

TOPICAL OPHTHALMIC PEPTIDE FORMULATION

FIELD OF THE INVENTION

5 This invention belongs to the field of medicine, and in particular it relates to a pharmaceutical formulation in the form of a topical ophthalmic composition of somatostatin and somatostatin analogues.

BACKGROUND OF THE INVENTION

Diseases of the retina and vitreous body are the principle causes of blindness, and among them are outstanding diabetic retinopathy and age-related macular degeneration.

10 Although there are references in the prior art of the potential use of somatostatin and analogues in the treatment of posterior segment eye diseases, based on the evidence of the overexpression of somatostatin receptors in these disorders, there are no clear examples of therapeutic effect.

The presence of endogenous somatostatin and somatostatin receptors in the different parts of the human eye has recently been described. Therefore, for example, the somatostatin receptors sstr1, sstr2 and sstr5 have been detected in the lacrimal glands, conjunctiva, cornea and nasolacrimal ducts [Minsel *et al.*, *Endocrinology*, 2009, 150(5) : 2254-2263]. In the posterior segment of the eye the presence of genes corresponding to the somatostatin receptors sstr1, sstr2, sstr3, sstr4 and sstr5 in the retina, ciliary body and choroids in healthy human eyes have been described [Klisovic *et al.*, *Investigative Ophthalmology & Visual Science*, 2001, 42(10) : 2193-2201], the presence of sstr2 and sstr3 mRNA in the retina [van Hagen *et al.*, *European Journal of Endocrinology*, 2000, 143 : S43-S51; Cervia *et al.*, *Molecular and Cellular Endocrinology*, 2008, 286 : 112-122] and also the presence of endogenous somatostatin in the vitreous humor and the retina [Simó *et al.*, *Diabetes Care*, 2002, 25(12) : 2282-2286]. It is also known in the prior art that there is a lower expression of somatostatin in the retina in the first phases of diabetic retinopathy, possibly associated to retinal

neurodegeneration [Carrasco et al., *Diabetes Care*, 2007, 30(11) : 1-7].

There are different routes of administration of drugs for the treatment of posterior segment eye diseases such as systemic, topical, intraocular and periocular administration.

Document WO 02/09739 A1 describes the use of somatostatin or an analogue thereof for the treatment or prevention of ocular disorders. Among the routes of administration named in this document, the preferred route is systemic parenteral administration, subcutaneous or intramuscular, and there is no indication in said document of a formulation in the form of eye-drops for the administration of somatostatin or an analogue of it. However, in the case of systemic administration it is difficult to reach therapeutic concentrations in the posterior segment of the eye due to the hemato-retinal barrier, which limits the penetration of medication into the eye. Another general disadvantage of systemic administration is that its effect is not local and it is not directed exclusively to the posterior segment of the eye. Furthermore, in the case of somatostatin, high doses would be necessary due to its instability in blood. In the case of the analogue octreotide, different clinical studies have assessed its effects through systemic subcutaneous administration, even if the results do not show a clear therapeutic effect [Wegewitz et al., *Current Pharmaceutical Design*, 2005, 11 : 2311-2330; Palii et al., *Investigative Ophthalmology & Visual Science*, 2008, 49(11) : 5094-5102].

An alternative to the low availability of the systemic route of administration in the posterior segment of the eye is the local administration in the posterior segment of the eye through intraocular injections and periocular injections such as subconjunctival, sub-Tenon and retrobulbar injections, or subconjunctival implants in this segment [Geroski et al., *Investigative Ophthalmology & Visual Science*, 2000, 41(5) : 961-964 ; Kiagiadaki et al. *Investigative Ophthalmology & Visual Science*, 2008, 49(7) : 3080-3089]. However, these forms of administration are not comfortable for the patient and present secondary effects whose frequency increases if they are carried out repeatedly. Furthermore, in the case of implants, they entail a surgical intervention. Although these type of treatments have shown to be effective in preclinical studies, they require the regular application of injections which in some cases have secondary effects such as retinal detachment, cataracts, rubeosis or endophthalmitis [Geroski et al., *Investigative Ophthalmology & Visual Science*, 2000, 41(5) : 961-964 ; Herrero-Vanrell et al, *Journal of Drug Delivery Science and Technology*, 2007, 17(1) : 11-17 ; Robertson et al., *Journal of Ocular Pharmacology and Therapeutics*, 1997, 13(2) : 171-177].

