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(54) **USE OF DEUTERIUM OXIDE FOR  
TREATING VIRAL DISEASES OF THE EYE**

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(57) **ABSTRACT**

The invention relates to the use of deuterium oxide for the prophylaxis and/or therapy of viral diseases of the eye.

Figure 1

Table 1

Virus Type / Virus Strain	Number in Treatment Group	Number in Control Group
Rhinoviruses / RV-16	8 ± 2	14 ± 3
HSV-1 / MacIntyre	2 ± 1	13 ± 2
Varicella Zoster /Ellen	3 ± 1	8 ± 1
Coronavirus /HCoV 229E	4 ± 2	13 ± 3
Influenza A/H1N1	6 ± 1	11 ± 1
Influenza B / Hong Kong	5 ± 2	9 ± 2
HPIV-1 / Washington	4 ± 2	8 ± 2
HRSV/Long	5 ± 1	12 ± 1
Coxsackie / A1	4 ± 1	9 ± 1
Human adenoviruses 3 / GZ1	2 ± 1	8 ± 2
Human enteroviruses 71 / SHZH98	5 ± 2	9 ± 2

**Figure 2****Table 2**

<b>Virus / Strain</b>	<b>Viral Count Treatment Group</b>	<b>Viral Count Control Group</b>
Rhinovirus /RV-16	$4 \pm 1$	$7 \pm 1$
Herpes HSV-2 / 764	$2 \pm 1$	$6 \pm 1$

**Figure 3****Table 3**

<b>Virus / Strain</b>	<b>Viral Count Treatment Group</b>	<b>Viral Count Control Group</b>
HSV-1 / MacIntyre	$2 \pm 1$	$10 \pm 1$
Herpes HSV-2 / 764	$3 \pm 1$	$5 \pm 1$
Varicella Zoster /Ellen	$4 \pm 1$	$9 \pm 2$
Human adenoviruses 3 / GZ1	$3 \pm 1$	$11 \pm 3$

Figure 4

Table 4

	Treatment A D2O Gel	Treatment B D2O Eye Drops	Control H2O Gel
Number of treated herpes incidences	7	6	7
Average total symptom score	$2.2 \pm 0.4$	$2.5 \pm 0.4$	$3.8 \pm 0.6$
Average max. size of herpes (mm)	$2.1 \pm 1$	$2.0 \pm 1$	$3.9 \pm 1.2$
Average time to complete external healing (days)	$1.5 \pm 0.6$	$1.2 \pm 0.5$	$3.3 \pm 0.8$
Average time to freedom from pain (days)	$1.0 \pm 0.3$	$0.8 \pm 0.5$	$3.2 \pm 0.5$
Average time between first symptom and application (hours)	$1.8 \pm 0.5$	$1.7 \pm 0.5$	$1.6 \pm 0.8$

## USE OF DEUTERIUM OXIDE FOR TREATING VIRAL DISEASES OF THE EYE

**[0001]** The present invention relates to the use of deuterium dioxide (D<sub>2</sub>O) for the prevention and/or treatment of virus-based diseases of the eye.

**[0002]** Diseases caused by viruses (virus infections) occur worldwide and represent a serious problem in medicine, in particular because of the high variability, adaptability, and mutation rate of viruses. Viruses are small particles of approx. 15 to 400 nm in diameter, which are not capable of replicating themselves alone but require a host cell for this. In accordance with their host specificity, a distinction is made between viruses that infect animals (invertebrates and vertebrates), plants, bacteria or algae, fungi, and protozoa. Virus infections in general are characterized by a high reproduction rate of the viral particles in the affected host cells, which can be described by an exponential or power law. The reproductive cycle of viruses occurs via the injection of their nucleic acid (viral RNA or viral DNA) into the host cell, in which the replication of the nucleic acid takes place by utilizing the replication apparatus of the host cell. A distinction is made here between the lytic and the lysogenic cycle. In the lytic cycle (active infection), after the injection of the nucleic acid, the replication of the viral nucleic acid takes place in the cell nucleus of the host cell, and assembly of the new viral particles in the cytoplasm, after which the host cell is finally lysed (destroyed) and the viruses are released. The viruses thus released infect other host cells. In the lysogenic cycle, the nucleic acid of the virus is integrated into the host cell genome, where it remains initially without destroying the host cell. Owing to external influences (e.g., UV radiation, addition of mutagenic substances), this lysogenic cycle can change into an aforementioned lytic cycle. In the case of RNA viruses, after infection therewith transcription of the RNA into DNA is necessary so that replication by the host cell can take place. This process takes place via reverse transcriptase, an enzyme which is encoded by viral genes and must first be synthesized in the host cell in order to transcribe the viral RNA into viral DNA, which is then in turn replicated by the DNA polymerase of the host cell.

**[0003]** Viruses are capable of infecting a broad spectrum of cells, organs, and hosts. Each virus species infects specifically preferred cells, such as, for example, cells of the stomach, intestine, skin, and eyes. This leads to many so-called virus-based diseases.

**[0004]** In this regard, virus-based diseases of the eye are a serious problem in pathology, in particular in man.

**[0005]** Viruses that cause such virus-based diseases of the eye comprise in particular herpesviruses, such as herpes simplex virus 1, herpes simplex virus 2, Epstein-Barr virus, varicella zoster virus and cytomegalovirus, adenoviruses, influenza viruses, parainfluenza viruses, picornaviruses, such as rhinoviruses, coxsackie A virus, coxsackie B virus, and echoviruses, molluscum contagiosum virus (poxvirus), vaccinia viruses, mumps viruses, measles viruses, rubella viruses, polioviruses, rabies viruses, human immunodeficiency viruses, and Rift Valley fever virus, but they are not limited thereto.

**[0006]** The viruses are transmitted mostly by droplet and smear infections. As a rule, viruses first infect skin cells, such as epithelial cells, mucous and mucous membrane cells, of the eye, followed by intense reproduction of the virus in the

host cell and death of the infected host cell. The host reacts with an immune response which leads to various symptomatic clinical pictures.

**[0007]** Virus-based diseases of the eye include, for example, keratoconjunctivitis or conjunctivitis, especially epidemic keratoconjunctivitis, keratoconjunctivitis sicca, hemorrhagic conjunctivitis, follicular conjunctivitis, pharyngoconjunctival fever, blepharitis, blepharoconjunctivitis, herpes simplex blepharitis, herpes simplex keratitis, dendritic keratitis, interstitial keratitis, endotheliitis/disciform keratitis, (varicella) zoster ophthalmicus, ophthalmia neonatorum (neonatal conjunctivitis), molluscum contagiosum, canaliculitis, acute dacryoadenitis, dacryocystitis, acute retinal necrosis, episcleritis, scleritis, choroiditis, chorioretinitis, iritis, iridocyclitis, retinitis, rhinoconjunctivitis, as well as uveitis, such as anterior uveitis, intermediate uveitis, posterior uveitis, panuveitis, and uveoretinitis. Furthermore, cataracts, ocular muscle paresis, and congenital glaucoma can be caused. Virus-based diseases of the eye often occur secondary to virus infections of the respiratory tract, e.g., influenza and rhinitis.

**[0008]** The symptoms and therefore the clinical picture caused by virus infections of the eye are similar for most viruses, with the exception of herpesviruses. Some of the virus-based diseases of the eye can be caused by different viruses, and some are caused preferentially by a specific virus type (also virus species). Keratoconjunctivitis, e.g., is caused in most cases by adenoviruses, but can also be caused by other viruses, such as cytomegaloviruses. Apart from the virus causing the infection, the symptoms depend on the severity of the infection and the immune defenses of the host.

**[0009]** Very often, virus-based diseases of the eye are caused by infections by alphaherpesviruses (HSV-1, varicella zoster) and adenoviruses. Some such specific viruses, their infection route, and pathological manifestation are explained in greater detail below by way of example:

### Infections by Herpesviridae

#### 1. Infections by Alphaherpesviruses

**[0010]** Herpesviruses are common in vertebrates, particularly in mammals, and above all in humans, horses, pigs, cattle, goats, sheep, cats, and dogs. Human herpesviruses (HHV) are differentiated into alpha-, beta-, and gammaherpesviruses (HHV-1 to HHV-8); viruses that can infect animals such as, for example, horses (equine herpesvirus), cattle (bovine herpesvirus), pigs (suid herpesvirus), cats (feline herpesvirus), dogs (canine herpesvirus), and poultry (gallid herpesvirus 1) also belong to the alpha and gamma viruses.

**[0011]** Among the human herpesviruses, i.e., those affecting humans, the alphaherpesviruses in particular are of major importance. Alphaherpesviruses include herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), and varicella zoster virus (VZV).

**[0012]** Human alphaherpesviruses initially reproduce in the skin of the eye according to the lytic cycle, especially in epithelial cells, as well as in the oral and nasal mucosa (primary infection) and usually cause local damage, namely, in the form of a lesion, mostly of vesicles. Viruses released during reproduction further infect certain nerve cells (neurons) by attaching to receptors on nerve endings, which leads to ganglia of the facial nerve (trigeminal). The viral DNA penetrates into an axon, and is transported into the cytoplasm of the nerve cells and finally into the cell nucleus thereof. The incorporation of the viral DNA into the genome of the nerve

cell occurs there and leads to a resting state (latency) in which only a few viral genes are expressed (lysogenic cycle). Various external stimuli can lead to renewed activation of the virus, which ultimately results in the destruction (lysis) of the nerve cell. The viral progeny arising during an activation are initially transported through the axon via the trigeminus to the site or region of the original infection and released at the nerve endings. The viruses again infect epithelial cells and endothelial cells there and in advanced cases in infection of the eye also the corneal stroma (secondary infections).

**[0013]** The above-described primary HSV-1 infections of the eye affect primarily the skin of the eyelid, the conjunctiva, and the outer layers of the cornea. In addition, the epithelium and mucous membranes of the lacrimal sacs, lacrimal canaliculi, and tear glands can be infected by the virus. The described secondary infections by HSV-1 affect both superficial and deeper epithelial layers, e.g., such as deep layers of the cornea, the choroid, sclera, and other regions of the uvea. The epithelial cells of these above-described epithelia are infected in particular by the virus.

**[0014]** HSV-1 infections of the eye can lead to various clinical pictures, in particular dacryocystitis, canaliculitis, dacryoadenitis, herpes simplex blepharitis, herpes simplex keratitis (dendritic keratitis, interstitial keratitis, endothelitis), disciform keratitis, uveitis, acute necrotizing retinitis, scleritis, and follicular conjunctivitis. The clinical pictures of the secondary infections are for the most part more serious than those of the primary infection.

## 2. Infections by Varicella Zoster Viruses

**[0015]** Infections of the eye with varicella zoster viruses occur secondary to chickenpox infections. Varicella zoster viruses also cause the disease zoster ophthalmicus (ocular herpes zoster). This disease usually begins with burning and pain on half of the face and forehead. Reddening of the skin and vesicle formation occur after a few days. In the area of the eye, swelling of the lids by vesicle formation on the eyelids, conjunctivitis, and keratitis may occur as a result of the virus infection.

