLC detection for chromatograms of the parent UP₄dC and UTP compounds at each time point to track the metabolism rates of each parent compound. Figure 6 shows that UP₄dC has a four-fold greater metabolic half-life than UTP under these experimental conditions.

Example 5. UP₄dC increases subretinal fluid reabsorption in pig models of large bullous retinal detachments.

Purpose

The present study is designed to evaluate the clinical benefit of UP₄dC on subretinal fluid reabsorption under normal surgical conditions in large experimental retinal detachments in pigs.

Methods

A central vitrectomy and a large retinal detachment extending 1-2 quadrants was created by injecting 300 μl of Balanced Salt Saline Plus (BSS+) into the subretinal space in one eye in each of 12 pigs. Either BSS+ without or with UP₄dC (5.6 mM) was injected into
the midvitreous cavity and observed over 3–7 days by indirect ophthalmoscopy, ultrasound and OCT-imaging.

Results

Complete reabsorption of subretinal fluid in the large retinal detachments was observed within 36 hours in all eyes treated with UP₄dC, and after 5–7 days in all control eyes. These results are shown in Figure 7.

Conclusion

Intravitreal application of UP₄dC enhanced the reabsorption rate of subretinal fluid in experimental animal models of small and large retinal detachments. UP₄dC may therefore be useful as a surgical adjunct to stimulate subretinal fluid reabsorption in retinal detachment or in limited macular translocation surgery in humans.

Example 6. Effects of subretinal and intravitreal UP₄dC in rabbit models

Methods

Surgical procedure for inducing subretinal blebs

New Zealand White rabbits weighing approximately 1.5 kg (2–3 month old) were anesthetized with an intramuscular injection of 0.3 ml ketamine hydrochloride (100 mg/ml) and 0.5 ml xylazine hydrochloride (100 mg/ml) per kilogram body weight. Ketamine hydrochloride was added as needed. For experiments requiring observation of the fundus, the pupil was dilated with scopolamine hydrobromide 0.25%, cycloglyl 1% and phenylephrine hydrochloride 2.5% eyedrops.

One local retinal detachment was created in each eye. A wire lid speculum was placed and a segmental conjunctival peritomy (of approximately 2 clock hours) was made at the 3 and 9 o’clock positions. Two scleral incisions were made with a 19 gauge MVR-blade 0.5 mm posterior to the limbus through the ciliary body. A self-retaining planoconcave contact lens was placed on the corneal surface. A chandelier light, which was used for illumination, (Grieshaber & Co., AG Schaffhausen, Switzerland) was carefully guided through one of the sclerotomy sites into the vitreous cavity to avoid touching the lens.

Retinal detachments were made with a beveled 36 gauge retinal needle (Grieshaber & Co., AG Schaffhausen, Switzerland) attached by an extension tube to a 1 ml syringe that was driven by a calibrated, mechanical syringe pump (model 351, Sage Instruments, Cambridge,
Mass). Under direct observation with an operating microscope, the retinal needle was inserted through the second sclerotomy and slowly advanced to either the nasal or temporal myelin wing. These sites were selected for injection as the myelin wing gives additional structural support when compared to the adjacent areas of thin, avascular retina. The intraocular pressure was maintained at a low level to allow a slow hydrodissection of the fragile retina from the RPE. The tip of the 36 gauge needle was carefully inserted under the myelin wing. A localized dome-shaped detachment of the retina was created by using a mechanical syringe pump to inject ~50 μl of phosphate buffered saline fluid into the subretinal space. The instruments were removed from the eye and the sclerotomy sites remained open to keep the intraocular pressure constant. Although these experimental retinal detachments have a very small retinal hole, they behave functionally like non-rhegmatogenous retinal detachments and have been used to study mechanisms of subretinal fluid reabsorption.

*Injection solution*

Modified phosphate buffered saline (MPBS) solution, used for all subretinal and intravitreal injections, was composed of 13.6 mM Na₂HPO₄, 6.2 mM NaH₂PO₄, 130.5 mM NaCl and 5 mM KCl, had an osmolarity of ~300 mOsm and a pH 7.2. UP₄dC (MW 862) was added to the MPBS solution to achieve a target drug concentration of 12 mM, 1.4 mM, 1.0 mM or 0.15 mM. The experimental and control solutions were kept at equal osmolarity. For concentrations of 1 mM UP₄dC or less an appropriate amount of NaCl was added to the MPBS solution to compensate for the osmolarity contribution of UP₄dC (1 mM UP₄dC contributes ~4-5 mOsm). For concentrations greater than 1 mM UP₄dC, solution isotonicity was maintained by reducing an equal osmolar of NaCl in the MPBS solution in place of the addition of UP₄dC. All experimental and control solutions for each dosing cohort were formulated such that the final osmolarities were ±2 mOsm of each other. Sterile solutions were provided and evaluated under investigator-masked conditions.