Another alternative to the previous routes of administration of drugs to the eye is topical ocular administration. However, the topical ocular channel, as a route of administration of medication whose target is in the posterior segment of the eye, has been limited up until now due to the estimation that the percentage of active substance capable of reaching the posterior segment is insignificant in the majority of cases. [Andrés-Guerrero et al., *Archivos de la Sociedad Española de Oftalmología*, 2008, 83 : 683-686].

Within the prior art several options for topical ocular administration of somatostatin and analogues have been mentioned. Therefore, for example, document US 2005/074497 A1 describes a hydrogel which contains an anti-angiogenic drug, such as octreotide, for the treatment of posterior segment eye disease. Octreotide is disclosed in this patent application inside of a vast list of drugs. Furthermore, no example of octreotide or any other drug is described in this document. The hydrogel is placed in contact with the eye by using a contact lens or alternatively it is attached to the eye using adhesives or by surgical intervention. Administration by eye drops is explicitly excluded in this document. However, administration using a contact lens entails the risk of the eye becoming infected by pathogens and additionally, it is not tolerated by patients with increased intraocular pressure.

Another possibility is topical administration in the form of an ointment or unguent in the inner part of the eyelid. However, ointments tend to be uncomfortable and lower visual acuity due to their excessive viscosity and slow absorption.

Document WO 99/24019 A1 also describes a dry, solid formulation made of different drugs, among them somatostatin, which is reconstituted in a liquid solution and is administered as ophthalmic drops. Although this document does not provide any example of a formulation of somatostatin, nor any indication of this or another drug which reaches the posterior segment of the eye applied using eye drops.

On the other hand, document US 5182258 A describes a formulation in the form of eye drops which, through the nasolacrimal system, is used for systemic administration, where the active principle being administered can be somatostatin, among many others. However, nothing is said in this document about the administration of this or another active principle for the treatment of posterior segment eye disease. The same occurs for other formulations in the prior art where octreotide is administered by topical route without a possible application for the treatment and/or

prevention of posterior segment eye diseases being indicated [*Danesi et al., Clinical Cancer Research, 1997, 3 : 265-272 ; Demir et al. Documenta Ophthalmologica, 2003, 107 : 87-92*].

Therefore, there is in the prior art the need to find a form of topical ocular administration of somatostatin or an analogue thereof for the local treatment and/or prevention of posterior segment eye diseases which resolves both the problems of comfort for the patient and low therapeutic availability in a pharmaceutically acceptable formulation.

The object of this invention is a topical ophthalmic composition, a liquid pharmaceutical formulation which is administered topically in the eye, of somatostatin or an analogue thereof, which reaches the posterior segment of the eye, joins the somatostatin receptors present there and which is applied for the treatment and/or prevention of posterior segment eye diseases which benefit from the binding of somatostatin or its analogues to the somatostatin receptors. This invention provides an unprecedented breakthrough in the topical ophthalmic administration of peptides, and particularly, somatostatin or its analogues.

DESCRIPTION OF THE INVENTION

Surprisingly, this invention resolves the previously described problems. In a first aspect, this invention relates to a topical ophthalmic composition which contains a peptide characterized in that a therapeutically effective amount of this peptide reaches the posterior segment of the eye. In a particular embodiment, the topical ophthalmic composition is selected from the group of eye drops, ointments and unguents. In a further aspect, this invention relates to eye drops which contain a peptide characterized in that a therapeutically effective quantity of this peptide reaches the posterior segment of the eye.

In the context of this invention the term "eye drops" relates to a pharmaceutical liquid formulation which is administered in the form of drops on the external surface of the eye and which has a local effect on the posterior segment of the eye.

In the context of this invention the term "posterior segment of the eye" includes choroids, retinal pigment epithelium, retina, macula, fovea, optic nerve and vitreous humor.

