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(54) Title: DEHYDROEPIANDROSTERONE THERAPY FOR THE TREATMENT OF EYE DISORDERS

(57) Abstract

A method for treating aging-related changes in tear film composition and chronic inflammatory conditions of the eye, collectively known as "dry eye syndrome", comprising the administration of the steroid hormone dehydroepiandrosterone (DHEA) and its derivatives. DHEA stimulates the meibomian gland to secrete lipids which maintain an effective tear film. Compositions for the administration of DHEA in the form of eyedrops are also described.

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DEHYDROEPIANDROSTERONE THERAPY FOR THE TREATMENT OF EYE DISORDERS

Field of the Invention

The present invention relates to methods and compositions comprising dehydroepiandrosterone (DHEA) for the treatment of irritative eye conditions, such as those collectively referred to as "dry eye syndrome."

Background of the Invention

Chronic eye irritation is a common complaint that accompanies aging, contact lens use, various inflammatory conditions, and auto-immune kerato-conjunctivitis, such as "Sjögren's Syndrome," which is common in elderly individuals. These conditions are collectively referred to as "dry eye syndrome" (DES). Severe DES, known as keratoconjunctivitis sicca (KCS), accounts for about 80%-90% of the diagnosed cases of DES. The most severe KCS represents sight-threatening dry eye and is found following facial burns, in association with Stevens-Johnson Syndrome, in seventh cranial nerve palsies, and in exophthalmic thyrotoxicosis. No effective medical therapy exists for these severe DES conditions.

In many instances, the symptoms associated with DES have been related to deficiencies in the production or function of the tear film, which covers the external

surface of the eye. DES may result from abnormalities in tear-film composition or spontaneous breakup of the film. Analysis of the tear film has revealed that it is made up of three distinct zones which differ in their compositions. These zones are:

1. a mucous or protein-containing zone or layer, which is closest to the eye surface and which coats the conjunctival membrane with a hydrophilic layer. The hydrophilic layer attracts water molecules and ensures uniform hydration of the conjunctival surface. In a healthy eye, the mucous layer is only a few hundredths of a micron thick;

2. an aqueous zone or layer, which contains mostly water and which constitutes the bulk of the tear film. This layer has a thickness of about 6-7 μm. Tears are a low-viscosity, aqueous solution containing organic salts, glucose, urea, some trace elements, and various biopolymers; and

3. a surface lipid zone or layer, which forms the interface between the tear film and the external environment. The thickness of the surface lipid layer has been estimated to be about 0.1 μm in the normal, open eye.

Physiological abnormalities associated with DES can affect any one of the zones of the tear film. Such abnormalities include tear-film instability, increased salt content and tear viscosity, debris, dehydrated mucin threads, reduced tear-film breakup time, increased corneal and conjunctival staining, filamentary keratitis, secondary infection, pain, and discomfort. Significant age-related changes have been described in the stability and composition of the tear film. By the age of 50, most individuals exhibit a loss in the ability to maintain full thickness of the tear film, which results in about a 50% decrease in the aqueous component of the film. Many DES patients have not lost the ability to produce the aqueous component

of tears, however, as evidenced by the complaint "epiphora" or excess, reflexive tear production, which is considered to be a response to painful dry spots on the cornea.

It has been suggested that the surface lipid layer of the tear film is an important element in controlling the evaporative loss of tear fluid and in maintaining the integrity and resilience of the intact film during the blinking process. Therefore, a change in the surface lipid layer is a possible factor in the development of DES, and observations suggest that loss or changes in the lipid component of the tear film may be the cause of DES associated with aging.

The lipid component of the tear film is secreted predominantly by the meibomian glands of the eyelids. These glands are histologically related to the sebaceous glands of the skin, although they are much larger. The meibomian glands are located in the tarsal conjunctiva of the upper and lower eyelids. The glands consist of grape-like clusters or "acini" connected to ducts which open onto the undersurface of the eyelid margins. With each blinking movement, the glands release a lipid-rich secretion which reconstitutes and maintains the surface lipid layer of the tear film. The main lipid classes secreted by the meibomian glands have been found to be waxy and cholesterol esters and cholesterol. Triglycerides, free fatty acids, and phospholipids have been found to exist in smaller quantities in some patients and to be absent in others. The variation in the secretions of the meibomian glands suggests that a loss of these lipid components may be a factor in the causation of DES. Therefore, stimulation of the meibomian glands to secrete these lipids may result in alleviation of the symptoms of DES.

Therapies for DES have previously been directed at the stimulation of tear production, and a multitude of

substances, such as melanocyte-stimulating hormone; agents which increase cyclic nucleotides; Vitamins A and B-12; and ocular irritants, such as Eldosin, have been proposed as agents to increase tear production. Therapies have recently been directed at reconstituting or supporting the tear film. These therapies include topical application of lipids in the form of charged phospholipid emulsions and topical combinations of hydrolyzed polyvinyl acetates and alcohols. However, none of these substances has been demonstrated, in animal or clinical trials, to have any effect on the normal physiology or function of meibomian glands. Furthermore, the effect of these substances on the meibomian glands appears to be nonspecific.