**[0016]** About 0.5% of all eye diseases are said to be due to Herpesviridae. Over 50% of all affected individuals suffer from relapses after the initial illness and therefore more serious secondary infections.

### Infection by Adenoviruses

**[0017]** Adenoviruses are DNA viruses, which infect both animals and humans. Of a total of 19 species, 6 are pathogenic to humans (human adenoviruses A-F). Human pathogenic types, causing virus-based diseases of the eye, are particularly the strains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15, 19, and 29.

**[0018]** Adenoviruses are notable for a high pH and temperature stability. They generally enter the body via the respiratory tract. The reproduction of the adenoviruses is not limited locally, however. They can reproduce according to the lytic replication cycle in epithelial cells of the pharynx, gastrointestinal tract, and eyes, primarily in the conjunctiva.

**[0019]** An infection of the eye with adenoviruses causes in particular keratoconjunctivitis, conjunctivitis, and severe keratitis, e.g., epidemic keratoconjunctivitis, as virus-based diseases of the eye. Adenoviruses are the most common cause of virus-based conjunctivitis. Symptoms are tearing, painful

eyes, sensitivity to light, and corneal erosions, which may last up to six weeks. Furthermore, adenoviruses may bring about pharyngoconjunctival fever.

**[0020]** The type and severity of the infection in this regard depends on the causative adenovirus type. For example, infections with adenoviruses 5, 8, and 19 lead to the most severe kerato-conjunctivitis of the eye. Infections with adenoviruses are highly contagious.

**[0021]** Antiviral active substances have been developed in past decades for the treatment of many virus infections, so-called virostatic agents, which are said to interfere in the reproductive cycle of the virus and to inhibit or block the viral infection of the host cell or the reproduction of the virus in the host cell.

**[0022]** Treatment of virus-based diseases of the eye with antiviral active substances, however, is currently possible solely in an infection by Herpesviridae, such as HSV-1, varicella zoster virus, and cytomegaloviruses, and influenza viruses (type A and type B). The current treatments will be discussed in greater detail hereafter:

### Antiviral Treatment of Herpesvirus-Based Diseases of the Eye

**[0023]** Herpes infections of the eye are treated with orally or topically administered virostatic agents, such as acyclovir (Zovirax®), valacyclovir (Valtrex®), (Goldblum D et al. *Comparison of oral antiviral therapy with valacyclovir or acyclovir after penetrating keratoplasty for herpetic keratitis*. British Journal of Ophthalmologic 2008; Vol. 92:1201-1205) ganciclovir, trifluridine (Michael H G, *Therapy of viral infections*. Bull. N.Y. Acad. Med. 2008; Vol. 59, No. 8: 711-720), idoxuridine (Virungent®) (Michael H G, *Therapy of viral infections*. Bull. N.Y. Acad. Med. 2008; Vol. 59, No. 8: 711-720), famciclovir (Famvir®), and cidofovir (Vistide®). These are nucleoside analogues, which are integrated into the genetic material of the virus and therefore inhibit RNA polymerase or DNA polymerase of the host during viral replication. This results in chain termination and thereby in the stopping of replication and viral reproduction. The virostatic agent to be administered depends, inter alia, on the herpesvirus species and the depth of the affected corneal layers in the eye. Superficial infections by HSV-1 are treated, for example, primarily topically with trifluridine, whereas deeper layers are often treated by application of an acyclovir ophthalmic ointment. Deeper layers of the cornea, infected by herpesviruses, can be reached in principle only with difficulty by the mentioned virostatic agents, however. If these layers are affected, therefore, in most cases a combination of virostatic agents with symptom-reducing pharmaceuticals, such as, e.g., anti-inflammatory corticosteroids, is necessary.

### Antiviral Treatment of Influenza Virus-Based Diseases of the Eye

**[0024]** Influenza A viruses and influenza B viruses are inhibited by neuraminidase inhibitors, such as zanamivir (Relenza®) and oseltamivir (Tamiflu®). The enzyme neuraminidase helps the newly formed virus cells to bud off from the host cell, so that they can infect other cells. Viral reproduction is suppressed by the inhibition of neuraminidase and therefore further infection is stopped. Neuraminidase inhibitors can also be used preventively to avoid a viral infection with influenza A and influenza B, particularly in the case of epi-

demics (Sugrue R J et al., *Antiviral Drugs for the Control of Pandemic Influenza Virus*. Annals Academy of Medicine, 2008, Vol. 37, 518-524).

**[0025]** M2 channel blockers (amantadine and rimantadine) are known, moreover, for influenza A virus. These prevent the "uncoating," i.e., the release of viral nucleocapsids into the cytoplasm of the host cell and therefore the infection the host cell, as a result of which viral reproduction is terminated (Sugrue R J et al., *Antiviral Drugs for the Control of Pandemic Influenza Virus*. Annals Academy of Medicine, 2008, Vol. 37, 518-524).

**[0026]** Both neuraminidase inhibitors and M2 channel blockers, however, because of their many adverse reactions are used only for the treatment of virus-based eye diseases, when a severe influenza infection has been diagnosed.

**[0027]** The listed antiviral treatment options for virus-based diseases of the eye have major disadvantages:

**[0028]** With systemic administration, the dose necessary for an effective treatment is associated relatively greatly with severe adverse reactions for the organism treated, such as, for example, nonspecific immune responses and autoimmune reactions. Many adverse reactions are known from the literature, e.g., for acyclovir, amantadine, and rimantadine. Neither long-term treatment nor repeated treatments are therefore advisable and to be asked of a patient;

**[0029]** With topical application, the amount of active substance (virostatic agent) that can be released per unit of time and become bioavailable in the area of the virus infection is very low. This low bioavailability of the virostatic agent is a major obstacle to an effective topical therapy. For the only poorly water-soluble acyclovir, e.g., the low bioavailability is due, for example, to the poor percutaneous transport of the active substance. Various chemical modifications of virostatic agents within the scope of prodrug concepts for improved delivery of the active substance in virostatic agents have also not led to any improvement in this phenomenon.

**[0030]** The described cytostatics are very specific and therefore cannot be used against different viruses for the treatment of virus-based diseases of the eye. In many virus-based diseases of the eye, e.g., viral conjunctivitis, the virus type, e.g., an influenza infection, cannot be determined from the symptoms. In these cases, a treatment with a virostatic agent that only acts on one virus type would very likely be unsuccessful and would moreover cause the described adverse reactions.

**[0031]** Other serious disadvantages are that the described antiviral treatment options for virus-based diseases of the eye can lead to other major adverse reactions and, as, for example, in the case of M2 channel blockers, could lead to resistant virus strains, which rules out future treatment of such illnesses with such active substances and possibly even with other active substances.

**[0032]** Only infections of the eyes caused by the described herpesviruses and influenza viruses are treated with the described antiviral treatments. There is no antiviral treatment for diseases of the eye caused by other viruses such as, e.g., adenoviruses, mumps viruses, measles viruses, rubella viruses, Epstein-Barr virus, parainfluenza viruses, picornaviruses (rhinoviruses, coxsackie A virus, coxsackie B virus, echoviruses), molluscum contagiosum virus (poxvirus), vaccinia viruses, polioviruses, rabies viruses, human immunodeficiency viruses, and Rift Valley fever virus.

**[0033]** Alternatives to currently known antiviral active substances, which could overcome the disadvantages known from the state of the art, are exclusively symptomatic treatments, e.g., of inflammatory reactions to the virus infection with topical vasoconstrictors (e.g., naphazoline) or steroids (e.g., vexol, flarex), but there is no therapeutic treatment for the disease itself.

**[0034]** Alternatives to known antiviral active substances for the prevention and/or treatment of virus-based diseases of the eye, which overcome the disadvantages known from the state of the art, are exclusively preventive vaccinations against the corresponding viruses. Effective vaccinations against viruses that can infect the eye, however, according to the current state of the art are known only for the influenza viruses (types A and B) and against the smallpox, rubella, measles, and mumps virus.

**[0035]** Most viruses such as, e.g., influenza viruses, moreover, are subject to constant mutations, so that new vaccines must be developed constantly. Moreover, the vaccinations are not effective in all vaccinated patients. Treatments for viral diseases accordingly cannot be replaced by vaccinations.

**[0036]** There are no other alternatives to the antiviral active substances or virostatic agents, disclosed in the state of the art, for the prevention and/or treatment of virus-based diseases of the eye, which overcome the disadvantages known from the state of the art.

**[0037]** There is therefore a need to develop improved and more tolerable antiviral active substances for the prevention and/or treatment of virus-based diseases of the eye, which interfere in the replication and/or reproductive cycle of the virus and which preferably already block or inhibit the viral infection of the host cell.

**[0038]** In addition, there is a need to identify antiviral active substances that inhibit the infection, replication, and/or reproduction of different viral strains or viral species simultaneously. This is particularly important because in most diseases of the eye the various viral strains or viral species that have triggered the infection cannot be differentiated symptomatically.

**[0039]** The object of the invention accordingly is to provide improved antiviral active substances for the prevention and/or treatment of virus-based diseases of the eye.

**[0040]** This object is attained by the present invention. The invention relates in its first subject to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye and in its second subject to the use of deuterium oxide for the production of a drug for the prevention and/or treatment of virus-based diseases of the eye.

**[0041]** A preferred embodiment of the invention relates to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye, whereby the virus-based diseases of the eye are conjunctivitis, keratoconjunctivitis, and/or uveitis.

**[0042]** Another preferred embodiment of the invention relates to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye, whereby the virus-based diseases of the eye are epidemic keratoconjunctivitis, keratoconjunctivitis sicca, hemorrhagic conjunctivitis, follicular conjunctivitis, pharyngoconjunctival fever, blepharitis, blepharoconjunctivitis, herpes simplex blepharitis, herpes simplex keratitis, dendritic keratitis, interstitial keratitis, endotheliitis, disciform keratitis, (varicella) zoster ophthalmicus, ophthalmia neonatorum, molluscum contagiosum, canaliculitis, dacryoadenitis, acute dacryoadenitis,



dacryocystitis, acute retinal necrosis, episcleritis, scleritis, choroiditis, chorioretinitis, iritis, iridocyclitis, retinitis, acute necrotizing retinitis, scleritis, anterior uveitis, intermediate uveitis, posterior uveitis, panuveitis, uveoretinitis, cataract, ocular muscle paresis, and/or congenital glaucoma.

**[0043]** Various diseases of the eye can also occur in parallel or successively. A preferred embodiment of the invention therefore relates to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye, whereby this concerns a combination of two or more virus-based diseases of the eye. Preferably, these two or more virus-based diseases of the eye occur simultaneously or successively, in particular as a consequence of the first or one of the preceding disease(s). Such a combination of two or more virus-based diseases of the eye in an especially preferred embodiment is rhinoconjunctivitis.

