COMPOUND FOR THE TREATMENT OF DISORDERS OR PATHOLOGIES OF THE OCULAR SURFACE

The present invention relates to the compound GSH-C4 or a pharmaceutically acceptable salt thereof as well as a composition comprising said compound, for use in the prevention and/or treatment of ocular disorders and/or pathologies, in particular dry eye syndrome, ocular surface disease (OSD), and ocular pathologies aggravated by the chronic use of drugs that induce oxidative stress and inflammation.

STATE OF THE TECHNOLOGY

According to the definition proposed by the *European Glaucoma Society* (EGS), open-angle glaucoma is a "chronic and progressive condition capable of causing irreversible loss of retinal ganglion cells, resulting in thinning of the retinal nerve fiber layer and loss of visual field".

The therapeutic strategies currently available for the management of patients with glaucoma can only delay the progression of the disease. Among these, the use of topical hypotensives represents the first line of therapy. However, the active ingredients, preservatives and excipients contained in these formulations can together cause the destruction of cellular structures, leading to alterations and instability of the tear film.

This vicious cycle underlies the condition known as glaucoma-related ocular surface disease (G-OSD), which, although underdiagnosed, is believed to be a comorbidity in 40-59% of glaucoma patients worldwide.

In particular, a large number of clinical and experimental studies have documented a strong correlation between the signs and symptoms of dry eye syndrome, with the number of drugs used for the treatment of glaucoma and/or lowering of intraocular pressure and, in particular, with the cumulative exposure to preservatives such as benzalkonium chloride (BAK).

Recently, Sedlak et al. demonstrated that the inflammatory state induced by the use of topical hypotensive agents (prostaglandin F2α analogues) in patients with ocular hypertension and glaucoma is associated with increased local oxidative stress, including a reduction in the activity of the enzyme glutathione peroxidase (*Sedlak L, Zych M, Wojnar W, Wygledowska-Promienska D. Effect of Topical Prostaglandin F2alpha Analogs on Selected Oxidative Stress Parameters in the Tear Film. Medicina (Kaunas). 2019;55*).

To counteract the above-mentioned drawbacks with reference to the use of conventional topical hypotensive drugs, the first line of treatment for dry eye syndrome involves the instillation of hyaluronic acid-based, preservative-free eyewashes in those

subjects who need them. However, although hyaluronic acid, thanks to its osmotic properties, is able to increase the hydration layer of the tear film, compositions containing this compound as the only active ingredient are often insufficient to interrupt the dry eye cycle.

In light of the above, the need to identify effective solutions to prevent or combat ocular disorders or pathologies such as dry eye syndrome and glaucoma-related ocular surface disease still appears to be very much felt in the sector.

SUMMARY OF THE INVENTION

The authors of the present invention have discovered that the GSH derivative, the compound butanoyl glutathione or GSH-C4, or a pharmaceutically acceptable salt thereof, can be used surprisingly effectively in the prevention, treatment or adjuvant treatment of ocular disorders or pathologies such as dry eye syndrome and glaucoma-related ocular surface disease (OSD), particularly when administered in solution at a concentration (w/w) of 0.1 to 1.5%; optionally in combination with 0.01-0.3% (w/w) hyaluronic acid.

As clearly illustrated by the results of the experimental tests conducted by the authors of the invention and reported in the present application, the compositions comprising GSH-C4 have proven to be surprisingly effective in the treatment of glaucoma patients subjected to topical hypotensive therapy with at least one prostaglandin analogue and having a diagnosis of dry eye, showing a significant action in improving tear breakup time and a positive impact on the corneal and conjunctival epithelium.

As reported in Example 2 of the present application, the authors of the invention surprisingly found, in parallel with the clinical improvement, a post-treatment accumulation of cytokines in the tears collected from the subjects under study. Without wishing to be tied to theory, this observation suggests that a potential mechanism of action able to explain the positive impact of glutathione GSH-C4 in the treatment of the tested patients, and in particular in the treatment of meibomian gland dysfunction (MGD) in the aforementioned patients, may consist, quite unexpectedly, in the induction of a low-grade local inflammation that promotes the wound healing process and corneal re-epithelialization.

Cytokines are known to play a role in activating inflammatory processes that support repair. Among the cytokines that are found to be modulated by the glutathione GSH-C4 enriched compositions tested by the authors of the invention is IL-33. IL-33 has been reported in the literature to be able to increase corneal tissue after epithelial

damage, promoting the number and function of corneal ILC2 in the healing process. Furthermore, as recognized in other contexts, for example at the skin level, the IL-33/ST2 axis acts on a broad range of innate immune cells and promotes macrophages towards a repair phenotype. Furthermore, the IL-33/ST2 axis is classified as an "alarm" for its ability to trigger both adaptive and innate immune defenses in cellular damage.

In support of the hypothesis of the proposed activation mechanism, linked to epithelial repair and protection from the oxidation state in conditions of cellular stress, in vitro tests were performed on primary human corneal cells cultured in the presence of artificial tears containing hyaluronic acid and GSH-C4, individually or added, using a model of epithelial injury and cellular oxidative stress induction. In line with the results obtained with the clinical trial, the results demonstrate the ability of the preparation combined with GSH-C4 to promote repair and/or the reduction of cellular oxidative stress precisely in the simulated conditions of stress induced by wounds and exposure to pro-oxidant molecules. These results, although preliminary, would support the therapeutic effect of the use of artificial tears containing hyaluronic acid and GSH-C4 precisely in those ocular pathologies already known for their characteristics characterized by oxidative and inflammatory stress, such as dry eye and glaucoma, characteristics dependent on their etiological nature aggravated by the concomitant and indispensable use of drugs that would determine a further accumulation of oxidative stress and inflammation, and which for these reasons benefit from the use of the composition of the invention.

Unlike cysteine, which freely enters cells and undergoes oxidation processes, becoming toxic and activating inflammatory responses, or reduced glutathione, which is unable to penetrate the cell membrane, GSH-C4 is also able to promote the synthesis of glutathione (GSH) at the intracellular level, is able to restore the cellular redox state and is expelled from the cells once oxidized. Extracellular GSH-C4 can be a substrate for the enzyme gamma-glutamyl transpeptidase (γ-glutamyl transferase, GGT) which transfers the glutamate portion to an acceptor amino acid that releases the dipeptide cysteinylglycine (Gys- Gly) , which is further cleaved by membrane-bound dipeptidases. The amino acids thus released are taken up and used by cells for the intracellular synthesis of GSH.