*Study design*

For each animal, one eye served as the experimental eye and the contralateral eye served as control. UP₄dC was delivered either subretinally or intravitreally to evaluate its effects on reabsorption of subretinal fluid in experimentally induced retinal detachments. In the first series of experiments, MPBS solution with or without UP₄dC (1 mM) was injected into the subretinal space. In the second series of experiments, a subretinal bleb containing
MPBS solution was created, and then 50 µl of MPBS solution with or without UP₄dC (12 mM, 1.4 mM, and 0.15 mM) was administered into the vitreous cavity with a 100 µl Hamilton syringe adjacent to the subretinal bleb. The surgeon was masked with respect to the content of the administered solutions.

Postoperative observation

The corneal epithelium was protected with a layer of methylcellulose to maintain corneal clarity. Fundus photographs were obtained with a fundus camera (TRC-W, TOPCON, Japan) in selected cases. The observer determined by indirect ophthalmoscopy the initial bleb size, and bleb size at 30-minute intervals for 3 hours. The vertical and horizontal dimensions of each subretinal bleb were recorded in disc diameters, using the adjacent optic disc as a reference marker. (The mean reference diameter of the optic disc is approximately 1 mm, as previously determined under a microscope in 10 enucleated eyes from albino rabbits). Bleb size at each evaluation time point was first quantified by multiplying the vertical and horizontal dimensions, and then expressing the resultant value as a dimensionless ratio relative to the initial size of each bleb. This dimensionless (normalized) bleb size at each evaluation time point was then plotted and analyzed as a function of time.

Results

Effects of subretinal and intravitreal UP₄dC

In the first series of experiments, an isotonic MPBS solution containing 1 mM UP₄dC was injected directly into the subretinal space. The contralateral eye received a subretinal injection of isotonic MPBS solution alone. Figure 8 shows that UP₄dC-containing subretinal blebs resolved significantly faster than blebs containing MPBS solution alone. Reattachment of the retina was observed at 120-150 minutes in the UP₄dC-treated blebs, whereas the control subretinal blebs did not resolve over the 3 hour observation period. There was a significant difference in subretinal reabsorption at 30, 60, and 90 minutes using repeated measures ANOVA (p<0.05).

In the second series of experiments, subretinal blebs were first induced with MPBS solution and then followed immediately with a 50 µl intravitreal injection of MPBS solution with or without UP₄dC (12, 1.4, and 0.15 mM) just adjacent to the bleb. Figures 9A & B shows that the two higher doses of UP₄dC significantly enhanced the rate of subretinal bleb reabsorption and retinal reattachment when compared with vehicle. There was a statistically significant difference in the reabsorption rate between the eye treated with UP₄dC and the vehicle alone at 30, 60, and
90 minutes (p<0.05). UP$_4$dC-treated eyes showed near complete retinal reattachment by ~90 min, whereas control blebs had not completely reabsorbed at 180 min.

Figure 9C shows the reabsorption rate of subretinal bleb in eyes treated with 0.15 mM intravitreal UP$_4$dC or vehicle alone. There was no statistically significant difference in the reabsorption of subretinal fluid at any time point in eyes with 0.15 mM UP$_4$dC compared to vehicle alone.

**Example 7. Effects of UP$_4$dC on retinal reattachment in rat models**

**Methods**

*In vivo preparation: Rat study design*

Retinal detachments were created in Long-Evans female rats by injecting 2 – 3 µl of modified phosphate buffer saline (MPBS) Ringer solution into the subretinal space; only one eye per rat was used. Using a CCD camera, images of the subretinal blebs were obtained at one min intervals for several hours. The acquisition of images is described in further detail below. In the control part of each experiment (at 0 to 30 min following creation of the retinal detachment), apparent bleb size reached a steady-state size, which remained unchanged during the course of anesthesia (several hours). MPBS solutions with or without UP$_4$dC (5 mM) were formulated and injected (3 µl) into the vitreous of the rat eye under masked and randomized conditions. The vials and their contents were indistinguishable. After vitreous injection, the apparent bleb size either increased or decreased monotonically or was constant over the next 60 min as judged by the experimenter using the seven rank scale (0-3) illustrated in Fig. 10. Ranks were assigned by observing the change in apparent bleb size between 30 - 90 minutes after drug or placebo vitreous injection. Animals were re-anesthetized the next day and a separate estimate of rank was obtained. A rank of negative 3 means that the retinal bleb was apparently flattened. A rank of positive three means that the bleb approximately doubled in size. A zero rank means that the apparent bleb size was unchanged over time. After all of the experiments were completed, the content of each vial was unmasked and compared with the experimenter’s conclusions based on the observation of images obtained between 30 and 90 minutes and at one day following administration of drug or placebo.

