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(54) **LIQUID FORMULATIONS FOR TREATMENT OF DISEASES OR CONDITIONS**
FLÜSSIGE FORMULIERUNGEN ZUR BEHANDLUNG VON ERKRANKUNGEN ODER LEIDEN
FORMULATIONS LIQUIDES POUR LE TRAITEMENT DE MALADIES OU AFFECTIONS

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Description

FIELD

[0001] Described herein are liquid formulations for treatment, prevention, inhibition, delaying onset of, or causing regression of a disease or condition by delivery of therapeutic agents to a subject, including but not limited to a human subject, including but not limited to the treatment of age-related macular degeneration ("AMD") by delivery of a liquid formulation comprising rapamycin (sirolimus), to the eye of a subject, including but not limited to a human subject.

BACKGROUND

[0002] The retina of the eye contains the cones and rods that detect light. In the center of the retina is the macula lutea, which is about 1/3 to 1/2 cm in diameter. The macula provides detailed vision, particularly in the center (the fovea), because the cones are higher in density. Blood vessels, ganglion cells, inner nuclear layer and cells, and the plexiform layers are all displaced to one side (rather than resting above the cones), thereby allowing light a more direct path to the cones.

[0003] Under the retina are the choroid, comprising a collection of blood vessels embedded within a fibrous tissue, and the deeply pigmented epithelium, which overlays the choroid layer. The choroidal blood vessels provide nutrition to the retina (particularly its visual cells).

[0004] There are a variety of retinal disorders for which there is currently no treatment or for which the current treatment is not optimal. Retinal disorders such as uveitis (an inflammation of the uveal tract: iris, ciliary body, and choroid), central retinal vein occlusive diseases (CRVO), branch retinal venous occlusion (BRVO), macular degeneration, macular edema, proliferative diabetic retinopathy, and retinal detachment generally are all retinal disorders that are difficult to treat with conventional therapies.

[0005] Age-related macular degeneration (AMD) is the major cause of severe visual loss in the United States for individuals over the age of 60. AMD occurs in either an atrophic or less commonly an exudative form. The atrophic form of AMD is also called "dry AMD," and the exudative form of AMD is also called "wet AMD."

[0006] In exudative AMD, blood vessels grow from the choriocapillaris through defects in Bruch's membrane, and in some cases the underlying retinal pigment epithelium. Organization of serous or hemorrhagic exudates escaping from these vessels results in fibrovascular scarring of the macular region with attendant degeneration of the neuroretina, detachment and tears of the retinal pigment epithelium, vitreous hemorrhage and permanent loss of central vision. This process is responsible for more than 80% of cases of significant visual loss in subjects with AMD. Current or forthcoming treatments include laser photocoagulation, photodynamic therapy, treatment with VEGF antibody fragments, treatment with pegylated aptamers, and treatment with certain small molecule agents.

[0007] Several studies have recently described the use of laser photocoagulation in the treatment of initial or recurrent neovascular lesions associated with AMD (Macular Photocoagulation Study Groups (1991) in Arch. Ophthal. 109:1220; Arch. Ophthal. 109:1232; Arch. Ophthal. 109:1242). Unfortunately, AMD subjects with subfoveal lesions subjected to laser treatment experienced a rather precipitous reduction in visual acuity (mean 3 lines) at 3 months follow-up. Moreover, at two years post-treatment treated eyes had only marginally better visual acuity than their untreated counterparts (means of 20/320 and 20/400, respectively). Another drawback of the procedure is that vision after surgery is immediately worse.

[0008] Photodynamic therapy (PDT) is a form of phototherapy, a term encompassing all treatments that use light to produce a beneficial reaction in a subject. Optimally, PDT destroys unwanted tissue while sparing normal tissue. Typically, a compound called a photosensitizer is administered to the subject. Usually, the photosensitizer alone has little or no effect on the subject. When light, often from a laser, is directed onto a tissue containing the photosensitizer, the photosensitizer is activated and begins destroying targeted tissue. Because the light provided to the subject is confined to a particularly targeted area, PDT can be used to selectively target abnormal tissue, thus sparing surrounding healthy tissue. PDT is currently used to treat retinal diseases such as AMD. PDT is currently the mainstay of treatment for subfoveal choroidal neovascularization in subjects with AMD (Photodynamic Therapy for Subfoveal Choroidal Neovascularization in Age Related Macular Degeneration with Verteporfin (TAP Study Group) Arch Ophthalmol. 1999 117:1329-1345.

[0009] Choroidal neovascularization (CNV) has proven to be recalcitrant to treatment in most cases. Conventional laser treatment can ablate CNV and help to preserve vision in selected cases not involving the center of the retina, but this is limited to only about 10% of the cases. Unfortunately, even with successful conventional laser photocoagulation, the neovascularization recurs in about 50-70% of eyes (50% over 3 years and >60% at 5 years). (Macular Photocoagulation Study Group, Arch. Ophthalmol. 204:694-701 (1986)). In addition, many subjects who develop CNV are not good candidates for laser therapy because the CNV is too large for laser treatment, or the location cannot be determined so that the physician cannot accurately aim the laser. Photodynamic therapy, although utilized in up to 50% of new cases of subfoveal CNV has only marginal benefits over natural history, and generally delays progression of visual loss rather than improving vision which is already decreased secondary to the subfoveal lesion. PDT is neither preventive or definitive. Several PDT treatments are usually required per subject and additionally, certain subtypes of CNV fare less well than others.

[0010] Thus, there remains along-felt need for methods, compositions, and formulations that may be used to optimally prevent or significantly inhibit choroidal neovascularization and to prevent and treat wet AMD.

[0011] In addition to AMD, choroidal neovascularization is associated with such retinal disorders as presumed ocular histoplasmosis syndrome, myopic degeneration, angioid streaks, idiopathic central serous chorioretinopathy, inflammatory conditions of the retina and or choroid, and ocular trauma. Angiogenic damage associated with neovascularization occurs in a wide range of disorders including diabetic retinopathy, venous occlusions, sickle cell retinopathy, retinopathy of prematurity, retinal detachment, ocular ischemia and trauma.

[0012] Uveitis is another retinal disorder that has proven difficult to treat using existing therapies. Uveitis is a general term that indicates an inflammation of any component of the uveal tract. The uveal tract of the eye consists of the iris, ciliary body, and choroid. Inflammation of the overlying retina, called retinitis, or of the optic nerve, called optic neuritis, may occur with or without accompanying uveitis.

[0013] Uveitis is most commonly classified anatomically as anterior, intermediate, posterior, or diffuse. Posterior uveitis signifies any of a number of forms of retinitis, choroiditis, or optic neuritis. Diffuse uveitis implies inflammation involving all parts of the eye, including anterior, intermediate, and posterior structures.

[0014] The symptoms and signs of uveitis may be subtle, and vary considerably depending on the site and severity of the inflammation. Regarding posterior uveitis, the most common symptoms include the presence of floaters and decreased vision. Cells in the vitreous humor, white or yellow-white lesions in the retina and/or underlying choroid, exudative retinal detachments, retinal vasculitis, and optic nerve edema may also be present in a subject suffering from posterior uveitis.

[0015] Ocular complications of uveitis may produce profound and irreversible loss of vision, especially when unrecognized or treated improperly. The most frequent complications of posterior uveitis include retinal detachment; neovascularization of the retina, optic nerve, or iris; and cystoid macular edema.

[0016] Macular edema (ME) can occur if the swelling, leaking, and hard exudates noted in background diabetic retinopathy (BDR) occur within the macula, the central 5% of the retina most critical to vision. Background diabetic retinopathy. (BDR) typically consists of retinal microaneurisms that result from changes in the retinal microcirculation. These microaneurisms are usually the earliest visible change in retinopathy seen on exam with an ophthalmoscope as scattered red spots in the retina where tiny, weakened blood vessels have ballooned out. The ocular findings in background diabetic retinopathy progress to cotton wool spots, intraretinal hemorrhages, leakage of fluid from the retinal capillaries, and retinal exudates. The increased vascular permeability is also related to elevated levels of local growth factors such as vascular endothelial growth factor. The macula is rich in cones, the nerve endings that detect color and upon which daytime vision depends. When increased retinal capillary permeability effects the macula, blurring occurs in the middle or just to the side of the central visual field, rather like looking through cellophane. Visual loss may progress over a period of months, and can be very annoying because of the inability to focus clearly. ME is a common cause of severe visual impairment.

[0017] There have been many attempts to treat CNV and its related diseases and conditions, as well as other conditions such as macular edema and chronic inflammation, with pharmaceuticals. For example, use of rapamycin to inhibit CNV and wet AMD has been described in U.S. Application No. 10/665,203. The use of rapamycin to treat inflammatory diseases of the eye has been described in US patent number 5,387,589, titled Method of Treating Ocular Inflammation, with inventor Prassad Kulkarni, assigned to University of Louisville Research Foundation.

[0018] WO2004/027027 discloses compositions and methods for inhibiting unwanted angiogenesis, specifically choroidal neovascularization that is associated with diseases such as age related macular degeneration. The composition comprises a therapeutic agent from the limus family, e.g. rapamycin. The compounds can be dissolved in polyethylene glycol.

[0019] Particularly for chronic diseases, including those described herein, there is a great need for long acting methods for delivering therapeutic agents to the eye, such as to the posterior segment to treat CNV in such diseases as AMD, macular edema, proliferative retinopathies, and chronic inflammation. Formulations with extended delivery of therapeutic agent are more comfortable and convenient for a subject, due to a diminished frequency of ocular injections of the therapeutic agent.

[0020] Direct delivery of therapeutic agents to the eye rather than systemic administration may be advantageous because the therapeutic agent concentration at the site of action is increased relative to the therapeutic agent concentration in a subject's circulatory system. Additionally, therapeutic agents may have undesirable side effects when delivered systemically to treat posterior segment disease. Thus, localized drug delivery may promote efficacy while decreasing side effects and systemic toxicity.

SUMMARY

[0021] The invention provides a liquid formulation comprising a therapeutic agent and a solvent component, wherein the liquid formulation which is a solution is administered to the vitreous of a subject's eye; the therapeutic agent is 2% (w/w) rapamycin; and the solvent component comprises a mixture of 4% (w/w) ethanol and 94% (w/w) polyethylene glycol 400.

[0022] Described herein are liquid formulations for administering to a human subject an amount of rapamycin effective to treat, prevent, inhibit, delay onset of, or cause regression of wet AMD.

[0023] As described in further detail in the Detailed Description section, the liquid formulations may also be used for delivery to a subject, including but not limited to a human subject or to the eye of a human subject of therapeutically effective amounts of rapamycin for the treatment, prevention, inhibition, delaying of the onset of, or causing the regression of wet AMD. In some variations, the liquid formulations are used to treat wet AMD. In some variations, the liquid formulations are used to prevent wet AMD. In some variations, the methods and formulations described herein are used to prevent the transition from dry AMD to wet AMD. The liquid formulations may also be used for delivery to a subject, including but not limited to a human subject or to the eye of a subject of therapeutically effective amounts of rapamycin for the treatment, prevention, inhibition, delaying of the onset of, or causing the regression of CNV. In some variations, the liquid formulations are used to treat CNV. The liquid formulations may also be used for delivery to a subject, including but not limited to a human subject or to the eye of a subject of therapeutically effective amounts of rapamycin for the treatment, prevention, inhibition, delaying of the onset of, or causing the regression of angiogenesis in the eye. In some variations, the liquid formulations are used to treat angiogenesis. Other diseases and conditions that may be treated, prevented, inhibited, have onset delayed, or caused to regress using rapamycin are described in the *Diseases and Conditions* section of the *Detailed Description.*

The invention provides the liquid formulation of the invention for use in the treatment of a disease or condition selected from diabetic retinopathy, macular degeneration, wet and dry AMD, retinopathy of prematurity (retrolental fibroplasia), infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, myopic degeneration, angioid streaks, ocular trauma, pseudoxanthoma elasticum, vein occlusion, artery occlusion, carotid obstructive disease, Sickle Cell anemia, Eales disease, myopia, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma, polypoidal choroidal vasculopathy, post-laser complications, complications of idiopathic central serous chorioretinopathy, complications of choroidal inflammatory conditions, rubeosis, diseases associated with rubeosis (neovascularization of the angle), neovascular glaucoma, uveitis and chronic uveitis, macular edema, proliferative retinopathies and diseases or conditions caused by the abnormal proliferation of fibrovascular or fibrous tissue, including all forms of proliferative vitreoretinopathy (including post-operative proliferative vitreoretinopathy), whether or not associated with diabetes.

Paragraphs [0024] and [0025] are deleted.

[0024] The liquid formulations described herein may form a non-dispersed mass when placed into a rabbit eye, including but not limited to the vitreous of a rabbit eye.

[0025] In some variations, the non-dispersed mass comprises a depot. In some variations, the non-dispersed mass consists of a depot.

[0026] For liquid formulations which form a non-dispersed mass, the non-dispersed mass may generally be any geometry or shape. The non-dispersed mass-forming liquid formulations may, for instance, appear as a compact spherical mass when placed in the vitreous. In some variations the liquid formulations described herein form a milky or whitish colored semi-contiguous or semi-solid non-dispersed mass relative to the medium in which it is placed, when placed in the vitreous.

[0027] The liquid formulations may generally be administered in any volume that has the desired effect. In one method a volume of a liquid formulation is administered to the vitreous and the liquid formulation is less than one half the volume of the vitreous.

[0028] Routes of administration that may be used to administer a liquid formulation include but are not limited to placement of the liquid formulation by placement, including by injection, into a medium, including but not limited to an aqueous medium in the body, including but not limited to intraocular or periocular injection. In some variations, the liquid formulation is administered subconjunctivally. In some variations, the liquid formulation is administered intravitreally.

[0029] The liquid formulations described herein may deliver rapamycin for an extended period of time. One nonlimiting example of such an extended release delivery system is a liquid formulation that delivers rapamycin to a subject, including but not limited to a human subject or to the eye of a subject in an amount sufficient to maintain an amount effective to treat, prevent, inhibit, delay onset of, or cause regression of wet age-related macular degeneration for an extended period of time. In some variations, the liquid formulation is used to treat wet age-related macular degeneration for an extended period of time. In some variations, the liquid formulation is used to prevent wet age-related macular degeneration for an extended period of time. In some variations, the liquid formulation is used to prevent transition of dry AMD to wet

AMD for an extended period of time. In one nonlimiting example, the liquid formulation delivers the rapamycin to the vitreous, sclera, retina, choroid, macula, or other tissues of a subject, including but not limited to a human subject in an amount sufficient to treat, prevent, inhibit, delay onset of, or cause regression of wet age-related macular degeneration for at least about three, about six, about nine, or about twelve months. In some variations, the level of rapamycin is sufficient to treat AMD. In some variations, the level of rapamycin is sufficient to prevent onset of wet AMD.

[0030] Other extended periods of release are described in the Detailed Description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0031]

FIGURES 1A-1C schematically depicts formation of a non-dispersed mass, after injection of a liquid formulation into the vitreous of an eye, as it is believed to occur in some variations.

FIGURE 2 depicts the level of rapamycin in the vitreous (ng/ml), retina choroid (ng/mg), and sclera (ng/mg) of rabbit eyes at 20, 40, 67, and 90 days after subconjunctival injection of a 1.256% solution of rapamycin in water, ethanol, and F127 (Lutrol).

FIGURE 3 depicts the level of rapamycin in the vitreous (ng/ml), retina choroid (ng/mg), and sclera (ng/mg) of rabbit eyes at 14, 35, 62, and 85 days after subconjunctival injection of a 5% solution of rapamycin in PEG 400 and ethanol. The level of rapamycin present in the vitreous (ng/ml) is also shown at 2 days after injection.

FIGURE 4 depicts the level of rapamycin in the vitreous (ng/ml), retina choroid (ng/mg), and sclera (ng/mg) of rabbit eyes at 14, 35, 62, and 90 days after intravitreal injection of a 5% solution of rapamycin in PEG 400 and ethanol. The level of rapamycin present in the vitreous (ng/ml) is also shown at 2 days after injection.

FIGURE 5 depicts images of rabbit eyes 8 days after intravitreal injection of 10 μl (Fig. 4A), 20 μl (Fig. 4B), and 40 μl (Fig. 4C) of a 6% rapamycin suspension in PEG400.

FIGURE 6 depicts the level of rapamycin in the vitreous (ng/ml), retina choroid tissues (ng/mg), and sclera (ng/mg) of rabbit eyes at 7, 32, 45, and 90 days after subconjunctival injection of a 4.2% solution of rapamycin in ethanol, PVP K90, PEG 400, and Eudragit RL 100.

FIGURE 7 depicts the level of rapamycin in the vitreous (ng/ml), retina choroid tissues (ng/mg), and sclera (ng/mg) of rabbit eyes at 14, 42, 63, and 91 days after subconjunctival injection of a 3% suspension of rapamycin in PEG 400.

FIGURE 8 depicts the level of rapamycin in the vitreous (ng/ml), retina choroid tissues (ng/mg) and sclera (ng/mg) of rabbit eyes at 14, 42, 63, and 91 days after intravitreal injection of a 3% suspension of rapamycin in PEG 400, and in the vitreous at 63 and 91 days after injection.

FIGURE 9 depicts the level of rapamycin in the vitreous (ng/ml), retina choroid tissues (ng/mg), and sclera (ng/mg) of rabbit eyes at 14, 42, 63, and 91 days after subconjunctival injection of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 10 depicts the level of rapamycin in the retina choroid tissues (ng/mg) and sclera (ng/mg) of rabbit eyes at 14, 42, 63, and 91 days after intravitreal injection of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 11 depicts the level of rapamycin in the vitreous (ng/ml) of rabbit eyes at 63 and 91 days after intravitreal injection of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 12 depicts the level of rapamycin in the vitreous (ng/ml) of rabbit eyes at 5, 30, 60, 90, and 120 days after subconjunctival injection of 20 μl, 40 μl, and 60 μl doses of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 13 depicts the level of rapamycin in the retina choroid tissues (ng/mg) of rabbit eyes at 5, 30, 60, 90, and 120 days after subconjunctival injection of 20 μl, 40 μl, and 60 μl doses of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 14 depicts the level of rapamycin in the vitreous (ng/ml) of rabbit eyes at 5, 30, 60, 90, and 120 days after intravitreal injection of 20 μl and 40 μl doses of a 2% solution of rapamycin in ethanol and PEG 400 and of a 100 μl dose of a 0.4 % rapamycin solution in ethanol and PEG 400.

FIGURE 15 depicts the level of rapamycin in the retina choroid tissues (ng/mg) of rabbit eyes at 5, 30, 60, 90, and 120 days after intravitreal injection of 20 μl and 40 μl doses of a 2% solution of rapamycin in ethanol and PEG 400 and of a 100 μl dose of a 0.4 % rapamycin solution in ethanol and PEG 400.

FIGURE 16 depicts the level of rapamycin in the vitreous (ng/ml) of rabbit eyes at 5 and 14 days after subconjunctival injection of a single 10 μl dose, a single 60 μl dose, two 30 μl doses, and three 30 μl doses of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 17 depicts the level of rapamycin in the retina choroid tissues (ng/mg) of rabbit eyes at 5 and 14 days after subconjunctival injection of a single 10 μl dose, a single 60 μl dose, two 30 μl doses, and three 30 μl doses of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 18 depicts the level of rapamycin in the vitreous (ng/ml) of rabbit eyes at 5, 14, and 30 days after subconjunctival injection of a single 10 μl dose, a single 30 μl dose, and three 30 μl doses of a 3% suspension of rapamycin in PEG 400.

FIGURE 19 depicts the level of rapamycin in the retina choroid tissues (ng/mg) of rabbit eyes at 5, 14, and 30 days after subconjunctival injection of a single 10 μl dose, a single 30 μl dose, and three 30 μl doses of a 3% suspension of rapamycin in PEG 400.

FIGURE 20 depicts the level of rapamycin in the retina choroid tissues (ng/mg) of rabbit eyes at 5, 30, and 90 days after intravitreal injection of 10 μl of a 0.2% solution of rapamycin in ethanol and PEG 400, of 10 μl of a 0.6% solution of rapamycin in ethanol and PEG 400, and of 10 μl of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 21 depicts the level of rapamycin in the vitreous (ng/ml) of rabbit eyes at 5, 30, and 90 days after intravitreal injection of 10 μl of a 0.2% solution of rapamycin in ethanol and PEG 400, of 10 μl of a 0.6% solution of rapamycin in ethanol and PEG 400, and of 10 μl of a 2% solution of rapamycin in ethanol and PEG 400.

FIGURE 22 depicts the level of rapamycin in the aqueous humor (ng/ml) of rabbit eyes, the cornea (ng/mg), and the retina choroid tissues (ng/mg) at 1, 4, 7, 11, 14, 21, 28, 35, 54, and 56 days after subconjunctival injection of 40 μl of a 2% solution of rapamycin in ethanol and PEG 400.

DETAILED DESCRIPTION

[0032] Described herein are liquid formulations relating to delivery of rapamycin to a subject, including but not limited to a human subject or to the eye of a subject. These liquid formulations may be used for the treatment, prevention, inhibition, delaying onset of, or causing regression of diseases and conditions of the eye including but not limited to diseases or conditions of the posterior segment, including but not limited to choroidal neovascularization; macular degeneration; age-related macular degeneration, including wet AMD and dry AMD; retinal angiogenesis; chronic uveitis; and other retinoproliferative conditions. In some variations, liquid formulations are used for the treatment of the aforementioned diseases or conditions of the eye.

[0033] Herein are described (1) the therapeutic agent that may be delivered to a subject, including but not limited to a human subject or an eye of a subject using the liquid formulations described herein, (2) the diseases and conditions that may be treated, prevented, inhibited, onset delayed, or regression caused by delivery of the therapeutic agent, (3) liquid formulations that may be used to deliver the therapeutic agent, (4) routes of administration for delivery of the liquid formulations, (5) extended delivery of rapamycin, and (6) description of the treatment of CNV and wet AMD by delivery of rapamycin to a subject, including but not limited to a human subject or to the eye of a subject for an extended period of time using the described compositions and liquid formulations.

[0034] The term "about," as used herein, refers to the level of accuracy that is obtained when the methods described herein, such as the methods in the examples, are used. However, by "about" a certain amount of a component of a formulation is meant 90-110% of the amount stated.

Therapeutic Agents

[0035] The terms rapamycin, rapa, and sirolimus are used interchangeably herein.

[0036] Rapamycin derivatives include 7-epi-rapamycin, 7-thiomethyl-rapamycin, 7-epi-trimethoxyphenyl-rapamycin, 7-epi-thiomethyl-rapamycin, 7-demethoxy-rapamycin, 32-demethoxy-rapamycin, 2-desmethyl-rapamycin, mono- and di-ester derivatives of rapamycin, 27-oximes of rapamycin; 42-oxo analog of rapamycin; bicyclic rapamycins; rapamycin dimers; silyl ethers of rapamycin; rapamycin arylsulfonates and sulfamates, mono-esters and di-esters at positions 31 and 42, 30-demethoxy rapamycin, and other derivatives described in Vezina et al., "Rapamycin (AY-22,989), A New Antifungal Antibiotic. I. Taxonomy Of The Producing Streptomycete And Isolation Of The Active Principle" J. Antibiot. (Tokyo) 28:721-726 (1975); Sehgal et al., "Rapamycin (AY-22,989), A New Antifungal Antibiotic. II. Fermentation, Isolation And Characterization" J. Antibiot. (Tokyo) 28:727-732 (1975); Sehgal et al., "Demethoxyrapamycin (AY-24,668), A New Antifungal Antibiotic" J. Antibiot. (Tokyo) 36:351-354 (1983); and Paiva et al., "Incorporation Of Acetate, Propionate, And Methionine Into Rapamycin By Streptomycetes hygroscopicus" J Nat Prod 54:167-177 (1991), WO 92/05179, EP 467606, Caufield et al., "Hydrogenated Rapamycin Derivatives" U.S. Pat. No. 5,023,262; Kao et al., "Bicyclic Rapamycins" U.S. Pat. No. 5,120,725; Kao et al., "Rapamycin Dimers" U.S. Pat. No. 5,120,727; Failli et al., "Silyl Ethers Of Rapamycin" U.S. Pat. No. 5,120,842; Failli et al., "Rapamycin 42-Sulfonates And 42-(N-carboalkoxy) Sulfamates Useful As Immunosuppressive Agents" U.S. Pat. No. 5,177,203; Nicolaou et al., "Total Synthesis Of Rapamycin" J. Am. Chem. Soc. 115: 4419-4420 (1993); Romo et al, "Total Synthesis Of (-) Rapamycin Using An Evans-Tishchenko Fragment Coupling" J. Am. Chem. Soc. 115:7906-7907 (1993); and Hayward et al, "Total Synthesis Of Rapamycin Via A Novel Titanium-Mediated Aldol Macrocyclization Reaction" J. Am. Chem. Soc., 115:9345-9346 (1993).

Diseases and conditions that may be treated, prevented, inhibited, onset delayed, or regression caused

[0037] Herein are described diseases and conditions that may be treated, prevented, inhibited, onset delayed, or regression caused using the liquid formulations and methods described herein. In some variations, the diseases or conditions are treated using the liquid formulations and methods described herein. Unless the context indicates otherwise, it is envisioned that the subjects on whom all of the methods of treatment may be performed include, but are not limited to, human subjects.

[0038] Generally, any diseases or condition of the eye susceptible to treatment, prevention, inhibition, delaying the onset of, or causing the regression of using the liquid formulations and methods described herein may be treated, prevented, inhibited, onset delayed, or regression caused treated or prevented. Examples of diseases or conditions of the eye include, but are not limited to, diseases or conditions associated with neovascularization including retinal and/or choroidal neovascularization.

[0039] Diseases or conditions associated with retinal and/or choroidal neovascularization that can be treated, prevented inhibited, have onset delayed, or be caused to regress using the liquid formulations and methods described herein include, but are not limited to, diabetic retinopathy, macular degeneration, wet and dry AMD, retinopathy of prematurity (retrolental fibroplasia), infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, myopic degeneration, angioid streaks, and ocular trauma. Other non-limiting examples of diseases and conditions of the eye that may be treated, prevented inhibited, have onset delayed, or be caused to regress using the liquid formulations and methods described herein include, but are not limited to, pseudoxanthoma elasticum, vein occlusion, artery occlusion, carotid obstructive disease, Sickle Cell anemia, Eales disease, myopia, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma, polypoidal choroidal vasculopathy, post-laser complications, complications of idiopathic central serous chorioretinopathy, complications of choroidal inflammatory conditions, rubeosis, diseases associated with rubeosis (neovascularization of the angle), neovascular glaucoma, uveitis and chronic uveitis, macular edema, proliferative retinopathies and diseases or conditions caused by the abnormal proliferation of fibrovascular or fibrous tissue, including all forms of proliferative vitreoretinopathy (including post-operative proliferative vitreoretinopathy), whether or not associated with diabetes.

[0040] In some variations, the formulations and pharmaceutical formulations described herein are used to prevent or delay onset of a disease or condition of the eye where the subject, including but not limited to a human subject, is at heightened risk of developing the disease or condition of the eye. A subject with a heightened risk of developing a disease or condition is a subject with one or more indications that the disease or condition is likely to develop in the particular subject. In some variations the subject with a heightened risk of developing wet AMD is a subject with dry AMD in at least one eye. In some variations the subject with a heightened risk of developing wet AMD in a fellow eye is a subject with wet AMD in the other eye. In some variations, the formulations and pharmaceutical formulations described herein are used to prevent or delay onset of CNV in a subject at heightened risk of developing CNV, including but not limited to prevention or delaying onset of CNV in the fellow eye of a subject, including but not limited to a human subject with AMD in one eye. In some variations, the formulations and pharmaceutical formulations described herein are used to prevent or delay onset of CNV in the fellow eye of a subject with wet AMD in one eye. In some variations the formulations and pharmaceutical formulations are administered periocularly, including without limitation subconjunctivally, to a human subject with vision of 20/40 or better. In some variations, the formulations and pharmaceutical formulations are administered periocularly, including without limitation subconjunctivally, to the eye of a human subject where the eye to which the formulation is administered has vision of 20/40 or better.

[0041] In some variations, the formulations and pharmaceutical formulations described herein are used to treat, prevent, or delay onset of AMD. In some variations, the formulations and pharmaceutical formulations described herein are used to treat, prevent, or delay onset of dry AMD. In some variations, subjects including but not limited to human subjects with non-central geographic atrophy are administered a formulation or pharmaceutical formulations described herein to treat, prevent, or delay onset of central geographic atrophy. In some variations the formulations and pharmaceutical formulations are administered periocularly, including without limitation subconjunctivally, to a human subject with vision of 20/40 or better. In some variations, the formulations and pharmaceutical formulations described herein are administered and the subject, including but not limited to a human subject is also treated with a second therapy for treating the disease or disorder. In some variations, the formulations and pharmaceutical formulations described herein are used to treat, prevent, or delay onset of wet or dry AMD and the subject, including but not limited to a human subject is also treated with laser therapy such as photodynamic laser therapy, either before, during, or after treatment with the formulations or pharmaceutical formulations described herein.

[0042] In some variations, the formulations and pharmaceutical formulations described herein are used to treat one or more of uveitis, allergic conjunctivitis, macular edema, glaucoma, or dry eye.

[0043] In some variations, the formulation or pharmaceutical formulation comprising rapamycin is administered to treat, prevent, or delay onset of dry eye. In some variations, the formulation or pharmaceutical formulation comprising rapamycinis administered to treat, prevent, or delay onset of allergic conjunctivitis.