This offers the advantage of restoring GSH levels surprisingly more effectively in those subjects undergoing anti-glaucoma therapy with topical hypotensive drugs, for whom the concentration of thiol-containing compounds on the ocular surface is reduced due to such therapies.

Therefore, objects of the present invention are:

- The compound GSH-C4 or a pharmaceutically acceptable salt thereof for use in the prevention and/or treatment or as an adjuvant to the treatment of ocular disorders and/or pathologies, in particular ocular surface disorders and/or pathologies; and
- A composition comprising GSH-C4 or a pharmaceutically acceptable salt thereof and one or more excipients and/or carriers and/or diluents, for use in the prevention and/or treatment or as an adjuvant to the treatment of ocular disorders and/or pathologies, in particular ocular surface disorders and/or pathologies.

DETAILED DESCRIPTION OF THE FIGURES

Figure 1. Schematic representation of the study design described in the Examples and its timelines.

Figure 2. Longitudinal change in OSDI score in Group 1 ("placebo first") and Group 2 ("glutathione first") tested.

Figure 3. Longitudinal change in the Faces score in Group 1 ("placebo first") and Group 2 ("glutathione first") tested.

Figure 4. Longitudinal change in Schirmer test values in Group 1 ("placebo first") and Group 2 ("glutathione first") tested.

Figure 5. Longitudinal change in tear film break *up time (*TBUT) in Group 1 ("placebo first") and Group 2 ("glutathione first") tested.

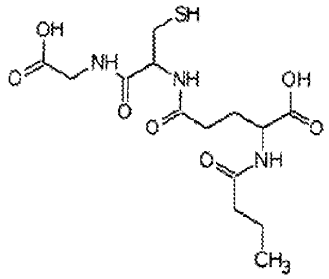
Figure 6. Longitudinal change in *National Eye Institute* staining score (NEI-SS) values in Group 1 ("placebo first") and Group 2 ("glutathione first") tested.

DETAILED DESCRIPTION

GLOSSARY

The terms used in this description, unless otherwise indicated, have the meaning commonly used in the state of the art.

In the present description and in the claims the term GSH-C4 alias butanoyl glutathione or n-butanoyl γ-glutamylcysteinylglycine (also known and denoted herein by INCI: butyroyl glutathione), indicates the molecule or compound having the formula:



wherein R is H,

and can be prepared as described in Example 2 of patent application WO2005063795 and/or as described in the examples of patent application WO2019102397A1.

The term “pharmaceutically acceptable salt” in reference to the GSH-C4 compound of the present invention is to be understood as including any salt of the GSH-C4 compound known in the art that is non-toxic or physiologically acceptable, e.g. alkali metal salts (e.g. sodium and potassium).

The term “Hyaluronic acid” has the meaning commonly used in literature and defines a natural polysaccharide, formed by a linear chain of glucuronic acid particles and N-acetylglucosamine. It has anti-inflammatory, mucoregulatory, anti-edema and restructuring functions and also performs a bacteriostatic function through the creation of a protective film on the mucosa, hinders the penetration of pathogenic agents and reduces the proliferation of pre-existing ones.

Hyaluronic acid has excellent viscoelasticity, high moisture retention capacity, high biocompatibility and hygroscopic properties (Gupta, 2019). In aqueous solution, hyaluronic acid is negatively charged and forms salts generally referred to as hyaluronan or hyaluronate (Laurent, 1989), which are highly hydrophilic and, consequently, surrounded by water molecules. In particular, low molecular weight sodium hyaluronate is able to hydrate and can reach a level of water mobilization up to a thousand times its own weight. Its physicochemical characteristics allow the aggregation of polymer chains with the formation of an extended network (Scott, 1991). Due to the rigidity of the molecule and its ability to attract water, hyaluronate occupies a large volume, forming a gel, resisting mechanical pressure. With increasing molecular weights (MW) and concentration, hyaluronic acid networks are strengthened and, as a result, hyaluronic acid solutions exhibit progressively increased viscosity and viscoelasticity (Kobayashi, 1994). Hyaluronic acid solutions are characterized by non-Newtonian, shear-thinning (pseudoplastic), and viscoelastic behavior. This unique rheological behavior is peculiar

and extremely important, as it determines many physiological roles and pharmaceutical, medical, food, and cosmetic applications of *hyaluronan* (Fallacara, 2018).

In this specification and the claims, the terms "glaucoma-related" and "glaucoma-associated" may be used interchangeably in the context of the invention.

In the context of the present invention, the term "glaucoma-related ocular surface disease" (also abbreviated herein as G-OSD) and "glaucoma-related dry eye syndrome" mean, respectively, ocular surface disease (OSD) and dry eye syndrome that are associated with or caused by therapy for the treatment of glaucoma and/or for the treatment of ocular hypertension in a subject with glaucoma and/or ocular hypertension, in particular by therapy for the treatment of increased intraocular pressure (or IOP).

In a preferred embodiment according to the invention, the expressions "glaucoma-related OSD" and "glaucoma-related dry eye syndrome" refer respectively to OSD and dry eye syndrome that are caused by an anti-glaucoma therapy that involves the administration to a subject with glaucoma and/or ocular hypertension of one or more drugs for the treatment of glaucoma and/or ocular hypertension, in particular drugs for lowering intraocular pressure through topical hypotensive therapy, for example a prostaglandin analogue.

As previously mentioned, a large number of clinical and experimental studies have documented a strong correlation between the signs and symptoms of OSD, and in particular dry eye syndrome, with the number of drugs used for the treatment of glaucoma and/or lowering of intraocular pressure and, in particular, with the cumulative exposure to preservatives such as benzalkonium chloride (BAK) present in such formulations. In fact, the active ingredients, preservatives and excipients contained in the topical hypotensive formulations commonly used for the treatment of glaucoma and/or ocular hypertension can together cause the destruction of cellular structures, leading to alterations and instability of the tear film.

The terms "glaucomatous subject" and "subject affected by glaucoma" may be used interchangeably in the context of the present invention. In a preferred aspect according to the present invention, the term "subject" means a mammal, in particular a human subject.

The term "anti-glaucoma therapy" is used in the context of the present invention to indicate a therapy for the treatment of glaucoma, i.e. a therapy aimed at eliminating or curing said pathology or at reducing and/or eliminating one or more symptoms or disorders related to it.

