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(54) **Title**: TREATMENT OF OCULAR DISEASE

(57) **Abstract**: Disclosed are methods for treating eye diseases or conditions characterized by vascular instability, vascular leakage, and neovacularization such as diabetic macular edema, age-related macular edema, choroidal neovascularization, diabetic retinopathy, trauma, ocular ischemia, and uveitis.

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TREATMENT OF OCULAR DISEASE

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority U.S. Provisional Application Serial No. 61/546,708 filed October 13, 2011. The entire content of U.S. Provisional Application Serial No. 61/546,708 is incorporated herein by reference.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety is a computer-readable sequence listing submitted concurrently herewith and identified as follows: One 92 KB ASCII (Text) file named "233106-331562_Seq_Listing_ST25.txt," created on October 12, 2012, at 12:42 pm.

FIELD

[0003] Methods for treating eye diseases or conditions characterized by vascular instability, vascular leakage, and neovacularization such as ocular edema, ocular neovascularization, diabetic macular edema, age-related macular degeneration, choroidal neovascularization, diabetic retinopathy, retinal vein occlusion (central or branch), ocular ischemia, ocular trauma, surgery induced edema, and uveitis.

BACKGROUND

[0004] The eye comprises several structurally and functionally distinct vascular beds, which supply ocular components critical to the maintenance of vision. These include the retinal and choroidal vasculatures, which supply the inner and outer portions of the retina, respectively, and the limbal vasculature located at the periphery of the cornea. Injuries and diseases that impair the normal structure or function of these vascular beds are among the leading causes of visual impairment and blindness. For example, diabetic retinopathy is the most common disease affecting the retinal vasculature, and is the leading cause of vision loss among the working age population in the United States. Vascularization of the cornea secondary to injury or disease is yet another category of ocular vascular disease that can lead to severe impairment of vision.

[0005] "Macular degeneration" is a general medical term that applies to any of several disease syndromes, which involve a gradual loss or impairment of eyesight due to cell and tissue degeneration of the yellow macular region in the center of the retina. Macular degeneration is often characterized as one of two types, non-exudative (dry form) or exudative (wet form). Although both types are bilateral and progressive, each type may reflect different pathological processes. The wet form of age-related macular degeneration (AMD) is the most common form of choroidal neovascularization and a leading cause of blindness in the elderly. AMD affects millions of Americans over the age of 60, and is the leading cause of new blindness among the elderly.

[0006] Choroidal neovascular membrane (CNVM) is a problem that is related to a wide variety of retinal diseases, but is most commonly linked to age-related macular degeneration. With CNVM, abnormal blood vessels stemming from the choroid (the blood vessel-rich tissue layer just beneath the retina) grow up through the retinal layers. These new vessels are very fragile and break easily, causing blood and fluid to pool within the layers of the retina.

[0007] Diabetes (diabetes mellitus) is a metabolic disease caused by the inability of the pancreas to produce insulin or to use the insulin that is produced. The most common types of diabetes are type 1 diabetes (often referred to as Juvenile Onset Diabetes Mellitus) and type 2 diabetes (often referred to as Adult Onset Diabetes Mellitus). Type 1 diabetes results from the body's failure to produce insulin due to loss of insulin producing cells, and presently requires the person to inject insulin. Type 2 diabetes generally results from insulin resistance, a condition in which cells fail to use insulin properly. Type 2 diabetes may have a component of insulin deficiency as well.

[0008] Diabetes is directly responsible for a large number of disease conditions, including conditions or diseases of the eye including diabetic retinopathy (DR) and diabetic macular edema (DME) which are leading causes of vision loss and blindness in most developed countries. The increasing number of individuals with diabetes worldwide suggests that DR and DME will continue to be major contributors to vision loss and associated functional impairment for years to come.

[0009] Diabetic retinopathy is a complication of diabetes that results from damage to the blood vessels of the light-sensitive tissue at the back of the eye (retina). At first, diabetic retinopathy may cause no symptoms or only mild vision problems. Eventually, however, diabetic retinopathy can result in blindness. Diabetic retinopathy can develop in anyone who has type 1 diabetes or type 2 diabetes.

[0010] At its earliest stage, non-proliferative retinopathy, microaneurysms occur in the retina's tiny blood vessels. As the disease progresses, more of these blood vessels become damaged or blocked and these areas of the retina send signals into the regional tissue to grow new blood vessels for nourishment. This stage is called proliferative retinopathy. The new blood vessels grow along the retina and along the surface of the clear, vitreous gel that fills the inside of the eye.

[0011] By themselves, these blood vessels do not cause symptoms or vision loss. However, they have thin, fragile walls and without timely treatment, these new blood vessels can leak blood (whole blood or a constituent thereof) which can result in severe vision loss and even blindness.

[0012] Also, fluid can leak into the center of the macula, the part of the eye where sharp, straight-ahead vision occurs. The fluid and the associated protein begin to deposit on or under the macula causing the patient's central vision to become distorted. This condition is called macular edema. It can occur at any stage of diabetic retinopathy, although it is more likely to occur as the disease progresses. About half of the people with proliferative retinopathy also have macular edema.

[0013] Uveitis is a condition in which the uvea becomes inflamed. The eye is shaped much like a tennis ball, hollow on the inside with three different layers of tissue surrounding a central cavity. The outermost is the sclera (white coat of the eye) and the innermost is the retina. The middle layer between the sclera and the retina is called the uvea. The uvea contains many of the blood vessels that nourish the eye. Complications of uveitis include glaucoma, cataracts or new blood vessel formation (neovascularization).

[0014] The currently available interventions for exudative (wet form) macular degeneration, diabetic retinopathy, diabetic macular edema, choroidal neovascular membrane, complications from uveitis or ocular trauma, include laser photocoagulation therapy, low dose radiation (teletherapy) and surgical removal of neovascular membranes (vitrectomy). Laser therapy has had limited success and selected choroidal neovascular membranes which initially respond to laser therapy have high disease recurrence rates. There is also a potential loss of vision resulting from laser therapy. Low dose radiation has been applied ineffectively to induce regression of choroidal neovascularization. Recently, vascular endothelial growth factor (VEGF) antagonists, ranibizumab and aflibercept, have been approved for use in age-related macular degeneration, diabetic macular edema and retinal vein occlusion (RVO).

[0015] (RVO) is the most common retinal vascular disease after diabetic retinopathy. Depending on the area of retinal venous drainage effectively occluded, it is broadly classified as either central retinal vein occlusion (CRVO), hemispheric retinal vein occlusion (HRVO), or branch retinal vein occlusion (BRVO). It has been observed that each of these has two subtypes. Presentation of RVO in general is with variable painless visual loss with any combination of fundal findings consisting of retinal vascular tortuosity, retinal hemorrhages (blot and flame shaped), cotton wool spots, optic disc swelling and macular edema. In a CRVO, retinal hemorrhages will be found in all four quadrants of the fundus, while these are restricted to either the superior or inferior fundal hemisphere in a HRVO. In a BRVO, hemorrhages are largely localized to the area drained by the occluded in the retinal vein.

[0016] There is therefore a long felt and substantial need for methods of treating diseases of the eye which are characterized by vascular instability, vascular leakage and neovascularization.

SUMMARY

[0017] Disclosed are agents that bind to the extracellular portion and inhibit human protein tyrosine phosphatase beta (HPTPβ). Also disclosed are methods for treating eye diseases or conditions characterized by vascular instability, vascular leakage, and neovacularization such as ocular edema, ocular neovascularization, diabetic macular edema, age-related macular degeneration, choroidal neovascularization, diabetic retinopathy, retinal vein occlusion (central or branch), ocular ischemia, ocular trauma, surgery induced edema, and uveitis.

BRIEF DESCRIPTION OF THE FIGURES

[0018] **Fig. 1.** The monoclonal antibody R15E6 recognizes Endogenous HPTPβ on endothelial cells. (Panel A) Endothelial cell lysates are immunoprecipitated with a control antibody (Lane 1), with R15E6 (Lane 2) or with a mixture of anti-Tie2 and anti-VEGFR2 antibodies (Lane 3). Immunoprecipitates are resolved by SDS-PAGE, transferred to a PVDF membrane and probed by western blot with a mixture of R15E6, anti-Tie2 and anti-VEGFR2 antibodies. A single major high molecular weight band consistent with HPTPβ is seen with R15E6 (Lane 2) and not with the control antibody (Lane 1) or the mixture of anti-Tie2 and anti-VEGFR2 (Lane 3). (Panel B) Endothelial cells are subjected to FACS analysis with R15E6 (white peak) or a control with no primary antibody (black peak). The robust shift in fluorescence indicates that R15E6 binds to HPTPβ on the surface of intact endothelial cells.

[0019] **Fig. 2.** The monoclonal antibody R15E6 enhances Tie2 Receptor Activation in HUVECs. Tie2 activation is measured in human endothelial cells as described in Example 4. R15E6 dose dependently enhances both basal and Ang1-induced Tie2 activation.

[0020] **Fig. 3.** Is a graphical representation of the mean area of choroidal neovascularization in C57BL/6 mice 14 days post laser injury in eyes treated with intravitreal injection of 1µg or 2µg of an anti-VE-PTP extracellular domain antibody in one eye versus similar treatment of the fellow eye with control.

[0021] **Fig. 4.** Shows the mean area (mm^2) of retinal neovascularization in C57BL/6 mice on day P17 after containment in a 75% oxygen atmosphere from P5 to P12 and intravitreal injection of an anti-VE-PTP extracellular domain antibody at P12 when the mice were returned to room air.

[0022] **Fig. 5.** Shows representative fluorescent micrographs of mouse retinas in the oxygen-induced retinopathy model after intravitreal injection of vehicle or 2 µg of an anti-VE-PTP extracellular domain antibody.

[0023] **Fig. 6.** Shows the mean area (mm^2) of retinal neovascularization in C57BL/6 mice on day P17 after containment in a 75% oxygen atmosphere from P5 to P12 followed by return to room air on P12 with subcutaneous administration of 1 mg/kg of an anti-VE-PTP extracellular domain antibody on days P12, 14 and 16.

[0024] **Fig. 7.** Shows the mean area (mm^2) of retinal neovascularization in C57BL/6 mice on day P17 after containment in a 75% oxygen atmosphere from P5 to P12 followed by return to room air on P12 with subcutaneous administration of 2 mg/kg of an anti-VE-PTP extracellular domain antibody on days P12, 14 and 16.

DETAILED DESCRIPTION

General Definitions

[0025] In this specification and in the claims that follow, reference will be made to a number of terms, which shall be defined to have the following meanings:

[0026] The term "HPTPβ-ECD binding agent" and “specific binding agent” are used interchangeably herein and refer to a molecule that specifically binds to the extracellular portion of HPTPβ, and variants and derivatives thereof, as defined herein, that inhibits the Tie2 dephosphorylase activity of HPTPβ.

[0027] “Agent” as used herein refers to a “HPTPβ binding agent” unless otherwise noted.

[0028] “Specifically binds HPTPβ-ECD” refers to the ability of a specific binding agent of the present invention to recognize and bind to an epitope of the extracellular domain of HPTPβ with higher affinity than to other related and/or unrelated molecules. Specific binding agents preferentially bind to HPTPβ in a complex mixture of proteins and/or macromolecules. The specific binding agent is preferably selective for HPTPβ. “Selective” means that the agent has significantly greater activity toward HPTPβ compared with other related and/or unrelated molecules, not that it is completely inactive with regard to other molecules. For example, a selective agent may show 10-fold, 100-fold, or 1000-fold selectivity toward HPTPβ than to other related or unrelated molecules.

[0029] The term “anti-HPTPβ-ECD antibodies” refers to antibodies or antibody fragments that bind to the extracellular domain of HPTPβ. Anti-HPTPβ-ECD antibodies are a type of HPTPβ-ECD binding agent as defined herein.