[0044] In some variations, the formulations and pharmaceutical formulations described herein are used to treat glaucoma. In some variations, the formulations and pharmaceutical formulations described herein for treating glaucoma are used as a surgical adjuvant to prevent, reduce or delay surgical complications. In some variations, the formulations and pharmaceutical formulations described herein for treating glaucoma are used to improve or prolong surgical implant success. In some variations, the formulations and pharmaceutical formulations described herein for treating glaucoma are used to improve or prolong success of an argon laser trabeculectomy or other glaucoma-related surgery. In some variations, the formulations and pharmaceutical formulations described herein have a neuroprotective effect and are used to treat glaucoma.

[0045] In some variations, the formulations and pharmaceutical formulations described herein are used to treat retinitis pigmentosa. In some variations, the formulations and pharmaceutical formulations described herein for treating glaucoma are used to treat, prevent, or delay onset of retinitis pigmentosa. In some variations, the formulations and pharmaceutical formulations described herein have a neuroprotective effect and are used to treat retinitis pigmentosa.

[0046] In some variations, the formulations and pharmaceutical formulations described herein are used to treat one or more of central retinal vein occlusive diseases (CRVO), branch retinal venous occlusion (BRVO), retinal vascular diseases and conditions, macular edema, diabetic macular edema, iris neovascularization, diabetic retinopathy, or corneal graft rejection. In some variations, the formulation or pharmaceutical formulation is administered to treat, prevent, or delay onset of one or more of these diseases or conditions. In some variations the formulations and pharmaceutical formulations are administered subconjunctivally to an eye with vision of 20/40 or better.

[0047] When used to treat, prevent, inhibit, delay the onset of, or cause regressions of uveitis, the formulations and pharmaceutical formulations described herein may be administered by a variety of routes as is known in the art, including but not limited to by ocular administration. Other routes of administration are known and are routine in the art. In some variations, the formulations described herein are used to treat uveitis.

[0048] One disease that may be treated, prevented, inhibited, have onset delayed, or be caused to regress using the liquid formulations and methods described herein is the wet form of AMD. In some variations wet AMD is treated using the liquid formulations and methods described herein. The wet form of AMD is characterized by blood vessels growing from their normal location in the choroid into an undesirable position under the retina. Leakage and bleeding from these new blood vessels results in vision loss and possibly blindness.

[0049] The liquid formulations, and methods described herein may also be used to prevent or slow the transition from the dry form of AMD (wherein the retinal pigment epithelium or RPE degenerates and leads to photoreceptor cell death and the formation of yellow deposits called drusen under the retina) to the wet form of AMD.

[0050] "Macular degeneration" is characterized by the excessive buildup of fibrous deposits in the macula and retina and the atrophy of the retinal pigment epithelium. As used herein, an eye "afflicted" with macular degeneration is understood to mean that the eye exhibits at least one detectable physical characteristic associated with the disease of macular degeneration. The administration of rapamycin appears to limit and regress angiogenesis, such as choroidal neovascularization in age-related macular degeneration (AMD), which may occur without treatment. As used herein, the term "angiogenesis" means the generation of new blood vessels ("neovascularization") into a tissue or organ. An "angiogenesis-mediated disease or condition" of the eye or retina is one in which new blood vessels are generated in a pathogenic manner in the eye or retina, resulting in dimunition or loss of vision or other problem, e.g., choroidal neovascularization associated with AMD.

[0051] The liquid formulations described herein may also be used to treat, prevent, inhibit, delay the onset of, or cause regression of various immune-related diseases and conditions, including but not limited to organ transplant rejection in a host, graft vs. host disease, autoimmune diseases, diseases of inflammation, hyperproliferative vascular disorders, solid tumors, and fungal infections. The liquid formulations described herein may be used to treat various immune-related diseases and conditions, including but not limited to organ transplant rejection in a host, graft vs. host disease, autoimmune diseases, diseases of inflammation, hyperproliferative vascular disorders, solid tumors, and fungal infections. The liquid formulations described herein may be used as immunosuppressants. The formulations and liquid formulations described herein may be used to treat, prevent, inhibit, or delay the onset of rejection of transplanted organs or tissues including but not limited to transplanted heart, liver, kidney, spleen, lung, small bowel, pancreas, and bone marrow. The liquid formulations described herein may be used to treat the onset of rejection of transplanted organs or tissues including but not limited to transplanted heart, liver, kidney, spleen, lung, small bowel, pancreas, and bone marrow. When used to treat, prevent, inhibit, delay the onset of, or cause regressions of immune-related diseases, including but not limited to transplant rejection, the liquid formulations described herein may be administered by a variety of routes as is known in the art.

[0052] As used herein, to "inhibit" a disease or condition by administration of a therapeutic agent means that the progress of at least one detectable physical characteristic or symptom of the disease or condition is slowed or stopped following administration of the therapeutic agent as compared to the progress of the disease or condition without administration of the therapeutic agent.

[0053] As used herein, to "prevent" a disease or condition by administration of a therapeutic agent means that the detectable physical characteristics or symptom of the disease or condition do not develop following administration of the therapeutic agent.

[0054] As used herein, to "delay onset of" a disease or condition by administration of a therapeutic agent means that at least one detectable physical characteristic or symptom of the disease or condition develops later in time following administration of the therapeutic agent as compared to the progress of the disease or condition without administration of the therapeutic agent.

[0055] As used herein, to "treat" a disease or condition by administration of a therapeutic agent means that the progress of at least one detectable physical characteristic or symptom of the disease or condition is slowed, stopped, or reversed following administration of the therapeutic agent as compared to the progress of the disease or condition without administration of the therapeutic agent.

[0056] As used herein, to "cause regression of" a disease or condition by administration of a therapeutic agent means that the progress of at least one detectable physical characteristic or symptom of the disease or condition is reversed to some extent following administration of the therapeutic agent.

[0057] A subject, including but not limited to a human subject, having a predisposition for or in need of prevention may be identified by the skilled practitioner by established methods and criteria in the field given the teachings herein. The skilled practitioner may also readily diagnose individuals as in need of inhibition or treatment based upon established criteria in the field for identifying angiogenesis and/or neovascularization given the teachings herein.

[0058] As used herein, a "subject" is generally any animal that may benefit from administration of the therapeutic agents described herein. In some variations the therapeutic agents are administered to a mammalian subject. In some variations the therapeutic agents are administered to a human subject. In some variations the therapeutic agents may be administered to a veterinary animal subject. In some variations the therapeutic agents may be administered to a model experimental animal subject.

[0059] Other diseases and conditions that may be treated, prevented, inhibited, have the onset delayed, or be caused to regress using the methods described herein include those disclosed in the following patents and publications, PCT publication WO 2004/027027, published April 1, 2004, titled Method of inhibiting choroidal neovascularization, assigned to Trustees of the University of Pennsylvania; US patent number 5,387,589, issued February 7, 1995, titled Method of Treating Ocular Inflammation, with inventor Prassad Kulkarni, assigned to University of Louisville Research Foundation; US patent number 6,376,517, issued April 23, 2003, titled Pipecolic acid derivatives for vision and memory disorders, assigned to GPI NIL Holdings, Inc; PCT publication WO 2004/028477, published April 8, 2004, titled Method subretinal administration of therapeutics including steroids: method for localizing pharmadynamic action at the choroid and retina; and related methods for treatment and or prevention of retinal diseases, assigned to Innorx, Inc; US patent number 6,416,777, issued July 9, 2002, titled Ophthalmic drug delivery device, assigned to Alcon Universal Ltd; US patent number 6,713,081, issued March 30, 2004, titled Ocular therapeutic agent delivery device and methods for making and using such devices, assigned to Department of Health and Human Services; and U.S. Pat. No. 5,536,729, issued July 16, 1996, titled Rapamycin Formulations for Oral Administration, assigned to American Home Products Corp., and U.S. Pat. App. Nos. 60/503,840 and 10/945,682.

Liquid formulations

[0060] The liquid formulations described herein contain rapamycin and are solutions.The liquid formulations form a non-dispersed mass relative to a surrounding medium when placed in the vitreous of a rabbit eye.

[0061] When a certain volume of a liquid formulation is administered, it is understood that there is some imprecision in the accuracy of various devices that may be used to administer the liquid formulation. Where a certain volume is specified, it is understood that this is the target volume. However, certain devices such as insulin syringes are inaccurate to greater than 10%, and sometimes inaccurate to greater than 20% or more. Hamilton HPLC type syringes are generally considered precise to within 10%, and are recommended for volumes below 10 μl are to be injected.

[0062] In some variations, a volume of a liquid formulation described herein may be administered to the vitreous of a rabbit eye or a subject's, including but not limiting a human subject's eye that is less than about 500 μl, less than about 400 μl, less than about 300 μl, less than about 200 μl, less than about 100 μl, less than about 90 μl, less than about 80 μl, less than about 70 μl, less than about 60 μl, less than about 50 μl, less than about 40 μl, less than about 30 μl, less than about 20 μl less than about 10 μl, less than about 5 μl, less than about 3 μl, or less than about 1 μl. In some variations, a volume of a liquid formulation described herein is administered to the vitreous of a rabbit eye or subject's, including but not limited to a human subject's eye that is less than about 20 μl. In some variations, a volume of a liquid formulation described herein may be administered to the vitreous that is less than about 10 μl. In some variations, a volume of a liquid formulation described herein may be administered to the vitreous of a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 0.1 μl and about 200 μl, between about 50 μl and about 200 μl, between about 50 μl and about 150 μl, between about 0.1μl and about 100 μl, between about 0.1μl and about 50 μl, between about 1μl and about 40 μl, between about 1μl and about 30 μl, between about 1μl and about 20 μl, between about 1μl and about 10 μl, or between about 1μl and about 5 μl. In some variations, a volume of a liquid formulation described herein may be administered to the vitreous of a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 1μl and about 10 μl. In some variations, a volume of a liquid formulation described herein may be administered to the vitreous of a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 1μl and about 5 μl. In some variations, a volume of a liquid formulation described herein may be administered to the vitreous of a rabbit eye or a subject's eye that is between about 1μl and about 5 μl. In some variations, a volume of a liquid formulation described herein may be administered to the vitreous of a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 0.1μl and about 200 μl.

[0063] In some variations, a total volume of a liquid formulation described herein may be subconjunctivally administered to a rabbit eye or a subject's, including but not limited to a human subject's eye that is less than about 1000 μl, less than about 900 μl, less than about 800 μl, less than about 700 μl, less than about 600 μl, less than about 500 μl, less than about 400 μl, less than about 300 μl, less than about 200 μl, less than about 100 μl, less than about 90 μl, less than about 80 μl, less than about 70 μl, less than about 60 μl, less than about 50 μl, less than about 40 μl, less than about 30 μl, less than about 20 μl, less than about 10 μl, less than about 5 μl, less than about 3 μl, or less than about 1 μl. In some variations, a volume of a liquid formulation described herein may be subconjunctivally administered to a rabbit eye or a subject's, including but not limited to a human subject's eye that is less than about 20 μl. In some variations, a volume of a liquid formulation described herein may be subconjunctivally administered to a rabbit eye or a subject's, including but not limited to a human subject's eye that is less than about 10 μl. In some variations, a volume of a liquid formulation described herein may be subconjunctivally administered to a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 0.1 μl and about 200 μl, between about 50 μl and about 200 μl, between about 200 μl and about 300 μl, between about 300 μl and about 400 μl, between about 400 μl and about 500 μl, between about 600 μl and about 700 μl, between about 700 μl and about 800 μl, between about 800 μl and about 900 μl, between about 900 μl and about 1000 μl, between about 50 μl and about 150 μl, between about 0.1 μl and about 100 μl, between about 0.1 μl and about 50 μl, between about 1 μl and about 40 μl, between about 1μl and about 30 μl, between about 1μl and about 20 μl, between about 1μl and about 10 μl, or between about 1μl and about 5 μl. In some variations, a volume of a liquid formulation described herein may be subconjunctivally administered to a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 1μl and about 10 μl. In some variations, a volume of a liquid formulation described herein may be subconjunctivally administered to a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 1μl and about 5 μl. In some variations, a volume of a liquid formulation described herein may be administered to subconjunctivally administered to a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 1μl and about 5 μl. In some variations, a volume of a liquid formulation described herein may be administered to subconjunctivally administered to a rabbit eye or a subject's, including but not limited to a human subject's eye that is between about 0.1μl and about 200 μl.

[0064] In some variations the liquid formulations described herein are for administration in multiple subconjunctival locations within a period of time, including without limitation within an hour of one another. Without being bound by theory, it is thought that such multiple administrations, such as multiple injections, allow for a greater total dose to be administered subconjunctivally than a single dose due to a potentially limited ability of the local ocular tissues to absorb larger volumes.

[0065] The liquid formulations may be used to maintain an amount of rapamycin in the vitreous effective to treat wet AMD. In one nonlimiting example, it is believed that a liquid formulation delivering rapamycin to maintain a concentration of rapamycin of about 10 pg/ml to about 2 μg/ml in the vitreous over a period of time may be used for the treatment of wet AMD. When the rapamycin is in a liquid formulation that forms a non-dispersed mass, the stated concentration of rapamycin represents the amount that is effectively treating the disease or condition of the eye, and not merely present in the form of the non-dispersed mass. In another nonlimiting example, it is believed that a delivery system delivering rapamycin to maintain a concentration of rapamycin of about 0.01 pg/mg to about 10 ng/mg in the retina choroid tissues over a period of time may be used for treatment of wet AMD. Other therapeutically effective amounts of therapeutic agent are also possible, and can be readily determined by one of skill in the art given the teachings herein.

[0066] The liquid formulations described herein may be used to deliver a dose of rapamycin to a subject, including but not limited to a human subject or to the eye of a subject. In one nonlimiting example, it is believed that a liquid formulation containing a dose of about 20 μg to about 4 mg may be used for the treatment of wet AMD.

[0067] The liquid formulations described herein are solutions, and comprise a therapeutic agent and a solvent component. The solvent component comprises ethanol and PEG 400.

[0068] In some variations the liquid formulations described herein contain no greater than about 250 μl of PEG 400. In some variations the liquid formulations described herein contain no greater than about 250 μl, no greater than about 200 μl, no greater than about 150 μl, no greater than about 125 μl, no greater than about 100 μl, no greater than about 75 μl, no greater than about 50 μl, no greater than about 25 μl, no greater than about 20 μl, no greater than about 15 μl, no greater than about 10 μl, no greater than about 7.5 μl, no greater than about 5 μl, no greater than about 2.5 μl, no greater than about 1.0 μl, or no greater than about 0.5 μl of PEG 400.

[0069] Some examples and Comparative examples of liquid formulations described herein were prepared and are listed in Table 1. Depending on their type, the listed formulations are denoted one or more of solutions ("S"), suspensions ("SP"), emulsions ("E") or in situ gelling ("ISG"). Median particle size is listed for some of the suspensions. As described herein, some liquid formulations form a non-dispersed mass after, for example, injection into an aqueous environment such as the vitreous of an eye. For those formulations injected into the vitreous of a rabbit eye, the right-hand column of Table 1 indicates whether or not a non-dispersed mass (NDM) formed after a specified volume was injected into the vitreous of the rabbit eye.

Liquid formulations which form a non-dispersed mass

[0070] One class of liquid formulations described herein forms a non-dispersed mass when placed in an aqueous medium. As used herein, a "non-dispersed mass" refers to the structure formed or shape assumed when the liquid formulation is placed into an environment, relative to the environment in which it is placed. Generally, a non-dispersed mass of a liquid formulation is anything other than a homogeneous distribution of the liquid formulation in the surrounding medium. The non-dispersed mass may, for instance, be indicated by visually inspecting the administered liquid formulation and characterizing its appearance relative to the surrounding medium.

[0071] In some variations, the aqueous medium is water. In some variations, the water is deionized, distilled, sterile, or tap water, including but not limited to tap water available at the place of business of MacuSight in Union City, California.

[0072] In some variations, the aqueous medium is an aqueous medium of a subject. In some variations the aqueous medium is an aqueous medium of the eye of a subject, including but not limited to the vitreous of an eye of a subject. In some variations the subject is a human subject. In some variations the subject is a rabbit.

[0073] In some variations the liquid formulation forms a non-dispersed mass when exposed to a certain temperature or range of temperatures, including but not limited to about room temperature, about ambient temperature, about 30°C, about 37°C, or about the temperature of the aqueous medium of the subject.

[0074] In some variations the liquid formulation forms a non-dispersed mass when exposed to a certain pH or range of pH, including but not limited to a pH between about 6 and about 8.

[0075] The liquid formulations described herein may generally be of any geometry or shape after administration to a subject or the eye of a subject, including but not limited to a human subject. In some variations, the non-dispersed mass is between about 0.1 and about 5 mm. In some variations, the non-dispersed mass is between about 1 and about 3 mm. The non-dispersed mass-forming liquid formulations may, for instance, appear as a compact spherical mass when administered to the vitreous. In some instances, the liquid formulation may appear as a non-dispersed mass relative to the surrounding medium, wherein the non-dispersed mass is less clearly defined and the geometry is more amorphous than spherical.

[0076] The non-dispersed mass-forming liquid formulations described herein may form a non-dispersed mass immediately upon placement in the medium or the non-dispersed mass may form some period of time after placement of the liquid formulation. In some variations the non-dispersed mass forms over the course of about 1, about 2, about 3, about 4, about 5, about 6, or about 7 days. In some variations the non-dispersed mass forms over the course of about 1 week, about 2 weeks, or about 3 weeks.

[0077] In some variations, the liquid formulations described herein that form a non-dispersed mass appear as a milky or whitish colored semi-contiguous or semi-solid non-dispersed mass relative to the medium in which it is placed.

[0078] One liquid formulation described herein forms a non-dispersed mass which has the form of a solid depot when the formulation is injected into any or all of water, the vitreous of a rabbit eye, or between the sclera and the conjunctiva of a rabbit eye. One liquid formulation described herein forms a non-dispersed mass which has the form of a semi-solid when the formulation is injected into any or all of water, the vitreous of a rabbit eye, or between the sclera and the conjunctiva of a rabbit eye. One liquid formulation described herein forms a non-dispersed mass which has the form of a polymeric matrix when the formulation is injected into any or all of water, the vitreous of a rabbit eye, or between the sclera and the conjunctiva of a rabbit eye. One liquid formulation described herein forms a non-dispersed mass which has the form of a gel, a hydrogel, or a gel-like substance when the formulation is injected into any or all of water, the vitreous of a rabbit eye, or between the sclera and the conjunctiva of a rabbit eye.

[0079] In some variations described herein the liquid formulation forms a non-dispersed mass relative to a surrounding medium where the surrounding medium is aqueous. An "aqueous medium" or "aqueous environment" is one that contains at least about 50% water. Examples of aqueous media include but are not limited to water, the vitreous, extracellular fluid, conjunctiva, sclera, between the sclera and the conjunctiva, aqueous humor, gastric fluid, and any tissue or body fluid comprised of at least about 50% of water. Aqueous media include but are not limited to gel structures, including but not limited to those of the conjunctiva and sclera.

[0080] In some variations, the liquid formulations described herein form a non-dispersed mass when a test volume of the liquid formulation is placed in the vitreous of a rabbit eye. In some variations the test volume administered to a rabbit eye, and the test volume is equal to the volume of the liquid formulation administered to a subject's, including but not limited to a human subject's eye.

[0081] In some variations, the test volume administered to a rabbit eye is equal to the volume administered to the subject's eye multiplied by a scale factor, and the scale factor is equal to the average volume of a rabbit eye divided by the average volume of a subject eye. The "average volume" of an eye, as used herein, refers to the average volume of an eye of a member of similar age of the species under consideration generally, as opposed to the average volume of any particular individual's eye.

[0082] In some variations, the test volume administered to the rabbit eye is between about 10 μl and about 50 μl. In some variations, the test volume administered to the rabbit eye is between about 1 μl and about 30 μl. In some variations, the test volume administered to the rabbit eye is between about 50 μl and about 100 μl. In some variations, the test volume administered to the rabbit eye is between about 25 μl and about 75 μl. In some variations, the test volume administered to the rabbit eye is about 30 μl.

[0083] Whether a liquid formulation exhibits a non-dispersed mass relative to a surrounding medium when present in a subject, including but not limited to a human subject or the eye of a subject may be determined by, for instance, mixing a therapeutic agent with a solvent, administering it to the vitreous of an eye of a subject, including but not limited to a human subject, and comparing the liquid formulation to the surrounding medium.

[0084] One liquid formulation that may be used for treating, preventing, inhibiting, delaying the onset of, or causing the regression of the diseases and conditions of a subject, including but not limited to a human subject, is a liquid formulation that forms a non-dispersed mass when placed into the vitreous of a rabbit eye. When used for treating, preventing, inhibiting, delaying the onset of, or causing the regression of the disease or condition of the subject, the liquid formulation is administered to the subject. The liquid formulation may or may not form a non-dispersed mass in the subject. One liquid formulation described herein forms a non-dispersed mass when administered to a subject and forms a non-dispersed mass when administered to a rabbit eye.

[0085] Without being bound by theory, it is believed that the low solubility of rapamycin in the vitreous contributes to the formation of a non-dispersed mass by some rapamycin-containing liquid formulations described herein. The vitreous is a clear gel composed almost entirely of water (up to 99%). Without being bound by theory, it is believed that as rapamycin in an injected formulation contacts the vitreous, the rapamycin precipitates.

[0086] Without being bound by theory, factors believed to affect the formation of and geometry of a non-dispersed mass include the concentration of rapamycin in the formulation, the viscosity of the formulation, ethanol content of the formulation, and the volume of injection. It is believed that maintaining a higher local concentration of rapamycin after injection of the formulation favors formation of a non-dispersed mass, as opposed to a lower local concentration of rapamycin after injection of the formulation. As volume is increased for a given dose, formation of a non-dispersed mass may become less favorable. Formation of a non-dispersed mass may become more favorable as rapamycin concentration is increased and/or as viscosity is increased. Ethanol content affects both the solubility of the rapamycin in the formulation and the viscosity of the formulation.

[0087] In one comparison, 100 μl of a solution of 0.4 % rapamycin, 4.0% ethanol, and 95.6% PEG 400 (a 400 μg dose) did not form a non-dispersed mass after injection into a rabbit eye. In contrast, 20 μl of a solution of 2.00 % rapamycin, 4.0% ethanol, and 94% PEG 400 (also a 400 μg dose) formed a compact spherical non-dispersed mass after injection into a rabbit eye.

[0088] Without being bound by theory, in the latter example, it is hypothesized that formation of the non-dispersed mass occurred as depicted in Figures 1A-1C and described as follows. Upon injection, due to its viscosity the liquid formulation formed a spherical globule 100 within the vitreous 110. Ethanol then diffused out of this globule, resulting in localized precipitation 120 of the rapamycin within the globule. Eventually, the polyethylene glycol also diffused out of the globule to leave a solid, compact non-dispersed mass of rapamycin 130.

[0089] In some variations, the non-dispersed masses described herein consists of at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95% by volume of therapeutic agent when injected into the vitreous of a rabbit eye.

[0090] In some variations, upon formation a non-dispersed mass comprising rapamycin, for example, delivers the drug continuously at approximately a constant rate for an extended period of time. Without being bound by theory, it is believed that delivery of rapamycin from a non-dispersed mass in the vitreous depends on dissolution of the rapamycin in the vitreous, which depends in turn on clearance of the drug from the vitreous to other tissues. Without being bound by theory, this release process is believed to maintain a steady-state concentration of rapamycin in the vitreous.

[0091] In some variations, formation of a non-dispersed mass reduces the toxicity of the injected liquid formulation compared to an equivalent dose that did not form a non-dispersed mass. In variations in which a liquid formulation injected into the vitreous does not form a non-dispersed mass, the drug (rapamycin) appears to disperse in the vitreous body. In some variations this may interfere with vision.

[0092] In some variations, it is believed that the liquid formulations will form a visually observable non-dispersed mass when injected into the eye of a subject, including but not limited to a human subject.

[0093] In some variations, liquid formulations are believed to form non-dispersed masses when injected subconjunctivally. In some variations it is believed that when subconjunctivally administered the liquid formulation forms a depot in the scleral tissue. That is, it is believed that the therapeutic agent is absorbed into the sclera proximate to the injection site and forms a local concentration of drug in the sclera.

Liquid formulations for delivery of therapeutic agents

[0094] The liquid formulations described herein may be used to deliver amounts of the therapeutic agent effective for treating, preventing, inhibiting, delaying on set of, or causing the regression of the diseases and conditions described in the *Diseases and Conditions* section. In some variations the liquid formulations described herein deliver the therapeutic agent over an extended period of time.

[0095] An "effective amount," which is also referred to herein as a "therapeutically effective amount," of a therapeutic agent for administration as described herein is that amount of the therapeutic agent that provides the therapeutic effect sought when administered to the subject, including but not limited to a human subject. The achieving of different therapeutic effects may require different effective amounts of therapeutic agent. For example, the therapeutically effective amount of a therapeutic agent used for preventing a disease or condition may be different from the therapeutically effective amount used for treating, inhibiting, delaying the onset of, or causing the regression of the disease or condition. In addition, the therapeutically effective amount may depend on the age, weight, and other health conditions of the subject as is well know to those versed in the disease or condition being addressed. Thus, the therapeutically effective amount may not be the same in every subject to which the therapeutic agent is administered.

[0096] An effective amount of a therapeutic agent for treating, preventing, inhibiting, delaying the onset of, or causing the regression of a specific disease or condition is also referred to herein as the amount of therapeutic agent effective to treat, prevent, inhibit, delay the onset of, or cause the regression of the disease or condition.

[0097] To determine whether a level of therapeutic agent is a "therapeutically effective amount" to treat, prevent, inhibit, delay on set of, or cause the regression of the diseases and conditions described in the *Diseases and Conditions* section, liquid formulations may be administered in animal models for the diseases or conditions of interest, and the effects may be observed. In addition, dose ranging human clinical trials may be conducted to determine the therapeutically effective amount of a therapeutic agent.

[0098] Generally, the therapeutic agent may be formulated in any liquid formulation capable of delivery of a therapeutically effective amount of the therapeutic agent to a subject or to the eye of a subject for the required delivery period.

[0099] In some variations, the formulations described herein are provided in one or more unit dose forms, wherein the unit dose form contains an amount of a liquid rapamycin formulation described herein that is effective to treat or prevent the disease or condition for which it is being administered.

[0100] In some embodiments, the unit dose form is prepared in the concentration at which it will be administered. In some variations, the unit dose form is diluted prior to administration to a subject. In some variations, a liquid formulation described herein is diluted in an aqueous medium prior to administration to a subject. In some variations the aqueous medium is an isotonic medium. In some variations, a liquid formulation described herein is diluted in an non-aqueous medium prior to administration to a subject.

[0101] In a further aspect, described herein are kits comprising one or more unit dose forms as described herein. The kit may comprise one or more of packaging and instructions for use to treat one or more diseases or conditions. The kit may comprise a diluent which is not in physical contact with the formulation or pharmaceutical formulation. The kit may comprise any of one or more unit dose forms described herein in one or more sealed vessels. The kit may comprise any of one or more sterile unit dose forms.

[0102] The unit dose form may be in a container, including but not limited to a sterile sealed container. The container may be a vial, ampule, or low volume applicator, including but not limited to a syringe. A low-volume applicator may be pre-filled with rapamycin for treatment of an ophthalmic disease or condition, including age-related macular degeneration. A pre-filled low-volume applicator may be pre-filled with a solution comprising 2% rapamycin, 94% PEG-400, 4% ethanol.

[0103] Described herein are kits comprising one or more containers. A kit may comprise one or more low-volume applicators pre-filled with a formulation described herein comprising formulations in liquid form comprising 2% rapamycin, 94% PEG-400, 4% ethanol. The kit may comprise one or more containers, including but not limited to pre-filled low-volume applicators, with instructions for its use. The kit may comprise one or more low-volume applicators pre-filled with rapamycin, with instructions for its use in treating a disease or condition of the eye.The containers described herein may be in a secondary packaging.

Routes of Administration

[0104] The liquid formulations described herein deliver rapamycin to a subject, including but not limited to a human subject.

[0105] In some variations, the liquid formulations described herein deliver rapamycin to an aqueous medium of a human subject.

[0106] In some variations, the liquid formulations described herein deliver rapamycin to an aqueous medium in or proximal to an area where a disease or condition is to be treated, prevented, inhibited, onset delayed, or regression caused.

[0107] In some variations, the liquid formulations described herein deliver rapamycin to an eye of a subject, including the macula and the retina choroid tissues, in an amount and for a duration effective to treat, prevent, inhibit, delay the onset of, or cause the regression of the diseases and conditions described in the *Diseases and Conditions* section.

[0108] "Retina choroid" and "retina choroid tissues," as used herein, are synonymous and refer to the combined retina and choroid tissues of the eye.

[0109] As a non-limiting example, the liquid formulations described herein may be administered to the vitreous, aqueous humor, sclera, conjunctiva, between the sclera and conjunctiva, the retina choroid tissues, macula, or other area in or proximate to the eye of a subject, either by direct administration to these tissues or by periocular routes, in amounts and for a duration effective to treat, prevent, inhibit, delay the onset of, or cause the regression of CNV and wet AMD. The effective amounts and durations may be different for each of treating, preventing, inhibiting, delaying the onset of, or causing the regression of CNV and wet AMD, and for each of the different sites of delivery.