**[0044]** “Virus-based diseases of the eye” within the meaning of the invention should be taken to mean diseases of the eye caused by a virus through a virus infection. The present invention discloses not only the treatment but also the prevention of virus-based diseases of the eye, as already defined above. Virus-based diseases of the eye according to the invention should therefore also be taken to mean such diseases of the eye that are to be assigned to a preventive treatment of virus-based diseases of the eye within the meaning of the invention. These include, for example, diseases typically preceding a virus-based disease of the eye. These include in particular keratoconjunctivitis sicca and indications and/or subforms derived herefrom, such as e.g., superficial keratitis and filiform keratitis, which are accordingly expressly designated as a virus-based disease of the eye according to the invention. A preferred novel use of D2O therefore relates to the administration of D2O in keratoconjunctivitis sicca. The disclosure in the present invention under the generally used term “keratoconjunctivitis” includes all forms of keratoconjunctivitis as virus-based diseases of the eye.

**[0045]** The term “eye” within the meaning of the invention can include all types of tissue, particularly skin types and cell types that can be affected by a virus. According to the invention, these are also designated as “organs of the eye” or “organs” or “eye organs to be treated” or “organs to be treated.” These include both superficial and deeper skin layers, primarily the eyelid skin, conjunctiva, the outer layers of the cornea, and deeper layers the cornea, the choroid, sclera, and other regions of the uvea, the epithelia and mucous membranes of the eye, such as the lacrimal sacs, lacrimal canaliculi, and tear glands. The epithelial cells of these above-described epithelia, which are also included in the term “eye,” are infected in particular by the virus.

**[0046]** Many of the aforementioned organs or organs of the eye represent regions of the eye accessible from the exterior. “Accessible from the exterior” within the meaning of the present invention means that the organs, particularly the mucous membranes thereof, can be reached by external application or administration of the active substance deuterium oxide (D2O) of the invention, i.e., not systemically.

**[0047]** Thus an “external” administration according to the invention can occur, for example, by means of a D2O-containing ophthalmic ointment (D2O ophthalmic ointment), D2O ophthalmic gel, D2O eye drops, or by irrigation of the corresponding organs or regions of the organs with D2O or by administration of liquid D2O or D2O gels or D2O hydrogels. A comprehensive and detailed description of the administration routes for D2O according to the invention is provided

below. An “internal” administration can occur according to the invention by injection of D2O into the eye and is done to reach posterior regions of the eye.

**[0048]** Organs or regions of such organs which are to be defined according to the invention as “accessible from the exterior” are, e.g., the eyelid, conjunctiva, the outer layers of the cornea, and the epithelia and mucous membranes of the lacrimal sacs, lacrimal canaliculi, and tear glands.

**[0049]** “Virus infection” within the meaning of the invention should be taken to mean the active or passive penetration of a virus into an organism, such as a plant, animal, or human, and the reproduction thereof in this organism. Such an organism is designated as a “host” according to the invention and includes vertebrates, in particular mammals, above all man, horses, pigs, cattle, goats, sheep, cats, and dogs. The cells of such a host are designated as “host cells.” An “organism to be treated” within the meaning of the present invention is such a host that either suffers from a virus-based disease of the eye and is therapeutically treated or is preventively treated with regard to such a disease, in this case man being a particularly preferred host. An “organ to be treated” within the meaning of the invention is an organ of such an above-defined organism to be treated.

**[0050]** Within the meaning of the invention, the terms “virus infection,” “infection by viruses,” “viral infection,” and “infection” are used synonymously and all refer to an infection caused by a virus.

**[0051]** Many viruses are known that cause a virus-based disease of the eye according to the invention. Some of these viruses and their infection route and pathological manifestation, therefore the disease caused by them, have already been described in greater detail in the present description. Therefore, a preferred embodiment of the invention relates to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye, whereby the virus is a virus from the family Herpesviridae, Poxviridae, Paramyxoviridae, Coronaviridae, Chordopoxviridae, Togaviridae, Orthomyxoviridae, Retroviridae, Adenoviridae, Picornaviridae, and/or Rhabdoviridae.

**[0052]** Especially preferably, the virus is a virus from the genus *Simplexvirus*, *Varicellovirus*, *Cytomegalovirus*, *Lymphocryptovirus*, *Chordopoxvirus*, *Rubivirus*, *Respirovirus*, *Morbillivirus*, *Rubulavirus*, *Adenovirus*, *Influenza A virus*, *Influenza B virus*, *Lentivirus*, *Mastadenovirus*, *Orthopoxvirus*, *Enterovirus*, *Rhinovirus*, *Lyssavirus*, and/or *Pneumovirus*.

**[0053]** Especially preferably the virus is a virus of the species herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella zoster virus (VZV), human cytomegalovirus (HCMV), Epstein-Barr virus, *Molluscipoxvirus*, vaccinia virus, *Rubivirus*, German measles virus, rubella virus, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, measles virus, mumps virus, human immunodeficiency virus, human adenovirus A, human adenovirus B, human adenovirus C, human adenovirus D, human adenovirus E, human adenovirus F, poliovirus, echovirus, Coxsackie A virus, Coxsackie B virus, rhinovirus, rabies virus, respiratory syncytial virus, and/or Rift Valley fever virus.

**[0054]** According to the invention, the terms “virus type” and “virus species” are used synonymously.

**[0055]** According to the invention, the terms “prevention and/or treatment” refer to any measure suitable for treating a virus-based disease of the eye which either represents a pre-

ventive treatment of such an emergent disease or of emergent symptoms thereof (prevention) or the treatment of the disease after onset and/or the avoidance of renewed onset of such a disease, for example, after a completed treatment period (therapy).

**[0056]** In general, an effective prevention and/or treatment of a virus-based disease of the eye occurs in particular by topical or systemic administration of a pharmaceutical agent or an antiviral agent.

**[0057]** Within the meaning of the invention, “topical” or “topical application” or “topical administration” should be taken to mean that a pharmaceutical or antiviral agent, according to the invention D2O, in contrast to systemic uptake, is taken up not into the bloodstream of the organism to be treated, but is only applied, placed, or introduced locally, especially superficially, onto/into an eye organ to be treated, particularly onto/into the epithelial cells or mucous membrane thereof, preferably as a formulation in the form of drops, i.e., in liquid form, such as, for example, as an eye rinse or eye drops, or a hydrogel, gel, ointment, or cream.

**[0058]** Within the meaning of the invention, “systemic” administration should be taken to mean that a pharmaceutical or antiviral agent is taken up in the bloodstream of the organism to be treated. In principle, topical administration of a pharmaceutical or antiviral agent has fewer adverse reactions than systemic administration, because with topical administration high active substance concentrations are only locally reached, i.e., in a defined and limited region of the organ, to be treated, of an organism, and the whole organism, more precisely its bloodstream, is not exposed to the active substance as is the case with systemic administration.

**[0059]** Such a pharmaceutical agent or antiviral agent, administered topically for the prevention and/or treatment of a virus-based disease of the eye, should have at least one, preferably several, of the following properties:

**[0060]** a) locally specific usability by topical administration over any period of time and with high permucosal transport kinetics of the active substance. This is achieved by the selective transport of the active substance into defined regions of the organ to be treated, in particular into cells, above all epithelial and mucosal cells, by choosing a suitable formulation of the active substance (for example, as a rinse);

**[0061]** b) retardation of the transepithelial transport of the active agent into the bloodstream, i.e., into blood vessels;

**[0062]** c) largely homogenous active substance distribution in the region of the site of action (organ to be treated) and the avoidance of local overconcentrations;

**[0063]** d) virostatic action, i.e., an action blocking or inhibiting the reproduction of viruses, on cells in the treated region of the organ (i.e., viral reproduction by the host cell's own replication apparatus is blocked or inhibited);

**[0064]** e) wide tolerance of the antiviral active substance by healthy cells (not infected by the virus) in skin tissue and the bloodstream to avoid adverse reactions, in particular also with regard to immune reactions.

**[0065]** It was established according to the invention that deuterium oxide (D2O) satisfies all the recited properties. In addition, D2O has clear advantages compared with the antiviral agent known in the state of the art, particularly virostatic agents, for example, far lower or no adverse reactions,

increased bioavailability, and universal applicability to all viruses causing infections of the eye.

**[0066]** According to the invention, it was established further that deuterium oxide is an effective antiviral agent with potential long-term action and is suitable for short-term, long-term, and repeated treatments of virus-based diseases of the eye. According to the invention, deuterium oxide, also referred to as D2O below, is a pharmaceutical or antiviral agent.

**[0067]** According to the invention, the term “pharmaceutical agent” is understood to be any inorganic or organic molecule, substance, or compound that has a pharmacological action. The term “pharmaceutical agent” is used herein synonymously with the term “drug.” Within the meaning of the invention, pharmaceutical agent also include antiviral agent, including D2O. Within the meaning of the invention, the term “antiviral agent” defines an active agent that is administered for treating virus-based diseases and in particular virus-based diseases of the eye. Preferably, such an antiviral agent is suitable for inhibiting or blocking the viral infection of the host cell and/or the replication of viral nucleic acid in the host cell and/or the reproduction of the virus. If an antiviral agent blocks or inhibits viral reproduction, the term “virostatic agent” is also used. The terms “pharmaceutical agent” and “antiviral agent” are used synonymously within the meaning of the invention.

**[0068]** Within the meaning of the invention, “cells” are vertebrate cells, in particular mammalian cells and above all human cells (cells of humans), namely, skin cells, above all epithelial cells, mucosal cells, and mucous membrane cells. According to the invention, “host cells” are cells that have been infected or can be infected by a virus. The defined cells and host cells of the invention are those of the eye.

**[0069]** The properties of D2O and the use thereof of the invention as an antiviral agent for the prevention and/or treatment of virus-based diseases of the eye are explained hereafter:

**[0070]** Deuterium oxide (D2O), often also called “heavy water,” is a substance extremely similar to “natural water” H2O in its physical properties. Deuterium oxide (D2O) and water (H2O) differ physically in the substitution of the hydrogen atoms of H2O by deuterium atoms, whereby D2O has an approx. 10% higher density and approx. 25% higher viscosity. Furthermore, the melting and boiling points of D2O are higher than for H2O. A detailed comparison of the properties is given in the Handbook of Chemistry and Physics, Section 6 (Handbook of Chemistry and Physics, David R. Lide, Editor, 79<sup>th</sup> edition, 1998, CRC Press, Boca Raton, USA).