In a particular embodiment, the peptide is selected from among somatostatin or a somatostatin analogue, preferably somatostatin or the somatostatin analogue are selected from the group formed by somatostatin-28, somatostatin-14, somatostatin-13, prosomatostatin, octreotide, lanreotide, vapreotide, pasireotide, seglitide, cortistatin and their pharmaceutically acceptable salts.

- 5 In another particular embodiment, somatostatin or the somatostatin analogue is substituted with an acyl group or a polyethylene glycol moiety. Preferably, the acyl group is selected from the group consisting of acetyl, tert-butanoyl, hexanoyl, 2-methylhexanoyl, cyclohexancarboxyl, octanoyl, decanoyl, lauroyl, myristoyl, palmitoyl, stearoyl, oleoyl and linoleoyl. Preferably, the polyethylene glycol moiety has a molecular weight between 200 and 35,000 daltons.

- 10 In another particular embodiment, the concentration of the peptide, preferably somatostatin or a somatostatin analogue, is between 0.1 $\mu\text{g/mL}$ and 100 mg/mL , preferably, between 1 $\mu\text{g/mL}$ and 10 mg/mL , more preferably between 10 $\mu\text{g/mL}$ and 1 mg/mL .

- In another particular embodiment, the pH of the eye drops is between 3 and 8, preferably between 4 and 7. To adjust the pH of the eye drops the acids and/or bases known by the person skilled in the art will be added to reach the previous pH values.
- 15

In another particular embodiment, the eye drop vehicle in the invention is an isotonic aqueous solution, such as the isotonic solution of sodium chloride or boric acid, or is a poly(vinyl alcohol) aqueous solution or mixtures thereof.

- 20 In another particular embodiment, the peptide of the composition of the invention can also be incorporated in liposomes, mixed liposomes, niosomes, ethosomes, nanoparticles, solid lipid nanoparticles, nanostructured lipid carriers, micelles, mixed micelles of surfactants, surfactant-phospholipid mixed micelles, nanospheres, lipospheres and nanocapsules.

- In another particular embodiment, the eye drops of this invention can optionally contain a preservative. A person skilled in the art recognizes the preservative agents of the prior art such as benzalkonium chloride, benzoic acid, alkyl parabens, alkyl benzoates, chlorobutanol, chlorocresol, cetyl alcohols, fatty alcohols such as hexadecyl alcohol, organometallic compounds of mercury
- 25

such as acetate, phenylmercury nitrate or borate, diazolidinyl urea, diisopropyl adipate, dimethyl polysiloxane, salts of EDTA, vitamin E and its mixtures.

5 In another particular embodiment, the eye drops of this invention can optionally contain an agent which increases the permeability of the peptide, preferably of the peptide somatostatin or a somatostatin analogue, to the posterior segment of the eye. Preferably the agent which increases the permeability is selected from the group formed by benzalkonium chloride, saponins, fatty acids, polyoxyethylene fatty ethers, alkyl esters of fatty acids, pyrrolidones, polyvinylpyrrolidone, pyruvic acids, pyroglutamic acids and their mixtures, among others.

10 In a second aspect, this invention relates to the eye drops of this invention for the treatment and/or prevention of posterior segment eye diseases. Preferably, the treatment and/or prevention of the posterior segment eye disease benefit from the binding of somatostatin or its analogues to the somatostatin receptors. More preferably, the posterior segment eye disease are selected from the group formed by non-proliferative diabetic retinopathy, proliferative diabetic retinopathy, age-related macular degeneration, neovascular retinal disease, ischemia-induced retinopathy, retinopathy of
15 prematurity, sickle-cell retinopathy, retinal vein occlusion, retinitis pigmentosa, choroidal neovascularization, uveitis, macular edema, cystoid macular edema. In the case of somatostatin, its greater stability in the vitreous humor of the posterior segment of the eye with regards to its half-life time in serum favors its action at a local level for the treatment and/or prevention of the previously mentioned posterior segment eye diseases.

20 In a third aspect, the present invention relates to a method of treatment and/or prevention of posterior segment eye diseases which comprises the topical administration of a therapeutically effective amount of a peptide by means of a topical ophthalmic composition selected from the group consisting of eye drops, ointments and unguents.