[0110] Intravitreal administration is more invasive than some other types of ocular procedures. Because of the potential risks of adverse effects, intravitreal administration may not be optimal for treatment of relatively healthy eyes. By contrast, periocular administration, such as subconjunctival administration, is much less invasive than intravitreal administration. When a therapeutic agent is delivered by a periocular route, it may be possible to treat patients with healthier eyes than could be treated using intravitreal administration. In some variations, subconjunctival injection is used to prevent or delay onset of a disease or condition of the eye, where the eye of the subject has visual acuity of 20/40 or better.

[0111] "Subconjunctival" placement or injection, as used herein, refers to placement or injection between the sclera and conjunctiva. Subconjunctival is sometimes referred to herein as "sub-conj" administration.

[0112] Routes of administration that may be used to administer a liquid formulation include but are not limited to placement of the liquid formulation, for example by injection, into an aqueous medium in the subject, including but not limited to placement, including but not limited to by injection, into the eye of a subject, including but not limited to a human subject.

[0113] Compositions and liquid formulations comprising therapeutic agent can be administered directly to the eye using a variety of procedures, including but not limited to procedures in which (1) the therapeutic agent is administered by injection using a syringe and hypodermic needle, (2) a specially designed device is used to inject the therapeutic agent, (3) prior to injection of the therapeutic agent, a pocket is surgically formed within the sclera to serve as a receptacle for the therapeutic agent or therapeutic agent composition. For example, in one administration procedure a surgeon forms a pocket within the sclera of the eye followed by injection of a solution or liquid formulation comprising the therapeutic agent into the pocket.

[0114] Other administration procedures include, but are not limited to procedures in which (1) a formulation of the therapeutic agent is injected through a specially designed curved cannula to place the therapeutic agent directly against a portion of the eye, (2) a compressed form of the therapeutic agent is placed directly against a portion of the eye, (3) the therapeutic agent is inserted into the sclera by a specially designed injector or inserter, (4) the liquid formulation comprising the therapeutic agent is incorporated within a polymer, (5) a surgeon makes a small conjunctival incision through which to pass a suture and any therapeutic agent delivery structure so as to secure the structure adjacent to the sclera, (6) a needle is used for injection directly into the vitreous of an eye, or into any other site described.

[0115] The liquid formulations described herein may be used directly, for example, by injection, as an elixir, for topical administration including but not limited to via eye drops, or in hard or soft gelatin or starch capsules. The capsules may be banded to prevent leakage.

Delivery by injection

[0116] One method that may be used to deliver the compositions and liquid formulations described herein is delivery by injection. In this method compositions and liquid formulations may be injected into a subject, including but not limited to a human subject, or into a position in or proximate to an eye of the subject for delivery to a subject or to the eye of a subject. Injection includes but is not limited to intraocular and periocular injection. Nonlimiting examples of positions that are in or proximate to an eye of a subject are as follows.

[0117] Injection of therapeutic agent into the vitreous may provide a high local concentration of therapeutic agent in the vitreous and retina. Further, it has been found that in the vitreous the clearance half-lives of drugs increases with molecular weight.

[0118] Intracameral injection, or injection into the anterior chamber of they eye, may also be used. In one example, up to about 100 μl may be injected intracamerally.

[0119] Periocular routes of delivery may deliver therapeutic agent to the retina without some of the risks of intravitreal delivery. Periocular routes include but are not limited to subconjunctival, subtenon, retrobulbar, peribulbar and posterior juxtascleral delivery. A "periocular" route of administration means placement near or around the eye. For a description of exemplary periocular routes for retinal drug delivery, see Periocular routes for retinal drug delivery, Raghava et al. (2004), Expert Opin. Drug Deliv. 1(1):99-114.

[0120] In some variations the liquid formulations described herein are administered intraocularly. Intraocular administration includes placement or injection within the eye, including in the vitreous.

[0121] Subconjunctival injection may be by injection of therapeutic agent underneath the conjunctiva, or between the sclera and conjunctiva. In one example, up to about 500 μl may be injected subconjunctivally. As one nonlimiting example, a needle of up to about 25 to about 30 gauge and about 30 mm long may be used. Local pressure to the subconjunctival site of therapeutic agent administration may elevate delivery of the therapeutic agent to the posterior segment by reducing local choroidal blood flow.

[0122] Subtenon injection may be by injection of therapeutic agent into the tenon's capsule around the upper portion of the eye and into the "belly" of the superior rectus muscle. In one example, up to about 4 ml may be injected subtenon. As one nonlimiting example, a blunt-tipped cannula about 2.5 cm long may be used.

[0123] Retrobulbar injection refers to injection into the conical compartment of the four rectus muscles and their intermuscular septa, behind the globe of the eye. In one example, up to about 5 ml may be injected retrobulbarly. As one nonlimiting example, a blunt needle of about 25 - or about 27-gauge may be used.

[0124] Peribulbar injection may be at a location external to the confines of the four rectus muscles and their intramuscular septa, i.e., outside of the muscle cone. A volume of, for example, up to about 10 ml may be injected peribulbarly. As one nonlimiting example, a blunt-tipped cannula about 1.25 inches long and about 25-gauge may be used.

[0125] Posterior juxtascleral delivery refers to placement of a therapeutic agent near and above the macula, in direct contact with the outer surface of the sclera, and without puncturing the eyeball. In one example, up to about 500 ml may be injected posterior juxtasclerally. As one nonlimiting example, a blunt-tipped curved cannula, specially designed at 56°, is used to place the therapeutic agent in an incision in the sclera.

[0126] In some variations the liquid formulations described herein are injected intraocularly. Intraocular injection includes injection within the eye.

[0127] Sites to which the compositions and liquid formulations may be administered include but are not limited to the vitreous, aqueous humor, sclera, conjunctiva, between the sclera and conjunctiva, the retina choroid tissues, macula, or other area in or proximate to the eye of a subject. Methods that may be used for placement of the compositions and liquid formulations include but are not limited to injection.

[0128] In one method that may be used, the therapeutic agent is dissolved in an solvent or solvent mixture and then injected into or proximate to the vitreous, aqueous humor, sclera, conjunctiva, between the sclera and conjunctiva, the retina choroid tissues, macula, other area in or proximate to the eye of a subject, or other medium of a subject, according to any of the procedures mentioned above. The therapeutic agent is rapamycin in a liquid formulation.

[0129] The liquid formulations may be used to deliver or maintain an amount of rapamycin in tissues of the eye, including without limitation retina, choroid, or the vitreous, which amount is effective to treat AMD. In one nonlimiting example, it is believed that a liquid formulation delivering rapamycin in an amount capable of providing a concentration of rapamycin of about 0.1 pg/ml to about 2 μg/ml in the vitreous may be used for treatment of wet AMD. In some nonlimiting examples, it is believed that a liquid formulation delivering a concentration of rapamycin of about 0.1 pg/mg to about 1 μg/mg in the retina choroid tissues may be used for treatment of wet AMD. Other effective concentrations are readily ascertainable by those of skill in the art based on the teachings described herein.

Method of preparing liquid formulations

[0130] One nonlimiting method that may be used for preparing the liquid formulations described herein is by mixing a solvent and a therapeutic agent together at room temperature or at slightly elevated temperature until a solution or suspension is obtained, with optional use of a sonicator, and then cooling the formulation. Other components including but not limited to those described above may then be mixed with the formulation. Other preparation methods that may be used are described herein including in the examples, and those of skill in the art will be able to select other preparation methods based on the teachings herein.

Extended delivery of therapeutic agents

[0131] Described herein are liquid formulations showing in vivo delivery or clearance profiles with one or more of the following characteristics. The delivery or clearance profiles are for clearance of the therapeutic agent in vivo after injection of the liquid formulations subconjunctivally or into the vitreous of a rabbit eye. In some variations, the delivery or clearance profiles are for clearance of rapamycin in vivo after injection of the liquid formulations subconjunctivally or into the vitreous of a rabbit eye. The volume of the rabbit vitreous is approximately 30-40% of the volume of the human vitreous. The amount of therapeutic agent is measured using techniques as described in Example 2, but without limitation to the formulation and therapeutic agent described in Example 2.

[0132] "Average percentage in vivo" level means that an average concentration of therapeutic agent is obtained across multiple rabbit eyes for a given timepoint, and the average concentration of therapeutic agent at one timepoint is divided by the average concentration of therapeutic agent at another timepoint.

[0133] The average concentration of a therapeutic agent in the tissue of a rabbit eye at a given time after administration of a formulation containing the therapeutic agent may be measured according to the following method. Where volumes below 10 μl are to be injected, a Hamilton syringe is used.

[0134] The liquid formulations are stored at a temperature of 2-8°C prior to use.

[0135] The experimental animals are specific pathogen free (SPF) New Zealand White rabbits. A mixed population of about 50% male, about 50% female is used. The rabbits are at least 12 weeks of age, usually at least 14 weeks of age, at the time of dosing. The rabbits each weigh at least 2.2 kg, usually at least 2.5 kg, at the time of dosing. Prior to the study, the animals are quarantined for at least one week and examined for general health parameters. Any unhealthy animals are not used in the study. At least 6 eyes are measured and averaged for a given timepoint.

[0136] Housing and sanitation are performed according to standard procedures used in the industry. The animals are provided approximately 150 grams of Teklad Certified Hi-Fiber Rabbit Diet daily, and are provided tap water ad libitum. No contaminants are known to exist in the water and no additional analysis outside that provided by the local water district is performed. Environmental Conditions are monitored.

[0137] Each animal undergoes a pre-treatment ophthalmic examination (slit lamp and ophthalmoscopy), performed by a board certified veterinary ophthalmologist. Ocular findings are scored according to the McDonald and Shadduck scoring system as described in Dermatoxicology, F. N. Marzulli and H.I. Maibach, 1977 "Eye Irritation," T.O. McDonald and J.A. Shadduck (pages 579-582). Observations are recorded using a standardized data collection sheet. Acceptance criteria for placement on study are as follows: scores of ≤ 1 for conjunctival congestion and swelling; scores of 0 for all other observation variables.

[0138] Gentamicin ophthalmic drops are placed into both eyes of each animal twice daily on the day prior to dosing, on the day of dosing (Day 1), and on the day after dosing (Day 2). Dosing is performed in two phases, the first including one set of animals and the second including the other animals. Animals are randomized separately into masked treatment groups prior to each phase of dosing according to modified Latin squares. Animals are fasted at least 8 hours prior to injection. The start time of the fast and time of injection are recorded.

[0139] Animals are weighed and anesthetized with an intravenous injection of a ketamine/xylazine cocktail (87 mg/mL ketamine, 13 mg/mL xylazine) at a volume of 0.1-0.2 mL/kg. Both eyes of each animal are prepared for injection as follows: approximately five minutes prior to injection, eyes are moistened with an ophthalmic Betadine solution. After five minutes, the Betadine is washed out of the eyes with sterile saline. Proparacaine hydrochloride 0.5% (1-2 drops) is delivered to each eye. For eyes to be intravitreally injected, 1% Tropicamide (1 drop) is delivered to each eye.

[0140] On Day 1, both eyes of each animal receive an injection of test or control article. Animals in selected groups are dosed a second time on Day 90 ± 1. Dosing is subconjunctival or intravitreal. Actual treatments, injection locations, and dose volumes are masked and revealed at the end of the study.

[0141] Subconjunctival injections are given using an insulin syringe and 30 gauge x 1/2-inch needle. The bulbar conjunctiva in the dorsotemporal quadrant is elevated using forceps. Test article is injected into the subconjunctival space.

[0142] Intravitreal injections are given using an Insulin syringe and 30 gauge x 1/2-inch needle. For each injection, the needle is introduced through the ventral-nasal quadrant of the eye, approximately 2-3 mm posterior to the limbus, with the bevel of the needle directed downward and posteriorly to avoid the lens. Test article is injected in a single bolus in the vitreous near the retina.

[0143] Animals are observed for mortality/morbidity twice daily. An animal determined to be moribund is euthanized with an intravenous injection of commercial euthanasia solution. Both eyes are removed and stored frozen at -70°C for possible future evaluation. If an animal is found dead prior to onset of rigor mortis, both eyes are removed and stored frozen at -70°C for possible future evaluation. Animals found after the onset of rigor mortis are not necropsied.

[0144] Animals are weighed at randomization, on Day 1 prior to dosing, and prior to euthanasia.

[0145] Ophthalmic observations (slit lamp and indirect ophthalmoscopy) are performed on all animals on Days 5 ± 1, 30 ± 1, 60 ± 1, 90 ± 1, and at later dates in some variations. Observations are performed by a board certified veterinary ophthalmologist. For animals to be dosed on Day 90 ± 1, ophthalmic observations are performed prior to dosing. Ocular findings are scored according to the McDonald and Shadduck scoring system as described in Dermatoxicology, F.N. Marzulli and H.I. Maibach, 1977 "Eye Irritation", T.O. McDonald and J.A. Shadduck (pages 579-582), and observations are recorded using a standardized data collection sheet.

[0146] Whole blood samples (1-3 mL per sample) are collected from each animal prior to necropsy in vacutainer tubes containing EDTA. Each tube is filled at least 2/3 full and thoroughly mixed for at least 30 seconds. Tubes are stored frozen until shipped on dry ice.

[0147] Animals are euthanized with an intravenous injection of commercial euthanasia solution. Euthanasia is performed according to standard procedures used in the industry.

[0148] For treatment groups dosed intravitreally or subconjunctivally with placebo, all eyes from each of these groups are placed into Davidsons solution for approximately 24 hours. Following the 24-hour period, the eyes are transferred to 70% ethanol; these globes are submitted for masked histopathological evaluation by a board certified veterinary pathologist. The time that eyes are placed into Davidsons and the time of removal are recorded.

[0149] For treatment groups dosed intravitreally or subconjunctivally with test article, some eyes from each of these groups are frozen at -70°C and submitted for pharmacokinetic analysis. The remaining eyes from each of these groups are placed into Davidsons solution for approximately 24 hours. Following the 24-hour period, the eyes are transferred to 70% ethanol; these globes are submitted for masked histopathological evaluation by a board certified veterinary pathologist. The time that eyes are placed into Davidsons and the time of removal are recorded.

[0150] Frozen samples submitted for pharmacokinetic analysis are dissected with disposable instruments. One set of instruments is used per eye, and then discarded. The samples are thawed at room temperature for 1 to 2 minutes to ensure that the frost around the tissue has been removed. The sclera is dissected into 4 quadrants, and the vitreous is removed. If a non-dispersed mass (NDM) is clearly visible within the vitreous, the vitreous is separated into two sections. The section with the NDM is approximately two-thirds of the vitreous. The section without the NDM is the portion of the vitreous that is the most distant from the NDM. The aqueous humor, lens, iris, and cornea are separated. The retina choroid tissue is removed using a forceps and collected for analysis. The conjunctiva is separated from the sclera.

[0151] The various tissue types are collected into separate individual pre-weighed vials which are then capped and weighed. The vials of tissue are stored at -80°C until analyzed.

[0152] The sirolimus content of the retina choroid, sclera, vitreous humor, and whole anticoagulated blood is determined by high-pressure liquid chromatography/tandem mass spectroscopy (HPLC/MS/MS) using 32-O-desmethoxyrapamycin as an internal standard. Where an NDM was observed in the vitreous, the section of the vitreous containing the NDM and the section of the vitreous not containing the NDM are analyzed separately.

[0153] The average concentration of a therapeutic agent over a period of time means for representative timepoints over the period of time the average concentration at each time point. For example, if the time period is 30 days, the average concentration may be measured at 5 day intervals: for the average concentration at day 5, the average of a number of measurements of concentration at day 5 would be calculated; for the average concentration at day 10, the average of a number of measurements of the concentration at day 10 would be calculated, etc.

[0154] For treatment, prevention, inhibition, delaying the onset of, or causing the regression of certain diseases or conditions, it may be desirable to maintain delivery of a therapeutically effective amount of the therapeutic agent for an extended period of time. Depending on the disease or condition being treated, prevented, inhibited, having onset delayed, or being caused to regress this extended period of time may be at least about 1 week, at least about 2 weeks, at least about 3 weeks, at least about 1 month, at least about 3 months, at least about 6 months, at least about 9 months, or at least about 1 year. Generally, however, any extended period of delivery may be possible. A therapeutically effective amount of agent may be delivered for an extended period by a liquid formulation or composition that maintains for the extended period a concentration of agent in a subject or an eye of a subject sufficient to deliver a therapeutically effective amount of agent for the extended time.

[0155] Delivery of a therapeutically effective amount of the therapeutic agent for an extended period may be achieved via placement of one composition or liquid formulation or may be achieved by application of two or more doses of composition or liquid formulations. As a non-limiting example of such multiple applications, maintenance of the therapeutic amount of rapamycin for 3 months for treatment, prevention, inhibition, delay of onset, or cause of regression of wet AMD may be achieved by application of one liquid formulation or composition delivering a therapeutic amount for 3 months or by sequential application of a plurality of liquid formulations or compositions. The optimal dosage regime will depend on the therapeutic amount of the therapeutic agent needing to be delivered, and the period over which it need be delivered. Those versed in such extended therapeutic agent delivery dosing will understand how to identify dosing regimes that may be used based on the teachings provided herein.

[0156] When using certain therapeutic agents or for the treatment, prevention, inhibition, delaying the onset of, or causing the regression of certain diseases, it may be desirable for delivery of the therapeutic agent not to commence immediately upon placement of the liquid formulation or composition into the eye region, but for delivery to commence after some delay. For example, but in no way limiting, such delayed release may be useful where the therapeutic agent inhibits or delays wound healing and delayed release is desirable to allow healing of any wounds occurring upon placement of the liquid formulation or composition. Depending on the therapeutic agent being delivered and/or the diseases and conditions being treated, prevented, inhibited, onset delayed, and regression caused this period of delay before delivery of the therapeutic agent commences may be about 1 hour, about 6 hours, about 12 hours, about 18 hours, about 1 day, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, about 7 days, about 8 days, about 9 days, about 10 days, about 11 days, about 12 days, about 13 days, about 14 days, about 21 days, about 28 days, about 35 days, or about 42 days. Other delay periods may be possible. Delayed release formulations that may be used are known to people versed in the technology.

Intravitreal and subconjunctival delivery of rapamycin for treatment, prevention, inhibition, delay of onset, or cause of regression of AMD

[0157] In one method described herein, a liquid formulation comprising rapamycin is delivered subconjunctivally or to the vitreous of an eye to prevent, treat, inhibit, delay onset of, or cause regression of angiogenesis in the eye, including but not limited to treating CNV as observed, for example, in AMD. In some variations, the liquid formulation is used to treat angiogenesis in the eye, including but not limited to treating CNV as observed, for example, in AMD. Rapamycin has been shown to inhibit CNV in rat and mice models, as described in U.S. Application No. 10/665,203. Rapamycin has been observed to inhibit Matrigel™ and laser-induced CNV when administered systemically and subretinally. Also, periocular injection of rapamycin inhibits laser-induced CNV.

[0158] As described herein, the dosage of the therapeutic agent will depend on the condition being addressed, whether the condition is to be treated, prevented, inhibited, have onset delayed, or be caused to regress, the particular therapeutic agent, and other clinical factors such as weight and condition of the subject and the route of administration of the therapeutic agent. It is to be understood that the methods, liquid formulations, and compositions described herein have application for both human and veterinary use, as well as uses in other possible animals. As described herein, tissue concentrations of therapeutic agents expressed in units of mass per volume generally refer to tissues that are primarily aqueous such as the vitreous, for example. Tissue concentrations of therapeutic agents expressed in unit of mass per mass generally refer to other tissues such as the sclera or retina choroid tissues, for example.

[0159] One concentration of rapamycin that may be used in the methods described herein is one that provides about 0.01 pg/ml or pg/mg or more of rapamycin at the tissue level. Another concentration that may be used is one that provides about 0.1 pg/ml or ng/mg or more at the tissue level. Another concentration that may be used is one that provides about 1 pg/ml or ng/mg or more at the tissue level. Another concentration that may be used is one that provides about 0.01 ng/ml or ng/mg or more at the tissue level. Another concentration that may be used is one that provides about 0.1 ng/ml or ng/mg or more at the tissue level. Another concentration that may be used is one that provides about 0.5 ng/ml or ng/mg or more at the tissue level. Another concentration that may be used is one that provides about 1 ng/ml or more at the tissue level. Another concentration that may be used is one that provides about 2 ng/ml or more at the tissue level. Another concentration that may be used is one that provides about 3 ng/ml or more at the tissue level. Another concentration that may be used is one that provides about 5 ng/ml or more at the tissue level. Another concentration that may be used is one that provides about 10 ng/ml or more at the tissue level. Another concentration that may be used is one that provides about 15 ng/ml or more at the tissue level. Another concentration that may be used is one that provides about 20 ng/ml or more at the tissue level. Another concentration that may be used is one that provides about 30 ng/ml or more at the tissue level. Another concentration that may be used is one that provides about 50 ng/ml or more at the tissue level. One of ordinary skill in the art would know how to arrive at an appropriate concentration depending on the route and duration of administration utilized, given the teachings herein.

[0160] Generally, the amount of rapamycin administered in a liquid formulation is an amount sufficient to treat, prevent, inhibit, delay the onset, or cause regression of the disease or condition of the eye for the required amount of time. In some variations the amount of rapamycin administered in the liquid formulation is an amount sufficient to treat the disease or condition of the eye for the required amount of time.

[0161] In some variations, a total amount of rapamycin less than about 5 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 5.0 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 4.5 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 4.0 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 3.5 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 3.0 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 2.5 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 2 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 1.2 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 1.0 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 0.8 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 0.6 mg is administered subconjunctivally. In some variations, a total amount of rapamycin less than about 0.4 mg is administered subconjunctivally. In some variations, a volume of a formulation is administered that contains an amount of rapamycin described herein.

[0162] In some variations, a liquid formulation containing an amount of rapamycin of between about 0.2 μg and about 4 mg is subconjunctivally administered to a human subject by administering between about 0.1 μl and about 200 μl of a liquid formulation described herein. In some variations, a liquid formulation containing an amount of rapamycin of between about 20 μg and about 2 mg is subconjunctivally administered to a human subject by administering between about 1 μl and about 100 μl of a liquid formulation described herein. In some variations, a liquid formulation containing an amount of rapamycin of between about 20 μg and about 1 mg is subconjunctivally administered to a human subject by administering between about 1 μl and about 50 μl of a liquid formulation described herein.In some variations, a liquid formulation containing an amount of rapamycin of between about 20 μg and about 500 μg is subconjunctivally administered to a human subject by administering between about 1 μl and about 25 μl of a liquid formulation described herein. In some variations, a liquid formulation containing an amount of rapamycin of between about 20 μg and about 300 μg is subconjunctivally administered to a human subject by administering between about 1 μl and about 15 μl of a liquid formulation described herein.

[0163] In some variations, a total amount of rapamycin less than about 200 μg is administered intravitreally. In some variations, a total amount of rapamycin less than about 200 μg is administered intravitreally. In some variations, a total amount of rapamycin less than about 300 μg is administered intravitreally. In some variations, a total amount of rapamycin less than about 400 μg is administered intravitreally. In some variations, a total amount of rapamycin less than about 500 μg is administered intravitreally. In some variations, a total amount of rapamycin less than about 600 μg is administered intravitreally. In some variations, a total amount of rapamycin less than about 800 μg is administered intravitreally. In some variations, a total amount of rapamycin less than about 1 mg is administered intravitreally. In some variations, a total amount of rapamycin less than about 2 mg is administered intravitreally. In some variations, a total amount of rapamycin less than about 2.5 mg is administered intravitreally. In some variations, a total amount of rapamycin less than about 3 mg is administered intravitreally. In some variations, a total amount of rapamycin less than about 3.5 mg is administered intravitreally. In some variations, a total amount of rapamycin less than about 4 mg is administered intravitreally. In some variations, a volume of a formulation is administered that contains an amount of rapamycin described herein.

[0164] In some variations, a liquid formulation containing an amount of rapamycin of between about 0.2 μg and about 4 mg is intravitreally administered to a human subject by administering between about 0.1 μl and about 200 μl of a liquid formulation described herein. In some variations, a liquid formulation containing an amount of rapamycin of between about 20 μg and about 2 mg is intravitreally administered to a human subject by administering between about 1 μl and about 100 μl of a liquid formulation described herein. In some variations, a liquid formulation containing an amount of rapamycin of between about 20 μg and about 1 mg is intravitreally administered to a human subject by administering between about 1 μl and about 50 μl of a liquid formulation described herein. In some variations, a liquid formulation containing an amount of rapamycin of between about 20 μg and about 500 μg is intravitreally administered to a human subject by administering between about 1 μl and about 25 μl of a liquid formulation described herein. In some variations, a liquid formulation containing an amount of rapamycin of between about 20 μg and about 300 μg is intravitreally administered to a human subject by administering between about 1 μl and about 15 μl of a liquid formulation described herein.

[0165] In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 1 μg and about 5 mg is administered to a human subject for treatment of wet AMD. In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 20 μg and about 4 mg is administered to a human subject for treatment of wet AMD. In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 20 μg and about 1.2 mg is administered to a human subject for treatment of wet AMD. In some variations an amount of rapamycin of between about 10 μg and about 0.5 mg is administered to a human subject for treatment of wet AMD. In some variations an amount of rapamycin of between about 10 μg and 90 μg is administered to a human subject for treatment of wet AMD. In some variations an amount of rapamycin of between about 60 μg and about 120 μg is administered to a human subject for treatment of wet AMD. In some variations an amount of rapamycin of between about 100 μg and about 400 μg is administered to a human subject for treatment of wet AMD. In some variations an amount of rapamycin of between about 400 μg and about 1 mg is administered to a human subject for treatment of wet AMD. In some variations an amount of rapamycin of between about 1 mg and about 5 mg is administered to a human subject for treatment of wet AMD. In some variations, an amount of rapamycin of between about 3 mg and about 7 mg is administered to a human subject for treatment of wet AMD. In some variations, an amount of rapamycin of between about 5 mg and about 10 mg is administered to a human subject for treatment of wet AMD.

[0166] In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 1 μg and about 5 mg is administered to a human subject for prevention of wet AMD. In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 20 μg and about 4 mg is administered to a human subject for prevention of wet AMD. In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 20 μg and about 1.2 mg is administered to a human subject for prevention of wet AMD. In some variations an amount of rapamycin of between about 10 μg and about 0.5 mg is administered to a human subject for prevention of wet AMD. In some variations an amount of rapamycin of between about 10 μg and 90 μg is administered to a human subject for prevention of wet AMD. In some variations an amount of rapamycin of between about 60 μg and about 120 μg is administered to a human subject for prevention of wet AMD. In some variations an amount of rapamycin of between about 100 μg and about 400 μg is administered to a human subject for prevention of wet AMD. In some variations an amount of rapamycin of between about 400 μg and about 1 mg is administered to a human subject for prevention of wet AMD. In some variations an amount of rapamycin of between about 1 mg and about 5 mg is administered to a human subject for prevention of wet AMD. In some variations, an amount of rapamycin of between about 3 mg and about 7 mg is administered to a human subject for prevention of wet AMD. In some variations, an amount of rapamycin of between about 5 mg and about 10 mg is administered to a human subject for prevention of wet AMD.

[0167] In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 1 μg and about 5 mg is administered to a human subject for treatment of dry AMD. In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 20 μg and about 4 mg is administered to a human subject for treatment of dry AMD. In some variations a liquid formulation as described herein containing an amount of rapamycin of between about 20 μg and about 1.2 mg is administered to a human subject for treatment of dry AMD. In some variations an amount of rapamycin of between about 10 μg and about 0.5 mg is administered to a human subject for treatment of dry AMD. In some variations an amount of rapamycin of between about 10 μg and 90 μg is administered to a human subject for treatment of dry AMD. In some variations an amount of rapamycin of between about 60 μg and about 120 μg is administered to a human subject for treatment of dry AMD. In some variations an amount of rapamycin of between about 100 μg and about 400 μg is administered to a human subject for treatment of dry AMD. In some variations an amount of rapamycin of between about 400 μg and about 1 mg is administered to a human subject for treatment of dry AMD. In some variations an amount of rapamycin of between about 1 mg and about 5 mg is administered to a human subject for treatment of dry AMD. In some variations, an amount of rapamycin of between about 3 mg and about 7 mg is administered to a human subject for treatment of dry AMD. In some variations, an amount of rapamycin of between about 5 mg and about 10 mg is administered to a human subject for treatment of dry AMD.

[0168] In some variations, a liquid formulation as described herein containing an amount of rapamycin of between about 1 μg and about 5 mg is administered to a human subject for treatment of angiogenesis, including but not limited to choroidal neovascularization. In some variations, an amount of rapamycin of between about 20 μg and about 4 mg is administered to the human subject; between about 20 μg and about 1.2 mg; between about 10 μg and about 0.5 mg is administered to a human subject for treatment of wet AMD, between about 10 μg and 90 μg, between about 60 μg and 120 μg is administered to the human subject; between about 100 μg and 400 μg, between about 400 μg and 1 mg is administered to the human subject; in some variations, an amount of rapamycin of between about 1 mg and 5 mg is administered to the human subject; in some variations, an amount of rapamycin of between about 3 mg and 7 mg is administered to the human subject; in some variations, an amount of rapamycin of between about 5 mg and 10 mg is administered to the human subject for treatment of angiogenesis, including but not limited to choroidal neovascularization.

[0169] In some variations, any one or more of the formulations described herein are for administrationintravitreally every 3 or more months, every 6 or more months, every 9 or more months, or every 12 or more months, or longer, to treat one or more of choroidal neovascularization, wet AMD, dry AMD, to prevent wet AMD, or to prevent progression of dry AMD to wet AMD. In some variations, any one or more of the formulations described herein are for administration subconjunctivally every 3 or more months, every 6 or more months, every 9 or more months, or every 12 or more months, or longer, to treat one or more of choroidal neovascularization, wet AMD, dry AMD, or to prevent wet AMD.