**Results**

The effects of intravitreal UP$_4$dC on retinal reattachment in 12 rats (1 eye/animal was dosed) are shown in Fig. 10. The experiments were carried out in a masked fashion to provide
a rigorous and objective evaluation of the effects of UP₄ dC on fluid reabsorption from experimentally produced subretinal blebs. In these experiments, after the creations of blebs, drug or placebo solutions were injected into vitreous of the rat eye in a masked fashion (vials and their contents were indistinguishable). After all 12 eyes were scored, the key was unmasked and compared with the summarized results based on the observations at 1 and 24 hours. The results summarized in Fig. 10 show a significant difference (p < 0.05) between UP₄ dC (open bars) and vehicle control (placebo, solid bars) on the scoring of subretinal blebs. After one hour of treatment, the UP₄ dC-treated eyes all showed a decrease in bleb size, whereas the control eyes all showed an increase in bleb size. The next day, the subretinal blebs from the UP₄ dC-treated eyes had almost completely disappeared, whereas the subretinal blebs from the vehicle-treated eyes remained essentially unchanged. In 4 out of 6 UP₄ dC-treated eyes, the retina appeared completely flat at the 24-hr time point.

Example 8. Primary Treatment for Subject with Macula-Off Rhegmatogenous Retinal Detachment

A patient presents with sudden onset of loss of central vision and is diagnosed with macula-off rhegmatogenous retinal detachment with a single break in the superior retina that is less than one clock hour in size. The patient's conjunctiva (cul de sacs) is sterilized with topical Betadine and by scrubbing and draping the face and lashes and lids. Local anesthesia is given via subconjunctival injection of xylocaine.

A patient is then given a single, slowly administered 50 μL-intravitreal injection of a sterile pharmaceutical composition by insertion of a 29 or 30 gauge needle from a 0.25 cc or 0.50 cc tuberculin syringe through the sclera in the pars plana region of the eye. The pharmaceutical composition consists of a metabolically resistant P₂Y₂ receptor agonist formulated to isotonicity (280 -300 mOsm) and physiological pH (7.0 -7.5) in saline.

The patient's eyes are bilaterally patched and the patient remains rested in a horizontal position for 4 hours, at which the eyes are examined for retinal reattachment. If the retina has not completely reattached at the four hour time point, the patient's eyes remain bilaterally patched until the next day (20-24 hours post dosing), at which point the retina is reexamined for reattachment. Following retinal reattachment, the retinal tear is suitably treated by conventional methods such as cryotherapy or laser photocoagulation.
The invention, and the manner and process of making and using it, are now described in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, to make and use the same. It is to be understood that the foregoing describes preferred embodiments of the present invention and that modifications may be made therein without departing from the scope of the present invention as set forth in the claims. To particularly point out and distinctly claim the subject matter regarded as invention, the following claims conclude this specification.
WHAT IS CLAIMED IS:

1. A method of treating edematous retinal disorders in a subject in need thereof, said method comprising:

   administering to said subject a pharmaceutical composition comprising a P2Y receptor agonist in an amount effective to stimulate the removal of pathological fluid accumulation in intra-retinal and subretinal spaces associated with edematous retinal disorders.

2. The method according to Claim 1, wherein said edematous retinal disorders are retinal detachment or retinal edema.

3. The method according to Claim 1, wherein said retinal disorders are selected from the group consisting of: diabetic macular edema, age-related macular degeneration, central serous retinopathy, and macular edema.

4. The method according to Claim 3, wherein said macular edema arises from uveitis, central and branch vein occlusion, retinitis pigmentosa, central serous retinopathy, CMV retinitis, or choroidal melanoma.

5. The method according to Claim 1, wherein said P2Y receptor agonist is administered to achieve an intravitreal concentration range of about 1 micromolar to about 500 micromolar.