**[0071]** Apart from the physically similar properties of H2O and D2O, there are significant physiological differences (see, inter alia, Kushner D J et al., *Pharmacological uses and perspectives of heavy water and denatured compounds*. Can J Physiol Pharmacol. 1999 Feb; 77(2): 79-88). Starting at a certain concentration in a cell, of more than 20-25% in animal cells, D2O has an effect on enzymatic reactions. Enzymatically controlled reactions are increasingly altered, in particular blocked or inhibited. The higher bond strength of the hydrogen bridge bonds, when the hydrogen atom in the bond is replaced by a deuterium atom, is regarded as a cause here. This higher bond strength occurs both in aqueous solutions of H2O and D2O and in the binding of water to organic molecules (Cuma M, Scheiner S, *Influence of Isotopic Substitution on Strength of Hydrogen Bonds of Common Organic Groups*. Journal of Physical Organic Chemistry, 1997, Vol-

ume 10, 383-395). This increased bond strength of hydrogen bridge bonds (H bridges) in the case where the hydrogen atom of the bond is replaced by a deuterium atom and the reduced rate of exchange of D<sub>2</sub>O compared with H<sub>2</sub>O (König, S., et al., *Molecular dynamics of water in oriented multilayers studied by quasi-elastic neutron scattering and deuterium-NMR relaxation*. 1994, J. Chem. Phys. 100, 3307-3316) has two direct consequences for the binding of D<sub>2</sub>O to binding sites suitable for H bridges: a) The sojourn probability of D<sub>2</sub>O on surfaces with bonding possibilities for H bridges is markedly increased compared with that of H<sub>2</sub>O. b) The hydration of the surfaces mentioned under a) increases and the detachment of D<sub>2</sub>O (e.g., by evaporation) is energetically impeded, which in turn increases the sustainability of the hydration.

**[0072]** According to the invention, “hydration” (also called hydration) should be taken to mean the deposition of D<sub>2</sub>O molecules, instead of or in addition to H<sub>2</sub>O molecules, onto a given surface, namely, the eye organ to be treated. A hydration within the meaning of the invention can also be called D<sub>2</sub>O hydration.

**[0073]** According to the invention, “degree of hydration” or “hydration level” is the time-averaged number of D<sub>2</sub>O molecules maximally binding via H bridges to a given surface, namely, the eye organs to be treated, instead of or in addition to H<sub>2</sub>O molecules. Within the meaning of the invention, the degree of hydration or hydration level can also be called the degree of D<sub>2</sub>O hydration or D<sub>2</sub>O hydration level.

**[0074]** “Sustainability of the hydration” in this case should be taken to mean the activation energy for the detachment of a D<sub>2</sub>O molecule or H<sub>2</sub>O molecule, bound by H bridges, from a surface, namely, the eye organ to be treated, a higher activation energy meaning higher sustainability. The sustainability of the hydration within the meaning of the invention can also be called the sustainability of the D<sub>2</sub>O hydration.

**[0075]** Mucous membranes, defined as organs (to be treated) of the eye according to the invention, are examples of particularly strongly hydrated membrane surfaces, in which the number of the H bridge bonding possibilities is greatly increased compared with other membranes (e.g., the skin surface) owing to the presence of glycolipids and glycoproteins. In this case, a slight increase in hydration and/or the sustainability of hydration has especially significant consequences which act, advantageously on the use of D<sub>2</sub>O according to the invention.

**[0076]** It is general technical knowledge that a cell takes up different quantities of H<sub>2</sub>O depending on the level of its specific metabolic activity. A cell that is infected by a virus, a so-called host cell, and in which reproduction of the virus takes place has a far higher metabolic activity than an uninfected cell of the same cell type in the surrounding region. The reason for this is that the host cell is bringing about not only its own replication, but also the replication of the virus. Because an increased metabolic activity of cells correlates with an increased water uptake, virus-infected host cells take up markedly more water (H<sub>2</sub>O) than uninfected cells. Because of the similar physical properties of H<sub>2</sub>O and D<sub>2</sub>O, D<sub>2</sub>O is taken up by cells parallel to H<sub>2</sub>O (if D<sub>2</sub>O and H<sub>2</sub>O are available) or instead of H<sub>2</sub>O (if only D<sub>2</sub>O is available).

Effect of D<sub>2</sub>O on the Replication and Reproduction of a Virus

**[0077]** As set forth above, it is known that enzymatic reactions in a cell can be altered by D<sub>2</sub>O at a sufficient concen-

tration. As explained above, cells, and virus-infected cells to an increased extent, take up D<sub>2</sub>O parallel to or instead of H<sub>2</sub>O. Accordingly, if D<sub>2</sub>O is administered to a virus-infected cell at a “sufficient concentration” according to the invention, i.e., more than 20% based on the total water content of a cell, this results in a blockade or inhibition of enzymatic reactions in the host cell. This includes in particular the blocking or inhibition of DNA polymerase (Takeda H et al., *Mechanisms of cytotoxic effects of heavy water (deuterium oxide: D<sub>2</sub>O) on cancer cells*. Anticancer Drugs, 1998 September; 9(8): 715-25). As a result of this, the DNA replication in a cell is blocked or inhibited. Uninfected, healthy cells in the region (area) surrounding the virus-infected cells take up D<sub>2</sub>O or H<sub>2</sub>O to a normal extent because of their lower metabolic activity in comparison with infected cells, so that no or only very slight, negligible blocking or inhibition of enzymatic reactions occurs in these.

**[0078]** According to the invention, it has now been established that if a cell is infected with a virus (host cell), the viral DNA is also not replicated owing to the blocking or inhibition of the host cell’s own DNA polymerase, because the replication thereof also occurs with the involvement of the host cell DNA polymerase. In the case of RNA viruses, the enzymatic blocking or inhibition by D<sub>2</sub>O also occurs for the synthesis of the reverse transcriptase which, encoded by virus genes, must be synthesized first in the host cell to transcribe the viral RNA into viral DNA, which is then in turn replicated by the DNA polymerase of the host cell. Blocking or inhibition of certain enzymatic reactions of the host cell by D<sub>2</sub>O therefore also blocks or inhibits the replication and therefore also the subsequent reproduction (as defined above) of a virus after a virus infection.

Effect of D<sub>2</sub>O on the Cell Division of Virus-Infected Cells

**[0079]** Another important aspect of the invention that is based on the described elevated binding property of D<sub>2</sub>O, is that when D<sub>2</sub>O is administered in sufficient concentration, i.e., more than 20%, based on the total water content of a cell, cell division is blocked or inhibited. This occurs most probably, in addition to the stated blocking or inhibition of DNA replication, by the blocking or inhibition of mitosis in the animal cell division cycle (Laissue J A et al., *Survival of tumor-bearing mice exposed to heavy water or heavy water plus methotrexate*. Cancer Research, 1982, Vol. 42(3): 1125-1129). According to the invention, this has crucial importance for the latency state in virus-based diseases of the eye in which the viruses are present in the resting state (latency) during the lysogenic cycle described in greater detail above. In this state, the viral genome is integrated into the host cell genome and transferred to the host cell progeny together with the host genome during division of the host cell. In a blocking or inhibition of cell division, such a transfer of the virus to new cells is prevented.

**[0080]** According to the invention, blocking or inhibition of viral reproduction thus occurs by the use of D<sub>2</sub>O for the prevention and/or treatment of virus-based diseases of the eye. This blocking or inhibition of viral reproduction by D<sub>2</sub>O as an antiviral agent occurs according to the invention in particular by:

**[0081]** blocking or inhibition of the replication of viral nucleic acid and thereby the reproduction of the virus and/or

[0082] blocking or inhibition of host cell division and thereby the reproduction of the virus in the lysogenic cycle.

[0083] The term “to block” or “blocking” according to the invention should be taken to mean that the replication of viral nucleic acids (viral RNA or viral DNA), viral reproduction, and/or the cell division rate of host cells of the invention are retarded and/or decreased, preferably up to 6%, preferably up to 10%, preferably up to 15%, also preferably up to 20%, more preferably up to 25%, more preferably up to 30%, also more preferably up to 35%, also more preferably up to 40%, also more preferably up to 45%, also more preferably up to 50%, still more preferably up to approx. 55%, still more preferably up to approx. 60%, further more preferably up to 65%, also more preferably up to 70%, also more preferably up to 75%, also more preferably up to 80%, also more preferably up to 85%, still more preferably up to 90%, and most preferably up to 94% compared with the replication or reproduction rate of the virus or the host cell division rate without administration of D2O.

[0084] The term “inhibit” or “inhibition” according to the invention means that the replication of viral nucleic acids (viral RNA or viral DNA), viral reproduction, and/or the cell division rate of host cells of the invention are prevented, preferably up to 95%, still more preferably up to 98%, and most preferably up to 100% (and therefore completely), compared with the replication or reproduction rate of the virus or the host cell division rate without administration of D2O.

[0085] Such an antiviral and virostatic effect of D2O based on the blocking or inhibition of the replication of virus nucleic acids (viral nucleic acids) and/or viral reproduction has not previously been described in the state of the art.

[0086] In addition, D2O has considerable advantages over known antiviral and virostatic agent for the treatment of virus-based diseases of the eye, namely, above all the following properties of D2O:

[0087] 1) D2O is not virus-specific and can block or inhibit the replication and/or reproduction of all viruses described according to the invention. This is particularly advantageous because most virus-based diseases of the eye, as described above, can be triggered by various viral species (also viral types) and in addition no symptomatic differentiation is possible. Accordingly, D2O as a so-called “broadband” virostatic agent can be administered for the prevention and/or treatment of all virus-based diseases of the eye.

[0088] 2) By means of the topical administration of D2O of the invention, e.g., by the application of D2O eye drops, D2O ophthalmic ointment, D2O ophthalmic cream, D2O ophthalmic ointment, or by irrigation with a D2O solution, a concentration high enough for therapeutic effectiveness (i.e., more than 20% based on the total water content of a cell) of D2O can be achieved in the epithelial cells of the eye organs to be treated without other, non-virus-infected organs of the organism being exposed to similarly high concentrations of D2O, as occurs with systemic administration. A critical problem, discussed in the state of the art, of achieving therapeutically effective D2O concentrations at the site of action (i.e., more than 20% based on the total water content of a cell) without severe adverse reactions for other, healthy organs or healthy surrounding tissue, is therefore solved.

[0089] 3) According to the invention, the physical state of D2O for topical administration is liquid or it is present in a solid formulation such as an ointment, cream, gel, or hydrogel. D2O is taken up by the epithelial cells of the eye by direct contact of the liquid D2O or a D2O-containing formulation.

[0090] 4) For the case that pure liquid D2O alone is administered (pure D2O), it should be stated that D2O has a unique advantage over all other liquid pharmaceutical agent. Like normal water (H2O), it can be transported into the epithelial cells of the eye, and owing to the strength and direction of the osmotic gradients and manipulation of these two factors, the penetration depth of D2O into epithelial cells can, moreover, be adapted to the therapeutic objective.

[0091] 5) As already described, the hydrogen bridge bond strength of deuterium atoms is higher than for hydrogen atoms, particularly in the binding of water to organic molecules. Topically administered D2O binds molecularly via hydrogen bridge bonds to the nearest available cell surface and thereby displaces the H2O absorbed there owing to its higher bond strength. Owing to this increased bond strength (and owing to the higher weight of the D2O molecule), the exchange frequency of D2O molecules with the H2O environment is in turn somewhat slower than for H2O (König, S., et al., *Molecular dynamics of water in oriented multilayers studied by quasi-elastic neutron scattering and deuterium-NMR relaxation*. 1994, J. Chem. Phys. 100, 3307-3316). This results in an increased sojourn probability of the D2O molecules directly on the cell surface, associated with increased internalization of D2O in the cell, as a result of which it can exert its action—the blocking or inhibition of the above-described enzymatic reactions of a virus-infected host cell and the division thereof. Because virus-infected cells have a higher uptake capacity for water or D2O than normal cells, it is ensured, moreover, that D2O is concentrated disproportionately in these cells compared with healthy cells, i.e., in a sufficient D2O concentration of more than 20% based on the total water content of the cell. Owing to these properties, the topical administration of D2O is exceptionally valuable.