[0170] In some variations, any one or more of the rapamycin formulations described herein are for administration intravitreally every 3 or more months, every 6 or more months, every 9 or more months, or every 12 or more months, or longer, to treat one or more of choroidal neovascularization, wet AMD, dry AMD, to prevent wet AMD, or to prevent progression of dry AMD to wet AMD. In some variations, any one or more of the rapamycin formulations described herein are for administration subconjunctivally every 3 or more months, every 6 or more months, every 9 or more months, or every 12 or more months, or longer, to treat one or more of choroidal neovascularization, wet AMD, dry AMD, or to prevent wet AMD. In some variations, the effect of the rapamycin persists beyond the period during which it is present in the ocular tissues.

[0171] Delivery of the therapeutic agents described herein may, for example, be delivered at a dosage range between about 1 ng/day and about 100 μg/day, or at dosages higher or lower than this range, depending on the route and duration of administration. In some variations of liquid formulation or composition used in the methods described herein, the therapeutic agents are delivered at a dosage range of between about 0.1 μg/day and about 10 μg/day. In some variations of liquid formulation or composition used in the methods described herein, the therapeutic agents are delivered at a dosage range of between about 1 μg/day and about 5 μg/day. Dosages of various therapeutic agents for treatment, prevention, inhibition, delay of onset, or cause of regression of various diseases and conditions described herein can be refined by the use of clinical trials.

[0172] The liquid formulations described herein may be used for delivery to the eye, as one nonlimiting example by ocular or periocular administration, of therapeutically effective amounts of rapamycin for extended periods of time to treat, prevent, inhibit, delay the onset of, or cause regression of CNV, and thus may be used to treat, prevent, inhibit, delay the onset of, or cause regression of wet AMD, or transition of dry AMD to wet AMD. It is believed that by changing certain characteristics of the liquid formulations described herein, including but not limited to the components of the liquid formulations, the location in the eye to which the liquid formulation is delivered, including without limitation subconjunctival or intravitreal placement, the liquid formulations may be used to deliver therapeutically effective amounts of rapamycin to the eye for a variety of extended time periods including delivery of therapeutic amounts for greater than about 1 week, for greater than about 2 weeks, for greater than about 3 weeks, for greater than about 1 month, for greater than about 3 months, for greater than about 6 months, for greater than about 9 months, for greater than about 1 year.

[0173] When a therapeutically effective amount of rapamycin is administered to a subject suffering from wet AMD, the rapamycin may treat, inhibit, or cause regression of the wet AMD. Different therapeutically effective amounts may be required for treatment, inhibition or causing regression. A subject suffering from wet AMD may have CNV lesions, and it is believed that administration of a therapeutically effective amount of rapamycin may have a variety of effects, including but not limited to causing regression of the CNV lesions, stabilizing the CNV lesion, and preventing progression of an active CNV lesion.

[0174] When a therapeutically effective amount of rapamycin is administered to a subject suffering from dry AMD, it is believed that the rapamycin may prevent or slow the progression of dry AMD to wet AMD.

EXAMPLES

[0175] Unless the context indicates otherwise, the error bars in the charts show one standard deviation. Where ethanol is used, it is 200 proof ethanol from Gold Shield Distributors, Hayward, CA. Where rapamycin is used, it is from LC laboratories, Woburn, MA, or Chunghwa Chemical Synthesis & Biotech Co., LTD (CCSB), Taipei Hsien, Taiwan, ROC. Where PEG 400 is used, it is from The Dow Chemical Company, New Milford, CT. Some of the graphs use the expression "uL" or "ug" to refer to μL or μg, respectively. Where a volume of 10 μL or less is administered, Hamilton HPLC syringes were used.

[0176] Examples 16 to 22 and 40 to 45 are Examples of the invention. The other Examples are Comparative Examples.

Example 1- Preparation and Characterization of a Rapamycin-Containing Solution

[0177] 1.256% rapamycin (percentage of the total weight) was dissolved in 9.676% ethanol (percentage of the total weight). An aqueous solution of 15% F127 (Lutrol) in sterile water was slowly added under continuous agitation. The final concentration was approximately 78.57% sterile water (percentage of the total weight) and approximately 10.50% F127 (Lutrol) (percentage of the total weight). This solution is listed as formulation #32 in Table 1. The solution was placed at 2°C until use.

Example 2 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0178] 50 μl of the solution described in Example 1 was injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits.

[0179] **Fig. 2** depicts the average concentration of rapamycin present in the vitreous (ng/ml), retina choroid (ng/mg), and sclera (ng/mg) on a logarithmic scale at 20, 40, 67, and 90 days after injection.

[0180] The analysis was by liquid chromatography mass spectroscopy (LCMS) using an internal standard.

[0181] At each timepoint, the average concentration of rapamycin was calculated by adding the concentrations of rapamycin obtained for each eye from each rabbit, and dividing the total by the number of eyes analyzed. In this experiment, each timepoint represents the average of either two eyes of each of two rabbits (four eyes at that timepoint) or the average of two eyes of one rabbits (two eyes at that timepoint).

[0182] The full vitreous was homogenized and analyzed. The average concentration of the vitreous was calculated by dividing the mass of rapamycin measured by the volume of vitreous analyzed. The sample did not include the site of administration; thus, this measurement indicated the level of rapamycin delivered to the vitreous via the solution.

[0183] The average level of rapamycin in the vitreous at 20, 40, 67, and 90 days after subconjunctival injection was about 4.425, 3.800,4.100, and 1.500 ng/ml, respectively.

[0184] The full retina choroid was homogenized and analyzed. The average concentration of the retina choroid was calculated by dividing the mass of rapamycin measured by the mass of retina choroid analyzed. The sample did not include the site of administration; thus, this measurement indicated the level of rapamycin delivered to the retina choroid via the solution.

[0185] The average level of rapamycin in the retina choroid at 20, 40, 67, and 90 days after subconjunctival injection was about 0.055, 0.209, 0.080, and 0.017 ng/mg, respectively.

[0186] The sclera was analyzed in the same way as the retina choroid. The scleral sample included the site of injection; thus, this measurement indicated clearance of rapamycin from the sclera.

[0187] The average level of rapamycin in the sclera at 20, 40, 67, and 90 days after subconjunctival injection was about 0.141, 0.271, 0.067, and 0.192 ng/mg, respectively.

Example 3 - Preparation and Characterization of a Rapamycin-Containing Solution

[0188] 5.233% rapamycin (per weight of the total of the formulation after all components were added) was dissolved in 0.4177 g of EtOH; the quantity of EtOH was reduced by forced evaporation (heat) to 0.1296g (6.344%, w/w). PEG 400 was added under continuous agitation. Final concentrations as a percentage of the total weight were approximately: rapamycin 5.233%, ethanol 6.344%, and PEG 400 88.424%. When contacted with the vitreous, the formulation formed a non-dispersed mass relative to the surrounding medium. This solution is listed as formulation #34 in Table 1.

Example 4 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0189] 25μl of the solution described in Example 3 were injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits.

[0190] **Fig. 3** depicts the level of rapamycin present in the vitreous (ng/ml), retina choroid (ng/mg), and sclera (ng/mg) on a logarithmic scale at 14, 35, 62, and 85 days after injection. The level of rapamycin present in the vitreous (ng/ml) is also shown at 2 days after injection.

[0191] The vitreous was homogenized and analyzed as described in Example 2, except on day 2 a single eye of each of three rabbits was analyzed; at day 14 two eyes from each of two rabbits were analyzed; at day 35 two eyes from a single rabbit were analyzed; at day 62 two eyes from a single rabbit were analyzed; and at day 85 one eye from a single rabbit plus two eyes from a second rabbit were analyzed.

[0192] The vitreous sample did not include the site of administration; thus, this measurement indicated the level of rapamycin delivered to the vitreous via the solution. The average level of rapamycin in the vitreous at 2, 14, 35, 62, and 85 days after subconjunctival injection was about 3.57, 53.65, 9.00, 4.700, and 0.600 ng/ml, respectively.

[0193] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken on the days as described for the vitreous above. No day 2 analysis was done. The retina choroid sample did not include the site of administration; thus, this measurement indicated the level of rapamycin delivered to the retina choroid via the solution. The average level of rapamycin in the retina choroid at 14, 35, 62, and 85 days after subconjunctival injection was about 0.4815, 1.725, 0.057, and 0.009 ng/mg, respectively.

[0194] The scleral sample was analyzed as described in Example 2, and the samples were taken on the days as described for the retina choroid as above. The scleral sample included the site of injection; thus, this measurement indicated clearance of rapamycin from the sclera. The average level of rapamycin in the sclera at 14, 35, 62, and 85 days after subconjunctival injection was about 34.5815, 0.135, 0.042, and 0.163666667 ng/mg, respectively.

Example 5 - Intravitreal Injection of a Rapamycin-Containing Solution

[0195] 25 μl of the solution described in Example 3 was injected into the vitreous of the eye of New Zealand white rabbits. **Fig. 4** depicts the level of rapamycin present in the vitreous (ng/ml), retina choroid (ng/mg), and sclera (ng/mg) on a logarithmic scale at 14, 35, 62, and 90 days after injection. The level of rapamycin present in the vitreous (ng/ml) is also shown at 2 days after injection.

[0196] The vitreous was homogenized and analyzed as described in Example 2, except on day 2 approximately 1 μl of a single eye of each of three rabbits was analyzed; at day 14 two eyes from each of two rabbits were analyzed; at day 35 two eyes from a single rabbit were analyzed; at day 62 two eyes from a single rabbit were analyzed; and at day 90 two eyes from each of two rabbits were analyzed.

[0197] Excepting the day 2 sample, the vitreous samples included the site of administration. An effort was made to avoid the administered solution where possible. However, the accuracy of the measured levels of rapamycin was potentially affected by sampling errors due to inadvertent inclusion of the administered solution.

[0198] The average level of rapamycin in the vitreous at 2, 14, 35, 62, and 90 days after intravitreal injection was about 11.4, 136538, 2850.3, 21820.35, and 27142.75 ng/ml, respectively.

[0199] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken on the days described for the vitreous above. No day 2 analysis was done. The retina choroid sample did not include the site of administration; thus, this measurement indicated the level of rapamycin delivered to the retina choroid via the solution. The average level of rapamycin in the retina choroid at 14, 35, 62, and 90 days after intravitreal injection was about 5.78975, 244.485, 0.105, and 1.782 ng/mg, respectively.

[0200] The scleral sample was analyzed as described in Example 2, and the samples were taken on the days as described for the retina choroid above. The scleral sample did not include the site of injection; thus, this measurement indicated level of rapamycin delivered to the sclera. The average level of rapamycin in the sclera at 14, 35, 62, and 90 days after intravitreal injection was about 0.5695, 12.34, 0.8505, and 0.71175 ng/mg, respectively.

Example 6 - Preparation and Characterization of a Rapamycin-Containing Suspension

[0201] 6% rapamycin (percentage of the total weight) was dispersed in 94% PEG400 (percentage of the total weight). This suspension is listed as formulation #55 in Table 1.

Example 7 - Intravitreal Injection of a Rapamycin-Containing Suspension

[0202] The solution prepared in Example 6 was injected intravitreally into the eyes of New Zealand white rabbits. **Fig. 5** depicts images of rabbit eyes after intravitreal injection of 10 μl (Fig. 5A), 20 μl (Fig. 5B), and 40 μl (Fig. 5C) of a 6% rapamycin suspension in PEG400. This resulted in an injected dose of about 0.6, about 1.2, and about 2.4 mg. The images were focused on the administered suspension. These images show that the suspension forms a non-dispersed mass relative to the surrounding vitreal medium.

Example 8 - Preparation and Characterization of a Rapamycin-Containing In Situ Galling Formulation

[0203] A liquid formulation of 4.2% rapamycin (obtained from LC laboratories in Woburn, MA, and Chunghwa Chemical Synthesis & BioTech. Co, Ltd in Taiwan), 4.3% ethanol (obtained from Gold Shield Chemical in Hayward, CA), 2.2% PVP K90 (obtained from BASF), 87.1% PEG 400 (obtained from DOW Chemical), and 2.2% Eudragit RL 100 (obtained from Rohm Pharma Polymers), where all percentages are by weight of the total.
[0204] Eudragit RL 100 was dissolved in ethanol. Sonication and heat may be required at this step. Ethanol - Eudragit was added to PEG 400. PVP was slowly added to the Eudragit-Ethanol-PEG solution, and a uniformly mixed solution was obtained. Vigorous mixing may be required for this step.
[0205] Rapamycin was added to and dissolved in the Eudragit-ethanol-PEG-PVP mix. Heat and sonication may be used. The formulation was mixed thoroughly (using a vortex or mixer) to achieve uniformity. This formulation is listed as #37 in Table 1.
[0206] When placed in deionized water or tap water, the liquid formulation formed a non-dispersed mass. The non-dispersed mass appeared as a gel-like substance.

Example 9 -Subconjunctival Injection of a Rapamycin-Containing Non-Dispersed Mass-Farming Formulation

[0207] 50 μl of the solution described in Example 8 was injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits.
[0208] **Fig. 6** depicts the average concentration of rapamycin present in the vitreous (ng/ml), retina choroid tissues (ng/mg), and sclera (ng/mg) on a logarithmic scale at 7, 32, 45, and 90 days after injection of the in situ gelling formulation.
[0209] The analysis was by LCMS (liquid chromatography - mass spectroscopy).
[0210] Where more than a single eye was analyzed, the average concentration of rapamycin was calculated by adding the concentrations of rapamycin obtained for each eye from each rabbit, and dividing the total by the number of eyes analyzed. In this experiment, the vitreous day 7 and the sclera day 7, 32, and 45 timepoints represent a single eye, as opposed to an average level. The remaining day 7, 32, and 45 timepoints represent the average of two eyes of one rabbit, and the day 90 timepoint represents the average of two eyes of each of two rabbits (four eyes total).
[0211] The full vitreous was homogenized and analyzed. The average concentration of the vitreous was calculated by dividing the mass of rapamycin measured by the volume of vitreous analyzed. The sample did not include the site of administration; thus, this measurement indicated the level of rapamycin delivered to the vitreous via the in situ gelling formulation.
[0212] The average level of rapamycin in the vitreous at 7, 32, 45, and 90 days after subconjunctival injection was about 13.9, about 7.4, about 1.35, and about 9.9 ng/ml, respectively.
[0213] The full retina choroid tissues were homogenized and analyzed. The average concentration of the retina choroid tissues was calculated by dividing the mass of rapamycin measured by the mass of retina choroid tissues analyzed. The sample did not include the site of administration; thus, this measurement indicated the level of rapamycin delivered to the retina choroid tissues via the in situ gelling formulation.
[0214] The average level of rapamycin in the retina choroid tissues at 7, 32, 45, and 90 days after subconjunctival injection was about 0.376, about 0.1875, about 0.136, and about 0.29 ng/mg, respectively.
[0215] The sclera was analyzed in the same way as the retina choroid tissues. The scleral sample may have included the injected liquid formulation; thus, this measurement was indicative of clearance of rapamycin from the sclera.
[0216] The average level of rapamycin in the sclera at 7, 32, 45, and 90 days after subconjunctival injection was about 2033, about 1653, about 3626, and about 420.5 ng/mg, respectively.

Example 10 - Preparation and characterization of a Rapamycin-Containing suspension.

[0217] A rapamycin containing suspension was formed by dispersing 150.5 mg of rapamycin (3.004 % by weight) in 4860.3 mg of PEG 400 (96.996% by weight). This formulation is listed as #49 in Table 1. 150.5 mg rapamycin (3.004 % by weight) and 4860.3 mg of PEG 400 (96.996% by weight) were placed in an amber vial. High Wear Resistant Zirconia Grinding Media (beads) of 3 mm diameter were added, up to three quarters of the total volume. The vial was sealed and placed in a Cole-Parmer milling apparatus for 48 hrs. The particle size median for rapamycin was 2.8386 mm and the mean was 3.1275 mm. The formulation was kept at 4C until use. Volumes of 20 μl and 40 μl each formed a non-dispersed mass when placed in the vitreous of a rabbit eye.

Example 11- Subconjunctival Injection of a Rapamycin-Containing Suspension.

[0218] 40 μl of the suspension described in Example 10 were injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 7** depicts the level of rapamycin in the vitreous (ng/ml), retina choroid (ng/mg), and the sclera (ng/mg) on a logarithmic scale at 14, 42, 63, and 91 days after injection.

[0219] The vitreous was homogenized and analyzed as described in Example 2. Two eyes from each of two rabbits were analyzed at each time point except for day 91, on which two eyes from one rabbit were analyzed. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 14, 42, 63, and 91 days after subconjunctival injection was about 4.031, 23.11, 53.27, and 13.94 ng/ml, respectively.

[0220] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 14, 42, 63, and 91 days after subconjunctival injection was about 0.1577, 4.965, 0.385, and 0.05 ng/mg, respectively.

[0221] The scleral sample was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The scleral sample included the site of injection. The average level of rapamycin in the sclera at 14, 42, 63, and 91 days after subconjunctival injection was about 1283, 476.3, 854.2, and 168.5 ng/mg, respectively.

Example 12 - Intravitreal Injection of a Rapamycin-Containing Suspension

[0222] 20 μl of the suspension described in Example 10 were injected into the vitreous of the eye of New Zealand white rabbits. The injected suspension formed a non-dispersed mass relative to the surrounding medium. **Fig. 8** depicts the level of rapamycin in the retina choroid (ng/mg) and the sclera (ng/mg) on a logarithmic scale at 14, 42, 63, and 91 days after injection and in the vitreous (ng/ml) at 63 and 91 days after injection.

[0223] The vitreous was homogenized and analyzed as described in Example 2. Two eyes from each of two rabbits were analyzed at each time point. The vitreous sample may have included the site of administration. The average level of rapamycin in the vitreous at 63 and 91 days after intravitreal injection was about 381,600 and 150,400 ng/ml, respectively.

[0224] The retina choroid was homogenized and analyzed as described in Example 2. Two eyes from each of two rabbits were analyzed at each time point. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 14, 42, 63, and 91 days after intravitreal injection was about 2.588, 4.249, 21.42, and 0.922 ng/mg, respectively.

[0225] The scleral sample was homogenized and analyzed as described in Example 2, with the samples taken as described for the retina choroid above. The scleral sample did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the sclera. The average level of rapamycin in the sclera at 14, 42, 63, and 91 days after intravitreal injection was about 0.7327, 6.053, 1.373, and 17.49 ng/mg, respectively.

Example 13 - Preparation and Characterization of a Rapamycin-Containing Solution.

[0226] A rapamycin containing solution was formed by placing 116.6 mg of rapamycin in ethanol and storing the mixture at 4°C for 6 hours. This solution was then mixed with 4647.5 mg of PEG 400 to give a solution having final concentrations by weight of 2.29% rapamycin, 6.05% ethanol, and 91.66% PEG 400. This solution is listed as formulation #51 in Table 1. A volume of 30 μl formed a non-dispersed mass when placed in the vitreous of rabbit eyes.

Example 14 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0227] 40 μl of the solution described in Example 13 were injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 9** depicts the level of rapamycin in the vitreous (ng/ml), retina choroid (ng/mg), and the sclera (ng/mg) on a linear scale at 14, 42, 63, and 91 days after injection.

[0228] The vitreous was homogenized and analyzed as described in Example 2. Two eyes from each of two rabbits were analyzed at each time point except for day 91, on which two eyes from one rabbit were analyzed. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 14, 42, 63, and 91 days after subconjunctival injection was about 1.804, 1.854, 1.785, and 1.255 ng/ml, respectively.

[0229] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 14, 42, 63, and 91 days after subconjunctival injection was about 1.221, 4.697, 0.1075, and 0.02 ng/mg, respectively.

[0230] The scleral sample was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The scleral sample included the site of injection. The average level of rapamycin in the sclera at 14, 42, 63, and 91 days after subconjunctival injection was about 1.987, 1.884, 0.56, and 10.84 ng/mg, respectively.

Example 15 - Intravitreal Injection of a Rapamycin-Containing Solution

[0231] 30 μl of the solution described in Example 13 were injected into the vitreous of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. **Fig. 10** depicts the level of rapamycin in the retina choroid (ng/mg) and the sclera (ng/mg) on a linear scale at 14, 42, 63, and 91 days after injection.

[0232] The retina choroid was homogenized and analyzed as described in Example 2. Two eyes from each of two rabbits were analyzed at each time point. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 14, 42, 63, and 91 days after intravitreal injection was about 5.515, 5.388, 0.3833, and 11.52 ng/mg, respectively.

[0233] The scleral sample was homogenized and analyzed as described in Example 2, with the samples taken as described for the retina choroid above. The scleral sample did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the sclera. The average level of rapamycin in the sclera at 14, 42, 63, and 91 days after intravitreal injection was about 1.077, 0.9239, 0.0975, and 2.0825 ng/mg, respectively.

[0234] **Fig. 11** depicts the level of rapamycin in the vitreous (ng/ml) on a linear scale at 63 and 91 days after injection. The vitreous was homogenized and analyzed as described in Example 2. Two eyes from each of two rabbits were analyzed at each time point. The vitreous sample may have included the site of administration. The average level of rapamycin in the vitreous at 63 and 91 days after intravitreal injection was about 299,900 and 196,600 ng/ml, respectively.

Example 16 - Reparation and Characterization of a Rapamycin-Containing Solution.

[0235] About 320 g of ethanol was sparged with N_2 for about 10 minutes, and then about 40 g of sirolimus was added to the ethanol. The mixture was sonicated for about 20 minutes, by the end of which all of the sirolimus had gone into solution to form a sirolimus stock solution. A diluent solvent was prepared by sonicating about 1880 g of PEG 400 for about 60 minutes, and then sparging the solvent with Nitrogen for about 10 minutes.

[0236] The sirolimus stock solution and the PEG 400 were then rotated at about room temperature in a rotary evaporator for about 10 minutes to mix the stock solution with the diluent solvent. After mixing, the solution was sparged with nitrogen for about 10 minutes and blanketed with nitrogen for about 5 minutes. After the solution was sparged and filled with nitrogen, about 240 g of excess ethanol was evaporated from the solution by increasing the solution temperature, maintaining a temperature that did not exceed 40°C for an extended period of time and continuing to rotate the solution for about 2.5 hours.

[0237] The resulting solution comprised about 40 g of sirolimus (about 2% by weight), about 80 g of ethanol (about 4% by weight), and about 1880 g of PEG 400 (about 94% by weight). This solution was sparged with nitrogen for about 10 minutes and blanketed with nitrogen for about 5 minutes. The solution was then filtered through a 0.2 micron filter. HPLC vials were filled with 2 ml each of the filtered solution to leave a head space in each container of about 400 μl. This head space was filled with nitrogen gas and capped.

Example 17 - Preparation and Characterization of a Rapamycin-Containing Solution.

[0238] Rapamycin, ethanol and PEG 400 were placed in a container to give final concentrations by weight of about 2.00% rapamycin, about 4.00% ethanol, and about 94.00% PEG 400. The mixture was capped and sonicated for 1-2 hours. The sonication generated heat, with temperatures of up to about 40 or 50°C. This solution is listed as formulation #100 in Table 1. Volumes of 1 μl, 3 μl, 20 μl, and 40 μl formed a non-dispersed mass in the vitreous of rabbit eyes.

Example 18 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0239] 20 μl of the solution described in Example 17 were injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 12** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5, 30, 60, 90, and 120 days after injection. **Fig. 13** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points. For comparison, **Fig. 12** and **Fig. 13** also depict results of similar studies, performed with 40 μl and 60 μl injections, described below in Example 19 and Example 20.

[0240] In **Figs. 12-15,** discussed in this and following examples, some outlier points have been omitted. Individual data points from the same study at the same time point were compared to each other. When the arithmetic mean of the data points was lower than their standard deviation, the data points that were higher or lower by an order of magnitude were considered as outliers.

[0241] The vitreous was homogenized and analyzed as described in Example 2. Between two and five rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5, 30, 60, 90, and 120 days after subconjunctival injection was about 1.81, 0.45, 0.39, 1.85, and 1.49 ng/ml, respectively.

[0242] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 30, 60, 90, and 120 days after subconjunctival injection was about 0.14, 0.03, 0.02, 0.02, and 0.01 ng/mg, respectively.

Example 19 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0243] 40 μl of the solution described in Example 17 were injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 12** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5, 30, 60, 90, and 120 days after injection. **Fig. 13** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0244] The vitreous was homogenized and analyzed as described in Example 2. Between two and five rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5, 30, 60, 90, and 120 days after subconjunctival injection was about 2.39, 0.65, 0.54, 2.07, and 1.92 ng/ml, respectively.

[0245] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 30, 60, 90, and 120 days after subconjunctival injection was about 0.47, 0.04, 0.01, 0.05, and 0.0 ng/mg, respectively.

Example 20 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0246] 60 μl of the solution described in Example 17 were injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 12** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5, 30, 60, 90, and 120 days after injection. **Fig. 13** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0247] The vitreous was homogenized and analyzed as described in Example 2. Between two and five rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5, 30, 60, 90, and 120 days after subconjunctival injection was about 8.65, 0.29, 0.18, 2.00, 1.41 ng/ml, respectively.

[0248] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 30, 60, 90, and 120 days after subconjunctival injection was about 0.63, 0.02, 0.02, 0.06, and 0.01 ng/mg, respectively.

Example 21- Intravitreal Injection of a Rapamycih-Containing Solution

[0249] 20 μl of the solution described in Example 17 were injected into the vitreous of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. **Fig. 14** depicts the level of rapamycin in the vitreous on a logarithmic scale 5, 30, 60, 90, and 120 days after injection. **Fig. 15** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points. For comparison, **Fig. 14** and **Fig. 15** also depict results of other studies described below in Example 22 and Example 24.

[0250] The vitreous was homogenized and analyzed as described in Example 2. Between two and five rabbit eyes were analyzed at each time point. The vitreous sample may have included the site of administration. The average level of rapamycin in the vitreous at 5, 30, 60, 90, and 120 days after intravitreal injection was about 162,100; 18,780; 57,830; 94,040; and 13,150 ng/ml, respectively.

[0251] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 30, 60, 90, and 120 days after intravitreal injection was about 2.84, 2.26, 0.17, 0.22, and 0.05 ng/mg, respectively.

Example 22 - Intravitreal Injection of a Rapamycin-Containing Solution

[0252] 40 μl of the solution described in Example 17 were injected into the vitreous of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. **Fig. 14** depicts the level of rapamycin in the vitreous on a logarithmic scale 5, 30, 60, 90, and 120 days after injection. **Fig. 15** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0253] The vitreous was homogenized and analyzed as described in Example 2. Between two and five rabbit eyes were analyzed at each time point. The vitreous sample may have included the site of administration. The average level of rapamycin in the vitreous at 5, 30, 60, 90, and 120 days after intravitreal injection was about 415,600; 4,830; 74,510; 301,300; and 7,854 ng/ml respectively.

[0254] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 30, 60, 90, and 120 days after intravitreal injection was about 5.36, 0.23, 1.27, 1.08, and 0.08 ng/mg, respectively.

Example 23 - Preparation and Characterization of a Rapamycin-Containing Solution.

[0255] Rapamycin, ethanol and PEG 400 were added to a container to give final concentrations by weight of about 0.4% rapamycin, 4.0% ethanol, and 95.6% PEG 400. The mixture was sonicated for 1-2 hours. Sonication resulted in elevated temperatures of up to about 40 to 50°C. This solution is listed as formulation #99 in Table 1.

Example 24 - Intravitreal Injection of a Rapamycin-Containing Solution

[0256] 100 μl of the solution described in Example 23 were injected into the vitreous of the eye of New Zealand white rabbits. The injected solution did not form a non-dispersed mass relative to the surrounding medium. **Fig. 14** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5, 30, 60, and 90 days after injection. **Fig. 15** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0257] The vitreous was homogenized and analyzed as described in Example 2. Between two and five rabbit eyes were analyzed at each time point. The vitreous sample may have included the site of administration. The average level of rapamycin in the vitreous at 5, 30, 60, and 90 days after intravitreal injection was about 151,000; 14,890; 4,743; and 1620 ng/ml respectively.

[0258] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 30, 60, and 90 days after intravitreal injection was about 1.21, 1.84, 0.04, and 0.71 ng/mg, respectively.

Example 25 - Preparation and Characterization of a Rapamycin-Containing Solution.

[0259] A rapamycin containing solution was formed by placing 102.4 mg of rapamycin in ethanol, adding 4719.3 mg of PEG 400, and vortexing. The resulting solution had final concentrations by weight of 2.036% rapamycin, 4.154%% ethanol, and 93.81% PEG 400. This solution is listed as formulation #139 in Table 1.

Example 26- Subconjunctival Injection of a Rapamycin-Containing Solution

[0260] 10 μl of the solution described in Example 25 were injected as a single dose between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 16** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5 and 14 days after injection. **Fig. 17** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points. For comparison, **Fig. 16** and **Fig. 17** also depict results of other studies described below in Examples 27-29.

[0261] The vitreous was homogenized and analyzed as described in Example 2. Four rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5 and 14 days after subconjunctival injection was about 2.45 and 20.13 ng/ml, respectively.

[0262] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5 and 14 days after subconjunctival injection was about 0.13 and 0.19 ng/mg, respectively.