6. The method according to Claim 1, wherein said P2Y receptor agonist is a dinucleoside polyphosphate compound of Formula I:

   ![Formula I](image)

   wherein:

   - B
   - B'
   - Y
   - Z
   - Y'
   - Z'
   - m
   - n
   - O
   - P
   - OH
   - O
   - O
   - PO
   - PO
   - X
   - Y
   - Z
   - Y'
   - Z'
   - m
   - n
X is oxygen, methylene, dihalomethylene, or imido;
n = 0, 1 or 2;
m = 0, 1 or 2;
n + m = 0, 1, 2, 3 or 4;
Z = OH or H;
Z' = OH or H;
Y = OH or H;
Y' = OH or H; and
B and B' are each independently a purine residue or a pyrimidine residue, as defined in
Formula Ia or Ib, linked through the 9- or 1-position, respectively:

\[
\text{Formula Ia}
\]

wherein:

R₁ is hydrogen, chlorine, amino, monosubstituted amino, disubstituted amino,
alkylthio, arylthio, or aralkylthio, wherein the substituent on sulfur contains up to a maximum
of 20 carbon atoms, with or without unsaturation;
R₂ is hydroxy, alkenyl, oxo, amino, mercapto, thione, alkylthio, arylthio, aralkylthio,
acylthio, alkyloxy, aryloxy, aralkyloxy, acyloxy, monosubstituted alkylamino, heterocyclic,
monosubstituted cycloalkylamino, monosubstituted aralkylamino, monosubstituted arylamino,
diaralkylamino, diarylamino, dialkylamino, acylamino, or diacylamino;
Rₓ is O, H or is absent;
R₂ and Rₓ are optionally taken together to form a 5-membered fused imidazole ring of
1, N⁶-etheno adenine derivatives, optionally substituted on the 4- or 5-positions of the etheno
moiety with alkyl, aryl or aralkyl moieties as defined below;
R₃ is hydrogen, azido, alkoxy, aryloxy, aralkyloxy, alkylthio, arylthio, or aralkylthio as defined below; or T(C₁₋₄alkyl)OCONH(C₁₋₄alkyl)W, wherein T and W are independently amino, mercapto, hydroxy or carboxyl; or pharmaceutically acceptable esters, amides or salts thereof; or absent;

J is carbon or nitrogen, with the provision that when J is nitrogen, R₃ is not present;

wherein the alkyls are straight-chain, branched or cyclic;

wherein the aryl groups are optionally substituted with lower alkyl, aryl, amino, mono- or dialkylamino, NO₂, N₃, cyano, carboxylic, amidio, sulfonamido, sulphanic acid, phosphate, or halo group;

Formula Ib

![Diagram]

wherein:

R₄ is hydroxy, oxo, mercapto, thione, amino, cyano, C₇₋₁₂arylalkoxy, C₁₋₄ alkylthio, C₁₋₆ alkoxy, C₁₋₆ alkylamino or diC₁₋₄alkylamino, wherein the alkyl groups are optionally linked to form a heterocycle;

R₅ is hydrogen, acetyl, benzoyl, C₁₋₄ alkyl, C₁₋₅ alkanoyl, aroyl, or absent;

R₆ is hydroxy, oxo, mercapto, thione, C₁₋₄alkoxy, C₇₋₁₂arylalkoxy, C₁₋₄alkylthio, S-phenyl, arythio, arylalkylthio, triazolyl amino, C₁₋₆alkylamino, C₁₋₅ disubstituted amino, or di-C₁₋₄alkylamino, wherein said dialkyl groups are optionally linked to form a heterocycle or linked to form a substituted ring such as morpholino, pyrrolo; or

R₅ and R₆ taken together form a 5-membered fused imidazole ring of 3,N₄-ethenocytosine derivatives between positions 3 and 4 of the pyrimidine ring, wherein said etheno moiety is optionally substituted on the 4- or 5-positions with C₁₋₄ alkyl, phenyl or phenyloxy; wherein at least one hydrogen of said C₁₋₄ alkyl, phenyl or phenyloxy is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy, C₁₋₄ alkoxy, C₁₋₄ alkyl, C₆₋₁₀ aryl, C₇₋₁₂ arylalkyl, carboxy, cyano, nitro, sulfonamido, sulfonate, phosphate, sulfonic acid, amino, C₁₋₄ alkylamino, and di-C₁₋₄ alkylamino, wherein said dialkyl groups are optionally linked to form a heterocycle;
R₇ is selected from the group consisting of hydrogen, hydroxy, cyano, nitro, C₁₋₆ alkyl or phenyl; substituted C₂₋₄ alkynyl, halogen, substituted C₁₋₄ alkyl, CF₃, C₂₋₃ alkenyl, C₂₋₃ alkynyl, allylamino, bromovinyl, ethyl propenoate, or propenoic acid and C₂₋₅ alkenyl; ; or
R₆ and R₇ together form a 5 or 6-membered saturated or unsaturated ring bonded through N or O or S at R₆, such ring optionally contains substituents that themselves contain functionalities;