Thus, current therapy for DES remains palliative and involves the use of hygroscopic biochemicals, such as polyvinyl alcohol and methylcellulose. These biochemicals are administered in the form of eyedrops. However, such preparations, while resulting in the temporary relief of symptoms, are non-physiological and often induce deleterious side effects, such as abnormalities in the corneal surface.

Therefore, there is a need for an improved treatment of DES. The treatment is preferably one which stimulates the natural processes of the eye to produce an adequate tear film which will prevent or reverse the degenerative changes in the tear film responsible for development of DES. The treatment is also preferably one which does not produce abnormalities or other side effects involving the corneal surface or other structures of the eye.

Summary of the Invention

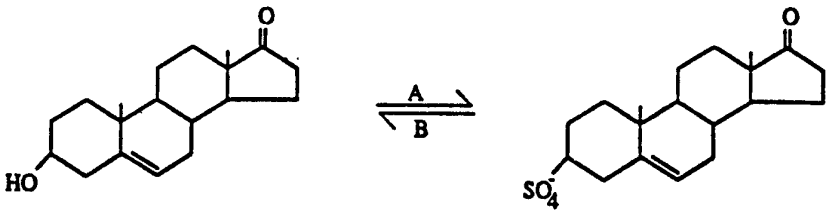
Compositions are provided in accordance with the present invention which are suitable for the treatment of DES and which comprise the hormone dehydroepiandrosterone (DHEA, 3β-hydroxy-5-androsten-17-one) and its derivatives. The compositions further comprise, in one embodiment of the invention, a solubilizing agent, a bacterial-growth-inhibiting agent, and a buffer. In a second embodiment of the invention, the DHEA or its derivatives are incorporated in a lipid emulsion, and, in a third embodiment, DHEA microcrystals are coated with a lipid mixture such as egg phosphatidylcholine and stearylamine.

Also provided in accordance with this invention are methods for the treatment of DES which comprise the administration of DHEA or its derivatives by topical or systemic administration. When topical administration of DHEA is used, the above-mentioned DHEA compositions are preferred. When administration of DHEA is by systemic means, other known compositions of DHEA are preferred.

Detailed Description

The present invention provides compositions comprising the steroid hormone DHEA in an "ophthalmic solution" which is suitable for the treatment of ocular tissues by topical application. The compositions are useful as a means of stimulating responsive glandular and cellular elements of the eye to produce and secrete lipids and proteins, which support and maintain the integrity of the tear film of the eye. Methods for treating DES with these compositions are also provided.

DHEA is an endogenous adrenal steroid which comprises the most abundant steroid species synthesized by the adrenal gland. DHEA is secreted in a "free," biologically active form, and a "sulfated," biologically inactive form (DHEA-S). DHEA-S circulates as part of the more abundant storage form of the hormone. DHEA is converted to DHEA-S by the action of steroid sulfo- transferase (A), which is found primarily in the adrenal gland and liver, and DHEA-S is converted to DHEA by the action of steroid sulfatase (B), which is found in a variety of tissues. The structures of DHEA and DHEA-S are given below:



Due to unknown causes, production and secretion of DHEA fall progressively in both males and females after young adulthood. The topical and systemic methods of treatment described herein provide hormonal replacement to reverse aging-related changes in the tear film composition and loss of tear-film function that are associated with DES. Purely pharmacologic use of DHEA to treat DES in young individuals due to various non-

aging etiologies is also envisioned by the present invention.

DHEA, like its parent compound cholesterol, is insoluble in water but is soluble in organic solvents such as hot alcohols, benzene, oils, fats, and aqueous solutions of bile salts. Treatment of the eye is preferably performed by the administration of eyedrops. However, for such administration, it is preferred that the DHEA be in a soluble form or in the form of microcrystals dispersed in solvents that are physiologically compatible with the eye. Therefore, preferred compositions of DHEA, for use in the present invention, solubilize the DHEA to facilitate its delivery, cellular availability, and retention in the ocular space, thereby maximizing its ophthalmologic therapeutic use.

In one embodiment of the present invention, a DHEA ophthalmic solution comprises a suspension of from about 0.1% (w/v) to about 1.5% (w/v) DHEA (supplied by Sigma Chemical Co., Catalog No. D4000) in a physiologically suitable carrier such as phosphate-buffered saline (137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4). The various forms of DHEA useful in the present invention are DHEA; DHEA-S; the free alcohol of DHEA; derivatives of DHEA such as DHEA-3-acetate (3β-Hydroxy-5-androsten-17-one acetate), DHEA-3-glucuronide (3β-Hydroxy-5-androsten-17-one 3 glucuronide), DHEA-hemisuccinate, DHEA-valerate, DHEA-enanthate, DHEA-fatty acid derivatives, 16-α-fluorinated, 16-α-brominated DHEA, and DHEA-salts; and other such DHEA compounds that retain the biological function of DHEA.