25 In a particular embodiment, the peptide is selected from among somatostatin or a somatostatin analogue, preferably somatostatin or the somatostatin analogue are selected from the group formed by somatostatin-28, somatostatin-14, somatostatin-13, prosomatostatin, octreotide, lanreotide, vapreotide, pasireotide, seglitide, cortistatin and their pharmaceutically acceptable salts.

In another particular embodiment, the frequency of administration can vary greatly, depending on

the needs of each subject and the severity of the disease to be treated or prevented, with a recommendation of an administration range from once a week to ten times a day, preferably from three times a week to three times a day, even more preferably once or twice a day.

In another particular embodiment, the method of treatment and/or prevention additionally comprises the administration of another therapeutic agent for the treatment and/or prevention of posterior segment eye diseases. Therapeutic agents for the treatment and/or prevention of the posterior segment eye diseases are for example, and in a non-limiting sense, those agents selected from the group of anti-Vascular Endothelial Growth Factors (VEGF), prostaglandin analogs, beta-adrenergic receptor antagonists, alpha-2-adrenergic agonists, carbonic anhydrase inhibitors, miotic agents, monoclonal antibodies, corticosteroids, glucocorticoid, kinase inhibitors, cycloplegics or antimetabolites. The administration of these therapeutic agents for the treatment and/or prevention of posterior segment eye diseases can be by topical, oral or parenteral route. In the context of this invention, the term "parenteral" includes intravitreous, intraocular, intracorneal, subcutaneous, intradermal, intravascular injections, such as intravenous, intramuscular and any another similar injection or infusion technique.

The following specific examples given here serve to illustrate the nature of this invention. These examples are included solely for illustrative purposes and should not be interpreted as limitations to the invention claimed herein.

DESCRIPTION OF THE FIGURES

Figure 1: Figure 1 shows the retinas of 2 mice (1 and 2) who received somatostatin in the right eye (+) and placebo in the left eye (-)

Figure 2: Figure 2 shows the difference between the amplitude of the second OP in control rats (Group 1); diabetic rats (Group 2); diabetic rats treated with somatostatin (Group 3) and diabetic rats treated with octreotide (Group 4)

Figure 3: Figure 3 shows the confocal microscopy images. Confocal GFAP immunohistochemistry 40X. A much higher GFAP expression (brightest labeling) was observed in the ganglion cell layer of

STZ-induced diabetic rats (Group 2). Treatment with somatostatin (Group3) or Octreotide (Group 4) prevented glial cell activation that correlated with GFAP levels similar to the control (Group1)

Figure 4: Figure 4 shows the confocal microscopy images. Confocal images of TUNEL 40X. Retinal degeneration correlated with a higher number of apoptotic cells (brightest labeling means TUNEL positive) specially in the ONL observed in STZ-induced diabetic rats (Group 2). Treatment with somatostatin (Group3) and octreotide (Group4) prevented retinal degeneration and showed a similar number of cells that found in healthy control rats (Group 1).

EXAMPLES

Example 1: Eye drops which contain somatostatin-14.

10 A pharmaceutical composition of somatostatin was prepared in the form of eye drops by adding 125 μ L of 0.9% sodium chloride to 5 mg of somatostatin-14.

Example 2: Evaluation of the concentration of topical ophthalmic somatostatin in the retina.

Eight-week old C57BL/6 mice were used. The handling and care of the animals was carried out following the Institut de Recerca del Hospital Universitari Vall d'Hebron protocol and the European Economic Community and A.R.V.O. (Association for Research in Vision and Ophthalmology) international guidelines (order 86/609/CEE). The animals were kept in individual cages in the animal facilities at a controlled temperature (20°) and humidity (60%), under constant cycles of light-darkness which last 12 hours and had free access to food and drink. The eye drops of somatostatin-14 from example 1 (1 drop of 5 μ L) were administered to the right eye and the placebo (saline solution) to the left eye. Two drops were administered per day, one in the morning and another in the evening, for 5 days. The animals were euthanized by cervical dislocation under anesthetic 2 hours after the last dosage. Immediately afterwards the eyeballs were extracted and were frozen at -80° until handling. The posterior segment (retina and vitreous humor) was separated from the rest of the eyeball by dissecting it under a magnifying glass. The quantity of somatostatin-14 in the retina was determined by Western blot. The proteins were extracted with the solution 50 mM Tris-HCl, pH 7.9, 300 mM KCl, 1.5 mM MgCl₂, 0.1% Nonidet P-40 and 20% glycerol