[0030] The term “VE-PTP” refers to the mouse ortholog of HPTPβ.

[0031] All percentages, ratios and proportions herein are by weight, unless otherwise specified. All temperatures are in degrees Celsius (^{O}C) unless otherwise specified.

[0032] Ranges may be expressed herein as from one particular value to another particular value, the endpoints are included in the range. For example for the range from “1mg to 50mg” includes the specific values 1mg and 50mg. The antecedent “about” indicates that the values are approximate. For example for the range from “about 1mg to about 50mg” indicates that the values are approximate values. Additionally, when such a range is expressed, the range includes the range “from 1mg to 50mg.” It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. For example the range “from 1mg to 50mg”, includes the range “from 30mg to 40mg.”

[0033] “Effective amount” means an amount of an active agent or combination of agents effective to ameliorate or prevent the symptoms, or prolong the survival of the patient being treated. An effective amount may vary according to factors known in the art, such as the disease state, age, sex and weight of the human or animal being treated. Although particular dosage regimes may be described in examples herein, a person skilled in the art would appreciate that the dosage regime may be altered to provide optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. In addition, the compositions of this disclosure can be administered as frequently as necessary to achieve a therapeutic amount. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0034] As used herein the term “inhibit” or “inhibiting” refers to a statistically significant and measurable reduction in activity, preferably a reduction of at least about 10% versus control, more preferably a reduction of about 50% or more, still more preferably a reduction of about 80% or more.

[0035] As used herein the term “increase” or “increasing” refers to a statistically significant and measurable increase in activity, preferably an increase of at least about 10% versus control, more preferably an increase of about 50% or more, still more preferably an increase of about 80% or more.

[0036] “HPTP beta” or “HPTPβ” are used interchangeably herein and are abbreviations for human protein tyrosine phosphatase beta.

[0037] As used herein, “subject” means an individual. Thus, the “subject” can include domesticated animals (e.g., cats, dogs, etc.), livestock (e.g., cattle, horses, pigs, sheep, goats, etc.), laboratory animals (e.g., mouse, rabbit, rat, guinea pig, etc.) and birds. “Subject” can also include a mammal, such as a primate or a human. “Subject” and “patient” are used interchangeably herein. Preferably the subject is a human.

[0038] By “reduce” or other forms of the word, such as “reducing” or “reduction,” is meant lowering of an event or characteristic (e.g., vascular leakage). It is understood that this is typically in relation to some standard or expected value, in other words it is relative, but that it is not always necessary for the standard or relative value to be referred to.

[0039] The terms “treatment”, “treating”, “treat” and the like, refer to obtaining a desired pharmacologic and/or physiologic effect such as mitigating a disease or a disorder in a host and/or reducing, inhibiting, or eliminating a particular characteristic or event associated with a disorder (e.g., ocular edema). Thus, the term "treatment" includes, preventing a disorder from occurring in a host, particularly when the host is predisposed to acquiring the disease, but has not yet been diagnosed with the disease; inhibiting the disorder; and/or alleviating or reversing the disorder. Insofar as the methods of the present invention are directed to preventing disorders, it is understood that the term "prevent" does not require that the disease state be completely thwarted. Rather, as used herein, the term preventing refers to the ability of the skilled artisan to identify a population that is susceptible to disorders, such that administration of the compounds of the present invention may occur prior to onset of a disease. The term does not imply that the disease state is completely avoided.

[0040] Unless otherwise specified, diabetic retinopathy includes all stages of non-proliferative retinopathy and proliferative retinopathy.

[0041] Throughout the description and claims of this specification the word “comprise” and other forms of the word, such as “comprising” and “comprises,” means including but not limited to, and is not intended to exclude, for example, other additives, components, integers, or steps.

[0042] As used in the description and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “a composition” includes one composition or mixtures of two or more such compositions.

[0043] Optional” or “optionally” means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

[0044] "Specifically binds HPTPβ" refers to the ability of an agent of the present invention to recognize and bind to an epitope of the extracellular domain of HPTPβ with higher affinity than to the other related and/or unrelated molecules. The agent is preferably selective for HPTPβ. "Specific" means that the agent has significantly greater activity toward HPTPβ compared with other related and/or unrelated molecules, not that it is completely inactive with regard to other molecules. For example, a selective agent may show 10-fold, 100-fold, or 1000-fold selectivity toward HPTPβ than to other related or unrelated molecule.

[0045] The term "epitope" refers to any portion of any molecule capable of being recognized by and bound by a agent at one or more of the agent's antigen binding regions. Epitopes usually consist of distinct, recognizable surface groupings such as amino acids, sugars, lipids, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. Epitopes as used herein may be conformational or linear.

[0046] "Peptibody" is a molecule comprising an antibody Fc domain attached to at least one peptide. The production of peptibodies is generally described in WO2002/24782.

[0047] "Fragment" refers to a portion of an agent. A fragment may retain the desired biological activity of the agent and may be considered to be an agent itself. For example a truncated protein in which the amino terminus and/or carboxy terminus and/or an internal amino acid residue is deleted is a fragment of the protein and an Fab of an immunoglobulin molecule is a fragment of the immunoglobulin. Such fragments may also be connected to a another molecule by way of a direct connection (e.g. a peptide or disulfide bond) or by way of a linker.

[0048] "Protein" is used herein interchangeably with peptide and polypeptide.

[0049] Peptides of the present invention include, but are not limited to amino acid sequences having from about 3 to about 75 amino acids, or from about 5 to about 50 amino acids, or from about 10 to about 25 amino acids. Peptides may be naturally occurring or artificial amino acid sequences.

[0050] A protein of the invention may be obtained by methods well known in the art, for example, using standard direct peptide synthesizing techniques such as via solid-phase synthesis.

If the gene sequence is known or can be deduced then the protein may be produced by standard recombinant methods. The proteins may be isolated or purified in a variety of ways known to one skilled in the art. Standard purification methods include precipitation with salts, electrophoretic, chromatographic techniques and the like.

[0051] Agents may be covalently or non-covalently conjugated to a vehicle. The term "vehicle" refers to a molecule that prevents degradation and/or increase half-life, reduces toxicity, reduces immunogenicity, or increases biological activity of the agent. Exemplary vehicles include, but are not limited, Fc domains of immunoglobulins and polymers, for example: polyethylene glycol (PEG), polylysine, dextran, a lipid, a cholesterol group (such as a steroid); a carbohydrate or oligosaccharide; or any natural or synthetic protein, or peptide that binds to a salvage receptor.

[0052] "Derivatives" include those binding agents that have been chemically modified in some manner distinct from insertion, deletion, or substitution variants. For example, wherein the binding agent is a protein, the carboxyl terminus may be capped with an amino group, such as NH_2.

[0053] In some embodiments one or more molecules are linked together to form the agent. For example antibody fragments may be connected by a linker. In general, the chemical structure of the linker is not critical as it serves primarily as a space. In one embodiment, the linker is made of amino acids linked together by way of peptide bonds. In another embodiment, the linker is a non-peptide linker such as a non-sterically hindering C_1-C_6 alkyl group. In another embodiment, the linker is a PEG linker. It will further be appreciated that the linker can be inserted in a number of locations on the molecule.

[0054] Variants of an agent are included within the scope of the present invention. "Variant" or "Variants" as used herein means an agent having a protein or nucleotide sequence which is substantially similar to the protein or nucleotide sequence of the non-variant agent and which shares a similar activity of the non-variant agent. A protein or nucleotide sequence may be altered in various ways to yield a variant encompassed by the present invention, including substitutions, deletions, truncations, insertions and other modifications. Methods for such manipulations are well known in the art. See, for example, Current Protocols in Molecular Biology (and updates) Ausubel et al., Eds (1996), John Wiley and Sons, New York: Methods in Molecular Biology, Vol. 182, In vitro Mutagenesis Protocols, 2^{nd} Edition, Barman Ed. (2002), Humana Press, and the references cited therein. For example, variants include peptides and polypeptides wherein amino acid residues are inserted into, deleted from and/or substituted into the known amino acid sequence for the binding agent. In one embodiment, the substitution of the amino acid is conservative in that it minimally alters the biochemical properties of the variant. In other embodiments, the variant may be an active fragment of a full-length protein, a chemically modified protein, a protein modified by addition of affinity or epitope tags, or fluorescent or other labeling moieties, whether accomplished by *in vivo* or *in vitro* enzymatic treatment of the protein, by chemical modification, or by the synthesis of the protein using modified amino acids.

[0055] Fusions proteins are also contemplated herein. Using known methods, one of skill in the art would be able to make fusion proteins of the proteins of the invention; that, while different from native form, may be useful. For example, the fusion partner may be a signal (or leader) polypeptide sequence that co-translationally or post-translationally directs transfer of the protein from its site of synthesis to another site (e.g., the yeast alpha-factor leader). Alternatively, it may be added to facilitate purification or identification of the protein of the invention (e.g., poly-His, Flag peptide, or fluorescent proteins).

[0056] Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The techniques and procedures are generally performed according to conventional methods known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. Unless specific definitions are provided, the nomenclature utilized in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those known and commonly used in the art. Standard techniques may be used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, delivery and treatment of patients.

Sequence Listing

[0057] Table 1.

SEQ ID NO:1	Full length Human HPTPβ nucleotide sequence (X54131)
SEQ ID NO:2	Full length Human HPTPβ amino acid sequence (P23467)

SEQ ID NO:3	Extracellular Portion of Human HPTPβ with $(His)_6$Gly Tag
SEQ ID NO:4	Extracellular Portion of Human HPTPβ
SEQ ID NO:5	Full length mouse VE-PTP nucleotide sequence
SEQ ID NO:6	Full length mouse VE-PTP amino acid sequence
SEQ ID NO:7	Extracellular portion of mouse VE-PTP amino acid sequence

HPTPβ-ECD binding agents

[0058] Agents useful in the present invention include, but are not limited to, antibodies, proteins, darpins, peptides, aptamers, adnectins, peptibodies, or nucleic acids that bind specifically to the extracellular portion of HPTPβ and inhibit at least one phosphatase activity of HPTPβ. As used herein, "phosphatase activity" includes enzymatic activity and biologic activity where biological activity is measured by assessing Tie2 phosphorylation.

[0059] Agents useful in the present invention further include: antibodies, or antigen binding fragments thereof which bind to the extracellular portion of HPTPβ wherein the antibody or antigen-binding fragment inhibits at least one phosphatase activity of HPTPβ. These agents include monoclonal and polyclonal antibodies. An agent may be a fragment of an antibody, wherein the fragment comprises the heavy and light chain variable regions, or the fragment is an $F(ab')_2$, or the fragment is a dimer or trimer of an Fab, Fv, scFv, or a dia-, tria-, or tetrabody derived from the antibody.

[0060] For example, the agent may be, without limitation, an antibody or antibody fragment that binds the extracellular portion of HPTPβ; or in particular an antibody that binds an FN3 repeat of HPTPβ, or more specifically an antibody that binds the first FN3 repeat of HPTPβ.

[0061] Agents further include: the monoclonal antibody R15E6 which is described in U.S. patent number 7,973,142, which is hereby incorporated in its entirety. (The mouse hybridoma, Balbc spleen cells (B cells) which may be used to produce the antibody are deposited with American Type Culture Collection (ATCC), P.O. Box 1549, Manassas, Va. 20108 USA on 4 May 2006, assigned ATCC No. PTA-7580) (Referred to herein as R15E6)), antibodies having the same or substantially the same biological characteristics of R15E6; antibody fragments of R15E6, wherein the fragment comprises the heavy and light chain variable regions; an $F(ab')_2$ of R15E6; dimers or trimers of an Fab, Fv, scFv; and dia-, tria-, or tetrabodies derived from R15E6.