A solubilizing agent such as polyethylene glycol or other suitable, physiologically acceptable solubilizing agent is added to the solution to solubilize the DHEA. When polyethylene glycol is used as the solubilizing agent, a molecular-weight range of

from about 300 to about 550 is preferred. The concentration of the solubilizing agent is preferably in the range of from about 1% (w/v) to about 5% (w/v). About 3% (w/v) polyethylene glycol 400 (supplied by Sigma Chemical Co., Catalog No. P3265) is particularly preferred for use in the present invention.

It is also preferred that an agent for inhibiting the growth of bacteria in the ophthalmic DHEA solution be included for long-term storage of the solution. Such agents, useful in the practice of the present invention, are benzalkonium chloride (Sigma Chemical Co., Catalog No. B1383) or POLYQUAD 0.001% (Alcon Labs.) or other physiologically acceptable agents. Preferably, the concentration of the bacterial growth inhibitor is in the range of from about 0.005% (w/v) to about 0.01% (w/v). Most preferably, 0.01% (w/v) benzalkonium chloride is used as the bacterial-growth inhibitor.

In a second embodiment of the present invention, a stable emulsion containing DHEA dispersed in a mixture of biocompatible lipids is provided. An emulsion is preferred in order to reduce the temporary irritation caused by crystalline DHEA suspended in a primarily aqueous medium. Emulsions also allow delivery of the DHEA in a carrier that does not disrupt the natural lipid component of the tear film. However, the lipid-based carrier causes visual blurring after administration and is therefore preferably administered prior to sleep.

A DHEA-lipid-emulsion suitable for use in the present invention comprises 0.5% (w/v) crystalline DHEA, about 0.7% (w/v) n-octanoic acid, about 0.3% (w/v) alpha-tocopherol acetate, about 0.35% (w/v) tidylinositol, and about 0.35% phosphatidylglycerol in an aqueous medium such as borate-buffered physiological saline, pH 7.2. POLYQUAD, or other suitable agent to

inhibit bacterial growth in the emulsion, is also added.

In a third embodiment of the present invention, a DHEA composition is provided for prolonged ocular retention of the added DHEA. Prolonged ocular retention is advantageous, since the pharmacologic action of the DHEA in stimulating the meibomian glands, and other responsive cells, is enhanced by sustained exposure of the tissue to the hormone. In this embodiment of the present invention, the DHEA composition comprises microcrystalline DHEA coated with a phospholipid mixture.

The DHEA-lipid-coated composition is prepared by dissolving DHEA, or its derivative as described above, in absolute ethanol or chloroform. The DHEA is recrystallized, from the ethanol, by the addition of distilled water. The crystals produced are of uniform size and morphology. Crystals are separated from the liquor by centrifugation and filtration.

The lipids useful for practice of the present invention are egg phosphatidylcholine, stearylamine, lecithin or other suitable lipids, or combinations thereof. To coat DHEA crystals, the lipids are dissolved in a lipid solubilizing agent such as anhydrous chloroform. Preferably, about 450 μmoles of egg phosphatidylcholine (Sigma Chemical Co., Catalog No. P2772) and about 50 μmoles of stearylamine (Sigma Chemical Co., Catalog No. S6755) are dissolved in about 2 to about 3 ml anhydrous chloroform. After the lipids are dissolved in the solvent, the solvent is evaporated in vacuo, leaving a residual, thin film of lipid.

The DHEA microcrystals, about 1phospha-00 mg (350 μmoles), are suspended in a buffer, such as about 5 mM Tris, pH 7.4, containing about 10 mM NaCl, or other pharmaceutically suitable buffer, and the suspension is added to the lipid film. The buffer-lipid-DHEA mixture is emulsified using means such as a vortex mixer and

sonication, using a Heat Systems Ultrasonics sonicator, at maximal output. The emulsification is preferably performed at room temperature, to facilitate the formation of the emulsion and the effective lipid coating of the DHEA crystals. The lipid-coated DHEA crystals are then separated from the emulsion by centrifugation or other suitable means of separation. When centrifugation is used, the resultant emulsion is centrifuged for about 16 hours at about 4°C and at about 120,000 x g. The lipid-coated-DHEA microcrystal pellet which is obtained, and which contains the lipid-coated microcrystals, is collected and resuspended in a buffer, such as about 5 mM Tris, pH 7.4, containing about 10 mM NaCl, or other pharmaceutically suitable buffer, to a DHEA concentration of from about 0.1% (w/v) to about 1.5% (w/v). A bacterial growth inhibitor, such as about 0.005% (w/v) to about 0.01% (w/v) benzalkonium chloride, or other suitable agent, is added to the solution to inhibit the growth of bacteria during storage.