which contained Complete™ protease inhibitor cocktail at 4° C for 12 hours. Subsequently the samples were centrifuged (12,000 rpm at 4° C) for 10 min. A Western blot was carried out on the proteins with rabbit antibodies against somatostatin-14 (ab53165, abcam). To standardize the concentration of somatostatin-14 obtained α -actin was used as a control. The specific antibody-antigen complexes were identified using goat antibodies against rabbit IgG marked with HRP or rabbit antibodies against goat IgG together with chemiluminescent substrates (Pierce Biotechnology Inc., Rockford, Illinois, USA) by exposure on radiographic films. A densitometric analysis was carried out (Densitometer GS-800, Bio-Rad Laboratories, Hercules, California, USA) and the results are expressed as arbitrary units. Figure 1 shows the retinas of 2 mice (1 and 2) who received somatostatin in the right eye (+) and placebo in the left eye (-). As can be seen the amount of somatostatin was greater in the treated eye on both animals. On control mice, who received the placebo in both eyes, no differences were detected in the concentration of somatostatin between both eyes.

Example 3: Stability of somatostatin in human vitreous humor and human serum.

Somatostatin was incubated with human serum or human vitreous humor at 90% at 37°C. Aliquots were extracted at different incubation times. Acetonitrile was added to precipitate the serum proteins, it was centrifuged and the supernatant was filtered and injected into the RP-HPLC (Gradient: 20-80% B in 30 min, B=0.07%TFA in acetonitrile). The disappearance of the initial product was analyzed using the area corresponding to the initial product and the half-life time was calculated. The half-life time of somatostatin is 2.7 hours in serum and 64 hours in vitreous humor.

Example 4: Eye drops which contain somatostatin-14.

A pharmaceutical composition of somatostatin was prepared in the form of eye drops by adding 1 mL of 0.9% sodium chloride aqueous solution to 10 mg of somatostatin-14. Finally, the solution was filtered through a 0.22 μ m sterilizing filter.

Example 5: Eye drops which contain octreotide.

A pharmaceutical composition of octreotide was prepared in the form of eye drops by adding 1 mL

of 0.9% sodium chloride aqueous solution to 10 mg of octreotide. Finally, the solution was filtered through a 0.22 μm sterilizing filter.

Example 6: Eye drops which contain vapreotide.

5 A pharmaceutical composition of vapreotide was prepared in the form of eye drops by adding 1 mL of 0.9% sodium chloride aqueous solution to 10 mg of vapreotide. Finally, the solution was filtered through a 0.22 μm sterilizing filter.

Example 7: Eye drops which contain cortistatin.

10 A pharmaceutical composition of cortistatin was prepared in the form of eye drops by adding 1 mL of 0.9% sodium chloride aqueous solution to 10 mg of cortistatin. Finally, the solution was filtered through a 0.22 μm sterilizing filter.

Example 8: Eye drops which contain somatostatin-14.

A pharmaceutical composition of somatostatin was prepared in the form of eye drops by adding 1 mL of 0.9% sodium chloride and 1.4% poly(vinyl alcohol) aqueous solution to 0.27 mg of somatostatin-14. Finally, the solution was filtered through a 0.22 μm sterilizing filter.

15 *Example 9: Eye drops which contain somatostatin-28.*

A pharmaceutical composition of somatostatin was prepared in the form of eye drops by adding 1 mL of 0.9% sodium chloride aqueous solution to 0.05 mg of somatostatin-28. Finally, the solution was filtered through a 0.22 μm sterilizing filter.