[0062] In particular, an agent suitable for use in the present invention is an antibody, antibody fragment, variant or derivatives thereof, either alone or in combination with other amino acid sequences, provided by known techniques. Such techniques include, but are not limited to enzymatic cleavage, chemical cleavage, peptide synthesis or recombinant techniques. The invention further embraces derivative agents, e.g. peptibodies.

[0063] Thus, one embodiment of an HPTPβ-ECD binding agent is an antibody, another embodiment is a protein, yet another embodiment is a peptide, and another embodiment is a darpin, another embodiment is an aptamer, another embodiment is a peptibody, still another embodiment is an adnectin, another embodiment is a nucleic acid. In some embodiments the HPTPβ-ECD binding agent is an monoclonal antibody, or is a polyclonal antibody. In particular embodiments, the HPTPβ-ECD binding agent is an antibody fragment that is capable of binding to HPTPβ-ECD. Preferably the HPTPβ-ECD binding agent is an antibody, or an antibody fragment, including but not limited to, an $F(ab')_2$, an Fab, a dimer of an Fab, an Fv, a dimer of an Fv, a scFv, a dimer of a scFv, a dimer an Fab, an Fv, a dimer of an Fv, a scFv, a dimer of a scFv, a trimer of an Fab, a trimer of an Fv, a trimer of a scFv, minibodies, a diabody , a triabody, a tetrabody, a linear antibody, a protein, a peptide, an aptamer, a peptibody, an adnectin, or a nucleic acid, that binds to the extracellular portion of HPTPβ. In certain embodiments the HPTPβ-ECD binding agent is and $F(ab')_2$ of a monoclonal antibody. In some embodiments the HPTPβ-ECD binding agent comprises a plurality of HPTPβ-ECD binding sites, for example where the HPTPβ-ECD binding agent is an intact antibody or an $F(ab')_2$, or a dimer of an Fab, or a trimer of an Fab. For example, in some embodiments an HPTPβ-ECD binding agent is able to bind to two HPTPβ molecules simultaneously at the same or different epitope, thereby bringing the two HPTPβ molecules into close proximity with one and other. In other embodiments the HPTPβ-ECD binding agent is able to bind to three HPTPβ molecules simultaneously at the same or different epitope, thereby bringing the three HPTPβ molecules into close proximity with one and other. In another embodiment, the HPTPβ-ECD binding agent is the monoclonal antibody produced by hybridoma cell line ATCC No. PTA-7680. In yet another embodiment, the HPTPβ-ECD binding agent is an antigen binding fragment of the monoclonal antibody produced by hybridoma cell line ATCC No. PTA-7680. In still another embodiment, the HPTPβ-ECD binding agent is an antibody having the same or substantially the same biological characteristics the monoclonal antibody produced by hybridoma cell line ATCC No. PTA-7680 or an antigen binding fragment thereof.

[0064] Any of the embodiments of HPTPβ-ECD binding agents disclosed in the present application, may be covalently or non-covalently conjugated to a vehicle. The term “vehicle” refers to a molecule that affects a biological property of an agent. For example, a vehicle may prevent degradation, and/or increase half-life, absorption, reduce toxicity, reduce immunogenicity, or increase biological activity of the agent. Exemplary vehicles include, but are not limited to, Fc domains of immunoglobulins; polymers, for example: polyethylene glycol (PEG), polylysine, dextran; lipids; cholesterol groups (such as a steroid); carbohydrates, dendrimers, oligosaccharides, or peptides that binds to a salvage receptor. In some embodiments the vehicle is polyethylene glycol (PEG), in other embodiments the vehicle is polylysine, in yet other embodiments the vehicle is dextran, in still other embodiments the vehicle is a lipid

[0065] Water soluble polymer attachments, such as polyethylene glycol, polyoxyethylene glycol, or polypropylene glycol, as described U.S. Pat. Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192; and 4,179,337, which are incorporated herein in their entirety. Still other useful polymers known in the art include monomethoxy-polyethylene glycol, dextran, cellulose, or other carbohydrate based polymers, poly-(N-vinyl pyrrolidone)-polyethylene glycol, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (e.g., glycerol) and polyvinyl alcohol, as well as mixtures of these polymers. Particularly preferred are peptibodies covalently modified with polyethylene glycol (PEG) subunits. Water soluble polymers may be bonded at specific positions, for example at the amino terminus of the peptibodies, or randomly attached to one or more side chains of the polypeptide. The use of PEG for improving the therapeutic capacity for agents, e.g. peptibodies, and for humanized antibodies in particular, is described in U.S. Pat. No. 6,133,426. The invention also contemplates derivatizing the peptide and/or vehicle portion of the agents. Such derivatives may improve the solubility, absorption, biological half-life, and the like of the agents. The moieties may alternatively eliminate or attenuate any undesirable side-effect of the agents and the like.

[0066] The term "antibody" (Ab) as used herein includes monoclonal antibodies, polyclonal antibodies, multi-specific antibodies (e.g. bispecific antibodies), single chain antibodies, e.g., antibodies from llama and camel, antibody fragments, e.g., variable regions and/or constant region fragments, so long as they exhibit a desired biological activity, e.g., antigen-binding activity. The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein.

[0067] An “antigen binding fragment” as used herein is a fragment of an agent that binds to a portion of HPTPβ and inhibits the activity of HPTPβ.

[0068] An "isolated antibody" is an antibody which has been identified, and/or separated, and/or recovered from its natural environment.

[0069] The basic four-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains (an IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called J chain, and therefore contain 10 antigen binding sites, while secreted IgA antibodies may polymerize to form polyvalent assemblages comprising 2-5 of the basic 4-chain units along with J chain). In the case of IgGs, the four-chain unit is generally about 150 kilo Daltons (kDa). Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the alpha and gamma chains and four C_H domains for mu and epsilon isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain (C_L) at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_{H1}). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see, e.g., Basic and Clinical Immunology, 8th edition, Daniel P. Stites, Abba I. Terr and Tristram G. Parslow (eds.), Appleton & Lange, 1994, page 71 and Chapter 6.

[0070] The L chain from any vertebrate species may be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins may be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha, delta, epsilon, gamma and mu, respectively. The gamma and alpha classes are further divided into subclasses on the basis of relatively minor differences in C_H sequence and function, e.g., humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2.

[0071] Members of the Camelidae family, e.g., llama, camel and dromedaries, contain a unique type of antibody, that are devoid of light chains, and further lack the C_{H1} domain (Muyldermans, S., Rev. Mol. Biotechnol., Vol. 74, pp. 277-302 (2001)). The variable region of these heavy chain antibodies are termed V_{HH} or VHH, and constitute the smallest available intact antigen-binding fragment (15 kDa) derived from a functional immunoglobulin.

[0072] The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines specificity of a particular antibody for its antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable domains. Instead, the V regions consist of relatively invariant stretches called framework regions (FR) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-12 amino acids long. The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

[0073] The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g., around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 1-35 (H1), 50-65 (H2) and 95-102 (H3) in the V_H; Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)) and/or those residues from a "hypervariable loop".

[0074] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. In contrast to polyclonal antibody preparations which include different antibodies directed against different epitopes, each monoclonal antibody is directed against a single epitope, i.e., a single antigenic determinant. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier "monoclonal" is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies useful in the present invention may be prepared by the hybridoma methodology or may be made using recombinant DNA methods in bacterial, eukaryotic animal or plant cells (see, e.g., U.S. Pat. No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries, using the available techniques, e.g., Clackson et al., Nature, Vol. 352, pp. 624-628 (1991).

[0075] The monoclonal antibodies herein include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (see U.S. Pat. No. 4,816,567; and Morrison et al., Proc. Natl. Acad. Sci. USA, Vol. 81, pp. 6851-6855 (1984)).

[0076] An "antibody fragment" comprises a portion of a multimeric antibody, preferably the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, dimers and trimers of Fab conjugates, Fv, scFv, minibodies; dia-, tria- and tetrabodies; linear antibodies (See Hudson et al., Nature Med. Vol. 9, pp. 129-134 (2003)).

[0077] "Fv" is the minimum antibody fragment which contains a complete antigen binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, and are therefore included in the definition of Fv.

[0078] A single-chain variable fragment (scFv) is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker.

[0079] Divalent (or bivalent) single-chain variable fragments (di-scFvs, bi-scFvs) can be engineered by linking two scFvs. This can be done by producing a single peptide chain with two V_H and two V_L regions, yielding tandem scFvs. Another possibility is the creation of scFvs with linker peptides that are too short for the two variable regions to fold together (about five amino acids), forcing scFvs to dimerize. This type is known as diabodies. Diabodies have been shown to have dissociation constants up to 40-fold lower than corresponding scFvs, meaning that they have a much higher affinity to their target. Consequently, diabody drugs could be dosed much lower than other therapeutic antibodies and are capable of highly specific targeting of tumors *in vivo*. Still shorter linkers (one or two amino acids) lead to the formation of trimers, so-called triabodies or tribodies. Tetrabodies are known and have been shown to exhibit an even higher affinity to their targets than diabodies.

[0080] The term "humanized antibody" or "human antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (e.g., a mouse) but in which at least a portion of the V_H and/or V_L sequence has been altered to be more "human-like", i.e., more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human V_H and V_L sequences to replace the corresponding nonhuman CDR sequences. Means for making chimeric, CDR-grafted and humanized antibodies are known to those of ordinary skill in the art (see, e.g., U.S. Pat. Nos. 4,816,567 and 5,225,539). One method for making human antibodies employs the use of transgenic animals, such as a transgenic mouse. These transgenic animals contain a substantial portion of the human antibody producing genome inserted into their own genome and the animal's own endogenous antibody production is rendered deficient in the production of antibodies. Methods for making such transgenic animals are known in the art. Such transgenic animals may be made using XenoMouse.RTM. technology or by using a "minilocus" approach. Methods for making XenoMice.RTM. are described in U.S. Pat. Nos. 6,162,963, 6,150,584, 6,114,598 and 6,075,181. Methods for making transgenic animals using the "minilocus" approach are described in U.S. Pat. Nos. 5,545,807, 5,545,806, 5,625,825, and WO 93/12227.

[0081] Humanization of a non-human antibody has become routine in recent years, and is now within the knowledge of one skilled in the art. Several companies provide services to make a humanized antibody, e.g., Xoma, Aries, Medarex, PDL and Cambridge Antibody Technologies. Humanization protocols are extensively described in technical literature, e.g., Kipriyanov and Le Gall, Molecular Biotechnol, Vol. 26, pp 39-60 (2004), Humana Press, Totowa, N.J.; Lo, Methods Mol. Biol., Vol. 248, pp 135-159 (2004), Humana Press, Totowa, N.J.; Wu et al., J. Mol. Biol. Vol. 294, pp. 151-162 (1999).

[0082] In certain embodiments, antibodies useful in the present invention may be expressed in cell lines other than hybridoma cell lines. Sequences encoding particular antibodies may be used for transformation of a suitable mammalian host cell by known methods for introducing polynucleotides into a host cell, including, for example packaging the polynucleotide in a virus (or into a viral vector) and transducing a host cell with the virus (or vector), or by transfection procedures known in the art, as exemplified by U.S. Pat. Nos. 4,399,216, 4,912,040, 4,740,461 and 4,959,455. The transformation procedure used may depend upon the host to be transformed. Methods for introduction of heterologous polynucleotides into mammalian cells are known in the art and include, but are not limited to, dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, encapsulation of the polynucleotide(s) in liposomes, mixing nucleic acid with positively-charged lipids, and direct microinjection of the DNA into nuclei.

[0083] A nucleic acid molecule encoding the amino acid sequence of a heavy chain constant region, a heavy chain variable region, a light chain constant region, or a light chain variable region of an antibody, or a fragment thereof in a suitable combination if desired, is/are inserted into an appropriate expression vector using standard ligation techniques. The antibody heavy chain or light chain constant region may be appended to the C-terminus of the appropriate variable region and is ligated into an expression vector. The vector is typically selected to be functional in the particular host cell employed (i.e., the vector is compatible with the host cell machinery such that amplification of the gene and/or expression of the gene may occur). For a review of expression vectors, see Methods Enzymol., Vol. 185, (Goeddel, ed.), 1990, Academic Press.