The above-described compositions comprising DHEA are useful for the treatment of DES when topical application is preferred. Such treatment is indicated in the various categories of DES patients as well as in veterinary practice with DES affected animals. Topical administration of the compositions is most conveniently by eyedrops. An effective treatment of DES is by the delivery of from about 0.5 mg to about 1.5 mg DHEA per treatment, and treatments are repeated from about 2 to about 3 times a day. Treatment with the DHEA is preferably continued for about 4 weeks. After about 4 weeks, the frequency of administration of the dosage is reduced to the minimum dose necessary to maintain an effective tear film. Due to variations in the patients being treated, the frequency will also vary from person to person. The minimum effective dose is qualitatively

determined for each patient by observing the properties of the tear film. Those properties of the tear film which are evaluated may include formal ophthalmologic evaluations, such as: slit lamp exam, to test for corneal and anterior chamber abnormalities; fluorescein tear film breakup time, to test for tear film stability; Rose-Bengal staining, to evaluate epithelial integrity; and the Schirmer test, to test for tear film production. These tests are described in detail in Example 3, below.

Alternatively, treatment of DES may be by systemic administration. In the case of systemic administration, especially in individuals with aging-related deficiencies in the levels of DHEA, any known compositions for the administration of DHEA are used. These compositions include systemic administration in the form of parental, sublingual, transdermal, intra-nasal, or oral delivery. DHEA can be incorporated into nanospheres, microspheres, or liposomes for injection, and can be incorporated into cyclodextrins for sublingual and aerosol delivery.

When delivered parenterally, the preferred treatment dose is from about 90 to about 150 mg per day, administered in about 3 separate doses. The treatment is preferably continued for about 4 weeks, and then the frequency is reduced as described above.

Another means for administering the DHEA for the treatment of DES is by oral administration. In the case of oral administration, any known compositions, such as capsules of crystalline DHEA, or its derivatives, are used. When delivered orally, the DHEA dose is preferably from about 90 to about 150 mg per day, administered in about 3 separate doses. The treatment is continued for about 4 weeks and then adjusted to the minimally effective dose, as described above.

Another form of administration of DHEA for the treatment of DES is coating contact lenses with a DHEA containing solution, such as lipid coated DHEA crystals as described above.

Example 1

Preparation of a Solubilized DHEA Ophthalmic Solution

1.5 gm microcrystalline DHEA was suspended in 100 ml 137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4. 3% (w/v) polyethylene glycol 400 and 0.01% (w/v) benzalkonium chloride were added. The suspension was stored at -20°C until needed. The suspension was shaken prior to use to redistribute the DHEA microcrystalline particles. The resultant composition comprised 1.5% (w/v) DHEA, 137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3% (w/v) polyethylene glycol 400, and 0.01% (w/v) benzalkonium chloride.

While the resulting suspension required shaking prior to use, to redistribute the DHEA microcrystalline particles, the suspension maintained constant microcrystalline particle size, as assessed by light microscopy over 2 months.

Example 2

Preparation of a Phospholipid-DHEA Ophthalmic Emulsion

A DHEA-lipid emulsion is prepared by adding about 500 mg crystalline DHEA to a mixture of about 0.7 ml n-octanoic acid (supplied by Sigma Chemical Co., Catalog No. C2875) and about 0.3 ml of alpha-tocopherol acetate (supplied by Sigma Chemical Co., Catalog No. T3001). The mixture is heated to about 55°C and stirred for about 10 min., or until the crystals are dissolved.

In a separate container, about 350 mg phosphatidylinositol (supplied by Sigma Chemical Co., Catalog No. P5766) and 350 mg phosphatidylglycerol (supplied by Sigma Chemical Co., Catalog No. P9524) are added to

about 100 ml borate-buffered physiological saline, pH 7.2. POLYQUAD is added to a final concentration of about 0.001% (w/v) to inhibit bacterial growth in the emulsion. The mixture is heated to about 55°C and stirred to facilitate the dispersion of the phospholipids, phosphatidylinositol, and phosphatidylglycerol in the saline.

The DHEA-octanoic-tocopherol mixture, at 55°C, is slowly added to the phospholipid mixture, at 55°C, with constant stirring. The mixture is then stirred for about 30 min., at about 55°C, to form a stable emulsion. The emulsion is the slowly cooled to about 24°C and transferred to sterile containers.