The terms "dry eye syndrome" and "dry eye disease" may be used interchangeably in the context of the present invention.

According to a preferred aspect of the invention, “therapeutically effective amount” means an amount of GSH-C4 compound or a composition comprising it according to any of the variants described herein, which allows to obtain an improvement or increase in tear break-up time at the site of application of the compound or composition at the ocular level.

In the context of the present invention, the expression “carriers, diluents and/or excipients suitable for the preparation of ophthalmic compositions” means carriers, diluents and/or excipients tolerated by the eye.

The term “assist in the treatment of an ocular disorder or pathology” refers, in the context of the present invention, to the ability of GSH-C4 or a composition comprising it according to any of the variations described herein, to be combined with a drug or a basic therapy for the treatment of said disorder or pathology, to reinforce or complement its action.

Throughout this specification and the claims, the term “comprising” may be replaced by “consisting of”.

DESCRIPTION

The present invention relates to the compound butanoyl glutathione (GSH-C4) or a pharmaceutically acceptable salt thereof for use in the prevention and/or treatment or adjuvant treatment of ocular disorders and/or pathologies, in particular ocular surface disorders and/or pathologies. The invention particularly provides the compound GSH-C4 or a pharmaceutically acceptable salt thereof for use in protecting the ocular surface from oxidative stress, dryness and/or inflammation in a subject in need thereof.

In one embodiment according to the present invention, said ocular disorders and/or pathologies are selected from dry eye syndrome, ocular surface disease (also referred to herein as OSD), and disorders or symptoms associated therewith, and ocular pathologies aggravated by the chronic use of drugs that induce oxidative stress and inflammation.

In a preferred embodiment according to the invention, said ocular disorders and/or pathologies comprise or are represented by glaucoma-related dry eye syndrome, glaucoma-related ocular surface disease (also referred to herein as G-OSD), and/or disorders or symptoms associated therewith.

As anyone in the field knows, dry eye syndrome or disease is a pathology caused by chronic dehydration of the conjunctiva and cornea, which causes irritation. It is mainly due to a quantitative reduction or qualitative alteration of the tear film, which covers the eye and, normally, lubricates and protects it. In particular, Dry eye syndrome can be

caused by either reduced tear production or increased tear evaporation or by both forms intersecting or alternating.

As is also known to those skilled in the art, OSD, and in particular G-OSD, includes or is associated with a group of disorders such as ocular *discomfort,* tear film instability, meibomian gland dysfunction, conjunctival inflammation, subconjunctival fibrosis, epithelial damage and allergic blepharitis, with dry eye syndrome being the most common cause of the disease.

Meibomian gland dysfunction or meibomitis (also abbreviated herein as MGD) specifically refers to a blockage or other abnormality in these glands that do not secrete enough lipids into the tears. Because the tear film on the surface of the eye evaporates too quickly, MGD is also often associated with dry eyes.

Non-limiting examples of disorders or symptoms that may be associated with dry eye syndrome and/or OSD, particularly glaucoma-related dry eye syndrome and/or OSD, include ocular burning, itching, irritation, foreign body sensation, blurred vision, marked sensitivity to light, ocular *discomfort,* tear film instability, meibomian gland dysfunction, conjunctival inflammation, subconjunctival fibrosis, epithelial damage, and allergic blepharitis.

Non-limiting examples of ocular conditions aggravated by chronic use of drugs that induce oxidative stress and inflammation include meibomian gland dysfunction, drug-induced toxic keratopathy, chronic blepharitis, neurotrophic keratitis, chronic drug-use keratitis, exposure keratopathy, chronic allergic conjunctivitis, chronic toxic conjunctivitis, ocular cicatricial pemphigoid, glaucoma-related ocular surface disease (G-OSD), ischemic optic neuropathy, exfoliative and pigmentary glaucoma, age-related macular degeneration, diabetic retinopathy, retinal drug toxicity, cystoid macular edema, chronic uveitis, drug-induced uveitis syndrome, neurogenic dry eye syndrome, and toxic optic neuropathies .

Therefore, in one aspect of the invention, the GSH-C4 compound or a pharmaceutically acceptable salt thereof may be used in the prevention and/or treatment of one or more disorders or symptoms selected from burning eyes, itching, irritation, foreign body sensation, blurred vision, marked sensitivity to light, ocular *discomfort* , tear film instability, meibomian gland dysfunction, conjunctival inflammation, subconjunctival fibrosis, epithelial damage, and allergic blepharitis.

In a particularly preferred aspect, the invention provides the compound GSH-C4 or a pharmaceutically acceptable salt thereof for use in the prevention, and/or treatment or adjuvant treatment of dry eye syndrome and/or OSD in a subject in need thereof, particularly a human subject.

According to the present invention, the compound GSH-C4 or a pharmaceutically acceptable salt thereof may be used for the prevention, and/or for the treatment or to assist in the treatment of dry eye syndrome and/or OSD, and/or a disorder or symptom associated therewith, in a glaucoma subject or in a subject undergoing anti-glaucoma therapy, in particular a human subject.

A glaucoma subject eligible for treatment with the GSH-C4 compound or a pharmaceutically acceptable salt thereof is a subject affected by any form of glaucoma, for example, congenital glaucoma, open-angle glaucoma, angle-closure glaucoma, or glaucoma sine hypertension, in particular a glaucoma subject who has been diagnosed with dry eye syndrome and/or OSD.

According to the invention, a subject particularly suitable for treatment with the GSH-C4 compound or a pharmaceutically acceptable salt thereof is a subject with glaucoma and/or with ocular hypertension who is undergoing therapy for the treatment of glaucoma with one or more anti-glaucoma drugs, for example one or more drugs for reducing intraocular pressure, preferably a subject with glaucoma and/or with ocular hypertension who is undergoing therapy with one or more topical hypotensive drugs, for example with at least one prostaglandin analogue.

According to the invention, the GSH-C4 compound or a pharmaceutically acceptable salt thereof is preferably administered to a subject in need thereof topically or intraocularly, even more preferably subconjunctivally.

The present invention also relates to a composition comprising GSH-C4 or a pharmaceutically acceptable salt thereof and one or more carriers, and/or diluents and/or excipients, for use in the prevention and/or treatment or in assisting the treatment of ocular disorders and/or pathologies, in particular ocular surface disorders and/or pathologies.

The invention provides in particular a composition comprising GSH-C4 or a pharmaceutically acceptable salt thereof and one or more carriers, and/or diluents and/or excipients, for use in the protection of the ocular surface in a subject in need of it from oxidative stress, dryness and/or inflammation.