R₈ is selected from the group consisting of hydrogen, amino, di-C₁₋₄ alkylamino, C₁₋₄ alkoxy, C₇₋₁₂ arylalkoxy, C₁₋₄ alkylthio, C₇₋₁₂ arylalkylthio, carboxamidomethyl, carboxymethyl, methoxy, methylthio, phenoxy, and phenylthio.

7. The method according to Claim 6, wherein said dinucleoside polyphosphates of general Formula I are dinucleoside triphosphates selected from a group consisting of: P³-(uridine 5′-)-triphosphate; P¹-(cytosine 5′-)-P³-(uridine 5′-)-triphosphate; P¹,P³-di(adenosine 5′-)-triphosphate; P¹-(adenosine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(adenosine 5′-)-P³-(cytosine 5′-)-triphosphate; P¹,P³-di(ethenoadenosine)-triphosphate; P¹-(uridine 5′-)-P³-(thymidine 5′-)-triphosphate; P¹-(adenosine 5′-)-P³-(inosine 5′-)-triphosphate; P¹,P³-di(uridine 5′-)-P²,P³-methylenetriphosphate; P¹,P³-di(uridine 5′-)-P²,P³-difluoromethylenetriphosphate; P¹,P³-di(uridine 5′-)-P²,P³-imidotriphosphate; P¹,P³-di(4-thiouridine 5′-)-triphosphate; P¹,P³-di(3,N⁴-etheno-5-monosubstituted)triphosphate; P¹,P³-di(imidazol[1,2-c]pyrimidine-5(6H)-one-2-(3-nitro)phenyl-6-β-D-ribofuranoside 5′-)-triphosphate, tetraammonium salt; P¹-(inosine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(4-thiouridine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(cytosine β-D-arabinofuranoside 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(uridine 5′-)-P³-(xanthosine 5′-)-triphosphate; P¹-(2′-deoxyuridine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(3′-azido-3′-deoxythymidine 5′-)-P³-(uridine 5′-)-triphosphate; P¹,P³-di(3′-azido-3′-deoxythymidine 5′-)-triphosphate; P¹,P³-di(3′-azido-3′-deoxythymidine 5′-)-triphosphate; 2′(3′)-benzoyl-P¹,P³-di(uridine 5′-)-triphosphate; P¹,P³-di(2′(3′)-benzoyl uridine 5′-)-triphosphate; P¹-(2′-deoxyguanosine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(2′-deoxyadenosine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(2′-deoxyinosine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(2′-deoxyctydine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(4-thiouridine 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(8-azaadenosine-5′-)-P³-(uridine 5′-)-triphosphate; P¹-(6-mercaptopurine riboside 5′-)-P³-(uridine 5′-)-triphosphate; P¹-(6-mercaptopurine riboside 5′-)-P³-(2′-deoxyuridine 5′-)-triphosphate; P¹-(4-thiouridine 5′-)-P³-(arabinocytidine 5′-)-triphosphate; P¹-(adenosine 5′-)-P³-(4-
thiomethyluridine 5′-) triphosphate; P⁴-(2′-deoxyadenosine 5′-)P³-(6-thiohexylpurine riboside 5′-) tetraphosphate; and P⁴-(6- eicosanyloxypurine riboside 5′-)P³-(uridine 5′-) triphosphate.

8. The method according to Claim 6, wherein said dinucleoside polyphosphates of general Formula I are compounds selected from a group consisting of: P⁴-(uridine 5′-)P³-(4-thiouridine 5′-) diphosphate; P⁴,P⁵-di(uridine 5′-)pentaphosphate; and P⁴,P⁶-di(uridine 5′-) hexaphosphate.