Example 3

Preparation of a Phospholipid-Coated-DHEA Ophthalmic Solution

150 mg crystalline DHEA was dissolved in 10 ml absolute ethanol. The DHEA was recrystallized, from the ethanol, by the addition of 1 ml distilled water. Microcrystals, from 5-20 microns in size, were separated from the liquor by centrifugation and filtration. Separately, 450 μmoles of egg phosphatidylcholine and 50 μmoles of stearylamine were dissolved in 2 ml anhydrous chloroform. The mixture was evaporated *in vacuo*, leaving a thin film of lipid on the surface of the container. 100 mg DHEA microcrystals were suspended in 5 ml buffer A (5 mM TRIS, pH 7.4, containing about 10 mM NaCl) and added to the lipid film. The buffer-lipid mixture was mixed at room temperature for 5 min. using a vortex mixer, then sonicated for 3 min. at room temperature using a Heat Systems Ultrasonics sonicator, at maximal output. The resultant mixture was then centrifuged for 16 hrs. at 4°C and at 120,000 x g in a Beckman ultra-centrifuge. The pellet which was obtained, and which contained the lipid-coated DHEA microcrystals, was resuspended in about 3 ml buffer A containing about 0.01% (w/v)

benzalkonium chloride. The solution contained a final concentration of 1% (w/v) DHEA.

Example 4

Use of DHEA in a Dog Model of Tear Film Disease

DHEA-containing eyedrops, prepared in accordance with the procedures of Example 1, were used to treat chronic inflammatory conjunctivitis, common in Pekingese dogs. This breed of dog, with characteristically prominent and bulging eyes (exophthalmos), is known to have a deficiency in complete lid closure and thinning of the lipid component of the tear film. Such a condition results in corneal and conjunctival changes similar to those seen in the human DES.

Four Pekingese dogs were used in the experiment. Two of the dogs were 11 years old and one was 7 years old. The fourth was 2 years old and had suffered from chronic bilateral conjunctivitis since it was one-month old. After an initial examination of the dogs had been performed, eyedrops were instilled 3 times a day, into each eye, by a single animal caretaker.

30-ml-dropper bottles for each of the right and left eyes of each dog were prepared. For each dog, one bottle contained DHEA drops (1.5% (w/v) DHEA, 137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3% (w/v) polyethylene glycol 400, and 0.01% (w/v) benzalkonium chloride). These eyedrops were instilled into the designated "treated" eye. The other bottle contained a vehicle-only solution (137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3% (w/v) polyethylene glycol 400, and 0.01% (w/v) benzalkonium chloride) and were instilled into the "control" eye. The animal caretaker did not know which were the "treated" eyes and which were the "control" eyes.

After 3 weeks, and again after 7 weeks, eyelid secretions were collected from each dog.

Lid secretions were manually expressed onto sterile absorbent cotton strips placed in the inferior and superior conjunctival recesses after locally anaesthetizing the eyes of the dogs with proparacaine and tetracaine. The saturated absorbent cotton strips were then immediately placed into 1.5 ml of an extraction solvent containing chloroform:methanol, in a ratio of 2:1, in sterile glass tubes. The samples were maintained on ice, in order to prevent oxidation and breakdown of the components of the collected tear film. If the samples were not processed immediately, they were stored at -20°C until needed.

The absorbent cotton strips were each washed into a separate tube with an additional volume of solvent (about 1 ml). The washed absorbent cotton strips were then discarded. The lipid solvent samples were centrifuged at 1500 rpm in a refrigerated centrifuge (Damon) at about 15°C for 10 min., to remove any particulate matter contaminating the samples. The lipid-solvent samples were then pipetted into fresh glass tubes and vacuum-evaporated. The volume of the sample was reduced to the point where precipitation of the dissolved lipids could begin to be observed.

Thin-layer chromatography (TLC) was then performed on the lipid samples to analyze and compare the lipid content of the tear film in the treated and the control eyes. Samples were applied to a 10 cm x 10 cm silica gel plate. The samples were each applied in a small (about 2 mm) spot, under a continuous cold-air stream, to evaporate the solvent. Six samples were applied to each plate. After the samples were applied to the plates, the plates were dried, then placed in a chromatography chamber with a saturated atmosphere of chromatography solvent. The TLC plates were developed by allowing the solvent to "run," by capillary action, to about 5 cm from the upper edge of the plate. The plates were first run in solvent system I, which

contained chloroform, methanol, and distilled water in a ratio of 65:30:5. The plates were removed from the chamber, dried, and then run again in solvent system I. The plates were removed, dried, and run to 4 cm from the upper edge, in solvent solution II, which contained n-hexane, diethyl ether, and acetic acid in a ratio of 80:20:1.5. The plates were removed from the chamber, dried, and rerun in solvent system II.

The plates were then removed from the chamber, dried, and sprayed with a 25% (v/v) sulfuric acid solution, and baked in a 110°C oven for at least 1 hr., to "stain" the separated lipids. Identification of phospholipids was accomplished by comparison to published tear-lipid separations, such as those described by Wollensak et al. in Graefe's Arch. Clin. Exp. Ophthalmol, 228, 78-82 (1990), incorporated herein by this reference.

The results obtained are summarized in Table I.