Preferably, a composition according to any of the variants described herein comprises GSH-C4 or a pharmaceutically acceptable salt thereof at a concentration of 0.01% to 1.5% by weight based on the total weight of the composition (w/w). The GSH-C4 or a pharmaceutically acceptable salt thereof may be at any concentration w/w within the range indicated above, such as 0.1%; 0.2%; 0.3%; 0.4%; 0.5%; 0.6%; 0.7%; 0.8%; 0.9%; 1.0%; 1.1%; 1.2%; 1.3%; 1.4%; 1.5% w/w.

According to some preferred embodiments, said GSH-C4 or a pharmaceutically acceptable salt thereof has a concentration of 0.4% to 1.2% w/w, or 0.6% to 1% w/w, or 0.8% w/w.

According to one aspect of the invention, a composition according to any of the variants set forth in this specification or in the claims is a pharmaceutical composition.

According to one aspect of the invention, a composition according to any of the variants set forth in this specification or in the claims is an ophthalmic composition or for ophthalmic use.

Preferably, a composition according to any of the embodiments of the present invention comprises GSH-C4 or a pharmaceutically acceptable salt thereof and one or more carriers, diluents and/or excipients suitable for the preparation of ophthalmic compositions or one or more pharmaceutically acceptable carriers, diluents and/or excipients.

Carrier compounds, diluents and/or excipients that may be employed in a composition according to any of the embodiments described herein may be selected from stabilizers, preservatives, solvents, pH adjusters, isotonic adjusters, chelating agents, cryoprotectant agents, diluent agents, binding agents, antioxidants, surfactants or combinations thereof.

Some examples of pharmaceutically acceptable and/or suitable carriers for the preparation of ophthalmic compositions include, for example, water, saline, phosphate buffered saline, glycerol, water-ethanol mixture and the like, as well as combinations thereof. In some cases, it is preferable to also include isotonic agents, for example sugars or polyalcohols, such as mannitol or sorbitol, in the composition of the invention.

Specific examples of excipients or carriers that can be used in a composition according to the invention are ascorbic acid, taurine, potassium hydroxide, monobasic and/or dibasic sodium phosphate, sodium chloride.

As mentioned, agents capable of increasing the shelf life of the composition may also be employed in the composition of the invention and include wetting agents, emulsifiers, preservatives or buffers.

The composition of the invention in any of the embodiments described herein may also contain microbial load stabilizing agents, such as benzalkonium chloride or the like. However, in a preferred aspect, a composition according to any of the embodiments described herein is free of preservatives, in particular it is free of benzalkonium chloride or BAK.

In a preferred embodiment, the composition may comprise excipients, such as polysaccharides, including hyaluronic acid (also abbreviated herein as HA), which exert

a mechanical action useful for the administration of the active ingredient GSH-C4 or its pharmaceutically acceptable salt.

In a preferred embodiment, the composition may comprise, in addition to excipients commonly used by those skilled in the art in the preparation of ophthalmic and/or pharmaceutical compositions, GSH-C4 or a pharmaceutically acceptable salt thereof at a concentration of 0.01% to 1.5% w/w and hyaluronic acid or a pharmaceutically acceptable salt thereof at a concentration of 0.01 to 0.3% w/w.

Advantageously, thanks to its excellent viscoelasticity, hyaluronic acid exerts a mechanical action useful for the administration of the active ingredient GSH-C4 or its pharmaceutically acceptable salt. The film-forming effect *of* hyaluronic acid, in addition to promoting ocular hydration by reducing *discomfort* in the presence of dryness, also allows the composition of the invention to achieve its intended use without the substances contained therein or the products deriving from the metabolism of the aforementioned substances being absorbed systemically by the body.

GSH-C4 or a pharmaceutically acceptable salt thereof may be present in the above embodiment of the composition at any concentration w/w within the range indicated above, such as 0.1%; 0.2%; 0.3%; 0.4%; 0.5%; 0.6%; 0.7%; 0.8%; 0.9%; 1.0%; 1.1%; 1.2%; 1.3%; 1.4%; 1.5% w/w. According to some preferred embodiments, said GSH-C4 or a pharmaceutically acceptable salt thereof has a concentration of 0.4% to 1.2% w/w, or 0.6% to 1% w/w, or 0.4% w/w or 0.8% w/w.

The hyaluronic acid or its pharmaceutically acceptable salt may be at any w/w concentration within the range above. In one embodiment, the hyaluronic acid or its pharmaceutically acceptable salt may be present in the composition at a concentration of 0.01 to 0.3% w/w.

In one embodiment the composition will therefore consist of GSH-C4 or a pharmaceutically acceptable salt thereof at a concentration of 0.01% to 1.5% (w/w) and hyaluronic acid or a pharmaceutically acceptable salt thereof at a concentration of 0.01 to 0.3% (w/w) and appropriate excipients. The excipients may vary depending on the type of formulation chosen according to what is commonly used by those skilled in the art.

The hyaluronic acid or its salt suitable for use in the preparation of the composition of the invention may be hyaluronic acid or its salt with high, medium or low molecular weight, in particular whose molecular weights are respectively within the following ranges: 1,800-2,200 KDa, 1,000-1,800 kDa, 100-500 KDa.

For the preparation of the composition, in any of the forms indicated in this description, hyaluronic acid and/or any hyaluronic acid salt suitable for ophthalmic use may be used, such as the sodium salt of hyaluronic acid (sodium hyaluronate or sodium hyaluronate).

According to one embodiment, the present invention refers to a composition formulated in liquid form according to the qualitative-quantitative composition reported in Table 1. The aforementioned composition is preferably in the form of eyewash, sterile and free from preservatives.

Table 1:

chemical name	Concentration % w/w
Demineralized water	q.s. 100
Sodium hyaluronate	0.2
Glutathione L-reduced C4	0.4
Ascorbic acid	0.1
Taurine	0.1
Potassium hydrate	0.18
Monobasic sodium phosphate	q.s. from pH 6.00 - 7.50
Sodium phosphate dibasic	
Sodium chloride	0.31

Preferably, the liquid composition indicated in Table 1 may be administered to an individual who needs it several times a day, with a dosage of 1-2 drops (gtt.) in the conjunctival sac.