[0092] 6) D2O is the only non-radioactive molecule which is very similar to H2O in its properties. Cells in general and in particular the cells of the invention cannot “distinguish” between the two molecules, so that D2O is transported into the cell by active and passive transport in the same way as H2O and reaches the cell nucleus. As a result, cell barriers of any type that prevent the penetration of other pharmaceutical agents are circumvented and defense mechanisms at the cellular level, such as internalization in lysosomes or the activation of MDR (multiple drug resistance) transporters, or at the organ level by the immune system, which could reduce or inhibit the effectiveness of the pharmaceutical agent D2O, are also largely turned off.

[0093] 7) A further advantage of D2O as an antiviral or virostatic agent is the fact that concentrations of less than 20% D2O (based on the total water content of the cell) in the cell exert no significant effects and therefore normal cells, which take up comparatively little D2O owing to their lower water permeability and/or water uptake com-

pared with the active, virus-infected cells, are barely exposed to the effects of D2O.

**[0094]** In an especially preferred embodiment of the present invention, according to the invention D2O is administered non-systemically.

**[0095]** An especially preferred embodiment of the invention is the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye, the deuterium oxide being administered topically. This type of topical administration occurs preferably in the form of drops, i.e., in liquid form, such as, for example, as eye drops (D2O eye drops) or as a rinse (D2O eye rinse), or in solid form as a cream (D2O cream), ointment (D2O ointment), gel (D2O gel), or hydrogel (D2O hydrogel). This prevents above all the adverse reactions caused by a systemic administration of antiviral or virostatic agent.

**[0096]** Another especially preferred embodiment of the invention is the use of deuterium oxide, whereby the deuterium oxide hydrates the eye or the eye organs to be treated, particularly the mucous membranes of the eye.

**[0097]** By direct administration according to the invention onto virus-infected organs of the eye or eye organs to be treated, in particular the mucous membranes of the eye, locally preventive or therapeutically active D2O concentrations and a locally preventive or therapeutically active hydration by D2O can be achieved and at the same time stresses on the system (i.e., the bloodstream) and the adverse effects on healthy tissue and organs of the eye that are not to be treated, as well as tissues of other surrounding or distant organs (such as, for example, the liver or kidneys), which could be caused by a high concentration of D2O of more than 20% D2O, based on the total water content of the cell, can be decreased or completely avoided. Furthermore, transport of D2O into the system can be prevented or limited by means well-known in the state of the art. Examples of these means are, inter alia, the selective manipulation of the osmotic gradients across the mucous membrane (i.e., between the systemic part and the mucous membrane surface) by decreasing the water potential of the administered D2O by means of substances suitable for altering said water potential, in particular physiologically acceptable salts, such as sodium chloride, water-soluble polymers, and other non-pharmaceutical substances.

**[0098]** According to the invention, D2O can be used alone as a pharmaceutical agent, more precisely as an antiviral or virostatic agent, or in combination with one or more other pharmaceutical agent(s) and/or one or more other non-pharmaceutical agent(s) (which can be used especially for optimizing the topical administration of D2O as a pharmaceutical agent onto mucous membranes).

**[0099]** Accordingly, a preferred embodiment of the present invention relates to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye, whereby D2O is used in combination with at least one additional pharmaceutical agent and/or at least one additional non-pharmaceutical agent.

**[0100]** Such a combination of D2O and at least one additional pharmaceutical agent and/or at least one additional non-pharmaceutical agent is referred to hereafter as a "novel combination."

**[0101]** All novel D2O uses and administrations disclosed in this description, for example, as a formulation, liquid, hydrogel, gel, cream, or ointment, or in a solvent, topically, etc., can also be applied without limitation to a novel combination,

unless otherwise specified. This applies to the use and administration of D2O in combination with H2O, also referred to hereafter as a "mixture of D2O and H2O."

**[0102]** Another preferred embodiment of the present invention relates to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye, whereby D2O is used in combination with at least one additional pharmaceutical agent, which is selected from the group consisting of virostatic agents, proteins, peptides, nucleic acids, and immunosuppressant agent.

**[0103]** Additional pharmaceutical active substances, preferred according to the invention, in a novel combination and the effect thereof are set forth below, whereby this enumeration is only by way of example and the present invention is not limited thereto:

#### Virostatic Agents

**[0104]** Active substances that block the reproduction of viruses are designated as virostatic agents. Virostatic agents block the activity of enzymes, for example, DNA polymerase, reverse transcriptase, or proteases, and thereby block or inhibit the replication of the virus or the processing of a synthesized long viral protein into smaller protein segments. Examples thereof are: amantadine and rimantadine.

#### Immunosuppressant Active Substances

**[0105]** The response of epithelial or skin tissue to the reproduction of viruses, particularly if inflammation is already present, can be improved and optimized by the addition of immunosuppressant active substances, for example, corticosteroids and/or other immunomodulators.

#### Proteins

**[0106]** Proteins that may be used according to the invention should be taken to mean proteins that interfere in a suitable manner in the infective, replication, or reproductive cycle of the virus in a host cell. In this connection, "in a suitable manner" means that the proteins block, preferably inhibit, the adsorption of the virus onto the host cell, the injection of viral nucleic acid into the host cell, the replication of the DNA of the host cell or of the viral nucleic acid, the processing of the viral nucleic acid, or assembly of the viral particles to a complete virus, or otherwise interfere in the reproductive cycle of the virus. Examples of these are protease inhibitors, uncoating inhibitors, penetration inhibitors, reverse transcription inhibitors, and DNA polymerase inhibitors.

#### Peptides

**[0107]** Peptides that may be used according to the invention should be taken to mean, for example, peptides that influence, in particular increase, the membrane permeability of the host cell membranes in a suitable manner. As a result, improved transport of D2O and optionally of the additional pharmaceutical or the non-pharmaceutical agents of the invention into the host cell can be achieved. An example of this is melittin. In addition, peptides that may be used as taught by the invention should be taken to mean all those peptides with effects analogous to those described above for proteins.

#### Nucleic Acids

**[0108]** A modification of the genetic information of the cells in the region of the site of action or a selective turning off

("gene silencing") of certain genes, for example, of DNA polymerase, of cells in the region of the site of action, i.e., of the eye organ to be treated, and thereby a modification of the proteome can be achieved by the addition of nucleic acids, parallel to the antiviral or virostatic action of D2O. "Gene silencing" can have the result, for example, that genes involved in DNA damage defense (for example, p53, BRCA1, BRCA2, ATM, and CHK2) are switched off and thereby the viruses whose reproduction has been impeded by D2O in the cells no longer revert to a latent stage even in the long term (after the end of the topical D2O administration), but are prevented in the long term from expressing viral DNA. Methods for "gene silencing" are well known to the person skilled in the art and described, for example, in Mello C C. Conte D, "Revealing the world of RNA interference" in *Nature* 431, 338-342 (Sep. 16, 2004). Preferably, the nucleic acids are DNA, preferably oligonucleotides, sense or antisense DNA, natural or synthetic, cDNA, genomic DNA, naked DNA, single- or double-stranded DNA, or circular DNA, or RNA, preferably antisense RNA, RNAi, siRNA, or other RNA molecules suitable for interference, which are not restricted in their length.

**[0109]** The concentration of additional pharmaceutical agents to be used as taught by the invention apart from D2O as a pharmaceutical agent based on the total solution of a novel combination is within the range of at least  $10^{-8}$  M to at least  $5 \cdot 10^{-2}$  M, preferably of at least  $10^{-7}$  M to  $10^{-3}$  M, and most preferably of at least  $10^{-6}$  M to at least  $10^{-2}$  M. An especially preferred concentration range is within the range of at least  $10^{-9}$  M to at least  $10^{-2}$  M.

**[0110]** A likewise preferred embodiment of the present invention relates to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eyes, whereby D2O is used in combination with at least one additional non-pharmaceutical agent, whereby the at least one additional non-pharmaceutical agent is selected from the group consisting of pharmaceutically acceptable inorganic or organic acids or bases, polymers, copolymers, block copolymers, monosaccharides, polysaccharides, ionic and nonionic surfactants, or lipids, as well as mixtures thereof, albumin, transferrin, and DNA repair proteins, such as kinase inhibitors.

**[0111]** The term "non-pharmaceutical agent" within the meaning of the invention refers to any pharmacologically acceptable and therapeutically useful molecule, substance, or compound that is not a pharmaceutical agent but is administered to an organism to be treated, preferably together with at least one pharmaceutical agent of the invention, for example, formulated in a formulation of the invention, in order to influence, in particular to improve, qualitative properties of the pharmaceutical agent(s). Preferably, the non-pharmaceutical agent exert no or no appreciable or at least no undesired pharmacological effect in regard to the intended prevention or treatment of virus-based diseases of the eye. Suitable non-pharmaceutical agents are, for example, pharmacologically acceptable salts, for example, sodium chloride, flavoring agents, vitamins, e.g., vitamin A or vitamin E, tocopherols, or similar vitamins or provitamins occurring in the human body, antioxidants such as, for example, ascorbic acid, and stabilizers and/or preservatives to prolong the use and storage period of a pharmaceutical agent or a formulation of the invention and other conventional non-pharmaceutical agents or excipients and additives known to the person skilled in the art.

**[0112]** Other non-pharmaceutical agent, preferred according to the invention, of a novel combination and the effect thereof and suitable concentrations are provided below, but this enumeration is only by way of example and the present invention is not limited thereto:

#### Water-Soluble Excipients and Additives

**[0113]** The physiological tolerance of D2O in cells of organs of the eye can be improved for non-virus-infected cells by addition of water-soluble excipients and additives, such as, e.g., pharmaceutically acceptable inorganic or organic acids, bases, salts, and/or buffer substances for adjusting the pH. Examples of preferred inorganic acids are selected from the group consisting of hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, and phosphoric acid, hydrochloric acid and sulfuric acid in particular being preferred. Examples of especially suitable organic acids are selected from the group consisting of malic acid, tartaric acid, maleic acid, succinic acid, acetic acid, formic acid, and propionic acid and particularly preferably ascorbic acid, fumaric acid, and citric acid. Optionally, mixtures of the recited acids may also be used, in particular of acids that apart from their acidic properties also have other properties, e.g., use as antioxidants, such as, for example, citric acid or ascorbic acid. Examples of pharmaceutically acceptable bases are alkali metal hydroxides, alkali metal carbonates, and alkali metal ions, preferably sodium. Mixtures of said substances may be used in particular for the adjustment and buffering of the pH; especially preferred in this case are potassium hydrogen phosphate and dipotassium hydrogen phosphate, sodium hydrogen phosphate and disodium hydrogen phosphate, as well as citric acid and sodium citrate. Preferred buffer substances within the meaning of the invention further are PBS, HEPES, TRIS, MOPS, and other physiologically acceptable buffer substances, in particular those with a pK value within the range of from 4.0 to 9.0. The concentration of these substances, based on the total solution of a novel combination, lies preferably within the range of micromolar to millimolar, particularly preferably within the range of 1-100 mM.