Identification of specific binding agents

[0084] Suitable HPTPβ-ECD binding agents may be identified using a variety of techniques known in the art. For example, candidate agents can be screened for binding to HPTPβ, and screened for activity. Generally, the candidate agents will first be screened for binding and those that show selective binding will then be screened to determine ability to inhibit the HPTPβ-mediated dephosphorylation of Tie2. In some cases however the candidate agents may be first screened in *vitro* for activity.

Determination of binding activity

[0085] The selection of a suitable assay for use in identification of a specific binding agent depends on the nature of the candidate agent to be screened. One of skill in the art would be able to choose the appropriate assays for the particular candidate agent.

[0086] For example, where the candidates are antibodies or peptibodies, which comprises an Fc moeity, FACS analysis as described in Example 3B allows the candidate agent to be selected based on its ability to bind to cells, which express HPTPβ. The cell may endogenously express HPTPβ or may be genetically engineered to express HPTPβ.

[0087] For other candidate agents such as aptamers, other techniques are known in the art. For example, aptamers which specifically bind to HPTPβ can be selected using a technique known as SELEX (systematic evolution of ligands by exponential enrichment) which selects specific aptamers through repeated rounds of in vitro selection.

Determination of inhibitor activity by Western blot

[0088] As exemplified in Example 4, in one suitable assay HUVECs are cultured in serum free media in the presence or absence of various concentrations of candidate agent and lysates of the cells are prepared, immunoprecipitated with a Tie2 antibody, resolved by polyacrylamide gel electrophoresis and transferred to a PVDF membrane. Membrane-bound immunoprecipitated proteins are then serially western blotted with an antiphosphotyrosine antibody to quantify Tie2 phosphorylation followed by a Tie2 antibody to quantify total Tie2. Tie2 phosphorylation is expressed as the ratio of the anti-phosphotyrosine signal over the total Tie2 signal. Greater levels of the anti-phosphotyrosine signal indicate greater HPTPβ inhibition by the candidate agent.

[0089] Candidate agents that can be screened include, but are not limited to, libraries of known agents, including natural products, such as plant or animal extracts, biologically active molecules including proteins, peptides including but not limited to members of random peptide libraries and combinatorial chemistry derived molecular library made of D- or L-configuration amino acids, antibodies including, but not limited to, polyclonal, monoclonal, chimeric, human, single chain antibodies, Fab, $F(ab)_2$ and Fab expression library fragments and eptiope-binding fragments thereof.

[0090] As used herein "antibody fragments" include, but are not limited, to a $F(ab')_2$, a dimer or trimer of an Fab, Fv, scFv, or a dia-, tria-, or tetrabody derived from an antibody.

METHODS

[0091] Disclosed are methods for the treatment of diseases or conditions of the eye, especially retinopathies, ocular edema and ocular neovascularization. Non-limiting examples of these diseases or conditions include diabetic macular edema, age-related macular degeneration (wet form), choroidal neovascularization, diabetic retinopathy, ocular ischemia, uveitis, retinal vein occlusion (central or branch), ocular trauma, surgery induced edema, surgery induced neovascularization, cystoid macular edema, ocular ischemia, uveitis, and the like. These diseases or conditions are characterized by changes in the ocular vasculature whether progressive or non-progressive, whether a result of an acute disease or condition, or a chronic disease or condition.

[0092] One aspect of the disclosed methods relates to diseases that are a direct or indirect result of diabetes, *inter alia*, diabetic macular edema and diabetic retinopathy. The ocular vasculature of the diabetic becomes unstable over time leading to conditions such as non-proliferative retinopathy, macular edema, and proliferative retinopathy. As fluid leaks into the center of the macula, the part of the eye where sharp, straight-ahead vision occurs, the buildup of fluid and the associated protein begin to deposit on or under the macula. This results in swelling that disturbs the subject's central vision. This condition is referred to as "macular edema." Another condition that may occur is non-proliferative retinopathy in which vascular changes, such as microaneurysms, may occur outside the macular region of the eye.

[0093] These conditions may or may not progress to diabetic proliferative retinopathy which is characterized by neovascularization. These new blood vessels are fragile and are susceptible to bleeding. The result is scaring of the retina, as well as occlusion or total blockage of the light pathway through the eye due to the over formation of new blood vessels. Typically, subjects having diabetic macular edema are suffering from the non-proliferative stage of diabetic retinopathy; however, it is not uncommon for subjects to only begin manifesting macular edema at the onset of the proliferative stage.

[0094] Diabetic retinopathy, if left untreated, can lead ultimately to blindness. Indeed, diabetic retinopathy is the leading cause of blindness in working-age populations.

[0095] Therefore, the disclosed methods relate to preventing, treating, controlling, abating, and/or otherwise minimizing ocular neovascularization in a subject having diabetes or a subject diagnosed with diabetes. In addition, subjects having or subjects diagnosed with diabetes can be alerted to or can be made aware of the risks of developing diabetes-related blindness, therefore the present methods can be used to prevent or delay the onset of non-proliferative retinopathy in subjects known to be at risk. Likewise, the present methods can be used for treating subjects having or being diagnosed with non-proliferative diabetic retinopathy to prevent progression of the condition.

[0096] The disclosed methods relate to preventing or controlling ocular neovascularization or treating a disease or condition that is related to the onset of ocular neovascularization by administering to a subject an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof.

[0097] One aspect of this method relates to treating or preventing ocular neovascularization by administering to a subject an effective amount of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. One embodiment of this aspect relates to a method for treating ocular neovascularization comprising administering to a subject a composition comprising an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carrier or compatible excipient.

[0098] Thus, one embodiment of the present disclosure is a method of treating or preventing ocular neovascularization in a subject, comprising administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. Another embodiment of the present disclosure is a method of treating or preventing ocular neovascularization in a subject, comprising administering an effective amount of a composition comprising an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carrier or compatible excipient. Yet another embodiment of the present disclosure is the use of an HPTPβ-ECD binding agent in the treatment of ocular neovascularization.

[0099] The disclosed methods also relate to preventing or controlling ocular edema or treating a disease or condition that is related to the onset of ocular edema by administering to a subject an HPTPβ-ECD binding agent.

[00100] One aspect of this method relates to treating or preventing ocular edema by administering to a subject an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. One embodiment of this aspect relates to a method for treating ocular edema comprising administering to a subject a composition comprising:

a. an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof; and
b. one or more carriers or compatible excipients.

[00101] Thus, one embodiment of the present disclosure is a method of treating or preventing ocular edema in a subject, comprising administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. Another embodiment of the present disclosure is a method of treating or preventing ocular edema in a subject, comprising administering an effective amount of a composition comprising HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients. An embodiment of the present disclosure is the use of an HPTPβ-ECD binding agent in the treatment of ocular edema.

[00102] Another disclosed method relates to preventing or controlling retinal edema or retinal neovascularization, or treating a disease or condition that is related to the onset of retinal edema or retinal neovascularization, by administering to a subject an HPTPβ-ECD binding agent. One aspect of this method relates to treating or preventing retinal edema or retinal neovascularization by administering to a subject an effective amount of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. One embodiment of this aspect relates to a method for treating retinal edema or retinal neovascularization comprising administering to a subject a composition comprising an effective amount of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients.

[00103] Thus, one embodiment of the present disclosure is a method of treating or preventing retinal edema in a subject, comprising administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. Another embodiment is a method of treating or preventing retinal neovascularization comprising administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. One embodiment of the present disclosure is a method of treating or preventing retinal edema in a subject, by administering a composition comprising an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients. Another embodiment is a method of treating or preventing retinal neovascularization by administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients. Another embodiment is the use of an HPTPβ-ECD binding agent in the treatment of retinal edema. A further embodiment is the use of an HPTPβ-ECD binding agent in the treatment of retinal neovascularization.

[00104] A further disclosed method relates to treating, preventing or controlling diabetic retinopathy, or treating a disease or condition that is related to the onset of diabetic retinopathy by administering to a subject an HPTPβ-ECD binding agent.

[00105] One aspect of this method relates to treating or preventing diabetic retinopathy by administering to a subject an effective amount of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. One embodiment of this aspect relates to a method for treating diabetic retinopathy comprising administering to a subject a composition comprising an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carrier or compatible excipient.

[00106] Thus, one embodiment of the present disclosure is a method of treating or preventing diabetic retinopathy in a subject, comprising administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. Another embodiment of the present disclosure is a method of treating or preventing diabetic retinopathy in a subject, by administering a composition comprising an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients. Yet another embodiment of the present disclosure is the use of an HPTPβ-ECD binding agent in the treatment of diabetic retinopathy.

[00107] A further disclosed method relates to a method for treating or preventing non-proliferative retinopathy comprising administering to a subject an effective amount of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof.

[00108] Another embodiment of this aspect relates to a method for treating or preventing non-proliferative retinopathy comprising administering to a subject a composition comprising an effective amount of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof; and one or more carrier or compatible excipient.

[00109] Thus, one embodiment of the present disclosure is a method of treating or preventing non-proliferative retinopathy in a subject, comprising administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. Another embodiment of the present disclosure is a method of treating or preventing non-proliferative retinopathy in a subject, by administering a composition comprising an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients. Yet another embodiment of the present disclosure is the use of an HPTPβ-ECD binding agent in the treatment of non-proliferative retinopathy.

[00110] Yet a further disclosed method relates to preventing or controlling diabetic macular edema, or treating a disease or condition that is related to the onset of diabetic macular edema by administering to a subject an HPTPβ-ECD binding agent.

[00111] One aspect of this method relates to treating or preventing diabetic macular edema by administering to a subject an effective amount of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. One embodiment of this aspect relates to a method for treating diabetic macular edema comprising administering to a subject a composition comprising: a) an effective amount of one or more of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof; and b)one or more carriers or compatible excipients.

[00112] Thus, one embodiment of the present disclosure is a method of treating or preventing diabetic macular edema in a subject, comprising administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. Another embodiment of the present disclosure is a method of treating or preventing diabetic macular edema in a subject, by administering a composition comprising an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients.

Yet another embodiment of the present disclosure is the use of an HPTPβ-ECD binding agent in the treatment of diabetic macular edema.

[00113] Another embodiment of the present disclosure is a method for treating, or preventing age-related wet form macular degeneration edema in a subject, comprising administering an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. Another embodiment of the present disclosure is a method of treating or preventing age-related wet form macular degeneration edema in a subject, by administering a composition comprising an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients. Yet another embodiment of the present disclosure is the use of an HPTPβ-ECD binding agent in the treatment of age-related wet form macular degeneration edema.

[00114] A further embodiment is a method for treating, preventing or controlling choroidal neovascularization, central retinal vein occlusion, branch retinal vein occlusion, ocular trauma, surgery induced edema, surgery induced neovascularization, cystoid macular edema, ocular ischemia, or uveitis, by administering to a subject an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof. Another embodiment is a method for treating, preventing or controlling choroidal neovascularization, central retinal vein occlusion, branch retinal vein occlusion, ocular trauma, surgery induced edema, surgery induced neovascularization, cystoid macular edema, ocular ischemia, retinal angiomatous proliferation, macular telangiectasia, or uveitis, by administering to a subject a composition comprising an effective amount of an HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof, and one or more carriers or compatible excipients. Yet another embodiment of the present disclosure is the use of an HPTPβ-ECD binding agent in the treatment of choroidal neovascularization, central retinal vein occlusion, branch retinal vein occlusion, ocular trauma, surgery induced edema, surgery induced neovascularization, cystoid macular edema, ocular ischemia, retinal angiomatous proliferation, macular telangiectasia or uveitis.