According to a further aspect of the present invention, a composition according to any of the embodiments described herein may include protective compounds that can facilitate the specific transport and/or release of GSH-C4 or its pharmaceutically acceptable salt to the eye. Such compounds may include any drug carrier system known in the art, for example biocompatible polymers, nano- or micro-particulate systems, liposomes, nanostructured materials, photosensitive capsules, nanoparticles, dendrimers, hybridized vesicles, cationic lipids, solid lipid nanoparticles (SLNs), nanostructured lipid carriers, solid lipid microparticles, or combinations thereof.

As mentioned, the composition according to any of the variants described herein may be used for the prevention, and/or for the treatment and/or to assist in the treatment of ocular disorders and/or pathologies, in particular ocular surface disorders and/or pathologies. In one embodiment according to the present invention, said ocular disorders or pathologies are selected from dry eye syndrome, ocular surface disease, and disorders or symptoms associated therewith.

In a preferred aspect, the invention particularly provides a composition according to any of the variations described herein for use in the prevention and/or treatment or adjuvant treatment of glaucoma-related dry eye syndrome, glaucoma-related ocular surface disease, and/or disorders or symptoms associated therewith.

Most preferably, a composition according to any of the variants described herein is for use in the prevention, and/or treatment or adjuvant treatment of dry eye syndrome and /or OSD in a subject in need thereof, particularly a human subject.

In particular, a composition according to any of the variants described herein may be used for the prevention, and/or for the treatment or to assist the treatment of dry eye syndrome and/or OSD, or a disorder or symptom associated therewith, in a glaucoma subject or in a subject undergoing anti-glaucoma therapy, in particular a human subject.

As previously mentioned, said glaucoma subject may be in particular a subject affected by any form of glaucoma, for example congenital glaucoma, open-angle glaucoma, closed-angle glaucoma, or glaucoma sine hypertension, in particular a glaucoma subject affected by dry eye syndrome and OSD.

A subject particularly suitable for treatment with a composition according to any of the embodiments described herein is a subject with glaucoma and/or with ocular hypertension who is undergoing therapy for the treatment of glaucoma with one or more anti-glaucoma drugs or drugs for reducing intraocular pressure, for example a subject with glaucoma and/or with ocular hypertension who is undergoing therapy with one or more topical hypotensive drugs, for example with at least one prostaglandin analogue.

The “Examples” section reports experiments performed with a liquid composition as reported in Table 1 according to the invention, on patients affected by glaucoma-related ocular surface disease undergoing topical hypotensive therapy and having dry eye syndrome, who have demonstrated that the composition of the invention advantageously allows to significantly improve the tear break-up time, with a positive impact on the corneal and conjunctival epithelium.

Therefore, the present invention also provides the compound GSH-C4 or a pharmaceutically acceptable salt thereof or a composition comprising it according to any of the variants described herein, for use in the treatment or in assisting the treatment of

a glaucoma subject, in particular undergoing topical hypotensive therapy, for whom dry eye syndrome and/or OSD has been diagnosed.

As mentioned, in one aspect of the invention, a composition according to any of the variants described herein may be used in the prevention and/or treatment of one or more of the disorders or symptoms associated with, or caused by, dry eye syndrome and/or OSD, e.g., burning eyes, foreign body sensation, blurred vision , marked sensitivity to light, ocular *discomfort* , tear film instability, meibomian gland dysfunction, conjunctival inflammation, subconjunctival fibrosis, epithelial damage, and/or allergic blepharitis.

In one embodiment, a composition comprising GSH-C4 or a pharmaceutically acceptable salt thereof as described and claimed herein may comprise other pharmacologically active compounds suitable for the treatment of ocular pathologies, in particular suitable for the treatment of glaucoma, dry eye syndrome and/or OSD, e.g. antibiotics, topical hypotensive or antihypertensive drugs or others.

However, in a preferred embodiment, GSH-C4 or a pharmaceutically acceptable salt thereof is the only pharmacologically active ingredient present in the composition.

Furthermore, according to a further aspect of the invention, the treatment of the ocular pathology can be carried out by administering GSH-C4 or a pharmaceutically acceptable salt thereof or a composition according to any of the variants described herein in association with other drugs conventionally used for the treatment of the pathology, such as for example antihypertensive or hypotensive drugs, at therapeutically effective dosages to a patient in need thereof.

Preferably, GSH-C4 or a pharmaceutically acceptable salt thereof or compositions according to any of the embodiments described herein may be administered to a subject in need thereof, optionally in combination with one or more additional active ingredients, such as antihypertensive or hypotensive drugs, topically and/or intraocularly, preferably subconjunctivalally.

In a preferred embodiment, GSH-C4 or a pharmaceutically acceptable salt thereof or compositions according to any of the embodiments described herein are administered into the conjunctival sac of a subject in need thereof.

GSH-C4 or a pharmaceutically acceptable salt thereof or the above compositions may be formulated in any form deemed suitable by the person skilled in the art for ophthalmic applications, for example in any form suitable for direct topical application to the ocular surface.

According to one aspect of the invention, a composition according to any of the variants described herein is in a form selected from the group consisting of: solution, suspension, gel, ointment, eye drops, artificial tears, eywash, spray, cream, pomade,

foam, powder. Such compositions may be prepared according to any of the techniques known to the person skilled in the art.

In a preferred aspect according to the invention, the composition according to any of the variants described herein is in the form of eyewash. Such formulation provides a simple and non-invasive mode of administration, thus encouraging *compliance* of a patient in need of treatment.

An embodiment according to the invention relates in particular to a composition in the form of eyewash for use in the treatment of dry eye syndrome and/or OSD or a disorder or symptom associated therewith, or in adjuvant treatment of dry eye syndrome and/or OSD, according to any of the variants described herein, comprising GSH-C4 or a pharmaceutically acceptable salt thereof and hyaluronic acid.

The composition in the form of eyewash may include one or more excipients, carriers, solvents, dispersion media, diluents, viscosifying agents, salts, adjuvants, or surfactants, provided they are physiologically compatible, such as excipients, carriers or solvents exemplified in this description.

Pharmaceutical compositions according to any of the embodiments described herein are sterile and stable under storage conditions.

GSH-C4 or a pharmaceutically acceptable salt thereof or a composition comprising it according to any of the embodiments described herein, may be administered to a subject in need thereof every 12 hours, morning and evening, or every 24 hours, preferably for a total duration of 7 days, or for a total duration of 15 days.

Preferably, a composition according to any of the embodiments described herein may be administered to a subject in need with a dosage of 1 or 2 drops (gtt.) in the conjunctival sac.