20 *Example 10: Eye drops which contain somatostatin-14, hydroxybenzoates and polyoxyethylene fatty ethers.*

A pharmaceutical composition of somatostatin was prepared in the form of eye drops by adding 1 mL of 0.9% sodium chloride aqueous solution to 50 μ g of methyl 4-hydroxybenzoate, 100 μ g of propyl 4-hydroxybenzoate, 50 μ g of polyoxyethylene-20-stearyl ether (Brij-78) and 1 mg of somatostatin-14. Finally, the solution was filtered through a 0.22 μ m sterilizing filter.

5 *Example 11: Eye drops which contain liposomes of somatostatin-14.*

A pharmaceutical composition of somatostatin was prepared in 0.9% sodium chloride containing 20 mg of phosphatidyl choline and 2.7 mg of somatostatin-14 per mL. Briefly, phosphatidyl choline was slowly added under stirring to the 0.9% sodium chloride aqueous solution containing somatostatin-14. The composition was further stirred for 15 minutes until a liposome suspension
10 was formed. Then liposomes were stepwise extruded through a polycarbonate filter with a 400 nm pore size, a 200 nm pore size filter and eventually ten times with a 100 nm pore size filter. Finally, the liposomal solution was filtered through a 0.22 μ m sterilizing filter.

Example 12: Eye drops which contain nanocapsules of somatostatin-14.

A 10% solution of poly lactic-co-glycolic acid (50:50) copolymer molecular weight 5000, 1g, in dichloromethane was prepared. Somatostatin-14 (100mg) was dissolved in 1mL of water. The somatostatin solution was added to the polymer solution and was emulsified using one ultrasound probe. Then, the nanoemulsion was filtered through a 0.22 μ m sterilizing filter. The resulting nanoemulsion was then dispersed in poly (vinylalcohol) 1% aqueous solution using ultrasound probe to reduce particle size until a nanoemulsion was obtained. The nanocapsules were extracted
15 in a 40% ethanol aqueous solution and they were purified by tangential flow ultrafiltration.

Finally, 70 mg of nanocapsules containing somatostatin (16 mg) were dispersed in 100 mL poly(vinyl alcohol) 1.4% aqueous solution.

Example 13: Ophthalmic ointment which contains somatostatin-14.

A pharmaceutical composition of somatostatin was prepared in the form of ophthalmic ointment by
25 adding 50 mL of white petrolatum USP to 200 mg of somatostatin-14.

Example 14: Effect of a topical ocular treatment with somatostatin and octreotide in the prevention of retinal neurodegeneration in diabetic rats.

Streptozotocin (STZ), 60 mg/Kg, was administered to Sprague Dawley rats. The animals were divided in four groups as detailed in Table 1:

Group /Identification	STZ Dose Level (mg/kg)	Treatment dose		
		Drops in Right eye per day	Drops in Left eye per day	
1/ Vehicle	0	1	2	4
2/ STZ + Vehicle	60	1	2	4
3/ STZ + Somatostatin	60	1	2	8
4/ STZ + Octreotide	60	1	2	8

(Vehicle =aqueous solution 0.9% NaCl)

Table 1: Study design

Two days after STZ was administered, animals were treated daily with one or two drops of vehicle (Group 2), eye drops of example 4 (Group 3) or eye drops of example 5 (Group 4) for additionally 14 days. Healthy control animals were only treated daily with one or two drops of vehicle (Group1).

Electroretinography is a technique used clinically to evaluate retinal function in diabetic retinopathy. Several parameters can be affected in early stages of the disease: implicit time, amplitude and oscillatory potentials [Tzekov *et al.*, *Survey of Ophthalmology*, 1999, 44(1) : 53-60]. The increment of the implicit time and diminishment of amplitude of the oscillatory potentials (OPs) are also observed in streptozotocin-induced diabetic rats [Hancok *et al.*, *Investigative Ophthalmology & Visual Science*, 2004, 45(3) : 1002-1008].

Electroretinography results showed that after 14 days of treatment, the implicit time of the b-wave at 0 db significantly raised compared to pretreatment in the diabetic group (Group 2) (15,5 Right eye ; 15,2 Left eye) and did not suffer a significant increased in control group (Group 1) (-2,3 Right eye; -7,5 Left eye) and groups treated with somatostatin composition of example 4 (Group 3) (9,0 Right eye; 4,9 Left eye) or octreotide composition of example 5 (Group 4) (8,8 Right eye; 1,1 Left eye).