#### Water-Soluble Polymeric Molecules

**[0114]** By addition of water-soluble, noncytotoxic molecules, such as, e.g., certain polymers (e.g., but not limited thereto, dextran, polyethylene glycol, agarose, cellulose, acrylic acid, and hyaluronic acid), copolymers, and block copolymers, an additional delay (retardation) in the passage of D2O into the epithelial cells of the eye with topical administration and also from epithelial cells into the system (bloodstream) can be achieved owing to their high water-binding capacity. In addition, the strength and direction of the osmotic gradient across the skin can be modified or improved and optimized by the ability of polymers to lower the chemical potential (water potential) of D2O. The concentration of said substances, based on the total solution, lies within the range of micromolar to molar, preferably within the range of 1-500 mM.

#### Water-Soluble Non-Polymeric Molecules

**[0115]** The osmotic conditions in the region of the topical D2O administration and the D2O transport and D2O retention in the epithelial cells of the eye can be modified or optimized by addition of water-soluble, non-polymeric molecules which modify the density and/or viscosity of D2O, for

example, but not limited thereto, monosaccharides and polysaccharides, in particular glucose, sucrose, dextrose, maltose, starch, and cellulose. The concentration of said substances, based on the total solution of a novel combination, preferably lies within the range of millimolar to molar, particularly preferably within the range of 1.0 mM to 1.5 M.

#### D2O Interfacial Tension-Modifying Molecules

**[0116]** The transport of D2O into the epithelial cells of the eye on topical administration and within these epithelial cells can be modified by addition of substances that modify the interfacial tension of D2O, for example, but not limited thereto, ionic and nonionic surfactants or lipids, in particular a mixture of surfactants and lipids. The concentration of said substances, based on the total solution of a novel combination, preferably lies within the range of micromolar to millimolar, particularly preferably within the range of 0.05-500 mM.

#### Water-Soluble Noncytotoxic Molecules

**[0117]** An additional increase in the D2O transport rate of the molecules, surrounded by a D2O hydration shell, into the target cells of the eye can be achieved by addition of water-soluble molecules that are known to be taken up to a particular extent by metabolically especially active cells, such as, e.g., virus-infected cells, for example, albumin or transferrin.

**[0118]** The concentration or dosage of D2O and optionally of the at least one additional pharmaceutical and/or non-pharmaceutical agent depends on various factors, for example, the nature of the treatment, nature of the disease, disease state of the patient (mammal), nature of the active substance, etc. Such parameters are known to the person skilled in the art and the determination of the specific dosages is subject to the know-how of the skilled person. Suitable concentration information is disclosed herein. Some exemplary information on suitable concentration ranges has already been given above but these are only intended as guide values.

**[0119]** The D2O usable as taught by the invention is preferably present as a liquid. D2O is preferably present in a solution, preferably with H2O (water) as solvent, and is also described herein as "mixture of D2O and H2O" or "D2O/H2O" if water is included, or as a "D2O solution" or "pure D2O" if no water is included. Pure D2O preferably contains D2O in a concentration range of 98.0 to 100%, preferably 98.5 to 99.9%, particularly preferably 99.7% based on the total water content of the solution. A novel mixture of D2O and H2O preferably contains D2O within a concentration range of from 1 to 99%, preferably 5 to 95%, further preferably 10 to 90%, also preferably 15 to 80%, more preferably 20 to 70%, also more preferably 30 to 60%, and most preferably 40 to 50%, where this information refers to the total water content of the mixture of D2O and H2O. The preparation of a D2O solution of the invention and analogously thereto of a novel combination is carried out, for example, by mixing of the components, in particular of D2O and optionally H2O, and optionally of the at least one additional pharmaceutical and/or non-pharmaceutical agent. Further, optionally a solvent can be added by mixing, as described below. The addition of H2O or the at least one additional pharmaceutical and/or non-pharmaceutical agent or the solvent to D2O occurs preferably in the liquid state. However, the preparation can be achieved by any suitable process. In the case that D2O

is used alone (i.e., not in combination with H2O or at least one additional pharmaceutical and/or non-pharmaceutical agent) and at a concentration of 100% or approx. 99.9%, based on the total volume of the solution (i.e., pure D2O), pure D2O is the D2O solution of the invention.

**[0120]** All novel uses and administrations of D2O, as disclosed in this description, for example, as a formulation, liquid, hydrogel, gel, cream, or ointment, or in a solvent, topically, etc., are also applicable without limitation to a novel mixture of D2O and H2O, unless otherwise specified. Likewise, uses and administrations of a novel combination can be applied without limitation to a novel mixture of D2O and H2O, and vice versa, unless specified otherwise.

**[0121]** In a preferred embodiment, the at least one additional pharmaceutical or additional non-pharmaceutical agent is bound to D2O. "Bound" within the meaning of the present invention means that the pharmaceutical or non-pharmaceutical agent is hydrated by D2O.

**[0122]** In another preferred embodiment, D2O or the novel combination is contained in a suitable solvent. A solvent according to the invention can be an inorganic or organic solvent. Suitable solvents of the present invention should preferably be physiologically well-tolerated by the organism (in particular mammal) to which the active substance with solvent is administered, i.e., trigger no adverse reactions, e.g., toxic adverse reactions. An especially preferred solvent is distilled water. Also preferred are ethanol-water mixtures; here the percentage by mass of ethanol in these mixtures is preferably within the range between 5% and 99% ethanol, also preferably within the range of 10% to 96% ethanol, more preferably between 50% and 92%, and most preferably between 69% and 91% ethanol.

**[0123]** D2O or a novel combination or a novel mixture of D2O and H2O can be "preformulated," for example, packaged in suitable means for the transport of pharmaceutical agent, so-called "drug delivery" systems, for example, in nanoparticles, vectors, preferably gene transfer vectors, viral or nonviral vectors, poly- or lipoplex vectors, liposomes, or hollow colloids (i.e., hollow spheres of colloidal size). Suitable further for transport are naked nucleic acids, in particular naked DNA. Suitable vectors, liposomes, hollow colloids, or nanoparticles and processes for the introduction of substances into such vectors, liposomes, hollow colloids, or nanoparticles are generally well-known in the state of the art and described, for example, in Cryan S -A., *Carrier-based Strategies for Targeting Protein and Peptide Drugs to the Lungs*. AAPS Journal, 2005, 07(01): E20-E41, and Sambrook et al., *Molecular Cloning A Laboratory Manual*. Cold Spring Harbor Laboratory (1989) NY. Polyethylenimine or cationic lipids such as, e.g., DOTAP can be used preferably as gene transfer vectors. Liposomes may be used preferably for packaging cytostatic agents; a detailed description is given, for example, in Koshkina N V et al., *Paclitaxel liposome aerosol treatment induces inhibition of pulmonary metastases in murine renal carcinoma model*. Clinical Cancer Research, 2001, 7, 3258-3262. Proteins as pharmaceutically active substances can be packaged preferably in biocompatible poly-lactic/glycolic acid polymers (PLGA) by means of supercritical liquids, emulsion processes, and spray drying.

**[0124]** According to the invention, D2O is preferably administered topically. This occurs by application, placing, or introduction of D2O onto/into the epithelial cells or mucous membranes of the eye organ to be treated preferably in the form of drops, i.e., in liquid form, such as, for example, as



drops, a rinse, or in solid form, for example, as a cream, ointment, gel, or hydrogel, or in the form of a formulation. The nature and duration of the topical administration of D2O for the prevention and/or treatment of virus-based diseases of the eye and the concentration of D2O and optionally additional pharmaceutical and/or non-pharmaceutical agent depend on the location and accessibility of the eye organ to be treated, apart from the severity of the disease and the patient's condition.

**[0125]** A preferred embodiment of the invention relates to the use of deuterium oxide for the prevention and/or treatment of virus-based diseases of the eye, whereby D2O is administered as a formulation. The formulation is administered topically by application to the eye organ to be treated, particularly to the epithelial cells or mucous membrane thereof. A novel formulation is especially preferably a liquid, cream, ointment, gel, or a hydrogel. Preferably above all, a novel formulation is an eye rinse (D2O eye rinse), eye drops (D2O eye drops), an ophthalmic ointment (D2O ophthalmic ointment), an ophthalmic gel (D2O ophthalmic gel), or an ophthalmic hydrogel (D2O ophthalmic hydrogel).

**[0126]** Such a "liquid" or liquid formulation within the meaning of the invention comprises formulations of pure D2O, a mixture of D2O and H2O, and a novel combination, provided it is in the liquid state. It is preferably administered in the form of drops or as a rinse.

**[0127]** Novel formulations, also D2O formulations, are suitable for the treatment of virus-based diseases of the eye. They are applied topically to the organ to be treated, in particular to the epithelial cells or mucous membranes thereof. To this end, they are preferably administered as a liquid, preferably as a rinse or drops, or as a cream, ointment, gel, or hydrogel:

**[0128]** D2O rinses are used for irrigating the eye. In this way, D2O can be applied and introduced topically and in high concentrations to the organ to be treated, in particular the epithelial cells or mucous membranes thereof. A D2O rinse is especially suitable, for example, for the treatment of keratoconjunctivitis sicca.

**[0129]** D2O eye drops may be used for direct local administration of D2O to the organ to be treated, especially the epithelial cells or mucous membranes thereof. D2O eye drops are dropped into the eye for this purpose. D2O nasal drops are especially suitable, for example, for the treatment of keratoconjunctivitis sicca.

**[0130]** D2O cream, D2O ointment, D2O gel, and D2O hydrogel are applied and introduced topically to the organ to be treated, particularly the epithelial cells or mucous membranes thereof.

**[0131]** An "ointment" according to the present invention is a drug preparation for external use comprising a base compound of spreadable material, such as vaseline, to which the actual pharmaceutical agents, such as D2O, and/or non-pharmaceutical agents are added, for example, by mixing.

**[0132]** A "cream" within the meaning of the present invention should be taken to mean an ointment of the invention, which may also contain other components, such as cosmetic agents, e.g., perfumes, colorants, and/or emulsifiers, e.g., lecithin. A distinction is generally made between a cream and a lotion, whereby this distinction depends mostly on the degree of viscosity. According to the invention, a cream should also be taken to mean a lotion.

**[0133]** A "gel" within the meaning of the present invention is the solution of a macromolecular substance, e.g., agarose,

acrylic acid, alginic acid, polysiloxanes, or acrylamide, whose concentration is so high that under suitable conditions and optionally with the addition of other substances (e.g., salts, acids, fillers, buffer substances) the dissolved macromolecules bind together into a sponge-like, three-dimensional skeleton with liquid within its void spaces. As a result, gels have a relatively firm consistency. The viscosity lies between liquid and solid. Such a liquid is preferably pure D2O or a novel mixture of D2O and H2O.