The present invention also provides GSH-C4 or a pharmaceutically acceptable salt thereof or a composition comprising it according to any of the embodiments described herein, for use in a method of treatment or in a method of adjuvant treatment of ocular disorders or pathologies, in particular glaucoma-related dry eye syndrome and/or G-OSD, which method comprises at least one step of administering a therapeutically effective amount of GSH-C4 or a pharmaceutically acceptable salt thereof or said composition to a subject in need thereof, in particular a human subject.

Also described herein is a method for the treatment of ocular disorders or pathologies, in particular dry eye syndrome and/or OSD, or a method for assisting the treatment of dry eye syndrome and/or OSD, comprising the administration to a subject in need thereof, and in particular a glaucoma subject, of an effective amount of GSH-C4

or a pharmaceutically acceptable salt thereof or a composition comprising it as described herein. The ocular disorders or pathologies subject to the aforementioned treatment may be selected from any of the disorders or pathologies exemplified in the present application.

As previously mentioned, a glaucoma subject eligible for treatment with GSH-C4 or a pharmaceutically acceptable salt thereof or any composition comprising it according to the invention is a subject affected by any form of glaucoma, for example, congenital glaucoma, open-angle glaucoma, angle-closure glaucoma, or sine-hypertension glaucoma, preferably a glaucoma subject diagnosed with dry eye syndrome and/or OSD.

In any method of treatment as described herein, the exact dosage and frequency of administration of GSH-C4 or a pharmaceutically acceptable salt thereof or a composition comprising it will depend upon the particular severity of the condition being treated, as well as upon the age, weight, and general physical condition of the particular subject, as is well known to one skilled in the art.

According to one aspect of the invention, the administration of GSH-C4 or a pharmaceutically acceptable salt thereof or a composition comprising it according to the invention can be carried out in multiple unit doses administrable at appropriate time intervals, preferably in one or more daily doses, using the same or a different administration method.

The invention also provides for the use of the compound GSH-C4 or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for use in the prevention and/or treatment or to assist in the treatment of ocular disorders or pathologies according to any of the variants described herein.

Below are examples which are intended to illustrate embodiments of the compositions disclosed in the present description, such examples are in no way to be considered as a limitation of the preceding description and subsequent claims.

EXAMPLES

All patients in the trials described below provided informed consent prior to participation in the trials.

Example 1

In the prospective, double-blind, interventional study described below, all patients were randomized in balanced blocks (1:1) into 2 groups:

- Group 1 (placebo first), receiving the control product (containing only 0.2% hyaluronic acid) for 4 weeks and, after a 1-week *wash-out period*, the

experimental product having the composition reported in Table 1 reported in the description (0.2% hyaluronic acid + 0.4% Butyroyl glutathione) for 4 weeks;

- Group 2 (glutathione first), receiving the experimental product with the composition reported in Table 1 in the description (comprising 0.2% hyaluronic acid + 0.4% Butyroyl glutathione) for 4 weeks and, after a 1-week *wash-out period*, the control product (containing only 0.2% hyaluronic acid) for 4 weeks.

The study and data collection complied with all national laws, were in accordance with the principles of the Declaration of Helsinki and were approved by the *Institutional Review Board* of the University of Rome "Tor Vergata" (RS53.22).

Inclusion and exclusion criteria

Patients of both sexes and aged >18 years with G-OSD (glaucoma-related ocular surface disease) were included in the study. Specifically, patients had to have a diagnosis of glaucoma or ocular hypertension defined by EGS criteria, be undergoing topical hypotensive therapy with at least one prostaglandin analog, and have a diagnosis of dry eye, defined by the simultaneous presence of: Ocular Surface Disease Index (OSDI) > 13; National Eye Institute fluorescein staining score > 2; Schirmer test, type I > 10 mm/5 min; duration of symptoms > 3 months; and Tear Film Breakup Time (TFBUT) < 10 seconds.

Patients were excluded if they had: any ocular surgery within 3 months prior to study entry; prior corneal surgery; current or prior corneal disease (e.g., corneal ulcer, corneal dystrophy, etc.); diagnosis of cicatricial conjunctivitis; prior mechanical/chemical corneal trauma; diagnosis of autoimmune disease (systemic lupus erythematosus, rheumatoid arthritis, Sjogren's syndrome, etc.); current contact lens use; allergy to one or more components of the drug; participation in another clinical study within 3 months prior to study entry.

Randomization and sample size calculation

Eligible participants were randomized (1:1) according to a block randomization design with a predetermined sequence of BABA-BAAB-AABB-ABBA. The randomization list was masked to study participants, sponsor, investigators, study monitors, and laboratory personnel until the database was locked. Treatment and control products were stored in identical cartons and labeled with protocol and kit numbers, ensuring that masked stakeholders were unable to determine product identification based on external appearance. The study was designed to include 16 participants/eyes (8 in each group), which was calculated to have 95% statistical power to detect a significant difference

between the hyaluronic acid + glutathione and hyaluronic acid alone groups, assuming a dispersion index of 20% and a two-sided α = 0.05.

Study mode

After randomization, recruited patients underwent a 9-week *follow-up period* that included one course of treatment with the investigational product and another course with the control product, both of 4 weeks in duration. A 1-week *wash-out period was observed* between the two courses. Patients were assessed every 2 weeks according to the schedule shown in Figure 1. At each *follow-up visit* , the following information was retrieved and entered into a CRF data collection form: OSDI score; National Eye Institute fluorescein staining score; subjective visual scale (Faces score), according to the Noble et al. model; Schirmer test, type I; TFBUT; and adverse events. To assess treatment compliance, the amount of drug remaining in the eyewash bottle returned by patients at the end of each treatment period was assessed. Additionally, tear collection was performed in each patient at visits V1, V3, V4 and V6 using Schirmer strips, as described in the literature.

Statistical analysis

Statistical analysis was performed using SPSS software (IBM, SPSS, v. 26.0.0.0). The distribution of values was assessed using the Shapiro-Wilk normality test. The Pearson chi-square test was used to compare categorical variables, correcting results for multiple comparisons using the conservative Bonferroni-Holm method. The Kruskal-Wallis test was used to compare continuous, nonparametric, unpaired variables. Confirmatory analysis was conducted following the principles outlined by Wellek and Blettner. A Kruskal-Wallis test was used to determine the difference in treatment effects, analyzing the within-subject difference in outcome between the two study periods. To exclude any carryover effects, the sum of the values measured in both periods was calculated for each subject and compared between the two sequence groups using the Kruskal-Wallis test.