Example 27 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0263] 60 μl of the solution described in Example 25 were injected as a single dose between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 16** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5 and 14 days after injection. **Fig. 17** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0264] The vitreous was homogenized and analyzed as described in Example 2. Four rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5 and 14 days after subconjunctival injection was about 17.98 and 87.03 ng/ml, respectively.

[0265] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5 and 14 days after subconjunctival injection was about 0.27 and 0.21 ng/mg, respectively.

Example 28 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0266] 60 μl of the solution described in Example 25 were injected as two 30 μl doses at two sites between the sclera and the conjunctiva of the eye of New Zealand white rabbits.

[0267] **Fig. 16** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5 and 14 days after injection. **Fig. 17** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0268] The vitreous was homogenized and analyzed as described in Example 2. Four rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5 and 14 days after subconjunctival injection was about 502.2 and 31.80 ng/ml, respectively.

[0269] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5 and 14 days after subconjunctival injection was about 0.80 and 0.15 ng/mg, respectively.

Example 29 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0270] 90 μl of the solution described in Example 25 were injected as three 30 μl doses at three sites between the sclera and the conjunctiva of the eye of New Zealand white rabbits.

[0271] **Fig. 16** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5 and 14 days after injection. **Fig. 17** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0272] The vitreous was homogenized and analyzed as described in Example 2. Four rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5 and 14 days after subconjunctival injection was about 39.05 and 13.63 ng/ml, respectively.

[0273] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5 and 14 days after subconjunctival injection was about 0.83 and 0.10 ng/mg, respectively.

Example 30 - Preparation and characterization of a Rapamycin-Containing suspension.

[0274] A rapamycin containing suspension was formed by placing 201.6 mg of rapamycin (3.000 % by weight) in 6518.8 mg of PEG 400 (97.000% by weight) and vortexing. The resulting particle size was not quantified but it was large, estimated at about 10 μm. This suspension is listed as formulation #147 in Table 1.

Example 31- Subconjunctival Injection of a Rapamycin-Containing Suspension

[0275] 10 μl of the suspension described in Example 30 were injected as a single dose between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 18** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5, 14, and 30 days after injection. **Fig. 19** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points. For comparison, **Fig. 18** and **Fig. 19** also depict results of other studies described below in Example 32 and Example 33.

[0276] The vitreous was homogenized and analyzed as described in Example 2. Four rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5, 14, and 30 days after subconjunctival injection was about 2.68, 0.90, and 5.43 ng/ml, respectively.

[0277] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 14, and 30 days after subconjunctival injection was about 0.20, 0.06, and 1.23 ng/mg, respectively.

Example 32 - Subconjunctival Injection of a Rapamycin-Containing Suspension

[0278] 30 μl of the solution described in Example 30 were injected as a single dose between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 18** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5, 14, and 30 days after injection. **Fig. 19** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0279] The vitreous was homogenized and analyzed as described in Example 2. Four rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5, 14, and 30 days after subconjunctival injection was about 84.55, 11.23, and 66.35 ng/ml, respectively.

[0280] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 14, and 30 days after subconjunctival injection was about 1.09, 0.19, and 1.02 ng/mg, respectively.

Example 33 - Subconjunctival Infection of a Rapamycin-Containing Suspension

[0281] 90 μl of the solution described in Example 30 were injected as three 30 μl doses at three sites between the sclera and the conjunctiva of the eye of New Zealand white rabbits.

[0282] **Fig. 18** depicts the level of rapamycin in the vitreous on a logarithmic scale at 5, 14, and 30 days after injection. **Fig. 19** depicts the level of rapamycin in the retina choroid on a logarithmic scale at the same time points.

[0283] The vitreous was homogenized and analyzed as described in Example 2. Four rabbit eyes were analyzed at each time point. The vitreous sample did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the vitreous. The average level of rapamycin in the vitreous at 5, 14, and 30 days after subconjunctival injection was about 29.95, 15.30, and 49.20 ng/ml, respectively.

[0284] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 14, and 30 days after subconjunctival injection was about 0.55, 1.31, and 5.74 ng/mg, respectively.

Example 34 - Preparation and Characterization of a Rapamycin-Containing Solution.

[0285] 10.3 mg of rapamycin was placed in ethanol, 4995.8 mg of PEG 400 was added, and the mixture was vortexed to give a solution having final concentrations by weight of 0.205% rapamycin, 0.544% ethanol, and 99.251% PEG 400. This solution is listed as formulation #140 in Table 1. A volume of 10 μl of this solution formed a non-dispersed mass when placed in the vitreous of a rabbit eye.

Example 35 - Intravitreal Infection of a Rapamycin-Containing Solution

[0286] 10 μl of the solution described in Example 34 were injected into the vitreous of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. **Fig. 20** depicts the level of rapamycin in the retina choroid on a logarithmic scale at 5 and 30 days after injection. **Fig. 21** depicts the level of rapamycin in the vitreous on a logarithmic scale at the same timepoints. For comparison, **Fig. 20** and **Fig. 21** also depict results of other studies described below in Example 37 and Example 39.

[0287] The vitreous was homogenized and analyzed as described in Example 2. Five rabbit eyes were analyzed at each time point. The vitreous sample may have included the site of administration. The average level of rapamycin in the vitreous at 5 and 30 days after intravitreal injection was about 12.02 and 0.92 ng/ml, respectively.

[0288] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5 and 30 days after intravitreal injection was about 0.08 and 0.02 ng/mg, respectively.

Example 36 - Preparation and Characterization of a Rapamycin-Containing Solution.

[0289] 31.5 mg of rapamycin was placed in ethanol, 4918.9 mg of PEG 400 was added, and the solution was vortexed. Final concentrations by weight were 0.628% rapamycin, 1.337% ethanol, and 98.035% PEG 400. This solution is listed as formulation #142 in Table 1. The formulation was stored at 4°C until use. A volume of 10 μl of this solution formed a non-dispersed mass when placed in the vitreous of a rabbit eye.

Example 37 - Intravitreal Injection of a Rapamycin-Containing Solution

[0290] 10 μl of the solution described in Example 36 were injected into the vitreous of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. **Fig. 20** depicts the level of rapamycin in the retina choroid on a logarithmic scale at 5 and 30 days after injection. **Fig. 21** depicts the level of rapamycin in the vitreous on a logarithmic scale at the same timepoints.

[0291] The vitreous was homogenized and analyzed as described in Example 2. Five rabbit eyes were analyzed at each time point. The vitreous sample may have included the site of administration. The average level of rapamycin in the vitreous at 5 and 30 days after intravitreal injection was about 87.46 and 44.34 ng/ml, respectively.

[0292] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5 and 30 days after intravitreal injection was about 1.40 and 0.01 ng/mg, respectively.

Example 38 - Preparation and Characterization of a Rapamycin-Containing Solution.

[0293] 103.5 mg of rapamycin was placed in ethanol, 4720.8 mg of PEG 400 was added, and the mixture was vortexed to give a solution having final concentrations by weight of 2.057% rapamycin, 4.116% ethanol, and 93.827% PEG 400. This solution is listed as formulation #144 in Table 1. A volume of 10 μl of this solution formed a non-dispersed mass in the vitreous of a rabbit eye.

Example 39 - Intravitreal Injection of a Rapamycin-Containing Solution

[0294] 10 μl of the solution described in Example 38 were injected into the vitreous of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. **Fig. 20** depicts the level of rapamycin in the retina choroid on a logarithmic scale at 5, 30, and 90 days after injection. **Fig. 21** depicts the level of rapamycin in the vitreous on a logarithmic scale at the same timepoints.

[0295] The vitreous was homogenized and analyzed as described in Example 2. Four rabbit eyes were analyzed at each time point. The vitreous sample may have included the site of administration. The average level of rapamycin in the vitreous at 5, 30, and 90 days after intravitreal injection was about 120,500; 55,160; and 0.55 ng/ml, respectively.

[0296] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above except that five rabbit eyes were analyzed at the 5 and 30 day time points. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 5, 30, and 90 days after intravitreal injection was about 4.75, 0.17, and 0.01 ng/mg, respectively.

Example 40 - Subconjunctival Infection of a Rapamycin-Containing Solution

[0297] 40 μl of the solution described in Example 17 were injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits. **Fig. 22** depicts on a logarithmic scale the level of rapamycin in the aqueous humor (ng/ml) at 1, 4, 7, 11, 14, 21, 28, 35, 54, and 56 days after injection, and the levels of rapamycin in the cornea (ng/mg) and the retina choroid (ng/mg) at 4, 14, 21, and 35 days after injection. The retina choroid level is labeled as "R/Choroid" in figure 22.

[0298] The aqueous humor was homogenized and then analyzed by liquid chromatography and mass spectroscopy. Four rabbit eyes were analyzed for each time point. The aqueous humor did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the aqueous humor. The average level of rapamycin in the aqueous humor at 1, 4, 7, 11, 14, 21, 28, 35, 54, and 56 days after injection was about 0.875, 1.0, 7.0, 0.725, 0.5, 0.525, 0.0, 0.125, 0.014, and 0.0485 ng/ml, respectively.

[0299] The cornea was homogenized and then analyzed by liquid chromatography and mass spectroscopy. The cornea did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the cornea. Four rabbit eyes were analyzed for each time point. The average level of rapamycin in the cornea at 4, 14, 21, and 35 days after injection was about 0.3225, 0.1, 0.0275, and 0.0125 ng/mg, respectively.

[0300] The retina choroid was homogenized and analyzed as described in Example 2, with the samples taken as described for the vitreous above. The retina choroid did not include the site of administration, so this measurement indicated the level of rapamycin delivered to the retina choroid. The average level of rapamycin in the retina choroid at 4,14, 21, and 35 days after injection was about 11.61, 0.2, 0.0275, and 2.655 ng/mg, respectively.

Example 41 - Intravitreal Injection of a Rapamycin-Containing Solution

[0301] 1.0 μl of the solution described in Example 17 was injected into the vitreous of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. Table 2 reports the average level of rapamycin in the aqueous humor one day after injection. For comparison, Table 2 also reports results of studies described in Examples 42-45 below.

[0302] The aqueous humor was homogenized and analyzed as described in Example 40. Two rabbit eyes were analyzed. The aqueous humor did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the aqueous humor. The average level of rapamycin in the aqueous humor at 1 day after injection was about 0.438 ng/ml with a standard deviation of about 0.141 ng/ml.

Example 42 - Intravitreal Injection of a Rapamycin-Containing Solution

[0303] 3.0 μl of the solution described in Example 17 were injected into the vitreous of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. Table 2 reports the average level of rapamycin in the aqueous humor one day after injection.

[0304] The aqueous humor was homogenized and analyzed as described in Example 40. Two rabbit eyes were analyzed. The aqueous humor did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the aqueous humor. The average level of rapamycin in the aqueous humor at 1 day after injection was about 0.355 ng/ml with a standard deviation of about 0.234 mg/ml.

Example 43 - Subconjunctival Injection of a Rapamycin-Containing Solution

[0305] 3.0 μl of the solution described in Example 17 were injected between the sclera and the conjunctiva of the eye of New Zealand white rabbits. The injected solution formed a non-dispersed mass relative to the surrounding medium. Table 2 reports the average level of rapamycin in the aqueous humor one day after injection.

[0306] The aqueous humor was homogenized and analyzed as described in Example 40. Two rabbit eyes were analyzed. The aqueous humor did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the aqueous humor. The average level of rapamycin in the aqueous humor at 1 day after injection was about 0.338 ng/ml with a standard deviation of about 0.122 ng/ml.

Example 44 - Anterior Chamber administration of a Rapamycin-Containing Solution

[0307] 5.0 μl of the solution described in Example 17 were injected into the anterior chamber of the eye of New Zealand white rabbits by injection into the front-end of the eye. The aqueous humor was withdrawn using a syringe. Table 2 reports the average level of rapamycin in the aqueous humor 14 days after injection.

[0308] The aqueous humor was homogenized and analyzed as described in Example 40. Two rabbit eyes were analyzed. The aqueous humor did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the aqueous humor. The average level of rapamycin in the aqueous humor at 14 days after injection was about 0.166 ng/ml with a standard deviation of about 0.183 ng/ml.

Example 45 - Anterior Chamber Administration of a Rapamycin-Containing Solution

[0309] 10 μl of the solution described in Example 17 were injected into the anterior chamber of the eye of New Zealand white rabbits. Table 2 reports the average level of rapamycin in the aqueous humor 14 days after injection.

[0310] The aqueous humor was homogenized and analyzed as described in Example 40. Two rabbit eyes were analyzed. The aqueous humor did not include the site of injection, so this measurement indicated the level of rapamycin delivered to the aqueous humor. The average level of rapamycin in the aqueous humor at 14 days after injection was about 0.004 ng/ml with a standard deviation of about 0.006 ng/ml.

Table 1 Liquid Formulations

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
1	DMSO = 2000mg (20%) Water = 8000mg (80%)	S		
2	F68 = 1000mg (10%) Water = 9000mg (90%)	S		
3	F68 = 3000mg (30%) Water = 7000mg (70%)	S		
4	F127 = 1000mg (10%) Water = 9000mg (90%)	S		
5	F127 = 1500mg (15%) Water = 8500mg (85%)	S		
6	Beta-cyclodextrin = 250mg (2.5%) Water = 9750mg (97.5%)	S		
7	Rapa = 10.2mg (0.101%) Pluronic, F68 = 1010mg (9.99%) Water = 9090mg (89.909%)	S		No, 50 μL
8	Rapa = 10.2mg (0.102%) Pluronic, F68 = 3000mg (29.969%) Water = 7000mg (69.929%)	S		No, 50 μL
9	Rapa = 10.5mg (0.104%) Pluronic, F127 = 1010mg (9.99%) Water = 9090mg (89.907%)	S		No, 50 μL
10	Rapa = 10.5mg (0.105%) Pluronic, F127 = 1500mg (14.984%) Water = 8925mg (84.9%)	S		No, 50 μL
11	Rapa = 10.7mg (0.105%) Beta-cyclodextrin = 255mg (2.497%) Water = 9945mq (97.398%)	S		No, 50 μL
12	Rapa = 6.4mg (0.0999%) CMC = 48mg (0.7493%) Polysorbitan 20 = 2.56mg (0.04%) Water = 6349.44mg (99.111%)	SP		
13	Rapa = 6.5mg (0.0999%) DMSO = 325mg (4.995%) Water = 6175mg (94.905%)	S		
14	Rapa = 13.5mg (0.0999%) CMC = 101.25mg (0.7493%)	SP		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	Polysorbitan 20 = 5.4mg (0.04%) Water = 13393.35mg (99.112%)			
15	Rapa = 11.Omg (0.2%) EtOH = 5500mg (99.8%)	S		
16	Rapa = 6.6mg (0.1 %) EtOH = 1054.6mg (15.933%) F127 = 833.64mg (12.595%) Water = 4723.96mg (71.372%)	S		
17	Rapa = 5 mg (0.1 %) Cavitron = 0.25 g (5%) Ethanol, 95% = 57 mg (1.1%) Sterile water = 4.753 g (93.8%)	S		
18	Rapa = 5 mg (0.1 %) Ethanol, 95% =150 mg (2.9%) PEG400 = 1.0 g (19.4%) Sterile water = 4.01 q (77.6%)	S		
19	Rapa = 5 mg (0.1 %) Ethanol, 95% =152 mg (3.2%) PEG400 = 1.5227 g (30.2%) Sterile water = 3.3592 g (66.67%)	S		Yes, 50 μL
20	Rapa = 6.6mg (0.1 %) EtOH = 505.1 mg (7.618%) F127 = 917.8mg (13.843%) Water = 5200.6mg (78.44%)	S		
21	Rapa = 6.6mg (0.1%) EtOH = 536mg (7.5%) Pluronic, F127 = 983.75mg (14.0%) Water = 5574.56mg (78.4%)	S		No, 50 μL
22	Rapa = 5.2mg (0.1023%) EtOH = 56.6mg (1.127%) Captisol = 2008.9mg (39.5%) Water = 3013.3mg (59.3%)	S		
23	Rapa = 6.9mg (0.201%) EtOH = 3418.0mag (99.799%)	S		
24	Rapa = 9.1mg (0.491%) EtOH = 90.9mg (4.908%) F127 = 262.8mg (14.191%) Water = 1489.1mg (80.409%)	S		
25	Rapa = 0mg (0%) EtOH = 310.2mg (5.144%) F127 = 858.1mg (14.228%) Water = 4862.6mg (80.628%)	S		
26	Rapa = 0mg (0%) EtOH = 613.1mg (10.19%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	F127 = 810.6mg (13.471%) Water = 4593.6mg (76.339%)			
27	Rapa = 53.5mg (1.095%) EtOH = 414.8mg (8.488%) F127 = 662:8mg (13.563%) Water = 3755.7mg (76.854%)	S		Yes, 50 μL
28	Rapa = 0.3 g (10%) PVP K90 = 0.35 g (12%) Eudragit RS30D = 2.35 g (78%)	ISG, SP		
29	Rapa = 0.2154 g (7.31 %) PVP K90 = 0.25 g (8.5%) Eudraqit RS30D = 2.48 q (84.19%)	ISG, SP		
30	Rapa = 53.9mg (1.103%) EtOH = 413.6mg (8.463%) Sterile water = 3843.5mg (78.647%) F127 (Lutrol) = 576.0mg (11.786%)	S		No, 50 μL
31	Rapa = 0mg (0%) EtOH = 411.9mg (8.513%) Sterile Water = 3849.3mg (79.554%) F127(Lutrol) = 577.4mg (11.933%)	S		
32	Rapa = 54.1 mg (1.256%) EtOH = 416.8mg (9.676%) Sterile Water = 3836.3mg (78.569%) F127(Lutrol) = 577.5mq (10.499%)	S		
33	Rapa = 80.7g (1.964%) EtOH = 65.0mg (0.158%) PEG400 = 4021.8mag (97.878%)	S		
34	Rapa 106.9g (5.233%) EtOH = 129.6mg (6.344%) PEG400 = 1806.5mg (88.424%)	S		Yes, 25 μL
35	Rapa = 0 mg (0%) PVP K90 = 0.204 g (2.3%) Ethanol, 100% = 0.4 g (4.5%) Eudragit RL100 =0.201g (2.3%) PEG 400 = 8.00 q (90.9%)	ISG, SP		
36	Rapa = 0 mg (0%) PVP K90 = 0.2 g (2.2%) Ethanol, 100% = 0.4 g (4.4%) PVAP = 0.4 g (4.4%) PEG 400 = 8.00 q (88.9%)	ISG, SP		
37	Rapa = 106.1 mg(4.2%) PVP K90 = 55.2 mg (2.2%) Ethanol, 100% = 108 mg (4.3%) Eudragit RL100 = 55 mg (2.2%)	ISG, SP		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	PEG 400 = 2.2 g (87.1%)			
38	Rapa = 399.6mg (9.965%) F68(Lutrol) = 40.6mg (1.012%) Sterile Water = 3569.7mg (89.022%)	S		Yes, 20 μL
39	Rapa = 53.8mg (1.1 %) EtOH = 415.2mg (8.489%) Sterile Water = 3844.2mg (78.594%) F127 = 578.0mg (11.817%)	S		
40	Rapa = 208.1mg (3.148%) PEG400 = 6403.4mq (96.852%)	S		Yes, 20 μL
41	Rapa = 200.4mg (5.148%) F68(Lutrol) = 20.8mg (0.534%) PEG400 = 3569.3mg (91.697%) EtOH (95%) = 102mg (2.62%)	SP		
42	Rapa = 200.4g (5.259%) PEG400 = 3561.4mg (93.46%) Tween 80 = 48.8mg(1.281%)	SP		
43	Rapa = 30.9 mg (1.03%) PEG 400 = 2.9624 g (98.97%)	S		No, 50 μL
44	Rapa = 61 mg (1.96%) Ethanol, 100% = 0.1860 g (6%) PEG 400 = 2.8588 g (92.04%)	S		Yes, 50 μL
45	Rapa = 90.7 mg (3.02%) Ethanol, 100% = 0.2722 g (9.06%) PEG 400 = 2.6423 g (87.94%)	S		Yes, 50 μL
46	Rapa = 101.6mg (4.997%) EtOH = 331.6mg (16.308%) PEG400 = 1600.1mq (78.695%)	S		
47	Rapa = 120.9g (3.189%) F68(Lutrol) = 42.4mg (1.118%) Sterile Water = 3627.7mg (95.692%)	SP		
48	Rapa = 100.1g (1.999%) EtOH = 305.1mg (6.092%) PEG400 = 4602.9mg (91.909%)	S		
49	Rapa = 150.5mg (3.004%) PEG400 = 4860.3mg (96.996%)	SP		Yes, 20 μL, 40 μL
50	Rapa = 153.4mg (3.055%) F68(Pluronic)= 50.6mg (1.008%) Sterile Water = 4816.6mg (95.937%)	SP		No, 20 μL
51	Rapa = 116.6mg (2.29%) EtOH = 306.6mg (6.05%) PEG400 = 4647.5mq (91.66%)	S		Yes, 30 μL
52	Rapa = 150.4 mg (2.994%)	SP		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	F68 Lutrol = 15.4 mg (0.306%) Sterile water = 4859.1 mg (96.7%)			
53	Rapa = 306.5 mg (6.088%) PEG 400 = 4727.7 mg (93.912%)	SP		
54	Rapa = 309.3 mg (6.146%) PEG 400 = 4723.3 mg (93.854%)	SP		
55	Rapa = 303.3 mg (6.061 %) PEG 400 = 4700.6 mg (93.939%)	SP		
56	Rapa = 305.4 mg (6.088%) PEG 400 = 4711.0 mg (93.912%)	SP		
57	Rapa = 306.9 mg (6.098%) PEG 400 = 4725.5 mq (93.902%)	SP		
58	Rapa = 302.5 mg (6.021%) PEG 400 = 4721.6 mg (93.979%)	SP		
59	Rapa = 304.5 mg (6.053%) PEG 400 = 4726.4 mg (93.947%)	SP		
60	Dexamethasone = 251.4 mg (5.011%) PEG 400 = 4765.2 mg (94.989%)	SP		
61	Dexamethasone = 252.4 mg (5%) PEG 400 = 4600 mg (92%) EtOH = 150 mg (3%)	SP		
62	Rapa = 32.2 mg (0.641%) PEG 400 = 4677.9 mg (93.096%) EtOH = 314.7 mg (6.263%)	S		
63	Rapa = 32.3 mg (6.6%) PEG 400 = 5516.3 mg (93.1 %) EtOH = 314.7 mg (6.263%)	S		
64	Rapa = 54.4 mg (1.007%) PEG 400 = 4638.9 mg (92.702%) EtOH = 314.8 mg (6.291%)	S		
65	Rapa = 50.8 mg (1.013%) PEG 400 = 4963.2 mg (98.987%)	S		
66	Rapa = 52.1 mg (1.035%) PEG 400 = 4868.6 mg (96.718%) EtOH = 113.1 mg (2.247%)	S		
67	Rapa = 50.5 mg PEG 400 = 4752.8 mg (94.953%) EtOH = 202.1 mg (4.038%)	S		Yes, 20 µL No, 40 µL, 100 µL
68	Rapa = 101.8 mg (2.030%) PEG 400 = 4712.4 mg (93.970%) EtOH = 200.6 mq (4.000%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
69	Rapa = 102.1 mg (2.036%) PEG 400 = 4605.5mg (91.847%) EtOH = 306.7 mg (6.117%)	S		
70	Rapa = 101.6 mg (2.025%) PEG 400 = 4510.6 mg (89.892%) EtOH = 405.6 mg (8.083%)	S		
71	Rapa = 75.9 mg (3.019%) PEG 400 = 2438.4 mg (96.981 %)	SP		
72	Rapa = 50.9 mg (2.034%) PEG 400 = 2350.1 mg (93.914%) EtOH = 101.4mq (4.052%)	S		
73	Rapa = 12.5 mg (0.620%) PEG 400 = 2004.8 mg (99.380%)	SP		
74	Rapa = 1.20949 g (2.0152%) EtOH = 2.401 g (4.000%) PEG 400 = 56.407 q (93.9848%)	s		
75	Rapa = 16.0 mg g (0.795%) EtOH = 80.0 mg (3.976%) PEG 400 = 1916.0 mq (95.2298%)	S		No, 50 µL
76	Rapa = 8.1 mg (0.400%) PEG 400 = 2014.5 mg (99.600%)	SP		
77	Rapa = 8.6 mg (0.428%) PEG 400 = 2002.5 mg (99.572%)	S		
78	Rapa = 8.2 mg (0.410%) PEG 400 = 1992.0 mg (99.590%)	S		
79	Rapa = 8.7 mg (0.433%) PEG 400 = 1998.8 mg (99.567%)	S		
80	Rapa = 8.6 mg (0.427%) PEG 400 = 2003.2 mg (99.573%)	S		
81	Rapa = 8.6 mg (0.428%) PEG 400 = 1999.3 mg (99.572%)	S		
82	Rapa = 9.0 mg (0.448%) PEG 400 = 2000.8 mg (99.552%)	S		
83	Rapa = 8.0 mg (0.397%) PEG 400 = 2008.8 mg (99.603%)	S		
84	Rapa = 8.5 mg (0.422%) PEG 400 = 2006.8 mg (99.578%)	S		
85	Rapa = 8.0 mg (0.399%) PEG 400 = 1998.2 mg (99.601%)	S		
86	Rapa = 8.5 mg (0.422%) PEG 400 = 2004.3 mg (99.578%)	S		
87	Rapa = 8.6 mg (0.428%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	PEG 400 = 2002.5 mg (99.572%)			
88	Rapa = 0.7 g (1.983%) EtOH = 1.4 g (3.966%) PEG 400 = 33.2 g (94.051%)	S		
89	Rapa = 0 g (0%) EtOH = 0.574 g (1.995%) PEG 400 = 28.2 g (98.005%)	S		
90	Rapa = 1.95 g (1.950%) EtOH = 4.05 g (4.050%) PEG 400 = 94.00 g (94000.%)	S		
91	Rapa = 0.0107 g (0.534%) EtOH = 0.0805 g (4.019%) PEG 400 = 1.912 g (95.447%)	S		No, 80 µL
92	Rapa = 0.0081 g (0.403%) EtOH = 0.0804 g (4.003%) PEG 400 = 1.920 g (95.594%)	S		No, 100 µL
93	Rapa = 1.992 g (2%) EtOH = 3.9419 (4%) PEG 400 = 93.95 q (94%)	S		
94	Rapa = 0.405 g (0.4%) EtOH = 4.24 g (4%) PEG 400 = 95.6 (95.6%)	S		
95	PEG 400 = 96 g (96%) EtOH = 3.9027 (4%)	S		
96	Rapa = 0.4020 g (0.402%) EtOH = 3.970 g (3.971%) PEG 400 = 95.600 g (95.627%)	S		
97	Rapa = 2.000 g (1.990%) EtOH = 4.000 g (3.980%) PEG 400 = 94.500 g (94.030%)	S		
98	PEG 400 = 96 g (96%) EtOH = 3.92 g (4%)	S		
99	Rapa = 0.4036 g (0.4%) EtOH = 3.9054 g (4%) PEG 400 = 95.6 (95.6%)	S		No, 100 µL
100	Rapa = 2.0025 g (2%) EtOH = 3.98 g (4%) PEG 400 = 94.00 q (94%)	S		Yes, 1 µL, 3 µL, 20 µL, 40 µL
101	Rapa = 9.5 mg (0.472%) EtOH = 90.3 mg (4.485%) PEG 600 = 1913.5 mg (95.043%)	S		
102	Rapa = 44.6 mg (2.21%) EtOH = 86.1.0 mg (4.26%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	PEG 600 = 1891.1 mg (93.53%)			
103	Rapa = 1.97 g (2%) EtOH = 4.10 g (4%) PEG 400 = 94.15 g (94%)	S		
104	Rapa = 1.95 g (2%) EtOH = 4.00 g (4%) PEG 400 = 94.0 g (94%)	S		
105	Rapa = 8.00 g (2%) PEG 400 = 376.0 g EtOH = 16.0 q (4%)	S		
106	Rapa = 6.00 g (2%) PEG 400 = 282.0 g (94%) EtOH = 12.0 g (4%)	S		
107	Rapa = 8.9 mg (0.4434%) EtOH = 80.3 mg (4.0006%) PEG 300 = 1918.0 mq (95.556%)	S		
108	Rapa = 40.8 mg (2.00886%) EtOH = 110.0mg (5.41605%) PEG 300 = 1880.2 mg (92.57509%)	S		
109	Rapa = 9.9 mg (0.488%) EtOH = 86.7mg (4.277%) PEG 400/300(50/50) = 1930.3 mg (95.235%)	S		
110	Dexamethasone = 142.5 mg (4.994%) PEG 400 = 2710.7 mg (95.006%)	SP	0.3305 μm	Yes, 30 μL
111	Dexamethasone = 134.3 mg (4.891%) PEG 400 = 2611.4 mg (95.109%)	SP	>10 μm	
112	Triamcinolone = 139.2 mg (5.087%) PEG 400 = 2597.4 mg (94.913%)	SP	3.98 μm	Yes, 30 μL
113	Triamcinolone = 135.3 mg (5.089%) PEG 400 = 2523.5 mg (94.911%)	SP	>10 μm	
114	EtOH = 206.4 mg (4.121%) PEG 400 = 4801.6 mg (95.879%)	S		No, 30 μL
115	Rapa = 43.0 mg (2.144%) PEG 400 = 1962.3mg (97.8567%)	SP	61.4390 μm	
116	Rapa = 40.0 mg (2.001 %) PEG 400 = 1959.1mg (97.999%)	SP	3.7128 μm	
117	Rapa = 42.9 mg (2.142%) PEG 400 = 1959.7mg (97.858%)	SP	2.7313 μm	