Table I

Dog No.	Age, Yrs.	Sex	Observations	Phospholipid Assay: DHEA-Treated Eye	Phospholipid Assay: Control Eye
1	2	F	Bilateral decreased inflammation	#1 not tested[1] #2 present[1]	Not tested Absent
2	11	M	DHEA eye more moist	#1a present[2] #1b present[2] #2 increased compared to 1a	Absent Absent Scant
3	11	F	3 days of conjunctivitis prior #2 test	#1a present #1b present #2 increased compared to 1a	Absent Absent Scant
4	7	M	Abnormal control eye; chronic inflammation	#1 scant #2 present	Present

[1] #1 refers to first sampling after 3 weeks of therapy
#2 refers to second sampling after 7 weeks of therapy

[2] a & b refer to "duplicate" testing on separate days

The phospholipid fraction from DHEA-treated eyes was compared to control eyes and was found to contain a much higher concentration of phospholipid. It was also noted that the lipid samples from DHEA-treated eyes began to precipitated while they were contained in a greater volume than the control eye samples, indicating a greater total quantity of lipids.

Example 5

Human Use of DHEA Eyedrops Treating DES

A 52-year-old woman who suffered for many years from bilateral dry, and sometimes painful, eyes, was

examined. The patient routinely used saline or lubricating drops containing methylcellulose which provide brief temporary relief of the DES symptoms. The patient's use of soft contact lenses aggravated her symptoms of dryness and pain. She had no history of recent conjunctival infection or of autoimmune disease. She was post-menopausal and took oral estrogen replacement. The patient had normal intraocular pressure in both eyes.

A trial treatment with DHEA-containing eyedrops, formulated in accordance with the procedures of Example 1, and containing 1.5% (w/v) DHEA, was begun. The treatment was performed as follows: One bottle containing DHEA drops (1.5% (w/v) DHEA, 137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3% (w/v) polyethylene glycol 400, and 0.01% (w/v) benzalkonium chloride) was given for use in the right eye only. A similar bottle was given for use in the left eye only. The bottle for the left eye contained 137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3% (w/v) polyethylene glycol 400, and 0.01% (w/v) benzalkonium chloride. The patient was not aware of which bottle contained the DHEA solution.

After one week of treatment, the right eye (treated) was described as being moister than the left eye (control). After 3 weeks of treatment, a formal ophthalmologic evaluation was performed, including slit lamp exam, to test for corneal and anterior chamber abnormalities; fluorescein tear film breakup time, to test for tear film stability; and the Schirmer test, to test for tear film production.

The fluorescein tear film breakup time was performed by adding to the eye fluorescein, a fluorescent dye, and observing the stained tear film with light passed through a cobalt-blue filter, as described by Norn, Acta Ophthalmol. (Kbh), 47, 865-880 (1969). Larger breakup times represent a more stable

tear film, and breakup times of about 10 seconds are considered normal.

The Schirmer test was performed as described by O. Schirmer in "Studies on the physiology and pathology of the secretion and drainage of tears," Albrecht von Graefes Arch. Klin. Ophthalmol., 56, 197-291 (1903). The test depends upon observing the extent of wetting of a strip of filter paper (standardized strips are available) and placing it over the lower lid for a specified time. First, a drop of topical local anesthetic is instilled into the eye to prevent reflex tearing. The strip is folded at a notched marking and is placed over the edge of the lateral one-third of the lid. The strip is usually left in place for 5 minutes while the patient looks straight ahead, in a dim light. The degree of wetting of the paper is measured in mm from the notch. Various end points for diagnostic significance have been reported in the literature. Probably, the safest cutoff value is 5 mm of wetting at 5 minutes in eyes previously treated with local anesthetic. Results are presented in Table II.

Table II

TEST	DHEA EYE			CONTROL EYE			
Tear film breakup, seconds	10	12	12	3	3	5	
Average	11.3						3.7
Normal	>10 seconds						
Schirmer tear production, mm	8	6		15	19		
Average	7						17
Normal	>5 mm						
Slit lamp exam of meibomian gland orifices	Profuse lipid visible			Scant lipid visible			

Slit lamp exam, performed by a trained ophthalmologist, revealed an increased quantity of meibomian secretions from the gland orifices on the lower lid of the right (treated) eye. The left lower lid was devoid of visible meibomian secretion. As indicated in Table II, tear film breakup time was more prolonged in the treated eye. This difference could not be attributed to poor tear production in the control eye, since the Schirmer test showed that the left (control) eye produced tears at twice the rate of the treated eye. These results indicate that DHEA treatment is effective in increasing tear film stability and symptomatic relief of DES.

Subsequent to this test treatment, the ophthalmic solutions were switched -- the left eye became the new DHEA "treated" eye, and the right eye

became the new "control" eye. Within one week, symptoms of dryness and pain had disappeared from the left eye. The left eye became more moist and comfortable during contact lens wear, and contact lens wear was possible for longer periods of time than before the treatment. The right eye remained asymptomatic for 3 weeks before demonstrating the return of pain and dryness.