Concerning amplitude, somatostatin prevented the diminishment of the b-wave amplitude at -30 db, -10 db and 0 db compared to the diabetic group and octreotide prevented the diminishment of the b-wave amplitude at -30 db but was effective only at the highest dose at -10 db and 0 db.

The analysis of the oscillatory potentials (OP) of the b-wave at 0 db indicates that somatostatin and octreotide presented a positive effect preventing the diminishment of the amplitude of the second OP (OP2). This effect was already observed on Day 8 and conserved till Day 14. Figure 2 shows the difference between the amplitude of the second OP in control rats (Group 1: 17,0 Right eye; 15,9 Left eye); diabetic rats (Group 2: -2,5 Right eye; -1,2 Left eye); diabetic rats treated with somatostatin (Group 3: 44,8 Right eye; 68,7 Left eye) and diabetic rats treated with octreotide (Group 4: 45,5 Right eye; 55,1 Left eye).

On day 14, the animals were sacrificed and ocular tissues paraffined. Glial activation and number of apoptotic cells were evaluated as markers of the first step in the development of diabetic retinopathy: retinal neurodegeneration [Carrasco *et al.*, *Diabetes Care*, 2007, 30(11): 1-7].

Glial activation was determined analysing Glial Fibrillar Protein (GFAP) immunofluorescence by confocal microscopy. Ocular sections of 7 μm thickness were fixed on high adherent coverslips (Visionbiosystems, Newcastle Upon Tyne, UK). They were desparaffined, rehydrated and washed in phosphate buffer saline (PBS). Unspecific unions were blocked incubating the samples for 1h in PBS 1% BSA (Bovine Serum Albumin) 0.5% Triton X-100. Thereafter, the primary rabbit anti-human GFAP antibody (Sigma, Madrid, Spain) diluted in the blocking buffer (1:100) was incubated for 36 h at 4°C. After three washings for 5 min with PBS, sections were incubated with a secondary antibody anti IgG human labelled with Alexa Fluor® 488 (Invitrogen, Eugene, OR) for 1h at room temperature. Labelled sections were washed and mounted with fluorescent medium containing 4,6-diamidino-2-phenylindole (DAPI) for staining cell nuclei (Vector Laboratories, Burlingame, CA). The sections positives for GFAP were captured in a confocal microscope (FV1000, Olympus, Hamburg, Germany) obtaining optical sections with a 488 nm laser for Alexa 488 and a 405 nm laser for DAPI. GFAP fluorescence was quantified in each image with the Fluoview ASW 1.4 software (Olympus, Hamburg, Germany). Results were normalized to the analysed area (21705.33 μm^2). Figure 3 shows the confocal microscopy images. A higher GFAP expression (brightest labeling) was observed in the ganglion cell layer of STZ-induced diabetic rats (Group 2). Treatment with somatostatin (Group3) or Octreotide (Group 4) prevented glial cell activation that correlated with GFAP levels similar to the control (Group1).

The efficacy of the topical ocular treatment with somatostatin (Group 3) and octreotide (Group 4) in the prevention of glial activation provoked by diabetes was determined by GFAP fluorescence values, indicator of glial activation. GFAP fluorescence value was 2095 ± 26 for healthy control Group 1; 6871 ± 159 for Group 2; 2514 ± 90 for Group 3 and 1696 ± 48 for Group 4.

The evaluation of the degree of apoptosis of the retina after 14 days of treatment was done by

using the TUNEL ("Terminal Transferase dUTP Nick-End Labeling") technique. The In Situ Cell Death Detection Kit (Roche Diagnostics, Mannheim, Germany) was used. Ocular sections were desparaffined, rehydrated and washed in PBS. Three confocal images (40X) corresponding to a 317,13 μm X 317,13 μm surface were obtained for each section. The total number of nuclei and nuclei positive for TUNEL were quantified with the Image J software (<http://rsbweb.nih.gov/ij>). Staining with propidium iodide (PI) was done for examining the nucleus morphology and discard false positives. Figure 4 shows the confocal microscopy images. Retinal degeneration correlated with a higher number of apoptotic cells (brightest labeling means TUNEL positive) observed in streptozotocin-induced diabetic rats (Group 2). Treatment with somatostatin (Group 3) and octreotide (Group 4) prevented retinal degeneration and showed a similar number of cells that found in healthy control rats (Group 1).