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
118	Rapa = 100.8 mg (2.013%) PEG 400 = 4906.0 mg (97.987%)	SP	4.1063 μm	
119	Rapa = 20.9 mg (0.42%) EtOH = 209.1 mg (4.17%) PEG 400 = 4784.9 mq (95.41%)	S		
120	Rapa = 20.6 mg (0.41 %) EtOH = 211.5mg (4.22%) Benz.Chl=19.1 mg (0.38%) PEG 400 = 4762.0 mg (94.99%)	S		
121	Rapa = 20.1 mg (0.40%) EtOH = 211.5mg (4.22%) Benz. Chl = 2.3 mg (0.05%) PEG 400 = 4782.3 mg (95.34%)	S		
122	Rapa = 8.0 g (2%) EtOH = 16.0g (4%) PEG 400 = 376.0 g (94%)	S		
123	Rapa = 351.3 mg (2.006%) EtOH = 2353.1mg (4.093%) PEG 400 = 16448.2 mg (93.901%)	S		
124	Rapa = 2.2035 g (2%) EtOH = 4.45 g (4%) PEG 400 = 103.7 g (94%(	S		
125	Rapa = 515.5 mg (2.021%) PEG 400 = 24,993.8 mg (97.979%)	SP	18.1453 μm	
126	Rapa = 0.3 g (2%) EtOH = 0.6 g (4%) PEG 400 = 14.1 g (94%) BHT = 0.0002 (0.002%)	S		
127	Rapa = 0.3 g (2%) EtOH = 0.6 g (4%) PEG 400 = 14.1 g (94%) BHT = 0.00037 (0.004%)	S		
128	Rapa = 0.3 g (2%) EtOH = 0.6 g (4%) PEG 400 = 14.1 g (94%) BHT = 0.0081 (0.05%)	S		
129	Rapa = 243.2 mg (1.869%) EtOH = 4.88.4 mg (3.753%) PEG 400 = 12283.3 mg (94.378%)	S		
130	Rapa = 0.404 g (2%) EtOH = 0.8 g (4%) PEG 400 = 18.8 g (94%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	BHT = 0.00051 (0.002%)			
131	Rapa = 0.6024 g (2%) EtOH = 1.2 g (4%) PEG 400 = 28.25 g (94%)	S		
132	Rapa = 2.001 g (2%) EtOH = 4.05 g (4%) PEG 400 = 94.45 g (94%)	S		
133	Rapa = 0.5155 g (2.057%) EtOH = 1.0198 g (4.070%) PEG 400 = 23.5225 g (93.873%)	S		
134	PEG 400 = 9.6 g (96%) EtOH = 0.4 q (4%)	S		
135	Rapa = 0.610 g (2%) EtOH = 1.2 g (4%) PEG 400 = 28.2 g (94%)	S		
136	Rapa = 24.6 mg (1.193%) EtOH = 91.1 mg (4.418%) Tyloxapol = 219.6 mg (10.649%) BSS = 1726.8 mg (83.740%)	S		
137	Rapa = 100.0 mg (1.993%) PEG 400 = 4916.9 mg (98.007%)	SP		
138	Rapa = 201.6 mg (4.005%) PEG 400 = 4831.5 mg (95.995%)	SP		
139	Rapa = 102.4 mg (2.036%) EtOH = 209.0 mg (4.154%) PEG 400 = 4719.3 mq (93.810%)	S		
140	Rapa = 10.3 mg (0.205%) EtOH = 27.4 mg (0.544%) PEG 400 = 4995.8 mg (99.251%)	S		Yes, 10 μL
141	Rapa = 10.6 mg (0.211%) EtOH = 208.4 mg (4.150%) PEG 400 = 4802.3 mg (95.639%)	S		No, 10 μL
142	Rapa = 31.5 mg (0.628%) EtOH = 67.1 mg (1.337%) PEG 400 = 4918.9mg (98.035%)	S		Yes, 10 μL
143	Rapa = 30.8 mg (0.613%) EtOH = 204.5 mg (4.073%) PEG 400 = 4786.1 mg (95.314%)	S		No, 10 μL, 100 μL
144	Rapa = 103.5 mg (2.057%) EtOH = 207.1 mg (4.116%) PEG 400 = 4720.8 mg (93.827%)	S		Yes, 10 μL
145	Rapa = 283.0 mg (2.020%) EtOH = 566.1 mg (4.041%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	PEG 400 = 13,160.8 mq (93.939%)			
146	Rapa = 280.1 mg (1.998%) EtOH = 565.2 mg (4.033%) PEG 400 = 13,171.7 mg (93.969%)	S		
147	Rapa = 201.6 mg (3.000%) PEG 400 = 6518.8 mg (97.000%)	SP		
148	Rapa = 31.9 mg (1.019%) Benzyl Alcohol = 1021.9 mg (20.070%) Sesame Oil = 4017.9 mg (78.911 %)	S		
149	Rapa = 51.5 mg (1.03%) Benzyl Alcohol = 259.9 mg (5.19%) Sesame Oil = 4694.3 mg (93.78%)	S		
150	Rapa = 5.96 g (2%) EtOH = 12.0 g (4%) PEG 400 = 282.0 g (94%)	S		
151	Rapa = 54.5 mg (1.07%) Benzyl Alcohol = 1014.3 mg (19.95%) Olive Oil = 4014.8 mg (78.98%)	S		
152	Rapa =0 mg (0.00%) Benzyl Alcohol = 269.4 mg (5.421%) Tyloxapol = 608.2 mg (12.238%) Sesame Oil = 4092.2 mg (82.341%)	S		
153	Rapa = 76.3 mg (1.75%) Benzyl Alcohol = 307.0 mg (7.06%) Tyloxapol = 607.8 mg (13.97%) Sesame Oil = 3000.5 mg (68.97%) Span 80 = 63.1 mg (1.45%) EtOH = 295.5 mg (6.79%)	S		
154	Form. # 150 = 200 g (99.998) BHT = 0.004 g (0.002%)	S		
155	Rapa = 51.0 mg (0.87%) EtOH = 642.3 mg (10.93%) Benzyl Alcohol = 431.8 mg (7.34%) Sesame Oil = 4753.7 mg (80.86%)	S		
156	Rapa = 51.4 mg (1.03%) Benzyl Alcohol = 518.4 mg (10.34%) Olive Oil = 4444.7 mg (88.64%)	S		
157	Rapa = 8.1 g (2%) EtOH = 16.0 g (4%) PEG 400 = 376.0 g (94%)	S		
158	Form. # 157 = 225.00 g (99.998%) BHT = 0.0045 g (0.002%)	S		
159	Rapa = 8.1 g (2%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	EtOH = 16.0 g (4%) PEG 400 = 376 q (94%)			
160	Form. # 159 = 112.0 g (99.998%) BHT = 0.00224 g (0.002%)	S		
161	Form. # 159 = 112.0 g (99.998%) BHT = 0.0019 g (0.002%)	S		
162	Rapa = 55.4 mg (1.10%) EtOH = 112.7 mg (2.25%) Benzyl Alcohol = 157.8 mg (3.15%) Cotton Seed Oil = 4688.0 mg (93.50%)	S		
163	Rapa = 5.005 g (1%) EtOH = 10.0 g (2%) PEG 400 = 485.5 g (97%)	S		
164	PEG 400 = 9.82 g (98%) EtOH = 0.235 g (2%)	S		
165	Form. # 163 = 100.25 g (99.998%) BHT = 0.0026g (0.002%)	S		
166	Rapa = 203.1 mg (2.025%) F68 = 30.3 mg (0.303%) Sterile Water = 9792.6 mg (97.672%)	SP	2.8651 µm	
167	Rapa = 201.4 mg (2.0005%) Tween 20 = 43.9 mg (0.436%) Sterile Water = 9822.8 mg (97.564%)	SP	1.0984 µm	
168	EtOH = 0.8301 g (4.144%) PEG 400 = 19.2014 q (95.856%)	S		
169	Form. # 168 = 300 µl	S		
170	Form. # 168 = 250 µl Form. # 154 = 50 µl	S		
171	Form. # 168 = 200 µl Form. # 154 = 100 µl	S		
172	Form. # 168 = 150 µl Form. # 154 = 150 µl	S		
173	Form. # 154 = 300 µl	S		
174	Rapa = 102.2 mg (2.041%) F68 = 16.0 mg (0.32%) Sterile Water = 4889.0 mq (97.639%)	SP	0.4165 µm	
175	Rapa = 101.1 mg (2.010%) Tween 20 = 27.7 mg (0.551%) Sterile Water = 4901.0 mg (97.439%)	SP	0.5294 µm	

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
176	BSS+ = 0 μl Sterile Water = 0 μl Form. # 154 = 1000 μl	S		
177	BSS+ = 200 μl Sterile Water = 0 μl Form. # 154 = 800 μl	SP		
178	BSS+ = 400 μl Form. # 154 = 600 μl	SP		
179	BSS+ = 500 μl Form. # 154 = 500 μl	SP		
180	BSS+ = 600 μl Form. # 154 = 400 μl	SP		
181	BSS+ = 800 μl Form. # 154 = 200 μl	SP		
182	Sterile Water = 200 μl Form. # 154 = 800 μl	SP		
183	Sterile Water = 400 μl Form. # 154 = 600 μl	SP		
184	Sterile Water = 500 μl Form. # 154 = 500 μl	SP		
185	Sterile Water = 600 μl Form. # 154 = 400 μl	SP		
186	Sterile Water = 800 μl Form. # 154 μl	SP		
187	BSS+ = 2536.9 mg(49.98%) Form. # 154 = 2538.7 mg (50.02%)	SP	60.2075 μm	
188	Sterile Water = 2515.6 mg (49.84%) Form. # 154 = 2532.2 mg (50.16%)	SP	617.5157 μm	
189	F68 = 12.6 mg (0.25%) Sterile Water = 2524.7 mg (49.79%) Form. # 154 = 2533.1 mg (49.96%)	SP	70.6089 μm	
190	Rapa = 2.0225 g (2%) EtOH = 3.65 g (4%) PEG 400 = 94.0 g (94%) BHT = 0.002 g (0.002%)	S		
191	F68 = 12.1 mg Sterile Water = 2558.9 mg Form. # 154 = 2556.4 mg	SP		
192	F68 = 19.8 mg Sterile Water = 2564.1 mg	SP		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	Form. # 154 = 25557.5 mg			
193	F68 = 25.3 mg Sterile Water = 2575.1 mg Form. # 154 = 2572.9 mg	SP		
194	F68 = 32.4 mg Sterile Water = 2572.1 mg Form. # 154 = 2562.1 mg	SP		
195	F68 = 38.3 mg Sterile Water = 2563.2 mg Form. # 154 = 2573.5 mg	SP		
196	F68 = 43.6 mg Sterile Water = 2541.1 mg Form. # 154 = 2556.0 mg	SP		
197	F68 = 51.2 mg Sterile Water = 2594.5 mg Form. # 154 = 2594.1 mg	SP		
198	PEG 400 = 1920 g (96%) EtOH = 80 q (4%)	S		
199	Form. # 168 = 1000 µl	S		
200	Form. # 168 =20 0 µl Form. # 154 = 800 µl	S		
201	Form. # 168 = 400 µl Form. # 154 = 600 µl	S		
202	Form. # 168 = 500 µl Form. # 154 = 500 µl	S		
203	Form. # 168 = 600 µl Form. # 154 = 400 µl	S		
204	Form. # 168 = 800 µl Form. # 154 = 200 µl	S		
205	PEG 400 = 200 µl Form. # 154 = 800 µl	S		
206	PEG 400 = 400 µl Form. # 154 = 600 µl	S		
207	PEG 400 = 500 µl Form. # 154 = 500 µl	S		
208	PEG 400 = 600 µl Form. # 154 = 400 µl	S		
209	PEG 400 = 800 µl Form. # 154 = 200 µl	S		
210	Phosal 50PG = 6735.0 mg (99.002%) Tween 80 = 67.9 mg (0.998%)	S		
211	Rapa = 2.0047 g (2%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	EtOH = 4.00 g (4%) PEG 400 = 94.05 g (94%)			
212	Phosal 50PG = 20.0662 g (98.999%) Tween 80 = 0.2029 g (1.001%)	S		
213	Form. # 154 = 100 µl Form. # 168 = 900 µl	S		
214	Form. # 154 = 100 µl Form. # 168 = 900 µl	S		
215	Form. # 154 = 100 µl Form. # 168 = 900 µl	S		
216	Form. # 154 = 100 µl PEG 400 = 900 µl	S		
217	Form. # 154 = 100 µl PEG 400 = 900 µl	S		
218	Form. # 154 = 100 µl PEG 400 = 900 µl	S		
219	Form. # 154 = 100 µl BSS+ = 900 µl	SP		
220	Form. # 154 = 100 µl BSS+ = 900 µl	SP		
221	Form. # 154 = 100 µl BSS+ = 900 µl	SP		
222	Form. # 154 = 1000 µl	S		
223	Form. # 154 = 1000 µl	S		
224	Form. # 154 = 100 µl Form. # 168 = 900 µl	S		
225	Form. # 154 = 100 µl Form. # 168 = 900 µl	S		
226	Form. # 154 = 100 µl Form. # 168 = 900 µl	S		
227	Form. # 154 = 100 µl PEG 400 = 900 µl	S		
228	Form. # 154 = 160 µl PEG 400 = 900 µl	S		
229	Form. # 154 = 100 µl PEG 400 = 900 µl	S		
230	Form. # 154 = 100 µl BSS+ = 900 µl	SP		
231	Form. # 154 = 100 µl BSS+ = 900 µl	SP		
232	Form. # 154 = 100 µl	SP		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	BSS+ = 900 µl			
233	Form. # 154 = 200 µl Form. # 168 = 800 µl	S		
234	Form. # 154 = 200 µl Form. # 168 = 800 µl	S		
235	Form. # 154 = 200 µl Form. # 168 = 800 µl	S		
236	Form. # 154 = 200 µl Form. # 168 = 800 µl	S		
237	Form. # 154 = 200 µl PEG 400 = 800 µl	S		
238	Form. # 154 = 200 µl PEG 400 = 800 µl	S		
239	Form. # 154 = 200 µl BSS+ = 800 µl	SP		
240	Form. # 154 = 200 µl BSS+ = 800 µl	SP		
241	Form. # 154 = 200 µl BSS+ = 800 µl	SP		
242	Form. # 154 = 100 µl Form. # 168 = 900 µl	S		No, 10 µL
243	Form. # 154= 100 µl PEG 400 = 900 µl	S		Yes, 10 µL
244	Form. # 154 = 100 µl BSS+ = 900 µl	SP		Yes, 10 µL
245	Form. # 154 = 100 µl BSS+/CMC(0.5%)= 900 µl	SP		
246	Form. # 154 = 400 µl Form. # 168 = 900 µl	S		No, 10 µL
247	Form. # 154 = 400 µl PEG 400 = 900 µl	S		Yes, 10 µL
248	Form. # 154 = 400 µl BSS+ = 900 µl	SP		Yes, 10 µL
249	Form. # 154 = 400 µl BSS+/CMC(0.5%)= 900 µl	SP		
250	Form. # 154 = 100 µl BSS+/CMC(0.5%)= 900 µl	SP		
251	Form. # 154 = 100 µl BSS+/CMC(0.5%)= 900 µl	SP		
252	Form. # 154 = 100 µl BSS+/CMC(0.5%)= 900 µl	SP		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
253	Form. # 154 = 200 µl BSS+/CMC(0.5%)= 800 µl	SP		
254	Form. # 154 = 200 µl BSS+/CMC(0.5%)= 800 µl	SP		
255	Form. # 154 = 200 µl BSS+/CMC(0.5%)= 800 µl	SP		
256	Form. # 154 = 400 µl BSS+/CMC(0.5%)= 900 µl	SP		
257	Form. # 154 = 400 µl BSS+/CMC(0.5%)= 900 µl	SP		
258	Form. # 154 = 400 µl BSS+/CMC(0.5%)= 900 µl	SP		
259	EtOH = 17.1 mg (0.57%) PEG 400 = 2997.3 mq (99.43%)	S		
260	EtOH = 40.8 mg (1.35%) PEG 400 = 2980.2 mg (98.65%)	S		
261	EtOH = 47.1 mg (1.57%) PEG 400 = 2950.1 mg (98.43%)	S		
262	Rapa = 2.0032 g (2%) EtOH = 3.92 g (4%) PEG 400 = 94.00 g (94%)	S		
263	Triamcinolone acetomide = 80.8 mg (4.04%) PEG 400 = 1920.8 mq (95.96%)	SP		
264	NFF-0007 filled in glove box	S		
265	PEG 400 = 9.598 g (96%) EtOH = 0.4052 (4%)	S		
266	Triamcinolone acetomide = 42.2 mg (4.123%) PEG 400 = 981.3 mg (95.877%)	SP		
267	Phosal 50PG = 20.0783 g (99.00835%) Tween 80 = 0.2011 g (0.99165%)	S		
268	PEG 400 = 96.1 g (96%) EtOH = 4.00 g (4%)	S		
269	Rapa = 0.4001 (2%) g EtOH = 0.80 g (4%) PEG 400 = 18.8 g (94%)	S		
270	Sterile Water = 9955.8 mg (99.27%) CMC High visc. = 47.8 mg (0.48%) Tween 80 = 25.4 mq (0.25%)	S		
271	Sterile Water = 9947.5 mg (99.00%) CMC Medium visc. = 75 mg (0.75%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	Tween 80 = 25.1 mg (0.25%)			
272	Rapa = 41 mg (2.01%) Form. # 270 = 2000 mg (97.99%)	SP		
273	Rapa = 40.2 mg (1.97%) MSF-03-172-07E = 2000 mg (98.03%)	SP		
274	NMP (Pharmasolve®)= 1280.5 mg (65.89%) PLGA 75/25 = 662.9 mg (34.11%)	S		
275	NMP (Pharmasolve®)= 1573.3 mg (80.50%) PLGA 75/25 = 381.0 mq (19.50%)	S		
276	NMP (Pharmasolve®)= 1009.7 mg 49.8%) PLGA 75/25 = 1001.6 mg (50.20%)	S		Yes, 10 μL
277	Sterile Water = 14934.0 mg (99.25%) CMC Medium visc. = 112.4 mg (0.75%)	S		
278	Propylene Glycol = 1893.7 mg (93.85%) EtOH = 83.8 mg (4.16%) Rapa = 40.2 mg (1.99%)	S		Yes, 10 μL
279	Propylene Glycol = 1946.2 mg (95.68%) Benzyl Alcohol = 47.1 mg (2.31 %) Rapa = 40.8 mg (2.01%)	S		Yes, 10 μL
280	PEG 300 = 1894.1 mg (93.74%) EtOH = 40.1 mg (1.98%) Rapa = 86.4 mg (4.28%)	S		Yes, 10 μL
281	PEG 300 = 1925.5 mg (95.88%) EtOH = 39.8 mg (1.98%) Rapa = 43.0 mg (2.14%)	S		Yes, 10 μL, 30 μL
282	Rapa = 100.6 mg (2.01%) MSF-03-176-02 = 4910.8 mg (97.99%)	SP		Yes, 10 μL, 30 μL
283	Rapa = 11.5 mg (0.57%) PEG 300 = 2012.5 mg (99.43%)	S		
284	Rapa = 10.3 mg (0.51%) PEG 400 = 2017.2 mg (99.49%)	S		
285	Rapa = 9.8 mg (0.486%) PEG 600 = 2005.9 mg (99.51%)	S		
286	Tacrolimus = 42.7 mg (2.11 %) EtOH = 46.0 mg (2.27%) PG = 1938.7 mg (95.62%)	S		
287	Tacrolimus = 40.7 mg (2.01%) EtOH = 43.0 mg (2.12%) PEG 300 = 1942.1 mg (95.87%)	S		
288	Tacrolimus = 40.3 mg (1.99%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
	EtOH = 43.8 mg (2.16%) PEG 400 = 1942.3 mg (95.85%)			
289	Tacrolimus = 40.8 mg (2.03%) EtOH = 44.5 mg (2.21%) PEG 600 = 1924.0mg (95.76%)	S		
290	Rapa = 61.0 mg (3.17%) NMP = 1226.54 mg (63.80%) PLGA 75/25 = 634.96 mg (33.03%)	S		
291	Rapa = 100.2 mg (5.13%) NMP = 1492.95 mg mg (76.37%) PLGA 75/25 = 361.65 mg (18.50%)	S		
292	Rapa = 62.9 mg (3.04%) NMP = 1103.8g mg (53.40%) PLGA 75/25 = 900.2 mg (43.56%)	S		
293	Rapa = 62.4 mg (3.00%) NMP = 1205.1 mg mg (58.11%) PLGA 75/25 = 806.4 mg (38.89%)	S		
294	Sterile Water+1 % CMC Med. = 4909.1 mg (97.99%) Rapa = 100.5 mg (2.01%)	SP		
295	Sterile Water+1 % CMC high. = 4903.8 mg (97.96%) Rapa = 101.9 mq (2.04%)	SP		
296	Rapa = 40.5 mg (2.03%) NMP = 1958.7 mq (97.97%)	S		
297	Rapa = 20.5mg (2.0%) DMA = 41.4mg (4.0%) PVP = 35.0mg (3.4%) H2O = 934.7mg (90.6%)	SP		
298	Rapa = 10.6mg (2.0%) DMA = 10.6mg (2.0%) PEG 400 = 506.1mg (96%)	S		
299	Rapa = 5.2mg (2.0%) 1% DMA in PEG 400 = 257.4 mg (98%)	SP		
300	Rapa = 20.0mg (2.0%) DMA = 7.8mg (0.8%) PEG 400 = 974mq (97.2%)	S		
301	Rapa = 20.1 mg (1.3%) DMA = 19.5mg (1.3%) PEG 400 = 1449.6mg (97.3%)	S		
302	Rapa = 20.0mg (2.0%) PVP = 10.8mg (1.1%) PEG 400 = 994.5mg (97.0%)	SP		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
303	Rapa = 20.4mg (2.0%) PVP = 24.5mg (2.4%) PEG 400 = 990.7mg (95.7%)	SP		
304	Rapa = 25.5mg (2.4%) PVP = 51.9mg (4.8%) PEG 400 = 1000.6mg (92.8%)	SP		
305	Rapa = 22.5mg (2.3%) BA = 27.5mg (2.7%) PEG 400 = 950.7mg (95.0%)	S		
306	Rapa = 30.2mg (2.3%) PVP = 240.9mg (18.6%) PEG 400 = 1021.2mg (79.0%)	SP		
307	Rapa = 8.7mg (3.1 %) 1% PVP in H2O = 273 mg (96.9%)	SP		
308	Rapa = 12.6mg (2.53%) 5% PVP in H2O = 501.6 mg (97.5%)	SP		
309	Rapa = 20.3mg (3.8%) 10% PVP in H2O = 513.9 mq (96.2%)	SP		
310	Rapa = 100.5mg (2.0%) DMA = 67.8mg (1.4%) PEG 400 = 4838.3mg (96.6%)	S		Yes, 10 μL
311	Rapa = 96.8mg (1.9%) BA = 157.5mg (3.2%) PEG 400 = 4748.7mg (94.9%)	S		Yes, 10 μL
312	Rapa = 105.8mg (2.1%) DMA = 5.63mg (0.1 %) PEG 400 = 4888.9mg (97.8%)	S		
314	Rapa = 100.3mg (2.0%) PVP = 251.4mg (5.0%) H2O = 4662.8mg (93.0%)	SP		
315	Rapa = 20.3mg (2.0%) DMA = 983.9mg (98%)	S		
316	Triamcinolone = 22.8mg (2.0%) DMA = 12.0mg (1.1 %) PEG 400 = 1104.5mq (96.9%)	S		Yes, 10 μL
317	Triamcinolone = 1.0mg (0.1%) EtOH = 49.30mg (4.0%) PEG 400 = 1191.9mg (96.0%)	S		
318	Triamcinolone = 18.7mg (0.9%) PEG 400 = 959.8mg (99.1%)	S		
319	Triamcinolone = 25.5mg (1.3%) EtOH = 83.0mg (4.1%) PEG 400 = 1905.6mg (94.6%)	S		

(continued)

Form. #	Composition (mg), % (w/w)	Formulation Type	Median particle size	Formation of NDM, Injection volume
320	Dexamethasone = 20.4mg (1.2%) EtOH = 71.7mg (4.1%) PEG 400 =1737.6mg (98.8%)	S		
321	Dexamethasone = 27.5mg (2.0%) DMA = 5.6mg (0.4%) PEG 400 = 1347.3mg (97.6%)	S		Yes, 10 μL
322	Rapa = 9.1 mg (0.152%) EtOH = 90.9mg (1.514%) F127 = 262.8mg (4.378%) Water = 1489.1mg (24.804%) Sesame oil = 4151.5mq (69.152%)	E		
323	Rapa = 24.4mg (0.625%) Phosal 50PG = 203.1mg (5.201%) EtOH = 166.8mg (4.272%) Labrafac CC = 1502.8mg (38.486%) Sesame oil = 2007.7mq (51.416%)	E		
324	Form. # 174 with 2mm beads	SP	0.4929 μm	
325	Form. # 175 with 2mm beads	SP	0.4804 μm	

Table 2 Aqueous Humor Rapa Concentration

Injection of 2% Rapa-PEG-EtOH Solution	Mean Rapa concentration (ng/mL)	Standard deviation (ng/mL)
1.0 μL intravitreal	0.438 (1 day after injection)	0.141
3.0 μL intravitreal	0.355 (1 day after injection)	0.234
3.0 μL sub-conj	0.338 (1 day after injection)	0.122
5.0 μL into anterior chamber	0.167 (14 days after injection)	0.183
10.0 μL into anterior chamber	0.004 (14 days after injection)	0.006

Claims

1. A liquid formulation comprising a therapeutic agent and a solvent component, wherein
the liquid formulation is a solution, which is administered to the vitreous of a subject's eye;
the therapeutic agent is 2% (w/w) rapamycin; and
the solvent component comprises a mixture of 4% (w/w) ethanol and 94% (w/w) polyethylene glycol 400.

2. The liquid formulation of claim 1 for use in the treatment of a disease or condition selected from diabetic retinopathy, macular degeneration, wet and dry AMD, retinopathy of prematurity (retrolental fibroplasia), infections causing a retinitis or choroiditis, presumed ocular histoplasmosis, myopic degeneration, angioid streaks, ocular trauma, pseudoxanthoma elasticum, vein occlusion, artery occlusion, carotid obstructive disease, Sickle Cell anemia, Eales disease, myopia, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, trauma, polypoidal choroidal vasculopathy, post-laser complications, complications of idiopathic central serous chorioretinopathy, complications of choroidal inflammatory conditions, rubeosis, diseases associated with rubeosis (neovascularization of the angle), neovascular glaucoma, uveitis and chronic uveitis, macular edema, proliferative retinopathies and diseases or con-

ditions caused by the abnormal proliferation of fibrovascular or fibrous tissue, including all forms of proliferative vitreoretinopathy (including post-operative proliferative vitreoretinopathy), whether or not associated with diabetes.

Patentansprüche

1. Flüssige Formulierung, umfassend ein therapeutisches Mittel und eine Lösungsmittel-Komponente, wobei die flüssige Formulierung eine Lösung ist, die an den Glaskörper des Auges eines Individuums verabreicht wird; das therapeutische Mittel 2% (w/w) Rapamycin ist; und die Lösungsmittel-Komponente ein Gemisch aus 4% (w/w) Ethanol ud 94% (w/w) Polyethylenglykol 400 umfasst.

2. Flüssige Formulierung gemäß Anspruch 1 zur Verwendung bei der Behandlung einer Erkrankung oder eines Zustands, der/die ausgewählt ist aus diabetischer Retinopathie, Makuladegeneration, feuchter und trockener AMD, Frühgeborenen-Retinopathie (retrolentaler Fibroplasie), eine Retinitis oder Choroiditis verursachenden Infektionen, vermuteter okularer Histoplasmose, myopischer Degeneration, angioiden Netzhautstreifen, okularem Trauma, Pseudoxanthoma elasticum, Venenverschluss, Arterienverschluss, Carotis-Verschlusskrankheit, Sichelzellenanämie, Eales-Krankheit, Myopie, chronischer Netzhautablösung, Hyperviskositätssyndrome, Toxoplasmose, Trauma, polydoidaler choroidaler Vaskulopathie, Komplikationen nach Laserbehandlungen, Komplikationen der idiopathischen zentralen serösen Chorioretinopathie, Komplikationen der choroidalen Entzündungszustände, Rubeosis, Erkrankungen in Zusammenhang mit Rubeosis (Neovaskularisation des Augenkammerwinkels), neovaskulärem Glaukom, Uveitis und chronischer Uveitis, Makulaödem, proliferativen Retinopathien und durch die abnormale Proliferation von fibrovaskulärem oder fibrösem Gewebe verursachten Erkrankungen oder Zuständen, einschließlich aller Formen von proliferativer Vitreoretinopathie (einschließlich postoperativer proliferativer Vitreoretinopathie), ob in Zusammenhang mit Diabetes oder nicht.

Revendications

1. Formulation liquide comprenant un agent thérapeutique et un solvant, dans laquelle la formulation liquide est une solution, qui est administrée au corps vitré de l'oeil d'un sujet ; l'agent thérapeutique est la rapamycine à 2 % (p/p) ; et le solvant comprend un mélange d'éthanol à 4 % (p/p) et de polyéthylène glycol 400 à 94 % (p/p).