[00115] Another embodiment is a composition for treating or preventing an ocular disorder, comprising an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carrier. Yet another embodiment is a composition for treating or preventing an ocular disorder, comprising an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable carrier composition wherein the ocular disorder is ocular neovascularization, ocular edema, retinal neovascularization, diabetic retinopathy, diabetic macular edema, age-related macular degeneration, choroidal neovascularization, central retinal vein occlusion, branch retinal vein occlusion, ocular trauma, surgery induced edema, surgery induced neovascularization, cystoid macular edema, ocular ischemia, non-proliferative retinopathy, retinal angiomatous proliferation, macular telangiectasia, or uveitis.

[00116] In some embodiments, the HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof is used for treating an ocular disorder. In some embodiments, the HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof is used for treating an ocular disorder, wherein the ocular disorder is ocular neovascularization, ocular edema, retinal neovascularization, diabetic retinopathy, diabetic macular edema, age-related macular degeneration, choroidal neovascularization, central retinal vein occlusion, branch retinal vein occlusion, ocular trauma, surgery induced edema, surgery induced neovascularization, cystoid macular edema, ocular ischemia, non-proliferative retinopathy, retinal angiomatous proliferation, macular telangiectasia or uveitis.

[00117] In still other embodiments, the HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof is used for the manufacture of a medicament for treating an ocular disorder. In some embodiments the ocular disorder is ocular neovascularization, ocular edema, retinal neovascularization, diabetic retinopathy, diabetic macular edema, age-related macular degeneration, choroidal neovascularization, central retinal vein occlusion, branch retinal vein occlusion, ocular trauma, surgery induced edema, surgery induced neovascularization, cystoid macular edema, ocular ischemia, non-proliferative retinopathy, retinal angiomatous proliferation, macular telangiectasia or uveitis.

Dosing

[00118] Effective dosages and schedules for administering the HPTPβ-ECD binding agent may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of the agent that must be administered will vary depending on, for example, the subject which will receive the agent, the route of administration, the particular type of agent used and other drugs being administered to the subject. For example, guidance in selecting appropriate doses for antibodies is found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. A typical dose of the agent used alone might range from about 0.01 mg/kg to up to 500 mg/kg of body weight or more per day, or from about 0.01 mg/kg to about 50 mg/kg, or from 0.1 mg/kg to about 50 mg/kg, or from about 0.1 mg/kg to up to about 10 mg/kg, or from about 0.2 mg/kg to about 1 mg/kg, depending on the factors mentioned above.

[00119] One embodiment relates to a method for treating ocular edema and/or neovascularization comprising administering to a subject from about 0.01 mg/kg to about 50 mg/kg of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. Another iteration of this embodiment relates to administering to a subject from about 0.1 mg/kg to about 10 mg/kg by weight of the subject being treated, an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. A further iteration of this embodiment relates to a method for treating or preventing diseases or conditions related to ocular edema and/or neovascularization comprising administering to a subject from about 1 mg/kg to about 10 mg/kg by weight of the subject an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. Yet another iteration of this embodiment relates to a method for treating or preventing diseases or conditions related to ocular edema and/or neovascularization comprising administering to a subject from about 5 mg/kg to about 10 mg/kg by weight of the subject an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. In a further iteration of this embodiment relates to a method for treating or preventing diseases or conditions related to ocular edema and/or neovascularization comprising administering to a subject from about 1 mg/kg to about 5 mg/kg by weight of the subject an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof. In a yet further iteration of this embodiment relates to a method for treating or preventing diseases or conditions related to ocular edema and/or neovascularization comprising administering to a subject from about 3 mg/kg to about 7 mg/kg by weight of the subject an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof.

[00120] The dosing schedules for administration of an HPTPβ-ECD binding agent include, but are not limited to, once daily, three-times weekly, twice weekly, once weekly, three times, twice monthly, once monthly and once every other month.

[00121] Further disclosed are methods of treating or preventing one or more of the diseases or conditions described herein above related to ocular edema and/or neovascularization that are the result of administration of another pharmaceutically active agent. As such, this aspect relates to a method comprising administering to a subject a composition comprising: a) an effective amount of an HPTPβ-ECD binding agent or pharmaceutically acceptable salt thereof; b) one or more additional pharmaceutically active agents; and c) one or more carriers or compatible excipients.

[00122] The methods of the present invention may be combined with the standard of care, including, but not limited to, laser treatment.

[00123] Non-limiting examples of pharmaceutically active agents suitable for combination with an HPTPβ-ECD binding agent include anti-infectives, i.e., aminoglycosides, antiviral agents, antimicrobials, anticholinergics/antispasmotics, antidiabetic agents, antihypertensive agents, antineoplastics, cardiovascular agents, central nervous system agents, coagulation modifiers, hormones, immunologic agents, immunosuppressive agents, ophthalmic preparations and the like.

[00124] The disclosed method also relates to the administration of the disclosed agents and compositions. Administration can be systemic via subcutaneous or i.v. administration; or the HPTP-β inhibitor will be administered directly to the eye, e.g., local. Local methods of administration include, for example, by eye drops, subconjunctival injections or implants, intravitreal injections or implants, sub-Tenon's injections or implants, incorporation in surgical irrigating solutions, etc.

[00125] The disclosed methods relate to administering an HPTPβ-ECD binding agent as part of a pharmaceutical composition. Compositions suitable for local administration are known to the art (see, for example, U.S. Pat. Publ. 2005/0059639). In various embodiments, compositions of the invention can comprise a liquid comprising an active agent in solution, in suspension, or both. As used herein, liquid compositions include gels. In one embodiment, the liquid composition is aqueous. Alternatively, the composition can take form of an ointment. In another embodiment, the composition is an *in situ* gellable aqueous composition. Such a composition can comprise a gelling agent in a concentration effective to promote gelling upon contact with the eye or lacrimal fluid in the exterior of the eye. Aqueous compositions of the invention have ophthalmically compatible pH and osmolality. The composition can comprise an ophthalmic depot formulation comprising an active agent for subconjunctival administration. The microparticles comprising active agent can be embedded in a biocompatible pharmaceutically acceptable polymer or a lipid encapsulating agent. The depot formulations may be adapted to release all or substantially all the active material over an extended period of time. The polymer or lipid matrix, if present, may be adapted to degrade sufficiently to be transported from the site of administration after release of all or substantially all the active agent. The depot formulation can be a liquid formulation, comprising a pharmaceutical acceptable polymer and a dissolved or dispersed active agent. Upon injection, the polymer forms a depot at the injections site, e.g., by gelifying or precipitating. The composition can comprise a solid article that can be inserted in a suitable location in the eye, such as between the eye and eyelid or in the conjuctival sac, where the article releases the active agent. Solid articles suitable for implantation in the eye in such fashion generally comprise polymers and can be bioerodible or non-bioerodible.

[00126] In one embodiment of the disclosed methods, a human subject with at least one visually impaired eye is treated with 2-4000 μg of an HPTPβ-ECD binding agent via intravitreal injection. Improvement of clinical symptoms are monitored by one or more methods known to the art, for example, indirect ophthalmoscopy, fundus photography, fluorescein angiopathy, electroretinography, external eye examination, slit lamp biomicroscopy, applanation tonometry, pachymetry, optical coherence tomography and autorefaction. Subsequent doses can be administered weekly or monthly, e.g., with a frequency of 2-8 weeks or 1-12 months apart.

[00127] The disclosed methods include administration of the disclosed agents in combination with a pharmaceutically acceptable carrier. “Pharmaceutically acceptable” means a material that is not biologically or otherwise undesirable, i.e., the material may be administered to a subject without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical formulation in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art. In another aspect, many of the disclosed agents can be used prophylactically, i.e., as a preventive agent, either neat or with a pharmaceutically acceptable carrier. The ionic liquid compositions disclosed herein can be conveniently formulated into pharmaceutical compositions composed of neat ionic liquid or in association with a pharmaceutically acceptable carrier. See Remington's Pharmaceutical Sciences, 18th ed., Gennaro, AR. Ed., Mack Publishing, Easton Pa. (1990), which discloses typical carriers and conventional methods of preparing pharmaceutical compositions that can be used in conjunction with the preparation of formulations of the agents described herein and which is incorporated by reference herein. Such pharmaceutical carriers, most typically, would be standard carriers for administration of compositions to humans and non-humans, including solutions such as sterile water, saline and buffered solutions at physiological pH. Other agents can be administered according to standard procedures used by those skilled in the art. For example, pharmaceutical compositions can also include one or more additional active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics and the like.

[00128] Examples of pharmaceutically-acceptable carriers include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the disclosed agents, which matrices are in the form of shaped articles, e.g., films, liposomes, microparticles, or microcapsules. It will be apparent to those persons skilled in the art that certain carriers can be more preferable depending upon, for instance, the route of administration and concentration of composition being administered. Other agents can be administered according to standard procedures used by those skilled in the art.

[00129] Pharmaceutical formulations can include additional carriers, as well as thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the agents disclosed herein. Pharmaceutical formulations can also include one or more additional active ingredients such as antimicrobial agents, anti-inflammatory agents, anesthetics and the like.

[00130] For the purposes of the present disclosure the term "excipient" and "carrier" are used interchangeably throughout the description of the present disclosure and said terms are defined herein as, "ingredients which are used in the practice of formulating a safe and effective pharmaceutical composition."

[00131] The formulator will understand that excipients are used primarily to serve in delivering a safe, stable and functional pharmaceutical, serving not only as part of the overall vehicle for delivery but also as a means for achieving effective absorption by the recipient of the active ingredient. An excipient may fill a role as simple and direct as being an inert filler, or an excipient as used herein may be part of a pH stabilizing system. The formulator can also take advantage of the fact the agents of the present invention have improved cellular potency, pharmacokinetic properties.

[00132] The disclosed agents can also be present in liquids, emulsions, or suspensions for delivery of active therapeutic agents. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc., an active agent as described herein and optional pharmaceutical adjuvants in an excipient, such as, for example, water, saline aqueous dextrose, glycerol, ethanol and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered can also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art, for example see Remington's Pharmaceutical Sciences, referenced above.

KITS

[00133] Also disclosed are kits comprising the agents and compositions to be delivered into a human, mammal, or cell. The kits can comprise one or more packaged unit doses of a composition comprising one or more agents to be delivered into a human, mammal, or cell. The unit dosage ampoules or multi-dose containers, in which the agents to be delivered are packaged prior to use, can comprise a hermetically sealed container enclosing unit dose of the composition, or multiples unit doses. The agents can be packaged as a sterile formulation, and the hermetically sealed container is designed to preserve sterility of the formulation until use.

EXAMPLES

EXAMPLE 1
Production of HPTPβ Extracellular Domain Protein

[00134] Full length HPTPβ cDNA (SEQ ID NO:1) is cloned from a human placental library according to the manufacturer's (Origene) instructions. A cDNA encoding the entire soluble extracellular domain (ECD) of HPTPβ is cloned by PCR from the full length cDNA coding for amino acids 1-1621 with an added c-terminal His-His-His-His-His-His-Gly (6His-Gly) (SEQ ID NO:3). The resulting cDNA is cloned into mammalian expression vectors for transient (pShuttle-CMV) or stable (pcDNA3.1(-)) expression in HEK293 cells. To obtain purified HPTPβ ECD (βED), HEK293 cells transfected with a βECD expression vector are incubated in OptiMEM-serum free (Gibco) for 24 hours under normal growth conditions. The conditioned media is then recovered, centrifuged to remove debris, and 1 mL of washed Ni-NTA agarose (Qiagen) (500μL packed material) is added to each 10μL of cleared media and allowed to rock overnight at 4° C. On the following day, the mixture is loaded into a column and washed with 20 bed volumes of 50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8. The purified HPTPβ extracellular domain protein (SEQ ID NO:4) is then eluted with 200 μL/elution in 50 mM NaH_2PO_4, 300 mM NaCl, 250 mM Imidazole, pH 8. Fractions are analyzed for protein content using reducing-denaturing SDS-polyacrylimide gel electrophoresis and detected by silver stain (Invitrogen) and confirmed by mass spectrometry.