Table 2 shows the percentage of apoptotic cells respect to the total number of cells in different retinal layers such as retinal pigment epithelium (RPE), outer nuclear layer (ONL), inner nuclear layer (INL) and ganglion cell layer (GCL). The increment in the percentage of apoptotic cells is significant in diabetic rats (Group 2). Topical ocular treatment with somatostatin (Group 3) or octreotide (Group 4) reduced the number of apoptotic cells till control levels (Group 1).

% Apoptotic cells				
	RPE	ONL	INL	GCL
Group 1	6	2	0	9
Group 2	28	10	4	35
Group 3	6	3	1	14
Group 4	11	5	0.1	7

Table 2: Percentage of apoptotic cells

Neurodegeneration of retinal cells is one of the first steps in the development of diabetic retinopathy. The results of electroretinography, glial activation and apoptosis indicated that topical ocular administration of somatostatin or octreotide for 14 days prevented retinal degeneration in streptozotocin-induced diabetic rats.

CLAIMS

1. Topical ophthalmic composition selected from the group of eye drops, ointments and unguents which contains a peptide, characterized in that a therapeutically effective amount of this peptide reaches the posterior segment of the eye.
- 5 2. A topical ophthalmic composition according to claim 1, said ophthalmic composition being an eye drop composition.
3. A topical ophthalmic composition according to any of claims 1 and 2, characterized in that said peptide is selected from somatostatin or a somatostatin analogue.
4. A topical ophthalmic composition according to claim 3, characterized in that said somatostatin or
10 somatostatin analogue is selected from the group formed by somatostatin-28, somatostatin-14, somatostatin-13, prosomatostatin, octreotide, lanreotide, vapreotide, pasireotide, seglitide, cortistatin and their pharmaceutically acceptable salts.
5. A topical ophthalmic composition according to claim 3, characterized in that said somatostatin or somatostatin analogue is substituted with an acyl group or a polyethylene glycol moiety.
- 15 6. A topical ophthalmic composition according to any of claims 1 or 2, characterized in that the concentration of said peptide ranges between 0.1 $\mu\text{g/mL}$ and 100 mg/mL.
7. A topical ophthalmic composition according to claim 6, characterized in that said concentration ranges between 1 $\mu\text{g/mL}$ and 10 mg/mL.
8. A topical ophthalmic composition according to claim 2, characterized in that the pH of the eye
20 drops is between 3 and 8.

9. A Topical ophthalmic composition according to claim 2, characterized in that the eye drop vehicle is an isotonic aqueous solution, a poly(vinyl alcohol) aqueous solution or mixtures thereof.

10. A Topical ophthalmic composition according to any of claims 1 or 2, said ophthalmic composition comprising a preservative.

5 11. A topical ophthalmic composition according to any of claims 1 or 2, said ophthalmic composition comprising an agent that increases the permeability of this peptide.

12. A topical ophthalmic composition according to any of claims 1 or 2, for the treatment and/or prevention of posterior segment eye diseases.

10 13. A topical ophthalmic composition according to claim 12, characterized in that said treatment and/or prevention benefits from the binding of somatostatin or somatostatin analogue to the somatostatin receptors.

15 14. A topical ophthalmic composition according to claim 12, characterized in that the posterior segment eye disease is selected from the group formed by nonproliferative diabetic retinopathy, proliferative diabetic retinopathy, age-related macular degeneration, retinal neovascularization, ischemia-induced retinopathy, retinopathy of prematurity, sickle-cell retinopathy, retinal vein occlusion, retinitis pigmentosa, choroidal neovascularization, uveitis, macular edema, cystoid macular edema.