2. Formulation liquide selon la revendication 1 pour son utilisation dans le traitement d'une maladie ou d'une affection sélectionnée parmi la rétinopathie diabétique, la dégénérescence maculaire, la DMLA de forme humide et sèche, la rétinopathie du prématuré (fibroplasie rétrolentale), des infections provoquant une rétinite ou une choroïdite, l'histoplasmose oculaire présumée, la dégénérescence myopique, des stries angioïdes, un traumatisme oculaire, le pseudoxanthome élastique, une occlusion veineuse, une occlusion artérielle, une maladie occlusive de l'artère carotide, la drépanocytose, la maladie d'Eales, la myopie, un décollement de rétine chronique, des syndromes d'hyperviscosité, la toxoplasmose, un traumatisme, la vasculopathie polypoïdale choroïdienne, des complications post-traitement au laser, des complications d'une choriorétinopathie séreuse centrale idiopathique, des complications d'affections inflammatoires choroïdiennes, la rubéose, des maladies associées à la rubéose (néovascularisation de l'angle), un glaucome néovasculaire, l'uvéite et l'uvéite chronique, l'oedème maculaire, des rétinopathies proliférantes et des maladies ou des affections provoquées par la prolifération anormale de tissu fibrovasculaire ou fibreux, comprenant toutes les formes de la vitréorétinopathie proliférante (comprenant la vitréorétinopathie proliférante post-opératoire), qu'elles soient associées ou non au diabète.

Figure 1A

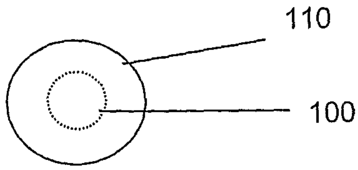


Figure 1B

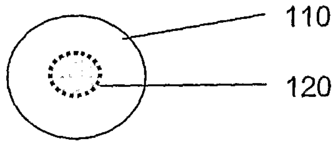
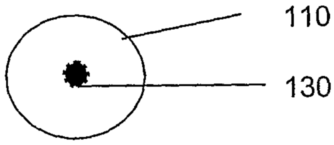


Figure 1C



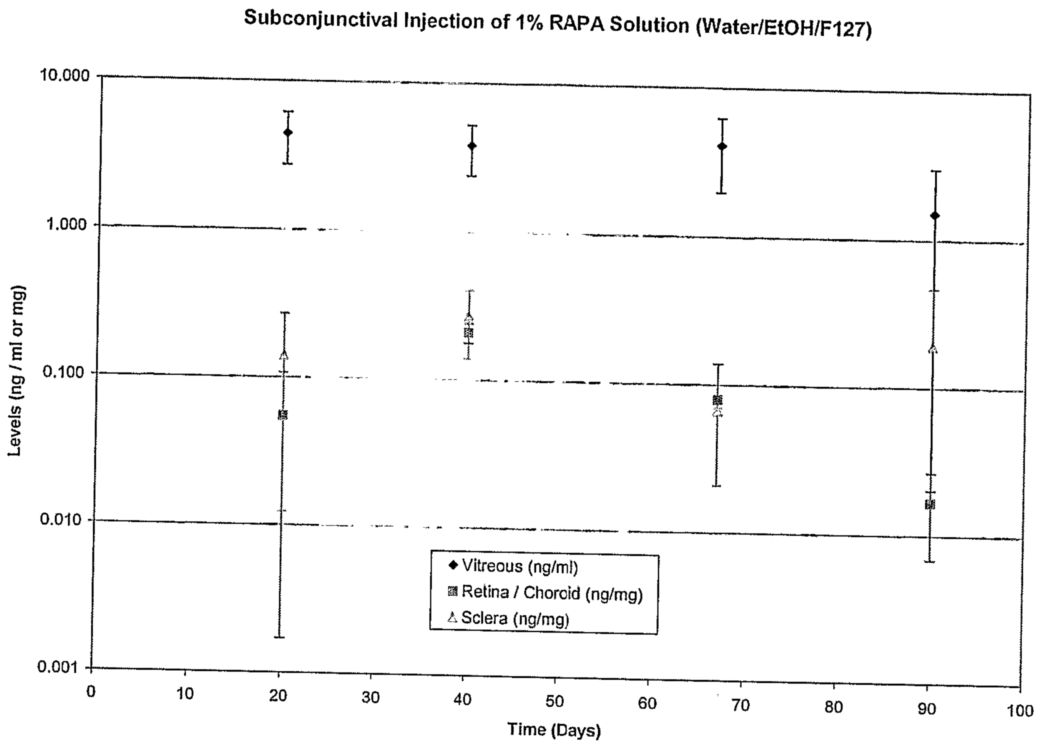

Subconjunctival Injection of 1% RAPA Solution (Water/EtOH/F127)
Levels (ng / ml or mg)
10.000
1.000
0.100
0.010
0.001
0
10
20
30
40
50
60
70
80
90
100
Time (Days)
Vitreous (ng/ml)
Retina / Chorcid (ng/mg)
Sclera (ng/mg)

Figure 2

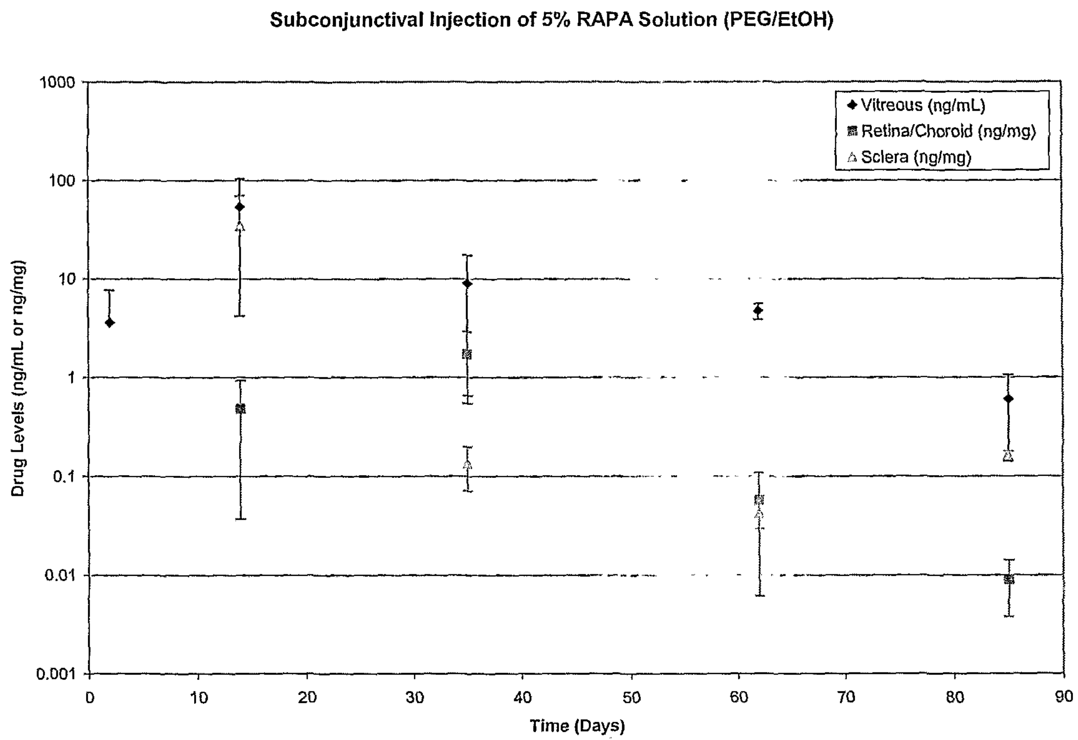

Subconjunctival Injection of 5% RAPA Solution (PEG/EtOH)
Vitreous (ng/mL)
Retina/Choroid (ng/mg)
Sclera (ng/mg)
Drug Levels (ng/mL or ng/mg)
1000
100
10
1
0.1
0.01
0.001
0
10
20
30
40
50
60
70
80
90
Time (Days)

Figure 3

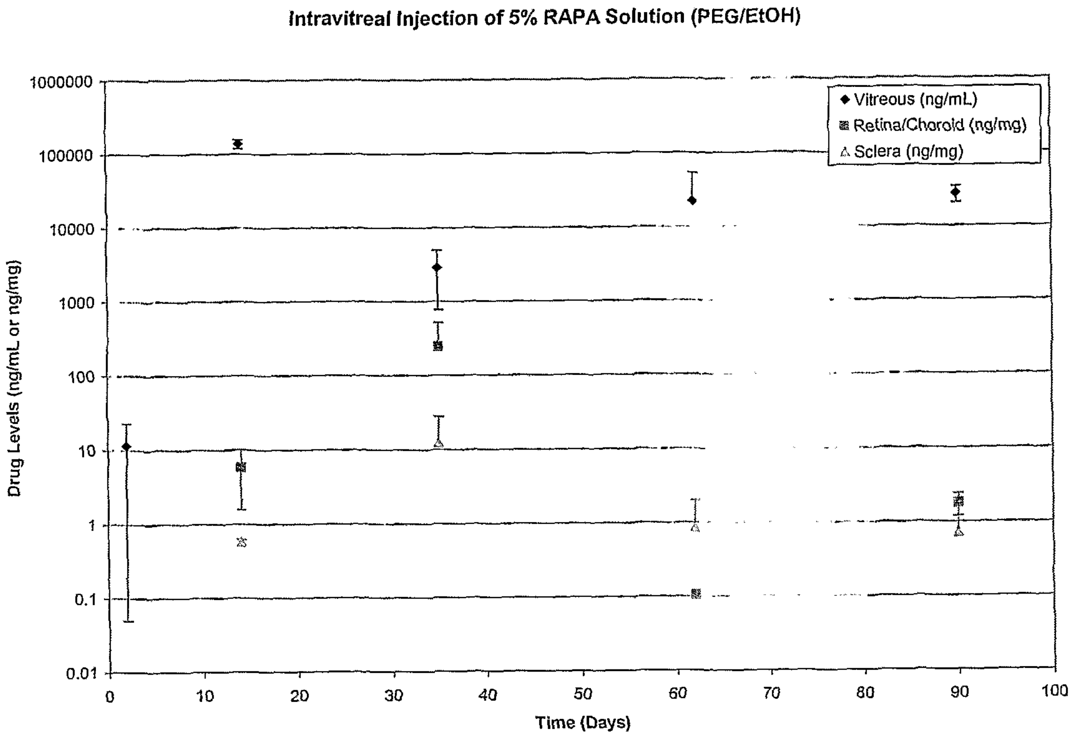
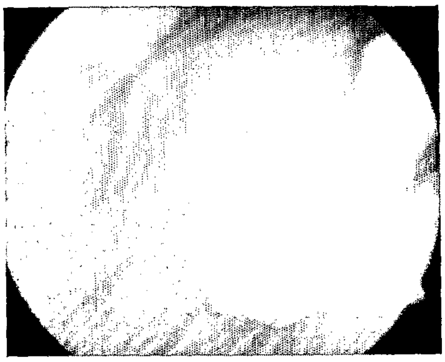
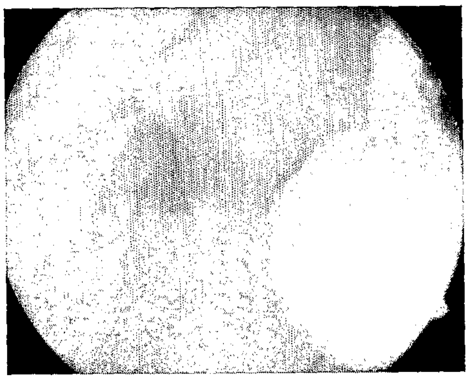

Intravitreal Injection of 5% RAPA Solution (PEG/EtOH)
1000000
100000
10000
1000
100
10
1
0.1
0.01
Drug Levels (ng/mL or ng/mg)
0
10
20
30
40
50
60
70
80
90
100
Time (Days)
Vitreous (ng/mL)
Retina/Choroid (ng/mg)
Sclera (ng/mg)

Figure 4



A.



B.

C.

Figure 5

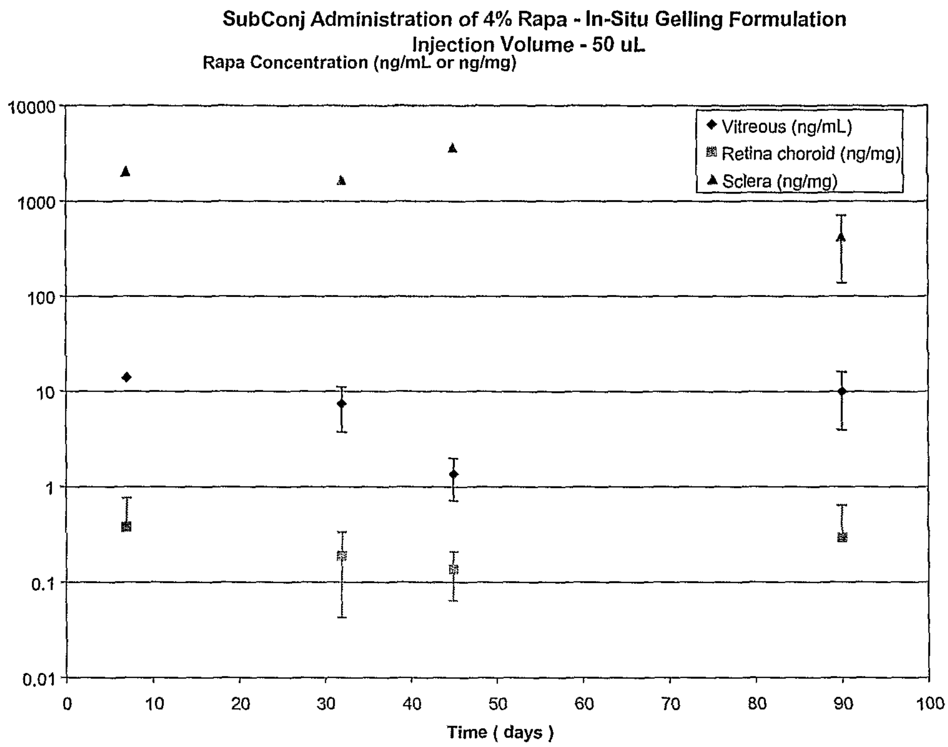

SubConj Administration of 4% Rapa - In-Situ Gelling Formulation
Injection Volume - 50 uL
Rapa Concentration (ng/mL or ng/mg)
10000
1000
100
10
1
0.1
0.01
0
10
20
30
40
50
60
70
80
90
100
Time (days)
Vitreous (ng/mL)
Retina choroid (ng/mg)
Sclera (ng/mg)

Figure 6

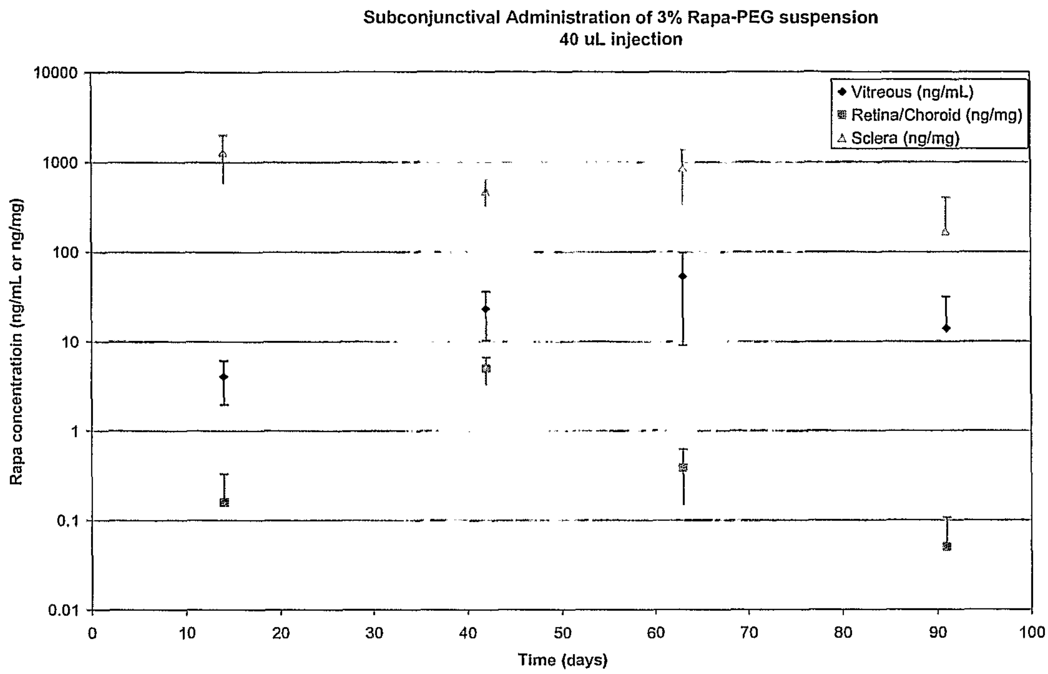

Subconjunctival Administration of 3% Rapa-PEG suspension
40 uL injection
Vitreous (ng/mL)
Retina/Choroid (ng/mg)
Sclera (ng/mg)
Rapa concentratioin (ng/mL or ng/mg)
10000
1000
100
10
1
0.1
0.01
0
10
20
30
40
50
60
70
80
90
100
Time (days)

Figure 7

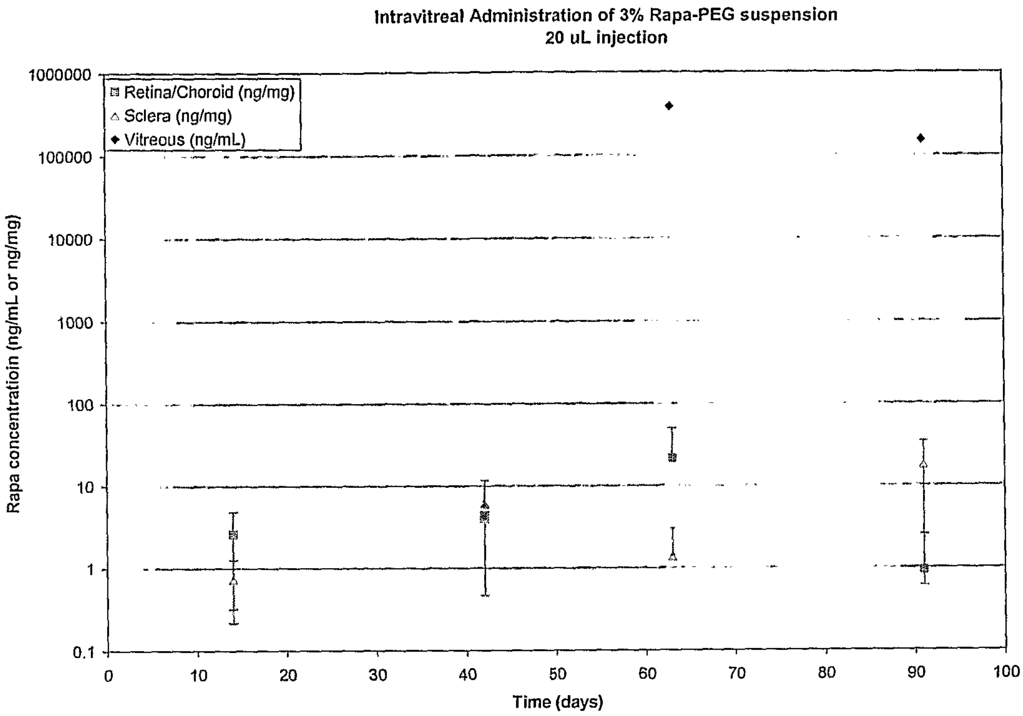

Intravitreal Administration of 3% Rapa-PEG suspension
20 uL injection
Retina/Choroid (ng/mg)
Sclera (ng/mg)
Vitreous (ng/mL)
Rapa concentratioin (ng/mL or ng/mg)
1000000
100000
10000
1000
100
10
1
0.1
0
10
20
30
40
50
60
70
80
90
100
Time (days)

Figure 8

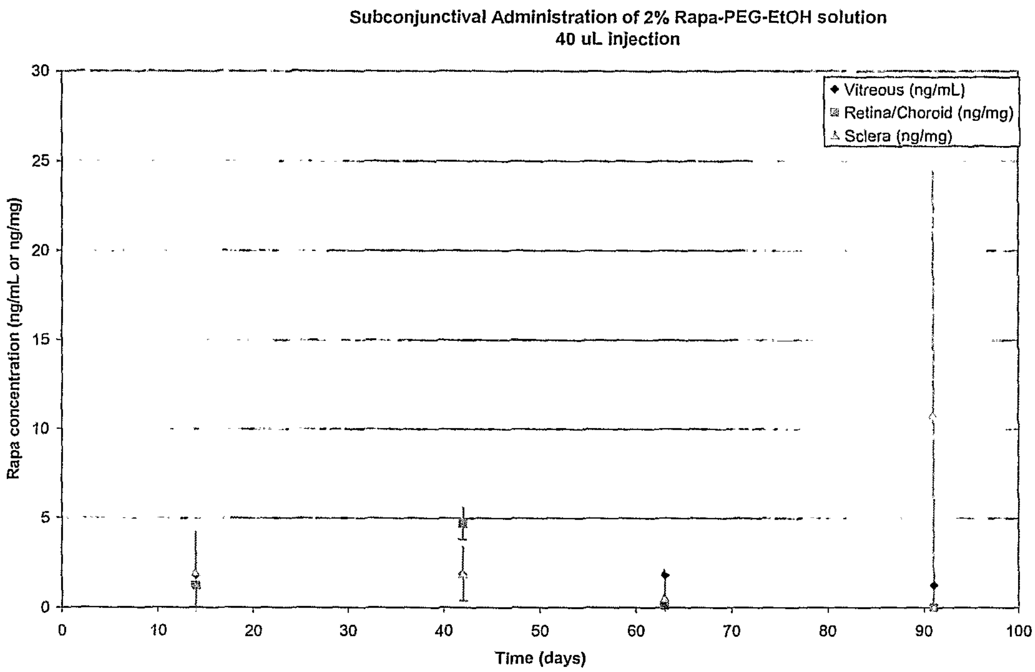

Subconjunctival Administration of 2% Rapa-PEG-EtOH solution
40 uL injection
Vitreous (ng/mL)
Retina/Choroid (ng/mg)
Sclera (ng/mg)
Rapa concentration (ng/mL or ng/mg)
Time (days)
0
5
10
15
20
25
30
0
10
20
30
40
50
60
70
80
90
100

Figure 9

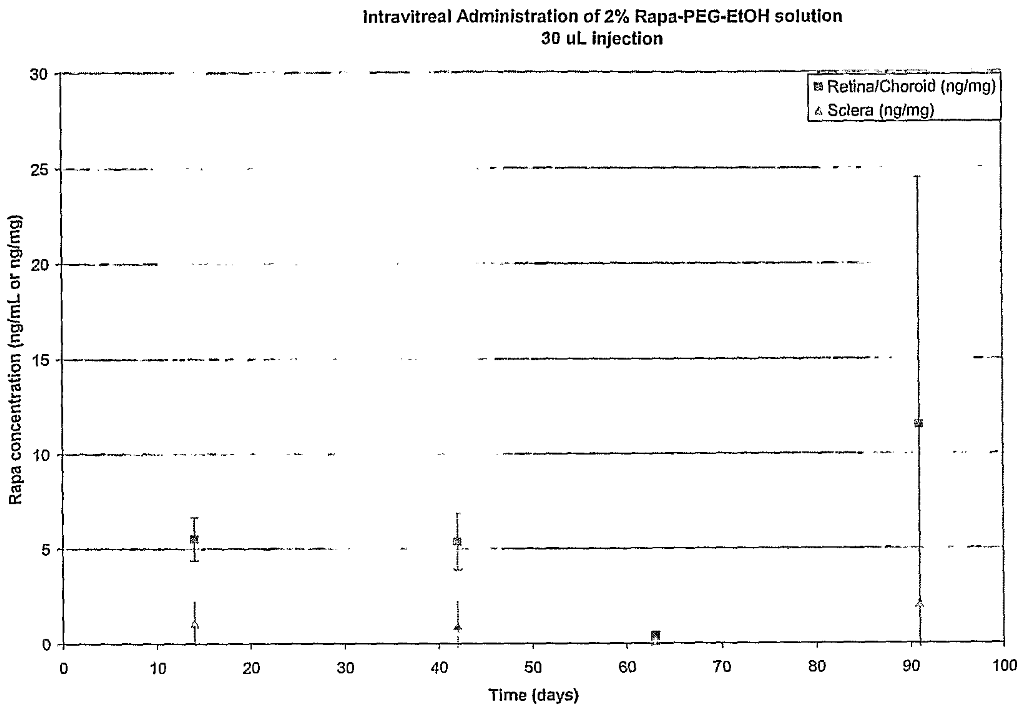

Intravitreal Administration of 2% Rapa-PEG-EtOH solution
30 uL injection
Retina/Choroid (ng/mg)
Sclera (ng/mg)
Rapa concentration (ng/mL or ng/mg)
0
5
10
15
20
25
30
0
10
20
30
40
50
60
70
80
90
100
Time (days)

Figure 10

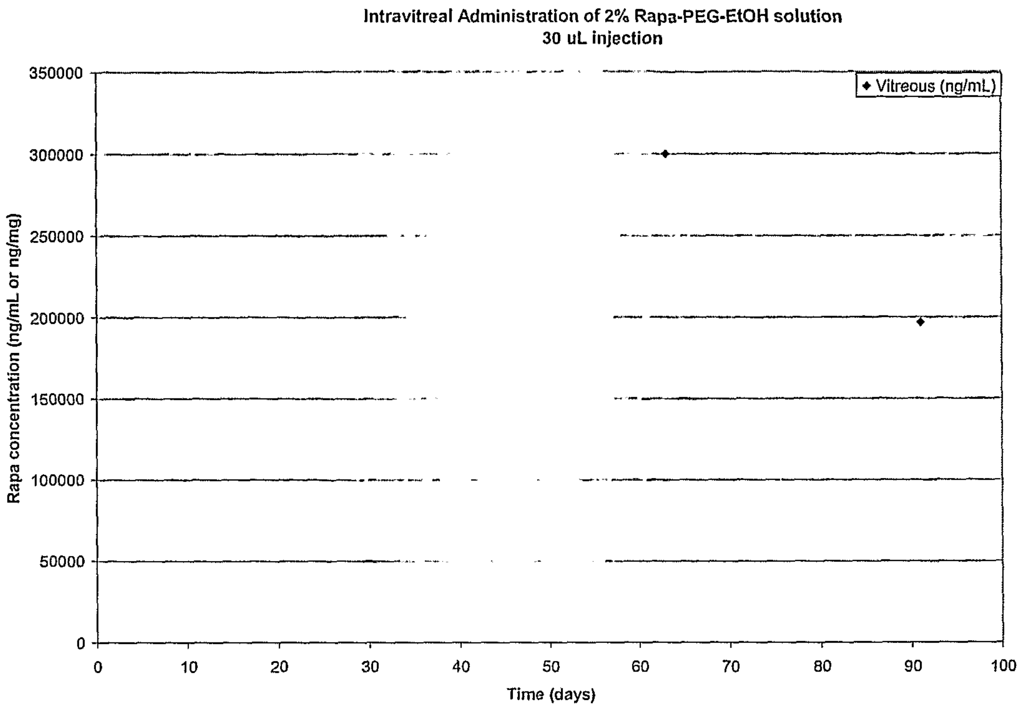

Intravitreal Administration of 2% Rapa-PEG-EtOH solution
30 uL injection
Vitreous (ng/mL)
Rapa concentration (ng/mL or ng/mg)
350000
300000
250000
200000
150000
100000
50000
0
0
10
20
30
40
50
60
70
80
90
100
Time (days)

Figure 11

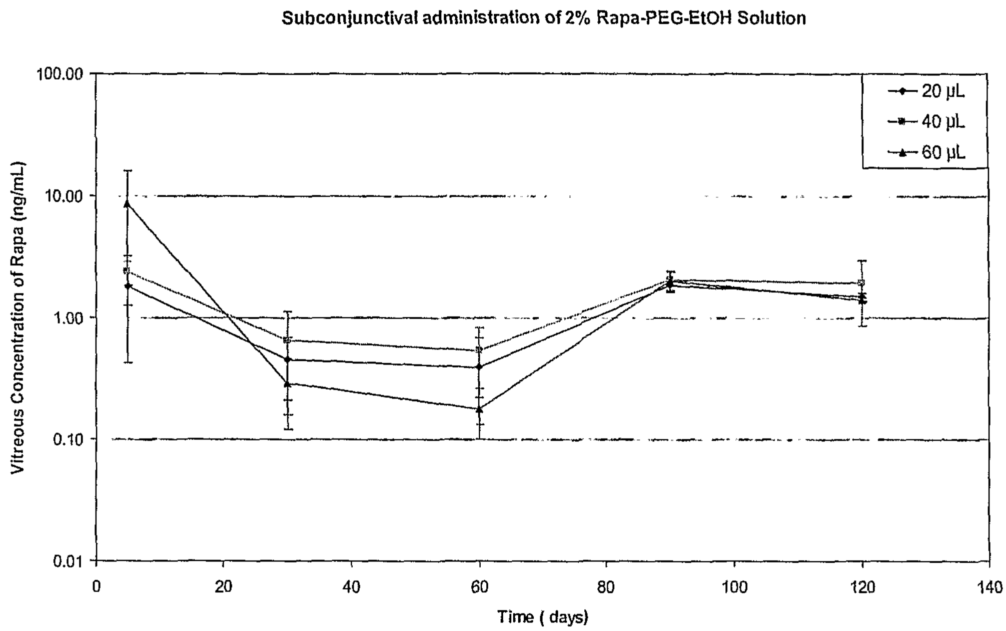

Subconjunctival administration of 2% Rapa-PEG-EtOH Solution
Vitreous Concentration of Rapa (ng/mL)
100.00
10.00
1.00
0.10
0.01
0
20
40
60
80
100
120
140
Time (days)
20 µL
40 µL
60 µL