9. The method according to Claim 6, wherein said dinucleoside polyphosphates of general Formula I are dinucleoside tetraphosphates selected from the group consisting of P⁴,P⁴-di( uridine 5′-)tetraphosphate; P⁴, (cytosine 5′-)P⁴-(uridine 5′-)tetraphosphate; P⁴,P⁴-di(adenosine 5′-)tetraphosphate; P⁴-(adenosine 5′-)P⁴-(uridine 5′-)tetraphosphate; P⁴-(uridine 5′-)P⁴-(cytosine 5′-)tetraphosphate; P⁴,P⁴-di(ethenoadenosine)tetraphosphate; P⁴-(uridine 5′-)P⁴-(thymidine 5′-) tetraphosphate; P⁴-(adenosine 5′-)P⁴-(inosine 5′-) tetraphosphate; P⁴, (uridine 5′-P⁴-di(uridine 5′-)P²,P³-methylene tetraphosphate; P⁴,P⁴-di(uridine 5′-) tetraphosphate; P⁴,P⁴-di(4-thiouridine 5′-) tetraphosphate; P⁴,(3,N⁴-etheno cytidine 5′-) tetraphosphate; P⁴,P⁴-di(imidazo[1,2-c]pyrimidine-5(6H)-one-2-(3-nitro)-phenyl-6-β-D-ribofuranoside 5′-) tetraphosphate, tetraammonium salt; P⁴-(inosine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(4-thiouridine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴, (cytosine β-D-arabinofuranoside 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(uridine 5′-)P⁴-(xanthosine 5′-)tetraphosphate; P⁴-(2′-deoxyuridine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(3′-azido-3′-deoxythymidine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴,P⁴-di(3′-azido-3′-deoxythymidine 5′-)tetraphosphate; P⁴,P⁴-di(3′-azido-3′-deoxythymidine 5′-)tetraphosphate; 2′(3′)-benzoyl-P⁴, (uridine 5′-)tetraphosphate; P⁴,P⁴-di(2′,3′)-benzoyl uridine 5′-) tetraphosphate; P⁴-(2′-deoxyguanosine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(2′-deoxyadenosine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(2′-deoxyinosine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(2′-deoxycytidine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(4-thiouridine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(8-aza adenosine 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(6-mercaptopurine riboside 5′-)P⁴-(uridine 5′-) tetraphosphate; P⁴-(6-mercapto purine riboside 5′-)P⁴-(2′-deoxyuridine 5′-) tetraphosphate; P⁴-(4-thiouridine 5′-)P⁴-(arabinocytidine 5′-) tetraphosphate; P⁴-(adenosine 5′-)P⁴-(4-thiomethyluridine 5′-) tetraphosphate; P⁴-(2′-
deoxyadenosine 5'-)P\(^4\)-(6-thiohexylpurine riboside 5') tetraphosphate, and P\(^1\)-(6-eicosanyloxypurine riboside 5')P\(^4\)-(uridine 5') tetraphosphate.

10. The method according to Claim 1, wherein said P2Y receptor agonist is co-administered with a primary treatment or adjunctive agents used to manage edematous retinal disorders.

11. The method according to Claim 10, wherein said primary treatment is selected from the group consisting of: surgery, grid and focal laser photocoagulation and pharmacotherapy.

12. The method according to Claim 11, wherein said surgery is selected from the group consisting of: scleral buckling, pneumatic retinopexy, vitrectomy, and macular translocation.

13. The method according to Claim 11, wherein said pharmacotherapy is selected from the group consisting of: corticosteroids, carbonic anhydrase inhibitors, antiinflammatory agents, and pharmaceuticals that promote digestion of collagen and fibrous tissues that connect vitreous and retina.

14. The method according to Claim 6, wherein said dinucleoside polyphosphate compound is prepared in a formulation selected from the group consisting of: aqueous, gel, gellike, and solid formulations.

15. The method according to Claim 14, wherein said gel or gel-like formulations is selected from the group consisting of: OE hyaluronic acid and hyaluronic acid-containing formulations approved for intraocular surgical use.

16. The method according to Claim 1, wherein said administering is topical administration of said pharmaceutical composition via a carrier vehicle selected from the group consisting of: drops of liquid, liquid wash, gels, ointments, sprays and liposomes.

17. The method according to Claim 16, wherein said topical administration comprises infusion of said pharmaceutical composition to said ocular surface via a device selected from the group consisting of: of a pump-catheter system, a continuous or selective release device,
and a contact lens.

18. The method according to Claim 1, wherein said administering is systemic administration of said pharmaceutical composition.

19. The method according to Claim 18, wherein said systemic administration of said pharmaceutical composition is administering a liquid or liquid suspension via nose drops, nasal spray, or nebulized liquid to oral or nasopharyngeal airways of said subject, or administering an oral form, an injection form, a suppository form to said subject, or administering an intra-operative instillation of a gel, a cream, a powder, a foam, a crystal, a liposome, a spray, or a liquid suspension form to said subject, such that a therapeutically effective amount of said compound contacts the ocular tissues of said subject via systemic absorption and circulation.