**[0134]** A "hydrogel" within the meaning of the invention is a gel that is characterized by an especially high water uptake capacity; within the meaning of the invention, it consists preferably 20-99%, more preferably 70-99%, and especially preferably 80-99% of water, but without exhibiting the rheological properties of a classic liquid. In an especially preferred embodiment, the hydrogel is transparent/translucent and at the same time spreadable, without its morphology and integrity being detrimentally affected by the spreading of the gel.

**[0135]** The preparation of a formulation usable according to the invention, in particular of an ointment, cream, or gel, is described exemplarily in the examples. If such a formulation contains additional pharmaceutical and/or non-pharmaceutical agents, these are preferably added by mixing of the formulation. It may occur, however, by any standard method known in the state of the art. Such methods and also the concentrations to be selected of the components or substances to be used are known to the person skilled in the art.

**[0136]** The concentrations of D2O in a formulation usable according to the invention preferably lie within the following ranges:

**[0137]** for a cream or ointment, preferably within the range of 0.1 to 98% by weight, preferably 5 to 85% by weight, also preferably 10 to 80% by weight, particularly preferably 15 to 70% by weight, more preferably 20 to 60% by weight, and most preferably 25 to 50% by weight, and

**[0138]** for a gel, preferably 0.1 to 99.8% by weight, preferably 10 to 99% by weight, also preferably 15 to 80% by weight, particularly preferably 20 to 70% by weight, more preferably 30 to 70% by weight, and most preferably 35 to 65% by weight.

**[0139]** The person skilled in the art will select the suitable concentration in particular depending on the particular indication, the state of the organism (patient) to be treated, the severity of the disease, etc.

**[0140]** In an especially preferred embodiment, a formulation usable according to the invention contains further at least one inorganic or organic solvent. The solvent is preferably selected from the group consisting of ethanol, water, and glycerol, as well as mixtures thereof.

**[0141]** All uses of the invention disclosed in this description also apply without limitation to a novel formulation, unless specified otherwise. Likewise, uses and administrations of a novel combination or a novel mixture of D2O and H2O apply without limitation to a novel formulation and vice versa, unless specified otherwise.

**[0142]** The present invention will be explained further on the basis of figures and the following examples, whereby these serve only for illustration and do not limit the subjects of the invention.

#### FIGURES

**[0143]** FIG. 1 shows Table 1, which in turn shows the number of viruses in a type-1 cell culture of the cell line



SV-40 (human corneal epithelial cell line), prepared according to Example 4, at the time 72 hours after infection for various viruses. The viral count was determined by electron microscopy by counting and averaging from 20 non-lysed cells each in the treatment group and the control group.

[0144] FIG. 2 shows Table 2, which in turn shows the number of viruses in a modified type-1 cell culture of the cell line SV-40 (human corneal epithelial cell line), prepared according to Example 5, at the time 72 hours after infection for various viruses. The viral count was determined by electron microscopy by counting and averaging from 20 non-lysed cells each in the treatment group and the control group.

[0145] FIG. 3 shows Table 3, which in turn shows the number of viruses in a type-2 cell culture of the cell line CCL 20.2 (human conjunctival epithelial cell line), prepared according to Example 6, at the time 72 hours after infection for various viruses. The viral count was determined by electron microscopy by counting and averaging from 20 non-lysed cells each in the treatment group and the control group.

[0146] FIG. 4 shows Table 4, which in turn shows the results of the effectiveness of D2O hydrogel (treatment A), D2O eye drops (treatment B), and H2O hydrogel (control) for the treatment of ocular herpes in humans according to Example 25. The "total symptom score" was averaged over the number of administrations per herpes incidence; for the "maximum size of the herpes," "time between first symptoms and first administration," "time to externally complete healing," and "time to freedom from pain" averaging was performed over the total number of herpes incidences treated per group (treatment or control). The provided errors are the standard deviations.

## EXAMPLES

### Example 1

#### Preparation of an Isotonic D2O or H2O Solution for Rinsing or as Eye Drops

[0147] Deuterium oxide (D2O) with an enrichment of 98% or purified water (H2O) was combined with 160 mM NaCl and the mixture was adjusted to a pH of 7.0 with 20 mM phosphate buffer (sodium dihydrogen phosphate and disodium hydrogen phosphate). The solutions were sterilized by processes that are the state of the art and stored until use under sterile conditions and protected from light.

### Example 2

#### Preparation of a D2O or H2O Hydrogel Based on Acrylic Acid

[0148] In separate batches, 2.0% by weight Carbopol 980 (manufacturer: Noveon Inc., 9911 Brecksville Rd., Cleveland Ohio 44141-3247, USA) was dissolved in pure D2O (98% D enrichment) or in pure H2O by stirring and then titrated to a pH of 6.8 by pipetting with 10 M NaOH solution and buffered to this pH with 20 mM phosphate buffer (sodium dihydrogen phosphate and disodium hydrogen phosphate). Next, the colorless, transparent, and optically clear acrylic acid gels (Carbopol gels) (D2O Carbopol gel, H2O Carbopol gel), formed by NaOH addition as a result of crosslinking of the polyacrylic acid via its carboxyl groups with the alkaline hydroxy

groups, were sterilized (autoclaved) and stored, aseptically packaged, at room temperature until further use, but for at least 24 hours.

### Example 3

#### Preparation of a D2O-Containing Cream

[0149] D2O was added slowly to 50 g of Asche base cream (manufacturer: Asche Chiesi GmbH, Hamburg, Germany) with constant stirring at 40° C., until a percentage by weight of 38% D2O (based on the starting weight of the cream) in the homogenous mixture was achieved. The cream was then cooled to room temperature and stored hermetically sealed.

### Example 4

#### Cell Culture Type 1

[0150] Cells of the SV-40 cell line (human corneal epithelial cell line), prepared according to Araki-Sasaki K. et al. ("An SV40-Immortalized Human Corneal Epithelial Cell Line and Its Characterization," Investigative Ophthalmology & Visual Science, March 1995, Vol. 36, No. 3), were seeded in 10 mm cell culture dishes at a cell density of  $8 \times 10^4$  cells/cm<sup>2</sup>. The medium used for this purpose was Dulbecco's Modified Eagle Medium, DMEM (Gibco/BRL, Life Technologies Inc., Grand Island, N.Y., USA) with 10% fetal calf serum, FCS (Hyclone, Logan, Utah, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Sigma Chemical Co., St. Louis, Mo., USA). At a temperature of 37° C., the cells were grown to confluence at 95% atmospheric humidity and 5% CO<sub>2</sub>. On the day of infection of the cell culture with viruses, the cell culture medium was changed and the new medium (DMEM with 2% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin) contained the viruses at a concentration that corresponded to a multiplicity of infection (MOI) of 3. The probability that a cell is infected with at least one viral particle at this MOI was 95%. Infection and incubation occurred at a temperature and humidity analogous to those given above.

### Example 5

#### Modified Cell Culture Type 1

[0151] Cells of the SV-40 cell line (human corneal epithelial cell line), prepared according to Araki-Sasaki K. et al. ("An SV40-Immortalized Human Corneal Epithelial Cell Line and Its Characterization," Investigative Ophthalmology & Visual Science, March 1995, Vol. 36, No. 3), were seeded in 10 mm cell culture dishes at a cell density of  $8 \times 10^4$  cells/cm<sup>2</sup>. The medium used for this purpose was Dulbecco's Modified Eagle Medium, DMEM (Gibco/BRL, Life Technologies Inc., Grand Island, N.Y., USA) with 10% fetal calf serum, FCS (Hyclone, Logan, Utah, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Sigma Chemical Co., St. Louis, Mo., USA). At a temperature of 37° C., the cells were grown to confluence at 95% atmospheric humidity and 5% CO<sub>2</sub>. After the confluence was reached and 24 hours before infection of the cell culture with viruses, the cell culture medium was changed and the new medium (DMEM with 2% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin) was diluted with 30% D2O, 98% D-enrichment (treated culture), or diluted with 30% H2O (control culture). At the time of infection, the medium was replaced again by a medium of identical composition, which contained the viruses in concentrations that correspond to a multiplicity of infection of 3.

Infection and incubation occurred at a temperature and humidity analogous to those given above.

#### Example 6

##### Cell Culture Type 2

**[0152]** Cells of the CCL 20.2 cell line (human conjunctival epithelial cell line) from the American Type Culture Collection (Manassas, Va., USA) were seeded in 10 mm cell culture dishes at a cell density of  $8 \times 10^4$  cells/cm<sup>2</sup>. The medium used for this purpose was Dulbecco's Modified Eagle Medium, DMEM (Gibco/BRL, Life Technologies Inc., Grand Island, N.Y., USA) with 10% fetal calf serum, FCS (Hyclone, Logan, Utah, USA), 100 U/mL penicillin, and 100 µg/mL streptomycin (Sigma Chemical Co., St. Louis, Mo., USA). At a temperature of 37° C., the cells were grown to confluence at 95% atmospheric humidity and 5% CO<sub>2</sub>. On the day of infection of the cell culture with viruses, the cell culture medium was changed and the new medium (DMEM with 2% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin) contained the viruses at a concentration corresponding to a multiplicity of infection (MOI) of 3. The probability that a cell is infected with at least one viral particle at this MOI was 95%. Infection and incubation occurred at a temperature and humidity analogous to those given above.

#### Example 7

##### Determination of the Viral Count in the Cell Culture After Incubation

**[0153]** After an incubation period of 3 days, the average number of viruses per cell was analyzed for the treated culture and the control culture. The viral count was determined by transmission electron microscopy by standard fixation and staining methods (glutaraldehyde and osmium tetroxide). Before fixation, the cell culture medium was removed and the supernatant (after centrifugation at 20,000 g for 30 minutes) was stored for further analyses. In each case, 20 randomly selected, non-lysed cells from the treated and control culture were evaluated by electron microscopy in regard to their viral particle content.