Results

Sixteen participants were included in the present study. The mean age of the entire group was 71.3 ± 9.6 years, and 33.3% were female. There was no significant difference in mean age between group 1 and group 2, which had a mean age of 68.1 ± 10.2 and 74.5 ± 8.4 years, respectively (p = 0.2898). Table 2 below presents the clinical and demographic characteristics of the selected group. One participant from group 1 was

excluded from the study after reporting ocular side effects, specifically eye irritation. This participant was later found to have been using placebo eyewashes when the symptoms occurred.

Patient-reported outcome measures (PROMs)

Overall, treatment with the glutathione-C4 composition had a significant impact on the OSDI score (p = 0.0064) (Figure 2). However, a statistically significant decrease in OSDI scores was observed only in Group 2 from baseline to the intermediate visit (p = 0.0078) (Figure 2). On the other hand, there was a statistical trend towards a decrease in OSDI scores in Group 1 (p = 0.0559) (Figure 2).

Similarly, there was a significant improvement in FACES score associated with treatment with the glutathione-C4-containing composition (p = 0.0228). Although a statistically significant median reduction of -1 from baseline to midpoint was observed in Group 2 *(p = 0.0312), only a modest decrease in FACES score from midpoint to final timepoint* was observed in Group 1 (p = 0.0625) (Figure 3).

Analysis of all PROMs revealed no carryover effect (OSDI: p = 0.7924; FACI: p = 0.727).

Clinical outcome measures

In the selected cohort, treatment with the composition including glutathione-C4 did not have a significant impact on the Schirmer test (p = 0.5559). However, it is worth noting that a statistically significant median increase of 5 mm was observed in the Schirmer test for group 2 from baseline to midpoint (p = 0.0345) (Figure 4).

In contrast, a significant improvement in tear breakup time was observed with the glutathione-C4 composition treatment (p = 0.0029). In particular, a statistically significant median increase of 2.5 and 3.0 seconds in TBUT was observed in Group 2 and Group 1, respectively, after glutathione treatment (p = 0.0121 and p = 0.0432) (Figure 5).

Glutathione treatment was found to have a positive impact on the corneal and conjunctival epithelium (p = 0.0311), with a statistically significant decrease in the *National Eye Institute Staining Score* (also abbreviated as NEI-SS) observed in Group 2 from baseline to the *midpoint* (p = 0.0433). Additionally, there was a trend toward a reduction in Group 1 following glutathione treatment (p = 0.0625) (Figure 6).

No carryover effect was observed in any of the clinical endpoints analyzed (Schirmer test: p = 0.2668; TBUT: p = 0.0599; NEI-SS: p = 0.0923).

Table 2

	Placebo First (Group 1 or A)	Glutathione First (Group 2 or B)	p
Age	72.5 (50.0, 81.0)	74.5 (63.0, 86.0)	0.2898
No. of anti-glaucoma drops	2.0 (1, 3)	2.5 (1, 4)	0.9068
Average Deviation	-7.1 (-16.3, -1.4)	-9.5 (16.7, -0.65)	0.6126
Pattern Standard Deviation	8.3 (1.9, 13.8)	7.8 (3.05, 13.66)	0.8665
Ocular Surface Disease Index	21 (11.0, 35.0)	23 (12.0, 44.0)	0.5873
Score Faces	3.0 (2.0, 7.0)	3.5 (1.0, 7.0)	0.8129
Schirmer Test	11.5 (11.0, 13.0)	11.5 (11.0, 20.0)	>0.9999
Tear Break Up Time	6.0 (2.0, 8.0)	5.5 (3.0, 8.0)	0.9671

Table 2 – Baseline clinical characteristics of patients included in the analysis. Results are expressed as mean (minimum, minimum value).

Example 2 – Effect of glutathione GSH-C4 on cytokine production in tear fluid of test subjects

Tear sample collection

Tears from Group 1 and Group 2 patients as reported in Example 1 were collected at the ophthalmology department of the Policlinico di Roma Tor Vergata to assess the expression of inflammatory cytokines/chemokines in the tear film. Schirmer strips were used to collect tear fluid in all patients. After adding 200 µl of sterile saline to the eye, the strip was placed in the lower conjunctival sac and tears were allowed to diffuse into the strip. The Schirmer strips were then inserted into a 1.5 ml tube and placed on ice. To

isolate the tear fluid, a hole was made in the 1.5 ml tube and inserted into a new tube. The tear sample was recovered by centrifugation at 13,000 RPM x 20' at 4°C. Finally, the volume was measured.

Analysis of cytokines present in tear fluid

The concentration of 13 inflammatory cytokines/chemokines in tear fluid (TNF-α, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 p70, GM-CSF, IFN-γ) was assessed using a multiplex assay based on fluorescence microspheres (LEGENDplex™ Human Inflammation Panel 1 (13-plex) with Filter Plate). Before proceeding with the test, each tear sample was divided equally into two different tubes (based on the volume obtained) and assay buffer was added to each until a volume of 50 µl was reached. At the end of the protocol, 150 µl of PBS was added to each sample to proceed with the reading by flow cytometry (CytoFLEX, Beckman Coulter).

Statistical analysis

Statistical analysis of cytokine expression levels of the groups was performed using the parametric Student's t-test. Pairwise associations between continuous variables were tested using the Spearman correlation coefficient. Statistically significant comparisons were considered at $p < 0.05$. Data analyses were performed using SPSS statistical software (version 23.0 for Windows, USA).