Example 2

Generation of monoclonal antibodies to HPTPβ extracellular domain

[00135] Purified HPTPβ extracellular domain protein is produced, for example by the procedure described in Example 1. For production of the HPTPβ extracellular domain immunogen, the purified HPTPβ extracellular domain-6-His protein is conjugated to porcine thyroglobulin (Sigma) using EDC coupling chemistry (Hockfield, S. et al., (1993) Cold Spring Habor Laboratory Press. Vol. 1 pp. 111-201, Immunocytochemistry). The resulting HPTPβ extracellular domain-thyroglobulin conjugate is dialyzed against PBS, pH 7.4. Adult Balb/c mice are then immunized subcutaneously with the conjugate (100-200 μg) and complete Freund's adjuvant in a 1:1 mixture. After 2-3 weeks, the mice are injected intraperitoneally or subcutaneously with incomplete Freund's adjuvant and the conjugate in a 1:1 mixture. The injection is repeated at 4-6 weeks. Sera are collected from mice 7 days post-third-injection and assayed for immunoreactivity to HPTPβ extracellular domain antigen by ELISA and western blotting. Mice that display a good response to the antigen are boosted by a single intra-spleen injection with 50 μl of purified HPTPβ extracellular domain protein mixed 1:1 with Alum hydroxide using a 31 gauge extra long needle (Goding, J. W., (1996) Monoclonal Antibodies: Principles and Practices. Third Edition, Academic Press Limited. p.145). Briefly, mice are anesthetized with 2.5% avertin, and a 1 centimeter incision is created on the skin and left oblique body wall. The antigen mixture is administered by inserting the needle from the posterior portion to the anterior portion of the spleen in a longitudinal injection. The body wall is sutured and the skin is sealed with two small metal clips. Mice are monitored for safe recovery. Four days after surgery the mouse spleen is removed and single cell suspensions are made for fusion with mouse myeloma cells for the creation of hybridoma cell lines (Spitz, M., (1986) Methods In Enzymology, Vol. 121. Eds. John J, Lagone and Helen Van Vunakis. pp. 33-41 (Academic

Press, New York, NY)). Resulting hybridomas are cultured in Dulbeccos modified media (Gibco) supplemented with 15 % fetal calf serum (Hyclone) and hypoxathine, aminopterin and thymidine.

[00136] Screening for positive hybridomas begins 8 days after the fusion and continues for 15 days. Hybridomas producing anti-HPTPβ extracellular domain antibodies are identified by ELISA on two sets of 96-well plates: one coated with the histidine tagged-HPTPβ extracellular domain and another one coated with a histidine-tagged bacterial MurA protein as a negative control. The secondary antibody is a donkey anti-mouse IgG labeled with horseradish peroxidase (HRP) (Jackson Immunoresearch). Immunoreactivity is monitored in wells using color development initiated by ABTS tablets dissolved in TBS buffer, pH 7.5. The individual HRP reaction mixtures are terminated by adding 100 microliters of 1% SDS and reading absorbance at 405 nm with a spectrophotometer. Hybridomas producing antibodies that interact with HPTPβ extracellular domain-6His, and not with the murA-6His protein are used for further analysis. Limiting dilutions (0.8 cells per well) are performed twice on positive clones in 96 well plates, with clonality defined as having greater than 99% of the wells with positive reactivity. Isotypes of antibodies are determined using the iso-strip technology (Roche). To obtain purified antibody for further evaluation, tissue culture supernatants are affinity purified using a protein A or a protein G column.

[00137] Five monoclonal antibodies immunoreactive to HPTPβ–ECD protein were isolated and given the following nomenclature, R15E6, R12A7, R3A2, R11C3, R15G2 and R5A8. Based on its reaction with theHPTPβ–ECD protein in ELISA and in western blots, R15E6 was selected for further study.

EXAMPLE 3

The monoclonal antibody R15E6

[00138] The monoclonal antibody R15E6 was identified and characterized as described in Example 2 of the present application and in United States Pat. No., 7,973,142; the procedure and results are summarized below.

A. R15E6 binds endogenous HPTPβ as demonstrated by demonstrated by immunoprecipitation.

[00139] Materials: Human umbilical vein endothelial cells (HUVECs), EGM media, and trypsin neutralizing solution from Cambrex; OPTIMEM I (Gibco), bovine serum albumin (BSA; Santa Cruz), phosphate buffered saline (PBS; Gibco), Growth Factors including Angiopoietin 1 (Ang1), vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) (R&D Systems), Tie2 monoclonal antibody (Duke University/P&GP), VEGF receptor 2 (VEGFR2) polyclonal antibody (Whitaker et. al), protein A/G agarose (Santa Cruz), Tris-Glycine pre-cast gel electrophoresis/transfer system (6-8%) (Invitrogen), PVDF membranes (Invitrogen), lysis buffer (20 mm Tris-HCl, 137 mm NaCl, 10% glycerol, 1% triton-X-100, 2 mM EDTA, 1 mM NaOH, 1 mM NaF, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin).

[00140] Method: HUVECs were pre-treated for 30 min with antibody (in OPTIMEM) or OPTIMEM I alone. After removal of pre-treatment, cells were treated with Ang1 (100 ng/ml) for 6 minutes in PBS+0.2% BSA and lysed in lysis buffer. Lysates were run directly on a Tris-Glycine gel or immunoprecipitated with 2-5 μg/ml Tie-2 antibody or 10 μg/ml R15E6 antibody and protein A/G agarose. Immunoprecipitated samples were rinsed once with lysis buffer and boiled for 5 min in 1 x times sample buffer. Samples were resolved on a Tris-Glycine gel, transferred to a PVDF membrane, and detected by western blot using the indicated antibodies (pTYR Ab (PY99, Santa Cruz), Tie-2, VEGFR2 and/or R15E6).

[00141] Results: By IP/western blotting, R15E6 recognizes a major, high molecular weight band consistent with the size of HPTPβ (Fig. 1, Panel A, Lane 2). The less intense, lower molecular weight bands likely represent less glycosylated precursor forms of HPTPβ. An immunoprecipitation (IP) with control, non-immune IgG shows no bands in the molecular weight range of HPTPβ (Fig. 1, Panel A, Lane 1), and a combined Tie2/VEGFR2 IP shows bands of the expected molecular weight (Fig. 1, Panel A, Lane 3). This result demonstrates that R15E6 recognizes and is specific for HPTPβ.

B. R15E6 binds endogenous HPTPβ as demonstrated by FACS analysis

[00142] Materials: HUVECs, EGM media, and trypsin neutralizing solution from Cambrex; Secondary Alexfluor 488-tagged antibody from Molecular Probes; Hanks balanced salt solution (Gibco); FACSCAN flow cytometer and CellQuest software from Becton Dickenson.

[00143] Method: HUVECs are trypsinized, treated with trypsin neutralizing solution and rinsed with HBSS. R15E6 antibody (0.6 μg) is added to 250,000 cells in 50μl of HBSS and incubated on ice for 20 minutes. Cells were rinsed with 1 ml HBSS followed by adding 2 μg of fluorescent-conjugated secondary antibody for 20 minutes on ice. Cells were rinsed and resuspended in 1 ml HBSS then analyzed on the FACSCAN flow cytometer with CellQuest software. Control cells were treated with fluorescent-conjugated secondary antibody only.

[00144] Results: By FACS analysis, intact HUVECs, R15E6 causes a robust shift (>90% of cells) in the fluorescence signal compared to the secondary antibody alone (Fig. 1, Panel B). This result indicates that R15E6 binds to endogenous HPTPβ presented on the surface of intact endothelial cells.

EXAMPLE 4
R15E6 Enhances Tie2 Activation

[00145] R15E6 enhances Tie2 phosphorylation in the absence and presence of the angiopoietin 1 (Ang1), the Tie2 ligand.

[00146] Methods: HUVECs are cultured in serum free media as described above in the presence or absence of various concentrations of R15E6 and with or without added Ang1. Lysates are prepared, immunoprecipitated with a Tie2 antibody, resolved by polyacrylamide gel electrophoresis and transferred to a PVDF membrane. Membrane-bound immunoprecipitated proteins are then serially western blotted with an antiphosphotyrosine antibody to quantify Tie2 phosphorylation followed by a Tie2 antibody to quantify total Tie2. Tie2 phosphorylation is expressed as the ratio of the antiphosphotyrosine signal over the total Tie2 signal.

[00147] Results: R15E6 enhances Tie2 phosphorylation both in the absence and presence of Ang1 (Fig. 2). This result indicates that binding of R15E6 to HPTPβ on the surface of endothelial cells modulates its biological function resulting in enhanced activation of Tie2 in the absence or presence of ligand.

EXAMPLE 5
Generation of anti-VE-PTP extracellular domain antibodies

A. Production of mouse VE-PTP extracellular domain protein (VE-PTP-ECD)

[00148] VE-PTP –ECD may be produced by any suitable method. Such methods are well known in the art. For example, VE-PTP –ECD can be produced using a method similar to

Example 1 of the present disclosure where VE-PTP-ECD cDNA is used in place of cDNA encoding HPTPβ-ECD. SEQ ID NO:7 provides a nucleotide sequence that encodes VE-PTP-ECD. SEQ ID NO:8 provides the amino acid sequence of VE-PTP-ECD.

B. Generation of antibodies to VE-PTP ECD

[00149] Anti-VE-PTP antibodies are readily generated by methods that are well known in the art. For example, anti VE-PTP antibodies can be generated using the method of Example 2 of the present disclosure by substituting VE-PTP-ECD for the HPTPβ extracellular domain and immunizing rats with the resulting protein. The rat anti-mouse VE-PTP antibody used in the present studies was kindly provided by Dr. D. Vestweber (mAb 109). The antibody was generated as described in Baumer S. et al., Blood, 2006; 107: 4754-4762. Briefly, the antibody was generated by immunizing rats with a VE-PTP-Fc fusion protein. Immunization, hybridoma-fusion, and screening were conducted as described in Gotsch U., et al., J Cell Sci. 1997, Vol. 110, pp. 583-588 and Bosse R. and Vestweber D., Eur J Immunol. 1994, Vol. 24, pp. 3019-3024.

[00150] The fusion protein was constructed such that the first 8 fibronectin type III-like repeats ending with the amino acid proline at position 732 of VE-PTP were fused in frame with the Fc part of human IgG1 (starting with amino acid proline at position 239). This construct cloned into pcDNA3 (Invitrogen) was stably transfected into CHO cells, and the fusion protein was purified by protein A Sepharose affinity purification.

EXAMPLE 6

Intravitreal injections of an anti- VE-PTP ECD antibody

[00151] Laser-induced Choroidal Neovascularization Model: The choroidal neovascularization model is considered to represent a model of neovascular age-related macular degeneration. Choroidal NV was generated as previously described. See Tobe T, et al., Am. J. Pathol. 1998, Vol. 153, pp. 1641-1646. Adult C57BL/6 mice had laser-induced rupture of Bruch's membrane in three locations in each eye and were then given 1 μL intravitreal injections of 1 or 2 μg of a VE-PTP-ECD antibody (IgG2a), in one eye and vehicle (5% dextrose) in the fellow eye. These treatments were repeated on day 7. Fourteen days after laser, the mice were perfused with fluorescein-labeled dextran (2×10^6 average MW, Sigma, St. Louis, MO) and the extent of neovascularization was assessed in choroidal flat mounts by fluorescence microscopy. The area of CNV at each Bruch's membrane rupture site was measured by image analysis by an observer masked with respect to treatment group. The area of CNV is the average of the three rupture sites in one eye. As shown in Fig. 3, treatment with the VE-PTP-ECD antibody significantly reduced choroidal neovascularization at both 1 and 2 μg doses versus treatment with vehicle control.