Example 6

Effects of DHEA Eyedrops on Meibomian Gland Histology in the Rabbit

A female, white, New Zealand rabbit, approximately 3 years of age, was examined and found to have normal eyes and ocular adnexa prior to the trial. Once daily, for one month, DHEA-containing eyedrops were administered according to the following regimen: The left eye received 2 drops (approximately 0.1 ml) of a solution, as described in Example 1, comprising 1.5% (w/v) DHEA, 137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3% (w/v) polyethylene glycol 400, and 0.01% (w/v) benzalkonium chloride. The right eye received 2 drops (approximately 0.1 ml) of a vehicle-only solution (137 mM NaCl, 1.6 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4, 3% (w/v) polyethylene glycol 400, and 0.01% (w/v) benzalkonium chloride).

After one month of daily treatment, full-thickness, upper-lid biopsies of the DHEA-treated and control eyes were performed under general anaesthesia (intramuscular injection of ketamine and methohexital). Lid tissues, containing a section of the tarsal plate and conjunctiva, were immediately submerged in liquid nitrogen prior to histologic preparation. Frozen-section tissue blocks (10-micron tissue slices through meibomian gland structures) were cut at -20°C. Sections were stained with

hematoxylin and eosin (H and E), and others with Oil Red O lipid stain.

Light microscopic examination of the hematoxylin and eosin (H and E) preparation revealed meibomian gland clusters or acini, with lighter staining nuclei and hazy cytoplasm in the DHEA-treated eyelid, whereas the control eyelid showed distinct dark staining nuclei and a "clear" cytoplasm. These results indicate that changes in the cellular physiology of the meibomian glands is induced by treatment with DHEA and these changes were consistently seen in multiple gland sections and in all slides examined. No significant differences were seen in the Oil Red O stained slides.

These results are consistent with a change in metabolic activity specific for the meibomian glands occurring only in the DHEA-treated eye and, therefore, being induced by DHEA treatment.

Example 7

Effects of Topical DHEA on Tear Protein Content in the Pekingese Dog

DHEA-containing eyedrops, prepared in accordance with the procedures of Example 1, are used to treat chronic inflammatory conjunctivitis in three Pekingese dogs, to assess the influence of DHEA on the protein content of the tear film.

30-ml-dropper bottles for each of the right and left eyes of each dog are prepared. For each dog, one bottle contains DHEA drops for the designated "treated" eye, and the other bottle contains a vehicle-only solution for the "control" eye. The animal caretaker does not know which are the "treated" eyes and which are the "control" eyes.

After 3 weeks, and again after 7 weeks, tear film is collected from each dog with a microcapillary from the inferior fornix of the conjunctiva.

1-2 μl of tear fluid is necessary to perform the protein assay. The protein samples are denatured and reduced in the presence of urea (8M) and β-mercapto-ethanol (1% v/v) and separated by electrophoresis on cellulose acetate strips. The electrophoresis is performed in a buffer containing 12.11 g TRIS (hydroxymethyl)aminomethane, 16.32 g bicine, 3.5 ml mercaptoethanol, 480.48 g urea, pH 8.6, and distilled water to 1000 ml. The pH of the buffer is adjusted to 9.7 with HCl. The electrophoresis is conducted at 10V, 4 mA, for 40 min. At the completion of the electro- phoresis, the strips are fixed for 30 min. in 12.5% (w/v) trichloroacetic acid and then stained for 1 hour in Coomassie blue staining solution (70 ml acetic acid, 10 g Coomassie blue R 250, distilled water to 1000 ml). The stained strips are washed in 7% acetic acid for about 15 min., or longer if required, to remove excess stain from the strips. The strips are then heated at 110°C for about 30 min. to make the strips transparent.

Such protein assays indicate an increase in the concentration of the proteins in the DHEA-treated eye as compared to the control eye. Also, the protein species present in the DHEA-treated eye are more numerous. These results indicate that treatment with DHEA induces changes in the protein synthetic properties of the meibomian glands.

The above description of exemplary embodiments of the preparation of ophthalmic solutions comprising DHEA and the methods for treating DES are for illustrative purposes. Variations will be apparent to those skilled in the art; therefore, the present invention is not intended to be limited to the particular embodiments described above. The scope of the invention is defined in the following claims.

WHAT IS CLAIMED IS:

1. A composition for the treatment of dry eye syndrome comprising dehydroepiandrosterone in a formulation suitable for administration by eyedrops.

2. A composition as recited in claim 1 further comprising a solubilizing agent, a bacterial growth inhibiting agent, and a buffer.

3. A composition as recited in claim 1 wherein the dehydroepiandrosterone is present at a concentration of from about 0.1% (w/v) to about 1.5% (w/v).

4. A composition as recited in claim 1 wherein the dehydroepiandrosterone is present at a concentration of about 1% (w/v).

5. A composition as recited in claim 2 wherein the solubilizing agent is polyethylene glycol and the bacterial growth inhibiting agent is benzalkonium chloride.

6. A composition as recited in claim 5 wherein the polyethylene glycol is present at a concentration of from 1% (w/v) to 3% (w/v) and the benzalkonium chloride is present at a concentration of from 0.005% (w/v) to 0.01% (w/v).