Results

Patients' tears, one month after treatment with the composition including glutathione GSH-C4 as reported in Table 1 reported in the description , showed higher levels than the initial value of inflammatory cytokines, such as IL1-β, IFN-α2, IFN-γ, TNF-α, IL-6, IL-23 and the cytokine IL-33, known for dual roles in context-dependent regulations and depending on intra- or extra-cellular localization. A direct correlation of these cytokines with the level of absorption, calculated in mm, obtained by Schirmer's test was observed. Furthermore, the high expression of IL-1β correlated inversely with Faces Pain Scale. IN The increase in cytokines could play a role in the activation of inflammatory processes that support repair. Furthermore, IL-33 was reported to increase in corneal tissue after corneal epithelial injury, promoting the number and function of corneal ILC2s in the healing process (J. *Liu, C. Xiao, H. Wang et al., "Local group 2 innate lymphoid cells promote corneal regeneration after epithelial abrasion," The American J. ournal of Pathology, vol. 187,no. 6, pp. 1313–1326, 2017*). Furthermore, the IL-33/ST2 axis acts on a broad range of innate immune cells and promotes M2 polarization of macrophages toward a repairing phenotype, as recognized in other contexts such as skin lacerations (*Yin et al, IL-33 accelerates cutaneous wound healing involved in upregulation of alternatively activated macrophage Mol Immunol. 2013 Dec;56(4):347-53. doi:*

10.1016/j.molimm.2013.05.225). Furthermore, IL-33/ST2 acts on a broad range of innate immune cells and is classified as an “alarmin” for its ability to trigger both adaptive and innate immune defenses in the event of cellular damage [Rev in The Functional Roles of IL-33/ST2 Axis in Ocular Pathologies. Qian 2020]. Interestingly, the clinical changes in the group that received treatment with Glutathione in the first phase (group 2) were statistically significant. Similarly, significant modulations of cytokines were found in the same group in the phase with Glutathione at the beginning (“glutathione first”, group 2), in the contexts of clinical response.

In vitro tests were also performed on primary human corneal cells cultured in the presence of artificial tears containing hyaluronic acid and glutathione GSH-C4 , individually or added, using a model of induction of epithelial injury and cellular oxidative stress. In line with the results obtained with the clinical trial, the results demonstrate the ability of the composition of the invention to promote the repair and/or reduction of cellular oxidative stress precisely in the simulated conditions of stress induced by wounds and exposure to pro-oxidant molecules. These results, although preliminary, support the therapeutic efficacy of the use of artificial tears containing hyaluronic acid and glutathione GSH-C4 precisely in those ocular pathologies already known for their characteristics characterized by oxidative and inflammatory stress, such as dry eye and glaucoma, characteristics dependent on their etiological nature aggravated by the concomitant and indispensable use of drugs that would determine a further accumulation of oxidative stress and inflammation, and which for these reasons benefit from the use of the composition of the invention.

To confirm the modulation of cytokine production by the device also in the in vitro model, the evaluation of the concentration of cytokines and chemokines in the supernatants of corneal cell cultures was performed using a multiplex analysis test based on fluorescence microspheres and flow cytometry reading, a methodology used for the analysis of cytokines on tear fluid during the clinical trial.

CLAIMS

1. Butanoyl glutathione compound (GSH-C4) or a pharmaceutically acceptable salt thereof for use in the prevention and/or treatment or to aid in the treatment of ocular disorders and/or pathologies selected from dry eye syndrome, ocular surface disease (OSD), and ocular pathologies aggravated by chronic use of drugs that induce oxidative stress and inflammation.

2. The GSH-C4 compound or a pharmaceutically acceptable salt thereof for use according to claim 1, wherein said ocular disorders and/or pathologies are selected from glaucoma-related dry eye syndrome and/or glaucoma-related OSD (G-OSD), and said ocular pathologies aggravated by chronic use of drugs inducing oxidative stress and inflammation are selected from meibomian gland dysfunction, drug-induced toxic keratopathy, chronic blepharitis, neurotrophic keratitis, chronic drug-induced keratitis, exposure keratopathy, chronic allergic conjunctivitis, chronic toxic conjunctivitis, ocular cicatricial pemphigoid, glaucoma-related ocular surface disease (G-OSD), ischemic optic neuropathy, exfoliative and pigmentary glaucoma, age-related macular degeneration, diabetic retinopathy, retinal drug toxicity, macular edema cystoid, chronic uveitis, drug-induced uveitis syndrome, neurogenic dry eye syndrome, and toxic optic neuropathies.

3. A composition comprising the compound butanoyl glutathione (GSH-C4) or a pharmaceutically acceptable salt thereof and one or more carriers, and/or diluents and/or excipients, for use in the prevention, and/or treatment or to aid in the treatment of selected ocular disorders and/or pathologies from dry eye syndrome, ocular surface disease (OSD), and associated disorders.

4. The composition for use according to claim 3, wherein said composition comprises GSH-C4 or a pharmaceutically acceptable salt thereof at a concentration of 0.01% to 1.5% w/w.

5. The composition for use according to claim 4, wherein said GSH-C4 or a pharmaceutically acceptable salt thereof has a concentration of 0.4% to 1.2% w/w, or 0.6% to 1% w/w, or 0.8% w/w.

6. The composition for use according to anyone of claims 3 to 5, wherein said ocular disorders and/or pathologies are selected from glaucoma-related dry eye syndrome and/or glaucoma-related OSD (G-OSD), and said ocular pathologies aggravated by chronic use of drugs inducing oxidative stress and inflammation are selected from meibomian gland dysfunction, drug-induced toxic keratopathy, chronic blepharitis, neurotrophic keratitis, chronic drug-induced keratitis, exposure keratopathy, chronic allergic conjunctivitis, chronic toxic conjunctivitis, ocular cicatricial pemphigoid,

glaucoma-related ocular surface disease (G-OSD), ischemic optic neuropathy, exfoliative and pigmentary glaucoma, age-related macular degeneration, diabetic retinopathy, retinal drug toxicity, cystoid macular edema, chronic uveitis, of drug-induced uveitis, neurogenic dry eye syndrome, and toxic optic neuropathies.

7. The composition for use according to anyone of claims 3 to 6, wherein said composition is for topical or intraocular administration, preferably by subconjunctival route.

8. The composition for use according to anyone of claims 3 to 7 wherein said composition is in a form selected from solution, suspension, gel, ointment, eye drops, eyewash, spray, artificial tears, cream, pomade, foam, and powder, preferably in the form of eyewash.

9. The composition for use according to anyone of claims 3 to 8 further comprising hyaluronic acid or a pharmaceutically acceptable salt thereof at a concentration from 0.01 to 0.3% w/w.

10. The composition for use according to anyone of claims 3 to 9, wherein said composition comprises one or more additional pharmacologically active compounds in addition to GSH-C4 or a pharmaceutically acceptable salt thereof.

11. Butanoyl glutathione compound (GSH-C4) or a pharmaceutically acceptable salt thereof for use according to anyone of claims 1 to 2, or the composition for use according to anyone of claims 4 to 12, in a glaucomatous subject and/or suffering from ocular hypertension, in particular wherein said subject is undergoing anti-glaucoma therapy with one or more topical hypotensive drugs.