Example 7

Oxygen-Induced Ischemic Retinopathy

[00152] The oxygen-induced ischemic retinopathy model is considered to represent a model of proliferative diabetic retinopathy. Ischemic retinopathy was produced in C57BL/6 mice by a method described by Smith, L.E.H., et al. Oxygen-induced retinopathy in the mouse. Invest. Ophthalmol. Vis. Sci. 35, 101-111 (1994).

[00153] C57BL/6 mice at postnatal day 7 (P7) and their mothers were placed in an airtight chamber and exposed to hyperoxia (75 ± 3% oxygen) for five days. Oxygen was continuously monitored with a PROOX model 110 oxygen controller (Reming Bioinstruments Co., Redfield, NY). On P12, mice were returned to room air and under a dissecting microscope, a Harvard Pump Microinjection System and pulled glass pipettes were used to deliver a 1 μl intravitreal injection of 1 or 2 μg of a VE-PTP-ECD antibody was made in one eye and vehicle was injected in the fellow eye. At P17, the area of NV on the surface of the retina was measured at P17 as previously described. See Shen J, et al., Invest. Ophthalmol. Vis. Sci. 2007, Vol. 48, pp. 4335-4341. Briefly, mice were given an intraocular injection of 1 μl containing 0.5 μg rat anti-mouse PECAM antibody (Pharmingen, San Jose, CA). Twelve hours later, the mice were euthanized, the eyes fixed in 10% formalin. The retinas were dissected, incubated for 40 minutes in 1:500 goat anti-rat IgG conjugated with Alexa488 (Invitrogen, Carlsbad, CA), washed, and whole mounted. An observer masked with respect to treatment group examined the slides with a Nikon Fluorescence microscope and measured the area of NV per retina by computerized image analysis using ImagePro Plus software (Media Cybernetics, Silver Spring, MD). Fig. 4 shows that treatment with the VE-PTP-ECD antibody significantly reduced retinal neovascularization at both 1 and 2 μg doses versus treatment with vehicle control. Fig. 5 shows representative retinal whole mounts from a mouse treated with vehicle versus a mouse treated with 2 μg of the VE-PTP-ECD antibody.

Example 8

Subcutaneous injection of a VE-PTP-ECD antibody

[00154] The oxygen-induced ischemic retinopathy model was conducted as described in Example 7 (containment in a 75% oxygen atmosphere from P5 to P12) for intravitreal dosing except that the VE-PTP-ECD antibody (1 mg/kg) was dosed subcutaneously at P12 when the mice were returned to room air and again on days P14 and P16 (three total doses). Neovascularization was assessed as described above on day (P17). Fig. 6 shows that subcutaneous dosing of the VE-PTP-ECD antibody reduces the area of retinal neovascularization.

Example 9

[00155] The experiment described in Example 8 was repeated at a subcutaneous dose of 2 mg/kg. (Fig. 7)

[00156] While a number of embodiments of this disclosure are described, it is apparent that the basic examples may be altered to provide other embodiments that utilize or encompass the HPTPβ-ECD binding agent, methods and processes of this invention. The embodiments and examples are for illustrative purposes and are not to be interpreted as limiting the disclosure, but rather, the appended claims define the scope of this invention.

CLAIMS

1. A method for alleviating an ocular edema, the method comprising administering to a subject in need thereof a therapeutically-effective amount of a human protein tyrosine phosphatase beta-extracellular domain (HPTPβ-ECD) binding agent or a pharmaceutically-acceptable salt thereof.

2. The method of claim 1, wherein the ocular edema is retinal edema.

3. The method of claim 1, wherein the ocular edema is diabetic macular edema.

4. The method of any one of claims 1-3, wherein the HPTPβ-ECD binding agent is a monoclonal antibody or an antigen-binding fragment thereof.

5. The method of any one of claims 1-4, wherein the HPTPβ-ECD binding agent is a monoclonal antibody produced by hybridoma cell line ATCC No. PTA 7580.

6. The method of any one of claims 1-5, wherein the HPTPβ-ECD binding agent comprises a F(ab')2, a dimer of a Fab, a dimer of a Fv, or a dimer of a scFv.

7. The method of any one of claims 1-6, wherein the HPTPβ-ECD binding agent binds to a FN3 repeat in the HPTPβ-ECD.

8. The method of any one of claims 1-7, wherein a dose of the HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof is from about 0.01 mg/kg to about 500 mg/kg by weight of the subject.

9. The method of any one of claims 1-8, wherein a dose of the HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof is from about 0.1 mg/kg to about 10 mg/kg by weight of the subject.

10. The method of any one of claims 1-9, wherein the administration is intraocular.

11. The method of any one of claims 1-9, wherein the administration is subcutaneous.

12. The method of any one of claims 1-9, wherein the administration intravenous.

13. The method of any one of claims 1-12, wherein the HPTPβ-ECD binding agent is R15E6.

14. The method of any one of claims 1-13, wherein the HPTPβ-ECD binding agent is humanized.

15. A use of a HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for alleviating an ocular edema in a subject in need thereof.

16. The use of claim 15, wherein the ocular edema is retinal edema.

17. The use of claim 15, wherein the ocular edema is diabetic macular edema.

18. The use of any one of claims 15-17, wherein the HPTPβ-ECD binding agent is a monoclonal antibody or an antigen-binding fragment thereof.

19. The use of any one of claims 15-18, wherein the HPTPβ-ECD binding agent is a monoclonal antibody produced by hybridoma cell line ATCC No. PTA 7580.

20. The use of any one of claims 15-19, wherein the HPTPβ-ECD binding agent comprises a F(ab')2, a dimer of a Fab, a dimer of a Fv, or a dimer of a scFv.

21. The use of any one of claims 15-20, wherein the HPTPβ-ECD binding agent binds to a FN3 repeat in the HPTPβ-ECD.

22. The use of any one of claims 15-21, wherein a dose of the HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof is from about 0.01 mg/kg to about 500 mg/kg by weight of the subject.

23. The use of any one of claims 15-22, wherein a dose of the HPTPβ-ECD binding agent or a pharmaceutically acceptable salt thereof is from about 0.1 mg/kg to about 10 mg/kg by weight of the subject.

24. The use of any one of claims 15-23, wherein the medicament is formulated for administration by intraocular injection.

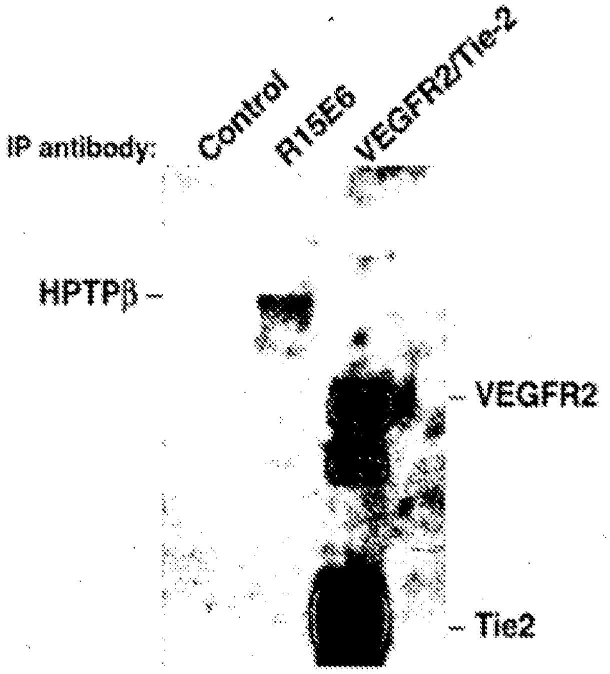
25. The use of any one of claims 15-23, wherein the medicament is formulated for administration by subcutaneous injection.

26. The use of any one of claims 15-23, wherein the medicament is formulated for administration by intravenous injection.

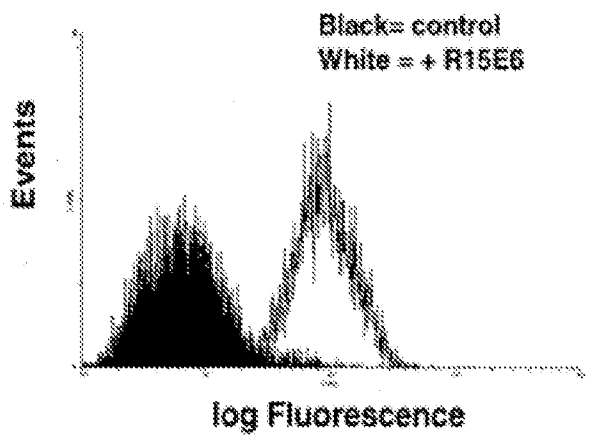
27. The use of any one of claims 15-26, wherein the HPTPβ-ECD binding agent is R15E6.

28. The use of any one of claims 15-27, wherein the HPTPβ-ECD binding agent is humanized.

Fig. 1

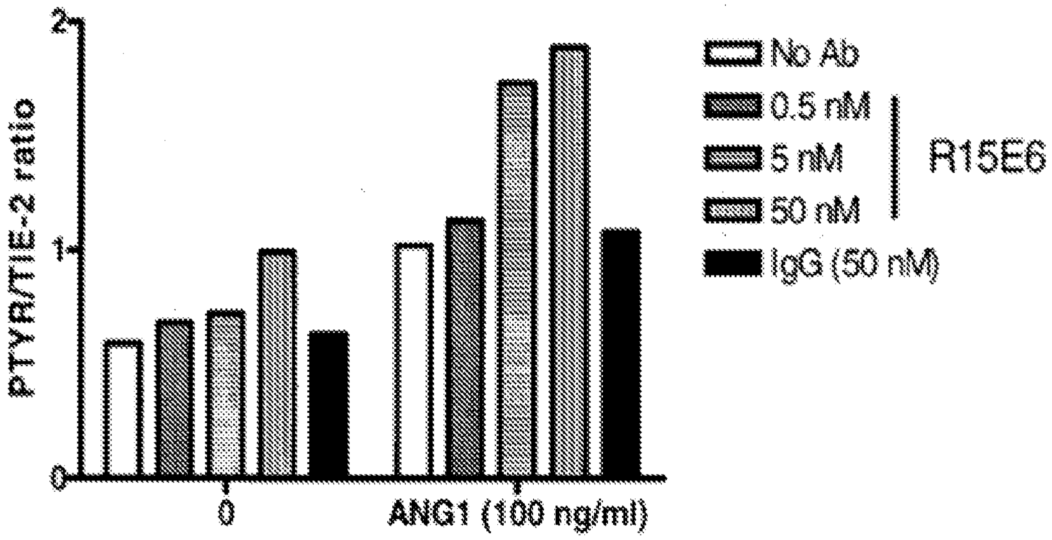

IP antibody:
Control
R15E6
VEGFR2/Tie-2
HPTPβ ~
~ VEGFR2
~ Tie2

A.


Black= control
White = + R15E6
Events
log Fluorescence

B.