#### Example 8

##### D2O Effectiveness with Herpesviruses HSV-1 in a Type-1 Cell Culture

**[0154]** 6 cell culture dishes of cell culture type 1 according to Example 4 were infected with herpesviruses of type 1, HSV-1 (strain MacIntyre VR-539 from the American Type Culture Collection, Manassas, Va., USA). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0155]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 9

##### D2O Effectiveness with Herpesviruses Type Varicella Zoster 1 in a Type-1 Cell Culture

**[0156]** 6 cell culture dishes of cell culture type 1 according to Example 4 were infected with herpesviruses of the zoster

type, varicella zoster (strain Ellen VR-586 from the American Type Culture Collection, Manassas, Va., USA). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0157]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 10

##### D2O Effectiveness with Coronaviruses Type Human Coronavirus HCoV 229E 1 in a Type-1 Cell Culture

**[0158]** 6 cell culture dishes of cell culture type 1 were infected according to Example 4 with human coronaviruses 229E. 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0159]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 11

##### D2O Effectiveness with Influenza Virus A1 in a Type-1 Cell Culture

**[0160]** 6 cell culture dishes of cell culture type 1 were infected according to Example 4 with influenza A virus, type H1N1. 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0161]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 12

##### D2O Effectiveness with Influenza Virus B1 in a Type-1 Cell Culture

**[0162]** 6 cell culture dishes of cell culture type 1 were infected according to Example 4 with influenza B virus/Hong Kong/5/72. 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0163]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 13

##### D2O Effectiveness with Human Parainfluenza Virus 1 in a Type-1 Cell Culture

**[0164]** 6 cell culture dishes of cell culture type 1 were infected according to Example 4 with human parainfluenza viruses 1, strain Washington 1957 (HPIV-1). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0165]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 14

##### D2O Effectiveness with Human Respiratory Syncytial Virus (HRSV) 1 in a Type-1 Cell Culture

**[0166]** 6 cell culture dishes of cell culture type 1 were infected according to Example 4 with HRSV, strain Long. 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0167]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 15

##### D2O Effectiveness with Human Coxsackievirus 1 in a Type-1 Cell Culture

**[0168]** 6 cell culture dishes of cell culture type 1 were infected according to Example 4 with human coxsackieviruses, type A1. 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0169]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 16

##### D2O Effectiveness with Human Adenoviruses Type 3 1 in a Type-1 Cell Culture

**[0170]** 6 cell culture dishes of cell culture type 1 were infected according to Example 4 with human adenoviruses type 3, strain GZ1. 120 minutes after infection, the cell culture medium combined with the viruses was removed and

replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0171]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 17

##### D2O Effectiveness with Human Enteroviruses Type EV71 1 in a Type-1 Cell Culture

**[0172]** 6 cell culture dishes of cell culture type 1 were infected according to Example 4 with human enteroviruses type EV71, strain SHZH98. 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0173]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 18

##### D2O Effectiveness with Rhinoviruses 1 in a Type-1 Cell Culture

**[0174]** 6 cell culture dishes of cell culture type 1 according to Example 4 were infected with rhinoviruses (strain RV-16 from the American Type Culture Collection, Manassas, Va., USA). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

**[0175]** The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 1.

#### Example 19

##### Preventive D2O Effectiveness in Rhinoviruses in a Modified Type-1 Cell Culture

**[0176]** 6 cell culture dishes of modified cell culture type 1 according to Example 5 were infected with rhinoviruses (strain RV-16 from the American Type Culture Collection, Manassas, Va., USA). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

[0177] The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 2.

#### Example 20

##### Preventive D2O Effectiveness in Herpesviruses Type 2 in a Modified Type-1 Cell Culture

[0178] 6 cell culture dishes of modified cell culture type 1 according to Example 5 were infected with herpesviruses type 2 (strain 734 from the American Type Culture Collection, Manassas, Va., USA). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

[0179] The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 2.

#### Example 21

##### D2O Effectiveness with Herpesviruses HSV-1 in a Type-2 Cell Culture

[0180] 6 cell culture dishes of cell culture type 2 according to Example 6 were infected with herpesviruses of type 1, HSV-1 (strain MacIntyre VR-539 from the American Type Culture Collection, Manassas, Va., USA). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

[0181] The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 3.

#### Example 22

##### D2O Effectiveness with Herpesviruses Type Varicella Zoster 1 in a Type-2 Cell Culture

[0182] 6 cell culture dishes of cell culture type 2 according to Example 6 were infected with herpesviruses of type zoster, varicella zoster (strain Ellen, VR-586 from the American Type Culture Collection, Manassas, Va., USA). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

[0183] The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 3.

#### Example 23

##### D2O Effectiveness with Herpesviruses HSV-2 in a Type-2 Cell Culture

[0184] 6 cell culture dishes of modified cell culture type 2 according to Example 6 were infected with herpesviruses

type 2 (strain 734 from the American Type Culture Collection, Manassas, Va., USA). 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

[0185] The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 3.

#### Example 24

##### D2O Effectiveness with Human Adenoviruses in a Type-2 Cell Culture

[0186] 6 cell culture dishes of cell culture type 2 were infected according to Example 6 with human adenoviruses type 3, strain GZ1. 120 minutes after infection, the cell culture medium combined with the viruses was removed and replaced as follows with a diluted cell culture medium: in the treated group (3 cell culture dishes), by 30% D2O and 70% DMEM with 10% FCS, 100 U/mL penicillin, and 100 µg/mL streptomycin. In the control group (3 cell culture dishes), the same amount of H2O was used instead of D2O for diluting the cell culture medium.

[0187] The determination of the viral count according to Example 7 for the treated and control cultures gave values which are shown in Table 3.

#### Example 25

##### D2O Effectiveness of D2O Hydrogel and D2O Eye Drops for the Treatment of Ocular Herpes in a Clinical Study

[0188] 33 healthy volunteers 20-65 years of age (15 female, 18 male) were selected for this study. All selected test subjects had a history of herpes in the eye region, which affected mainly the conjunctiva and in some cases also the cornea, with at least 5 events per year.

[0189] None of the test subjects used antiviral or immunomodulating therapeutic agents a week before or during the test which was limited to 3 months.

[0190] The 33 test subjects were randomized to 3 groups (treatment groups A and B and a control) with 11 subjects in each. Each test subject in treatment group A and the control group received a 20 g tube of a hydrogel prepared according to Example 2—treatment group A, a D2O hydrogel (prepared with 100% D2O), and the control group, an H2O hydrogel (prepared with 100% H2O) with the instruction to apply it to the affected mucous membrane site immediately upon the appearance of first ocular herpes symptoms. Treatment group B received a 30 mL bottle of a D2O eye drop solution prepared according to Example 1. The application was to be repeated in all groups every 2 hours for the duration of 3 days during waking hours. The application amount was approx. 0.5 mL of the hydrogel and 0.3 mL of the eye drops. The test subjects were instructed to keep an application log (patient diary) and to note each application in it and further also to enter for each application the following symptom details (symptom score), based on the previous application of the gel, on a scale from 1 (very limited/small) to 5 (very marked/large): a) itching b) pain, c) dryness, d) extent of the affected

conjunctiva area. These 4 quantities were averaged arithmetically in the data evaluation and this score was then averaged over the total number of the individual scores (=number of applications). Further, the date and time when first symptoms were noted, time of the start of treatment, time to externally complete healing of the herpes, time to freedom from pain, and (as far as possible) the location of the herpes were to be provided. For the data evaluation, only herpes events in the conjunctiva area were considered and there only those in which the test subjects were able to measure the extent approximately (using a provided measuring tape before a mirror). The period of the study extended over 3 months. Overall, evaluable data were obtained in this way from 7 subjects in the control group and 6 subjects in treatment group A and 7 subjects in treatment group B. The results are presented in Table 4.

1-17. (canceled)

18. A method for the prevention and/or treatment of a virus-based disease of an eye, wherein said method comprises contacting the eye with deuterium oxide.

19. The method, according to claim 18, wherein the virus-based disease of the eye is conjunctivitis, keratoconjunctivitis, and/or uveitis.

20. The method, according to claim 18, wherein the disease is selected from the group consisting of epidemic keratoconjunctivitis, keratoconjunctivitis sicca, hemorrhagic conjunctivitis, follicular conjunctivitis, pharyngoconjunctival fever, blepharitis, blepharoconjunctivitis, herpes simplex blepharitis, herpes simplex keratitis, dendritic keratitis, interstitial keratitis, endothelitis, disciform keratitis, (varicella) zoster ophthalmicus, ophthalmia neonatorum, molluscum contagiosum, canaliculitis, dacryoadenitis, acute dacryoadenitis, dacryocystitis, acute retinal necrosis, episcleritis, scleritis, choroiditis, chorioretinitis, iritis, iridocyclitis, retinitis, acute necrotizing retinitis, scleritis, anterior uveitis, intermediate uveitis, posterior uveitis, panuveitis, uveoretinitis, cataract, ocular muscle paresis, and congenital glaucoma.

21. The method, according to claim 18, used to treat a combination of two or more virus-based diseases of the eye.

22. The method, according to claim 21, wherein the two or more virus-based diseases of the eye occur simultaneously or successively.

23. The method, according to claim 18, used to treat rhinoconjunctivitis.

24. The method, according to claim 18, wherein the virus is of a family selected from the group consisting of Herpesviridae, Poxviridae, Paramyxoviridae, Coronaviridae, Chordopoxviridae, Togaviridae, Orthomyxoviridae, Retroviridae, Adenoviridae, Picornaviridae, and Rhabdoviridae.

25. The method, according to claim 18, wherein the virus is a virus of a genus selected from the group consisting of *Simplexvirus*, *Varicellovirus*, *Cytomegalovirus*, *Lymphocryptovirus*, *Chordopoxvirus*, *Rubivirus*, *Respirovirus*, *Morbillivirus*, *Rubulavirus*, *Adenovirus*, *Influenza A virus*, *Influenza B virus*, *Lentivirus*, *Mastadenovirus*, *Orthopoxvirus*, *Enterovirus*, *Rhinovirus*, *Lyssavirus*, and *Pneumovirus*.

26. The method, according to claim 18, wherein the virus is a virus of a species selected from the group consisting of herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella zoster virus (VZV), human cytomegalovirus (HCMV), Epstein-Barr virus, *molluscipoxvirus*, *vaccinia virus*, *rubivirus*, *rubella virus*, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, measles virus, mumps virus, human immunodeficiency virus, human adenovirus A, human adenovirus B, human adenovirus C, human adenovirus D, human adenovirus E, human adenovirus F, poliovirus, echovirus, coxsackie A virus, coxsackie B virus, rhinovirus, rabies virus, respiratory syncytial virus, and rift Valley fever virus.

27. The method, according to claim 18, wherein the deuterium oxide is administered topically.

28. The method, according to claim 18, wherein the deuterium oxide hydrates the mucous membranes of the eye.

29. The method, according to claim 18, wherein the deuterium oxide is used in combination with at least one other pharmaceutically active substance and/or at least one other non-pharmaceutically active substance.

30. The method, according to claim 29, wherein the at least one other pharmaceutically active substance is selected from the group consisting of viro-static agents, sympathomimetic agents, proteins, peptides, nucleic acids and immunosuppressive agents.

31. The method, according to claim 29, wherein the at least one other non-pharmaceutically active substance is selected from the group consisting of pharmaceutically acceptable inorganic or organic acids or bases; polymers; copolymers; block copolymers; monosaccharides; polysaccharides; ionic and nonionic surfactants, or lipids, as well as mixtures thereof; albumin; transferrin; and DNA repair proteins.

32. The method, according to claim 18, wherein the deuterium oxide is administered as a formulation.

33. The method, according to claim 32, wherein the formulation is a liquid, cream, ointment, gel, or hydrogel.

34. A method for the production of a drug for the prevention and/or treatment of virus-based disease of the eye wherein said method comprises the use of deuterium oxide.

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