20. The method according to Claim 18, wherein said systemic administration of said pharmaceutical composition is accomplished by administering an injectable form of said compound, such that a therapeutically effective amount of said compound contacts the ocular tissues of said subject via systemic absorption and circulation.

21. The method according to Claim 20, wherein said pharmaceutical composition is administered to said subject by injection into the vitreous or bolus, by sustained infusion into the vitreous, by sustained release into the vitreal cavity, by retrobulbar conjunctival injection, release, or infusion, by transcleral injection, by sustained transcleral release or infusion, by ocular surface instillation, or by acute and chronic injection or infusions.

22. The method according to Claim 21, wherein the injection into the vitreous is by single or multiple intravitreal injections at injection volumes of 50 -100 microliter.

23. The method according to Claim 21, wherein said P2Y receptor agonist is administered in an amount of between about 0.10 to about 4.0 milligrams of per eye.

24. The method according to Claim 1, wherein said pharmaceutical composition is administered in an ophthalmic formulation comprising an effective amount of said P2Y
receptor agonist, or pharmaceutically acceptable salts thereof, together with a physiologically compatible vehicle selected from the group consisting of: aqueous electrolyte solutions, polyethers, polyvinyls, polymers of acrylic acid, lanolin, and glucosaminoglycans; whereby said formulation stimulates the removal of pathological fluids from the subretinal and retinal spaces associated with edematous retinal disorders.

25. A compound of Formula I:

\[
\begin{align*}
  &B \quad O \quad \underbrace{\left[ O-P-O \right]}_{n} X \quad \underbrace{\left[ P-O \right]}_{m} O' \quad B' \\
  &Y \quad Z \quad n \quad m \quad Y' \quad Z'
\end{align*}
\]

wherein:
X is oxygen, methylene, dihalomethylene, or imido;
n = 0, 1 or 2;
m = 0, 1 or 2;
n + m = 0, 1, 2, 3 or 4;
Z = OH or H;
Z' = OH or H;
Y = OH or H;
Y' = OH or H; and
B and B' are each independently a purine residue or a pyrimidine residue, as defined in Formula Ia or Ib, linked through the 9- or 1-position, respectively:
wherein:

R₁ is hydrogen, chlorine, amino, monosubstituted amino, disubstituted amino, alkylthio, arylthio, or aralkylthio, wherein the substituent on sulfur contains up to a maximum of 20 carbon atoms, with or without unsaturation;

R₂ is hydroxy, alkenyl, oxo, amino, mercapto, thione, alkylthio, arylthio, aralkylthio, acylthio, alkoxy, aralkyloxy, acyloxy, monosubstituted alkylamino, heterocyclic, monosubstituted cycloalkylamino, monosubstituted aralkylamino, monosubstituted arylamino, diaralkylamino, diarylamino, dialkylamino, acylamino, or diacylamino;

R₃ is O, H or is absent;

R₂ and Rₓ are optionally taken together to form a 5-membered fused imidazole ring of 1,₅-etheno adenine derivatives, optionally substituted on the 4- or 5-positions of the etheno moiety with alkyl, aryl or aralkyl moieties as defined below;

Rₓ is hydrogen, azido, alkoxy, aralkyloxy, alkylthio, arylthio, or aralkylthio as defined below; or T(C₁₋₅alkyl)OCONH(C₁₋₅alkyl)W, wherein T and W are independently amino, mercapto, hydroxy or carboxyl; or pharmaceutically acceptable esters, amides or salts thereof; or absent;

J is carbon or nitrogen, with the provision that when J is nitrogen, R₃ is not present;

wherein the alkyls are straight-chain, branched or cyclic;

wherein the aryl groups are optionally substituted with lower alkyl, aryl, amino, mono- or dialkylamino, NO₂, N₃, cyano, carboxylic, amido, sulfonamido, sulphononic acid, phosphate, or halo group;
Formulas 1b

wherein:

- R₄ is hydroxy, oxo, mercapto, thione, amino, cyano, C₇-12arylalkoxy, C₁₋₆ alkythio, C₁₋₆ alkoxy, C₁₋₆ alkylamino or diC₁₋₄alkylamino, wherein the alkyl groups are optionally linked to form a heterocycle;
- R₅ is hydrogen, acetyl, benzoyl, C₁₋₆ alkyl, C₁₋₅ alkanoyl, aroyl, or absent;
- R₆ is hydroxy, oxo, mercapto, thione, C₁₋₄ alkoxy, C₇₋₁₂arylalkoxy, C₁₋₄ alkythio, S-phenyl, aroylthio, arylalkylthio, triazolyl amino, C₁₋₆ alkylamino, C₁₋₅ disubstituted amino, or di-C₁₋₄ alkylamino, wherein said dialkyl groups are optionally linked to form a heterocycle or linked to form a substituted ring such as morpholino, pyrrolo; or
- R₅ and R₆ taken together form a 5-membered fused imidazole ring of 3,N₈-ethenocytosine derivatives between positions 3 and 4 of the pyrimidine ring, wherein said etheno moiety is optionally substituted on the 4- or 5-positions with C₁₋₄ alkyl, phenyl or phenyloxy; wherein at least one hydrogen of said C₁₋₄ alkyl, phenyl or phenyloxy is optionally substituted with a moiety selected from the group consisting of halogen, hydroxy, C₁₋₄ alkoxy, C₁₋₄ alkyl, C₆₋₁₀ aryl, C₇₋₁₂ arylalkyl, carboxy, cyano, nitro, sulfamido, sulfonate, phosphate, sulfonic acid, amino, C₁₋₄ alkylamino, and di-C₁₋₄ alkylamino, wherein said dialkyl groups are optionally linked to form a heterocycle;
- R₇ is selected from the group consisting of hydrogen, hydroxy, cyano, nitro, C₁₋₆ alkyl or phenyl; substituted C₂₋₈ alkynyl, halogen, substituted C₁₋₄ alkyl, CF₃, C₂₋₃ alkenyl, C₂₋₃ alkynyl, allylalino, bromovinyl, ethyl propenoate, or propenoic acid and C₂₋₈ alkenyl; or
- R₆ and R₇ together form a 5 or 6-membered saturated or unsaturated ring bonded through N or O or S at R₆, such ring optionally contains substituents that themselves contain functionalities;
- R₈ is selected from the group consisting of hydrogen, amino, di-C₁₋₄ alkylamino, C₁₋₄ alkoxy, C₇₋₁₂arylalkoxy, C₁₋₄ alkythio, C₇₋₁₂ arylalkylthio, carboxamidomethyl, carboxymethyl, methoxy, methylthio, phenoxy, and phenylthio; provided that when R₈ is amino or substituted amino, R₇ is hydrogen; and
the furanosyl sugar moieties of Formula I are selected from the group consisting of 3'-deoxyribofuranosyl, 2',3'-dideoxyribofuranosyl, arabinofuranosyl, 3'-deoxyarabinofuranosyl, xylofuranosyl, 2'-deoxyxylofuranosyl, and lyxofuranosyl.

26. The compounds selected from the group consisting of \( P^1 \)-(6-mercaptopurine riboside 5')-\( P^4 \)-(uridine 5')-tetraphosphate, \( P^1 \)-(6-mercaptopurine riboside 5')-\( P^4 \)-(2'-deoxyuridine 5')-tetraphosphate, \( P^1 \)-(4-thiouridine 5')-\( P^4 \)-(arabinocytidine 5')-tetraphosphate, \( P^1 \)-(2'-deoxyadenosine 5')-\( P^4 \)-(6-thiohexylpurine riboside 5')-tetraphosphate, \( P^1 \)-(6-eicosanyloxypurine riboside 5')-\( P^4 \)-(uridine 5')-tetraphosphate, \( P^1 \)-(arabinoadenosine-5')-\( P^4 \)-(uridine-5')-tetraphosphate, \( P^1 \)-(lyxofuranosylthymine-5')-\( P^4 \)-(uridine-5')-tetraphosphate, and \( P^1 \)-(xylofuranosyluracil-5')-\( P^4 \)-(uridine-5')-tetraphosphate.
FIGURE 1
P2Y₂
Calcium Mobilization

% Maximal Response

UTP
INS37217

log [stimulant] M

FIGURE 2
P2Y<sub>2</sub>
Inositol Phosphate Generation

[Graph showing the relationship between log [stimulant] M and % Maximal Response for UTP and INS37217]

FIGURE 3
**FIGURE 6**

The graph shows the normalized concentration over time for two different substances: INS37217 and UTP. The half-life ($T_{1/2}$) for INS37217 is 3.9 hours, and for UTP, it is 1.0 hour.
Effects of INS37217 on Subretinal Fluid Reabsorption
Pig Macular Translocation Studies
Composite scores for each treatment group

- Filled Triangles: INS37217
- Unfilled Circles: Vehicle

**FIGURE 7**
FIGURE 8
Figure 9
FIGURE 10