Fig. 2



PTYR/TIE-2 ratio
2
1
0
0
ANG1 (100 ng/ml)
No Ab
0.5 nM
5 nM
50 nM
R15E6
IgG (50 nM)

Fig. 3

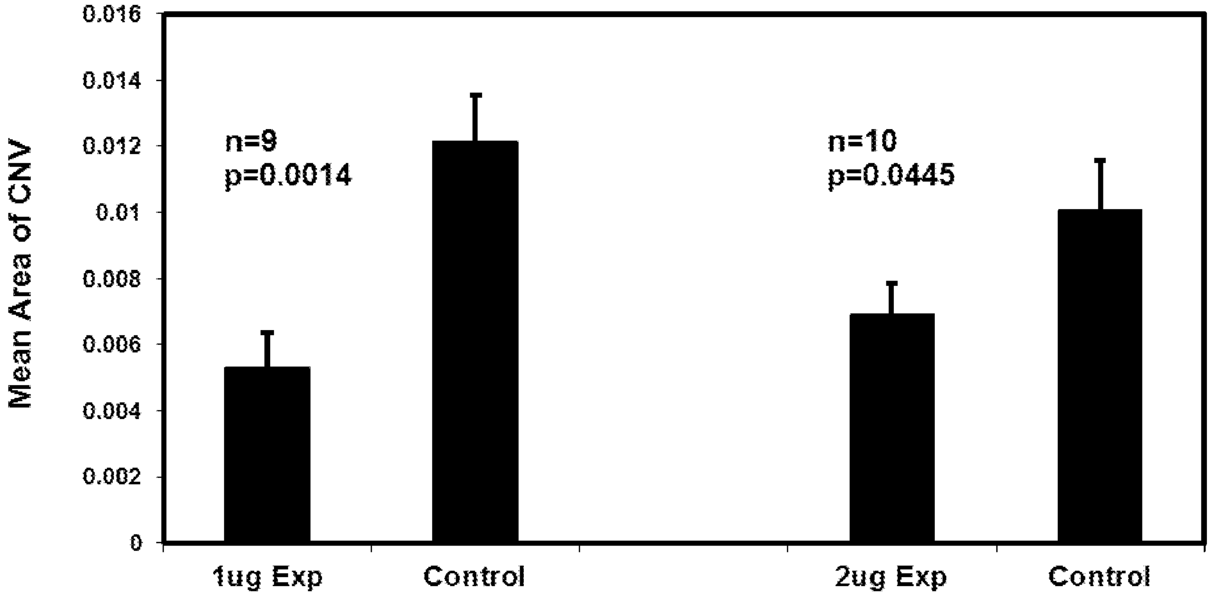

0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Mean Area of CNV
n=9
p=0.0014
n=10
p=0.0445
1ug Exp
Control
2ug Exp
Control

Fig. 4

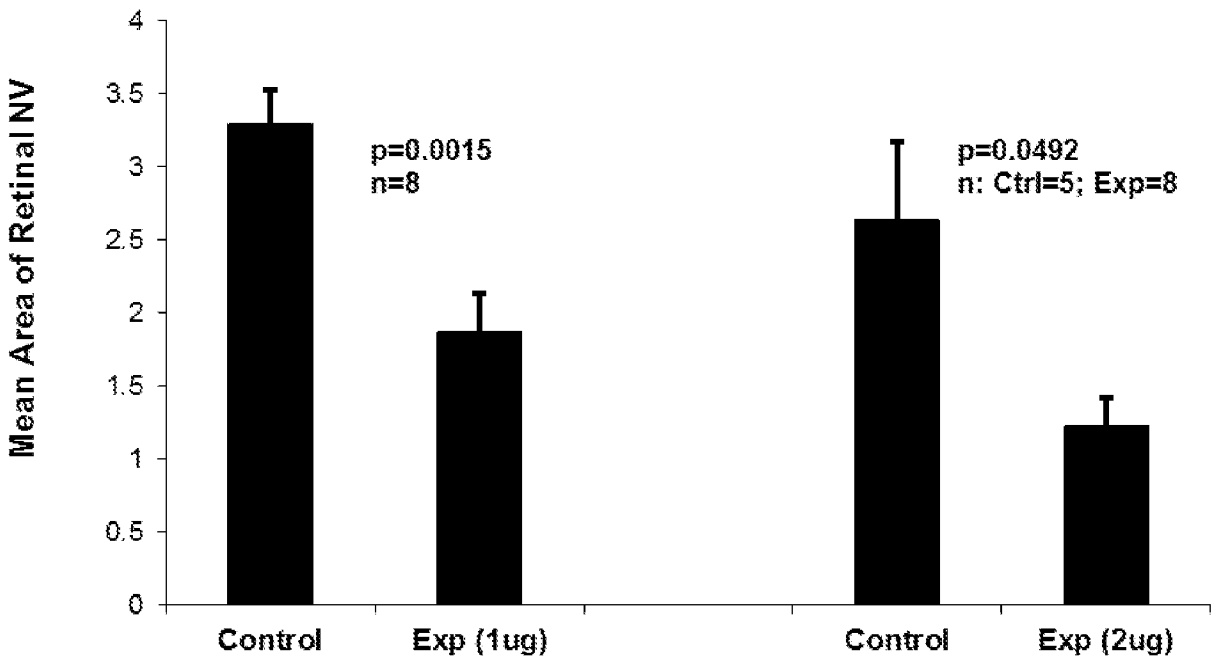

Mean Area of Retinal NV
4
3.5
3
2.5
2
1.5
1
0.5
0
p=0.0015
n=8
p=0.0492
n: Ctrl=5; Exp=8
Control
Exp (1ug)
Control
Exp (2ug)

Fig. 5

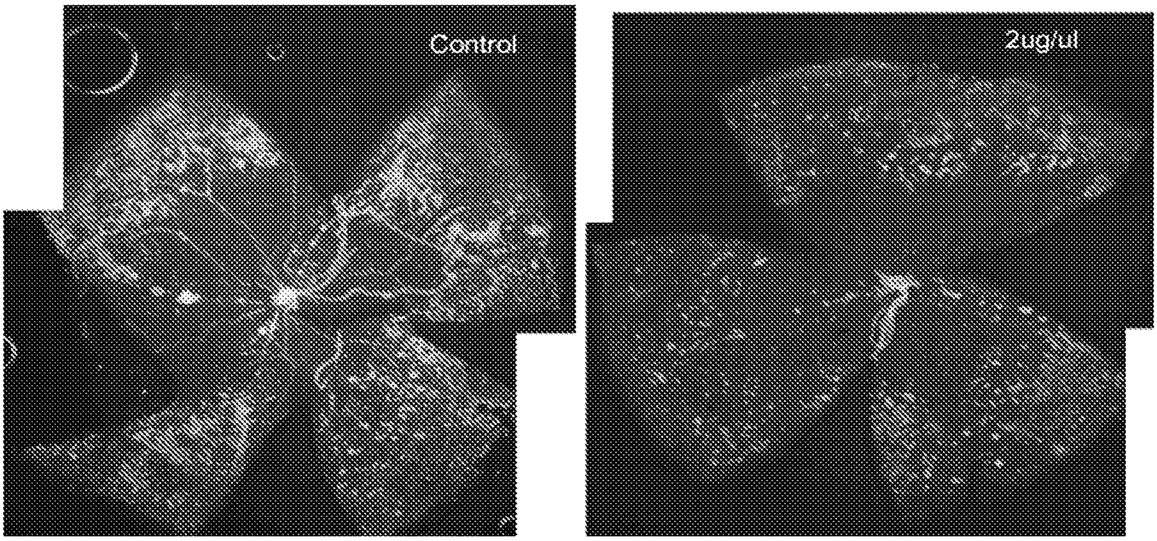


Fig. 6

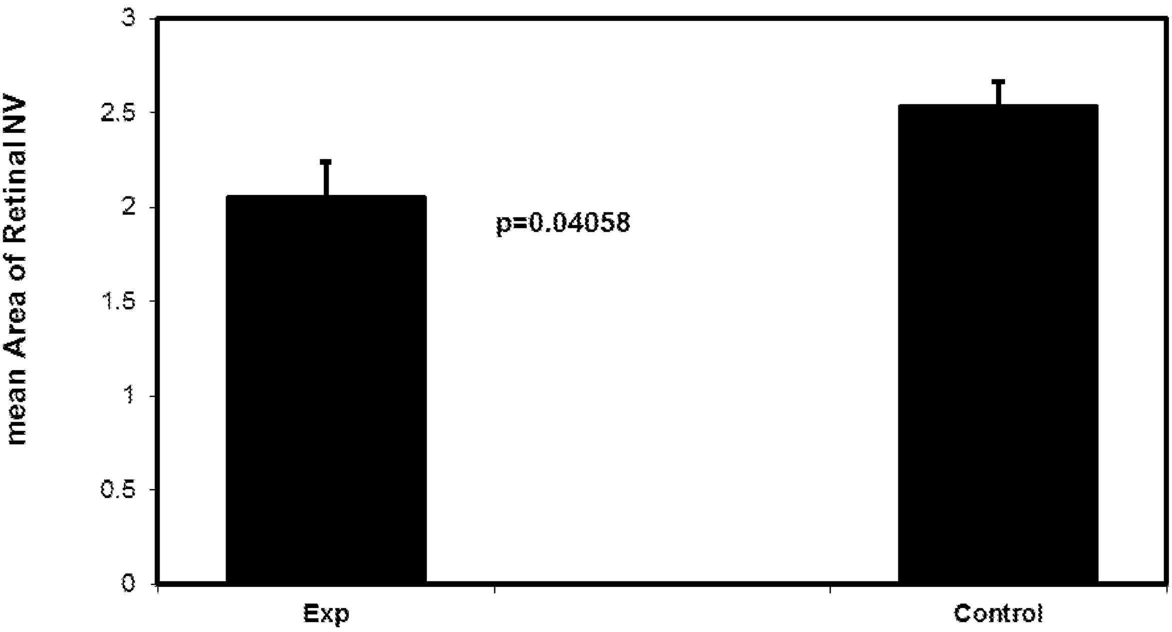
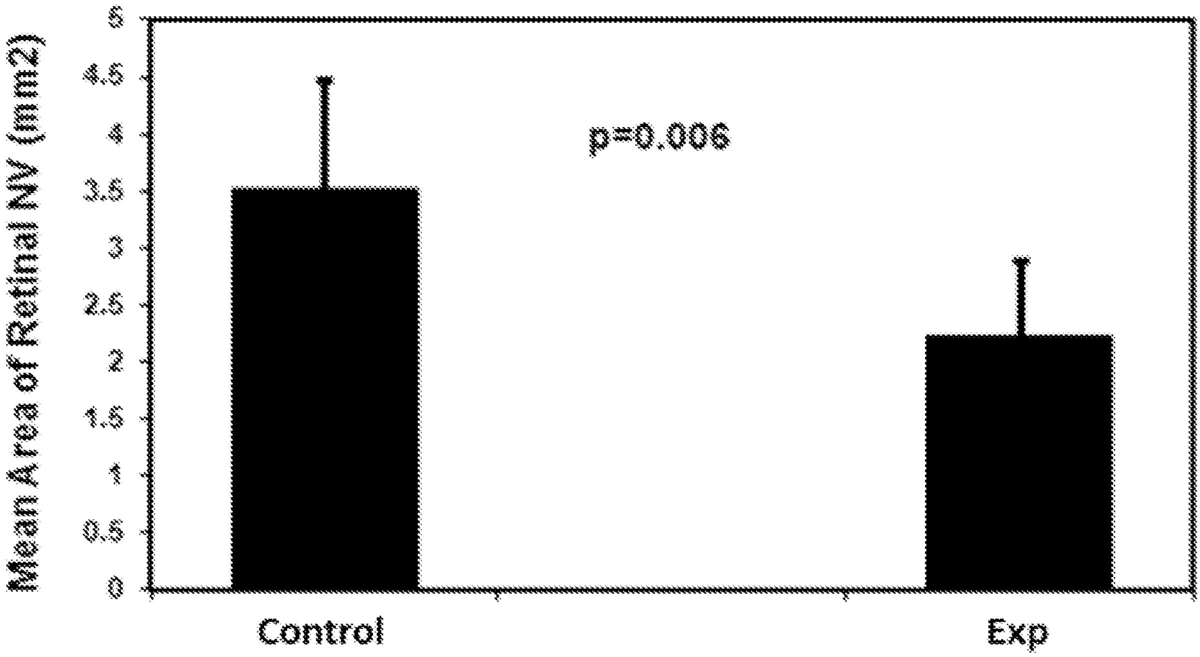


Fig. 7


Mean Area of Retinal NV (mm2)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
p=0.006
Control
Exp