Figure 12

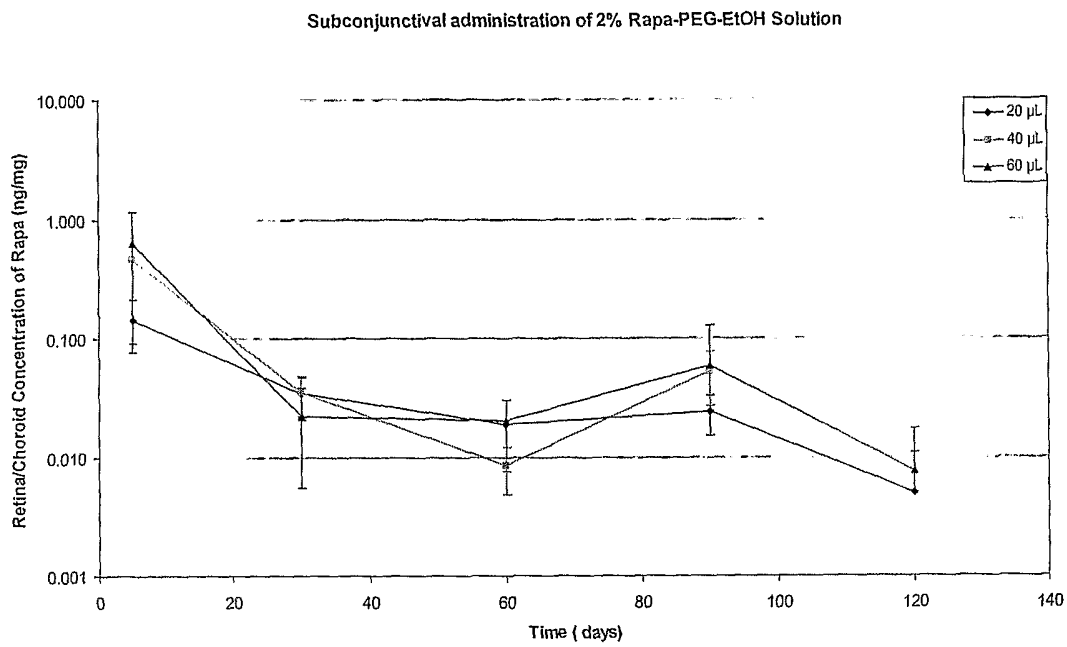

Subconjunctival administration of 2% Rapa-PEG-EtOH Solution
Retina/Choroid Concentration of Rapa (ng/mg)
10.000
1.000
0.100
0.010
0.001
0
20
40
60
80
100
120
140
Time (days)
20 µL
40 µL
60 µL

Figure 13

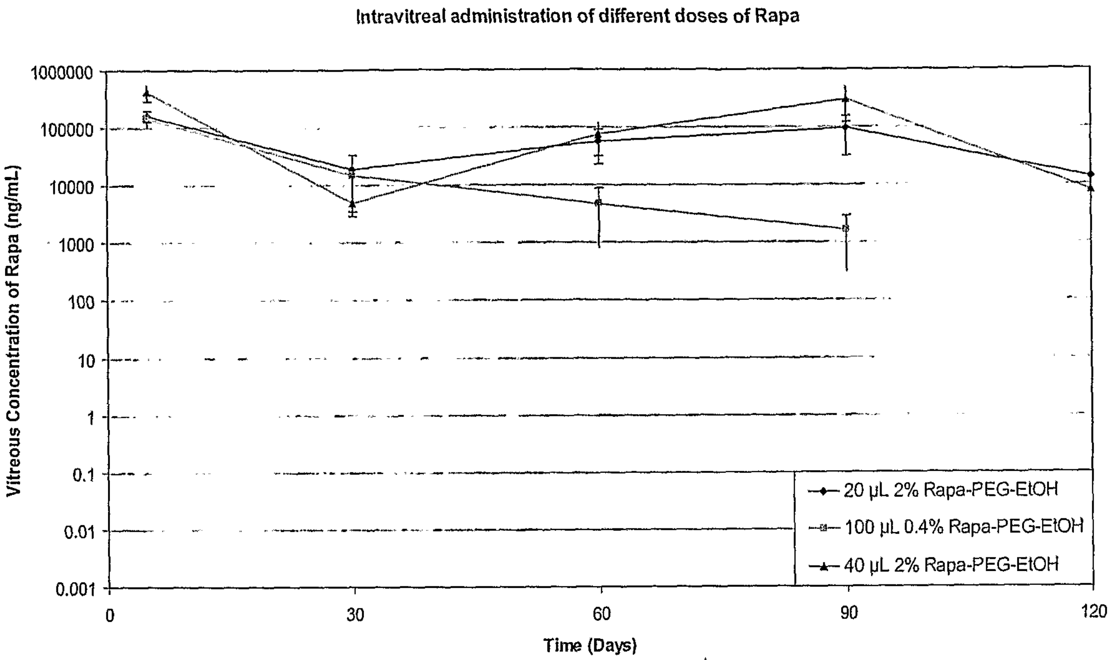

Intravitreal administration of different doses of Rapa
Vitreous Concentration of Rapa (ng/mL)
1000000
100000
10000
1000
100
10
1
0.1
0.01
0.001
0
30
60
90
120
Time (Days)
20 µL 2% Rapa-PEG-EtOH
100 µL 0.4% Rapa-PEG-EtOH
40 µL 2% Rapa-PEG-EtOH

Figure 14

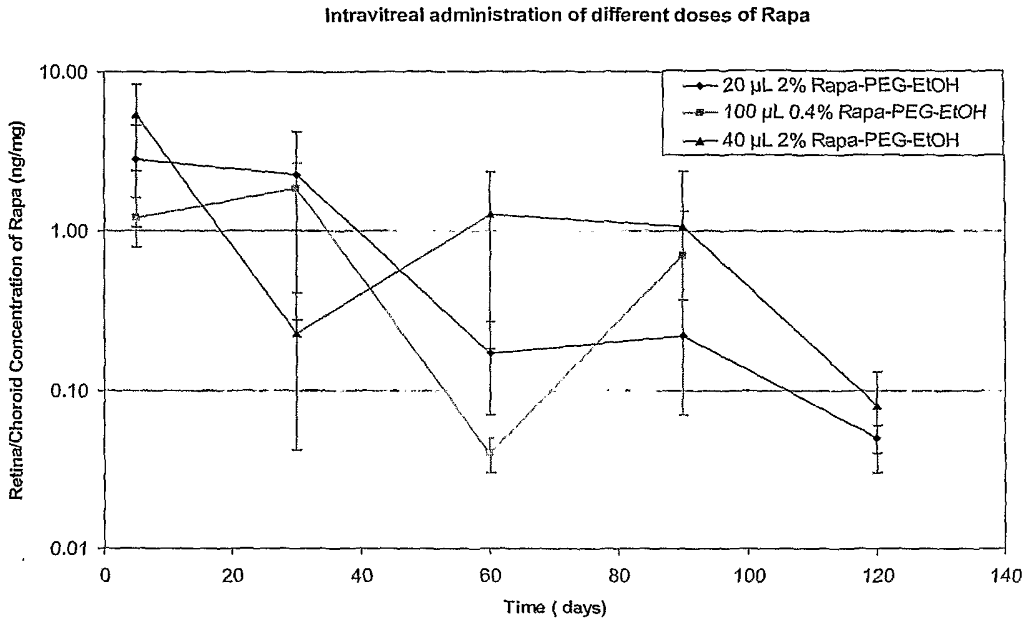

Intravitreal administration of different doses of Rapa
20 µL 2% Rapa-PEG-EtOH
100 µL 0.4% Rapa-PEG-EtOH
40 µL 2% Rapa-PEG-EtOH
Retina/Choroid Concentration of Rapa (ng/mg)
10.00
1.00
0.10
0.01
0
20
40
60
80
100
120
140
Time (days)

Figure 15

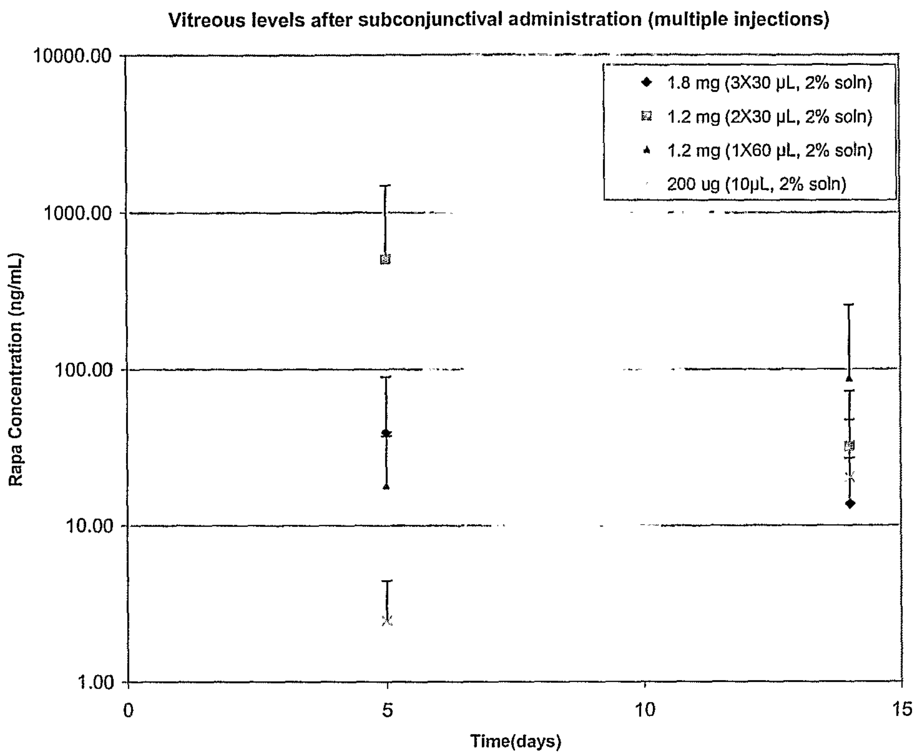

Vitreous levels after subconjunctival administration (multiple injections)
Rapa Concentration (ng/mL)
10000.00
1000.00
100.00
10.00
1.00
0
5
10
15
Time(days)
1.8 mg (3X30 µL, 2% soln)
1.2 mg (2X30 µL, 2% soln)
1.2 mg (1X60 µL, 2% soln)
200 ug (10µL, 2% soln)

Figure 16

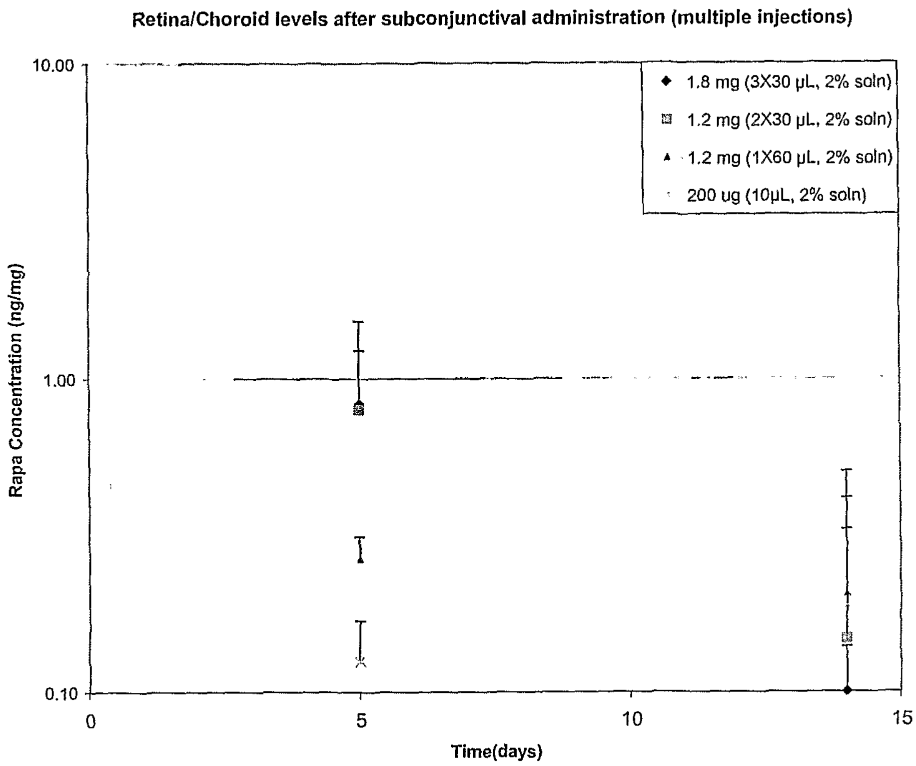

Retina/Choroid levels after subconjunctival administration (multiple injections)
10.00
1.00
0.10
Rapa Concentration (ng/mg)
0
5
10
15
Time(days)
1.8 mg (3X30 µL, 2% soln)
1.2 mg (2X30 µL, 2% soln)
1.2 mg (1X60 µL, 2% soln)
200 ug (10µL, 2% soln)

Figure 17

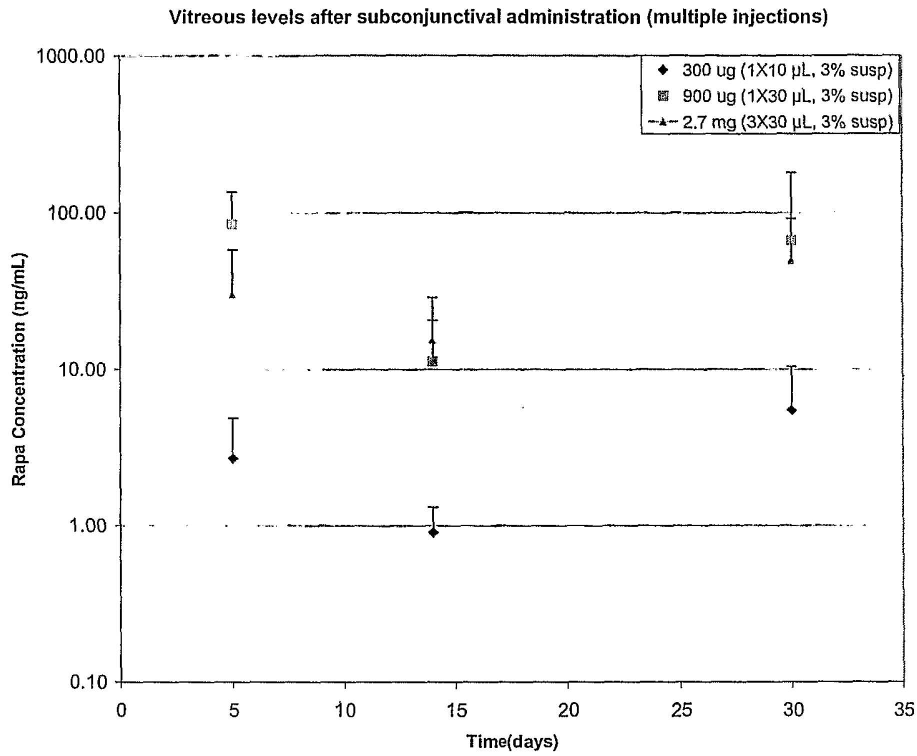

Vitreous levels after subconjunctival administration (multiple injections)
1000.00
100.00
10.00
1.00
0.10
Rapa Concentration (ng/mL)
0
5
10
15
20
25
30
35
Time(days)
300 ug (1X10 µL, 3% susp)
900 ug (1X30 µL, 3% susp)
2.7 mg (3X30 µL, 3% susp)

Figure 18

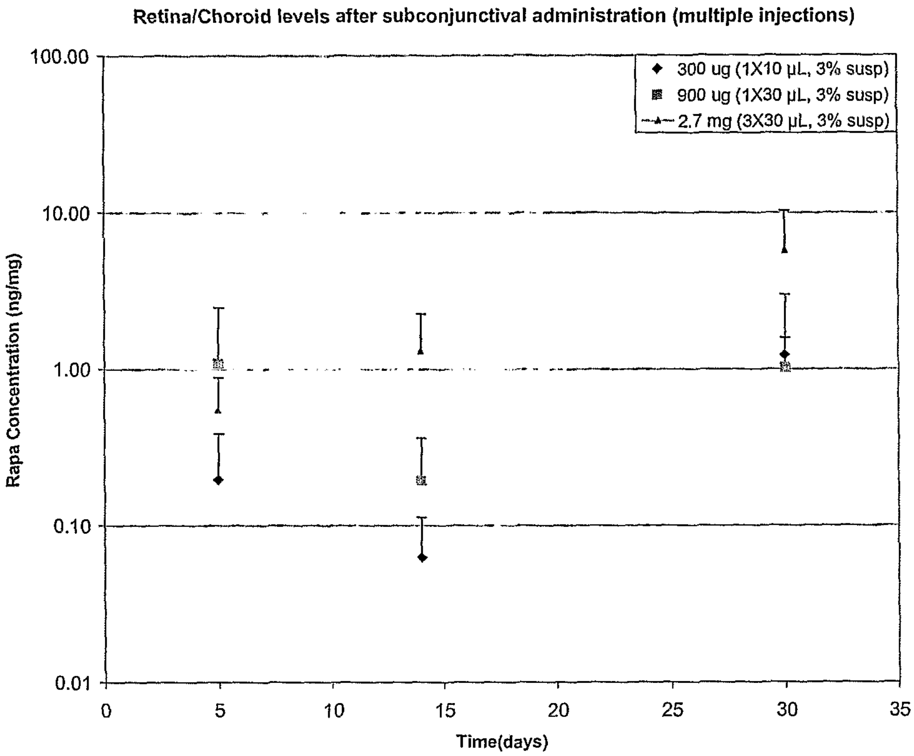

Retina/Choroid levels after subconjunctival administration (multiple injections)
300 ug (1X10 µL, 3% susp)
900 ug (1X30 µL, 3% susp)
2.7 mg (3X30 µL, 3% susp)
100.00
10.00
1.00
0.10
0.01
Rapa Concentration (ng/mg)
0
5
10
15
20
25
30
35
Time(days)

Figure 19

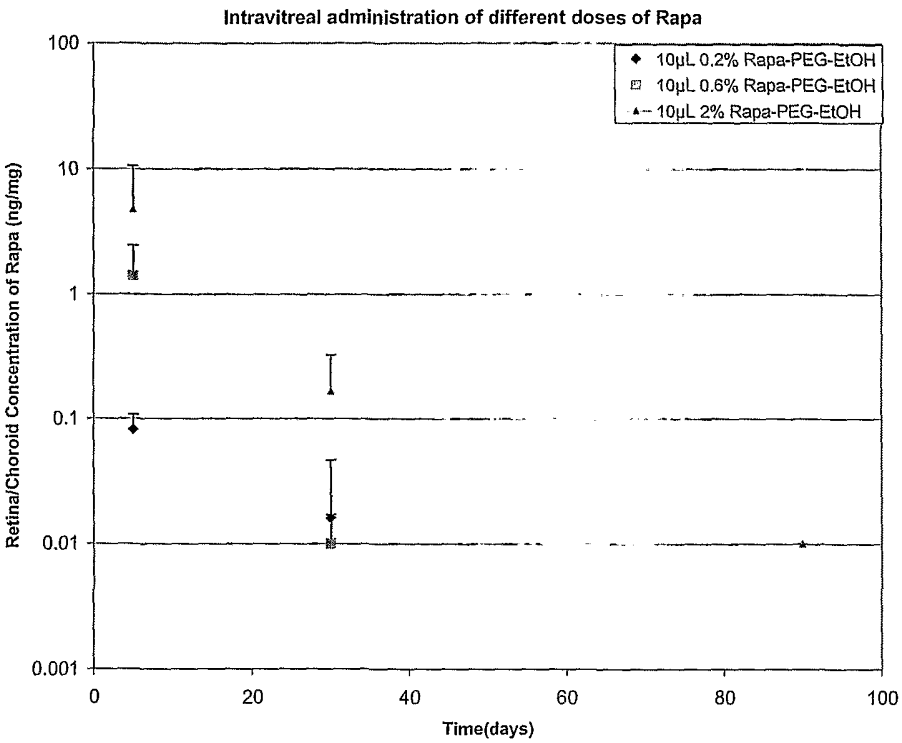

Intravitreal administration of different doses of Rapa
100
10
1
0.1
0.01
0.001
Retina/Choroid Concentration of Rapa (ng/mg)
10μL 0.2% Rapa-PEG-EtOH
10μL 0.6% Rapa-PEG-EtOH
10μL 2% Rapa-PEG-EtOH
0
20
40
60
80
100
Time(days)

Figure 20

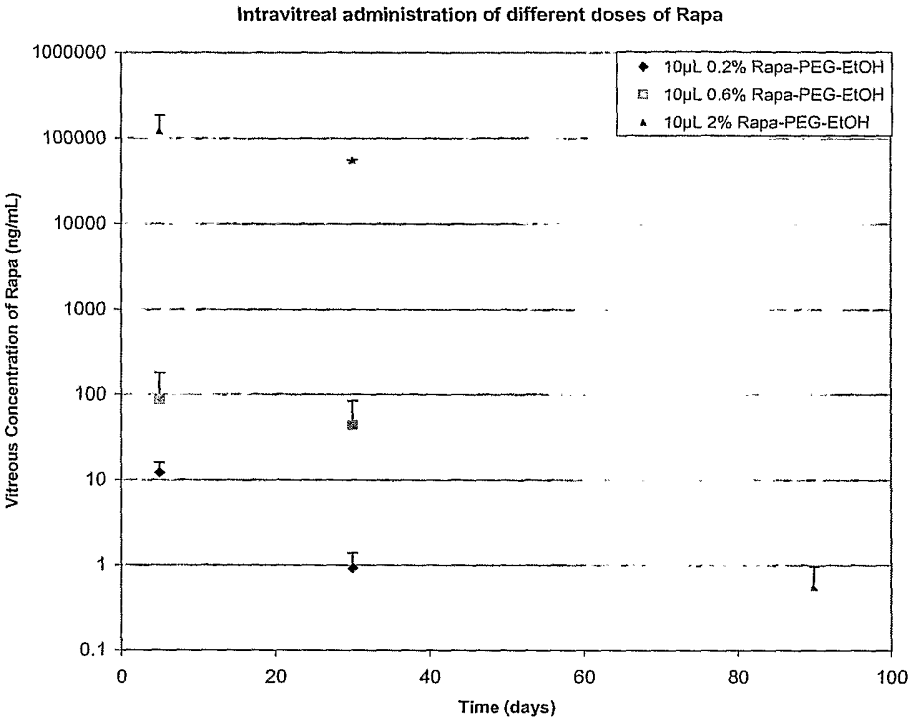

Intravitreal administration of different doses of Rapa
1000000
100000
10000
1000
100
10
1
0.1
Vitreous Concentration of Rapa (ng/mL)
0
20
40
60
80
100
Time (days)
10µL 0.2% Rapa-PEG-EtOH
10µL 0.6% Rapa-PEG-EtOH
10µL 2% Rapa-PEG-EtOH

Figure 21

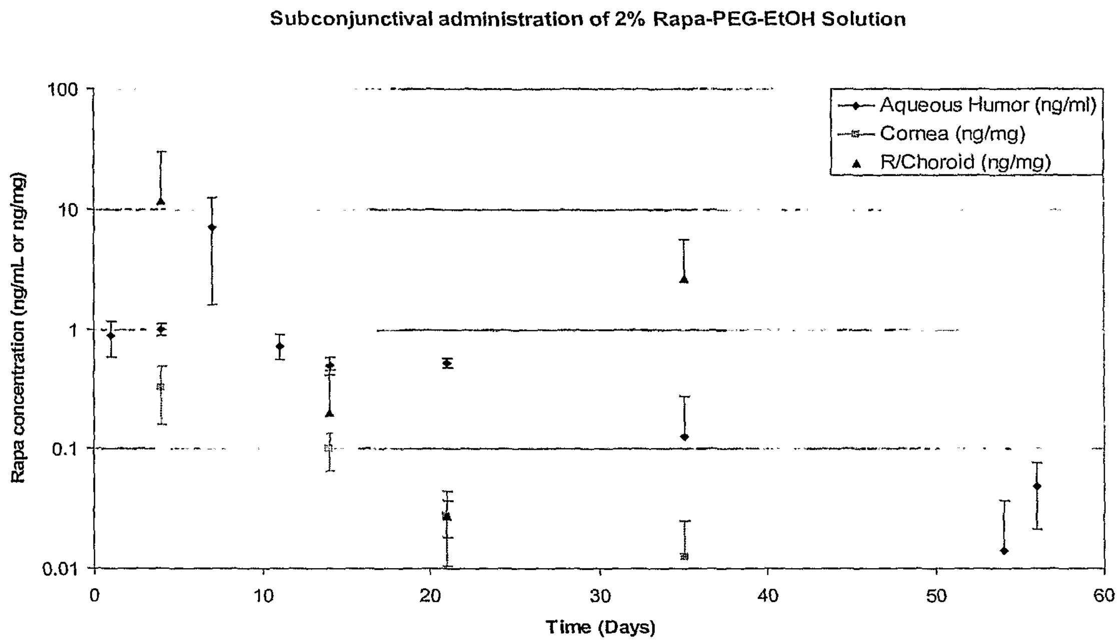

Subconjunctival administration of 2% Rapa-PEG-EtOH Solution
Rapa concentration (ng/mL or ng/mg)
100
10
1
0.1
0.01
0
10
20
30
40
50
60
Time (Days)
Aqueous Humor (ng/ml)
Cornea (ng/mg)
R/Choroid (ng/mg)

Figure 22

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

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- Photodynamic Therapy for Subfoveal Choroidal Neovascularization in Age Related Macular Degeneration with Verteporfin. *Arch Ophthalmol.,* 1999, vol. 117, 1329-1345 **[0008]**
- *Arch. Ophthalmol.,* 1986, vol. 204, 694-701 **[0009]**
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- **SEHGAL et al.** Rapamycin (AY-22,989), A New Antifungal Antibiotic. II. Fermentation, Isolation And Characterization. *J. Antibiot. (Tokyo),* 1975, vol. 28, 727-732 **[0036]**
- **SEHGAL et al.** Demethoxyrapamycin (AY-24,668), A New Antifungal Antibiotic. *J. Antibiot. (Tokyo),* 1983, vol. 36, 351-354 **[0036]**
- **PAIVA et al.** Incorporation Of Acetate, Propionate, And Methionine Into Rapamycin By Streptomycetes hygroscopicus. *J Nat Prod,* 1991, vol. 54, 167-177 **[0036]**
- **NICOLAOU et al.** Total Synthesis Of Rapamycin. *J. Am. Chem. Soc.,* 1993, vol. 115, 4419-4420 **[0036]**
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- **RAGHAVA et al.** Periocular routes for retinal drug delivery. *Expert Opin. Drug Deliv.,* 2004, vol. 1 (1), 99-114 **[0119]**
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- Eye Irritation. **F.N. MARZULLI ; H.I. MAIBACH.** Dermatoxicology. 1977, 579-582 **[0145]**

Szabadalmi igénypontok

1. Terápiás szert és oldószer komponenst tartalmazó folyékony formuláció, ahol a folyékony formuláció egy oldat, amelyet egy alany szemének üvegtestébe adagolunk;
a terápiás szer 2% (tömeg/tömeg) rapamicin; és
az oldószer komponens 4% (tömeg/tömeg) etanol és 94% (tömeg/tömeg) polietilén-glikol 400 keverékét tartalmazza.

2. Az 1. igénypont szerinti folyékony formuláció az alábbiak közül választott betegség vagy állapot kezelésében történő alkalmazásra: diabetikus retinopátia, makula degeneráció, nedves és száraz AMD (életkorral járó makuladegeneráció), koraszülött retinopátia (retrolentális fibroplázia), ideghártya gyulladást vagy érhártya gyulladást okozó fertőzések, feltételezett szemészeti hisztoplazmozis, miópia miatti degeneráció, angioid csíkok, okuláris trauma, pszeudoxantoma elasztikum, véna elzáródás, artéria elzáródás, nyaki verőeret elzáró betegség, sarlósejt anémia, Eales betegség, miópia, krónikus retinaleválás, hiperviszkózitási szindrómák, toxoplazmózis, trauma, polipszerű érhártya vaszkulopátia, lézerkezelés utáni komplikációk, idiopátiás központi savós érhártyaretinopátia komplikációi, érhártya gyulladásos állapotok komplikációi, rubeózis, rubeózissal (érújraképződés a szemzúgban) összefüggő betegségek, érújraképződés okozta glaukóma, uveitisz és krónikus uveitisz, makula ödéma, sejtburjánzással járó retinopátiák és betegségek vagy állapotok, amelyeket a fibrovaszkuláris vagy szálas szövetek abnormális burjánzása okoz, ideértve burjánzásos vitreoretinopátia összes formáját (ideértve az operáció utáni burjánzásos vitreoretinopátiát), akár összefüggésben vannak a diabétesszel, akár nincsenek.

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