7. A composition as recited in claim 1 wherein the dehydroepiandrosterone is emulsified in a composition comprising phospholipids.

8. A composition as recited in claim 7 wherein the emulsion comprises about 0.5% (w/v) crystalline DHEA, about 0.7% (v/v) n-octanoic acid, about 0.3% (v/v) alpha-tocopherol acetate, about 0.35% (w/v) phosphatidylinositol, and about 0.350% (w/v) phosphatidylglycerol in borate-buffered physiological saline, at a pH of 7.2, and about 0.001% (w/v) of a bacterial growth inhibitor.

9. A composition as recited in claim 1 wherein the dehydroepiandrosterone is coated with phospholipid.

10. A composition as recited in claim 9 wherein the phospholipid coat comprises egg phosphatidylcholine and stearylamine.

11. A composition as recited in claim 10 wherein the phospholipid coat comprises about 450 μmoles of egg phosphatidylcholine and about 50 μmoles of stearylamine per 350 μmoles of dehydroepiandrosterone.

12. A method for the treatment of dry eye syndrome comprising administering to a mammal a composition comprising dehydroepiandrosterone.

13. A method as recited in claim 12 wherein the composition is administered by eyedrops.

14. A method as recited in claim 13 wherein the composition comprises from about 0.1% (w/v) to about 1.5% (w/v) dehydroepiandrosterone.

15. A method as recited in claim 14 wherein the composition further comprises a solubilizing agent, a bacterial growth inhibiting agent, and a buffer.

16. A method as recited in claim 15 wherein the solubilizing agent is polyethylene glycol and the bacterial growth inhibiting agent is benzalkonium chloride.

17. A method as recited in claim 16 wherein the polyethylene glycol is present at a concentration of from 1% (w/v) to 3% (w/v) and the benzalkonium chloride is present at a concentration of 0.005% (w/v) to 0.01% (w/v).

18. A method as recited in claim 13 wherein the dehydroepiandrosterone is emulsified with phospholipid.

19. A method as recited in claim 18 wherein the emulsion comprises about 0.5% (w/v) crystalline DHEA, about 0.7% (v/v) n-octanoic acid, about 0.3% (v/v) alpha-tocopherol acetate, about 0.35% (w/v) phosphatidylinositol, and about 0.350% (w/v) phosphatidylglycerol in borate-buffered physiological saline, at a pH of 7.2, and about 0.001% (w/v) of a bacterial growth inhibitor.

20. A method as recited in claim 13 wherein the dehydroepiandrosterone is coated with phospholipid.

21. A method as recited in claim 20 wherein the phospholipid coat comprises egg phosphatidylcholine and stearylamine.

22. A method as recited in claim 21 wherein the phospholipid coat comprises about 450 μmoles of egg phosphatidylcholine and about 50 μmoles of stearylamine per 350 μmoles of dehydroepiandrosterone.

23. A method as recited in claim 12 wherein the dehydroepiandrosterone composition is administered systemically.

24. An aqueous eyedrop composition comprising solubilized dehydroepiandrosterone.

25. An aqueous eyedrop composition as recited in claim 24 wherein the dehydroepiandrosterone is solubilized by polyethylene glycol.

26. An aqueous eyedrop composition as recited in claim 24 wherein the dehydroepiandrosterone is emulsified with phospholipids.

27. An aqueous eyedrop composition as recited in claim 24 wherein the dehydroepiandrosterone is solubilized by coating the dehydroepiandrosterone with phospholipid.

28. A coating for contact lenses comprising DHEA.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/07096

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : A61K 31/56
US CL : 514/178,912
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : U.S.CL: 514/178,912

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Dialog: dehydroepian drosterone and dry (w) eye or ophthal? Steroids and dry eye.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Medline Abstract of Jikken Dobutsu, volume 38, issued January 1989. Higuchi N, "Effect of androgen on onset of diabetes in the KK mice treated with monosodium aspartate". See the entire abstract.	1-11 and 24-28
Y	Biosis Abstract of Okayama Igakkai Zasshi, volume 94, issued 1982. Kageyama K. "Clinical Studies on patients with Sjogrens syndrome 2-characteristics of patients with Sjorgrens syndrome and the effect of cortico steroid therapy". See the entire Abstract.	12-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 17 NOVEMBER 1992	Date of mailing of the international search report 09 JUN 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE	Authorized officer ZOHREH A. FAY NGUYEN NGOC-HO INTERNATIONAL DIVISION Telephone No. (703) 308-4604

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/07096

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Physician's Desk reference For Ophthalmology, 16 Ed., 1988, See pages 12 and 13.	1-28
Y	U S, A, 5,041,434 (Lubkin) 20 August 1991 See the entire patent.	12-23
X	U.S , A, 4,496,556 (Orentreich) 29 January 1985. See column 4, lines 5-18	1,3,4 and 28