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(54) Title: OCULAR THERAPY IN KERATOCONJUCTIVITIS SICCA USING TOPICALLY APPLIED ANDROGENS OR TGF-β

(57) Abstract

The topical application, at a dose rate less than or equal to 0.1 mg day, to the ocular surface or adjacent regions of the eye of a preparation containing a therapeutically effective amount of an androgen or androgen analogue or a therapeutically effective amount of TGF-β is disclosed as a method of relieving the chronic and acute manifestation of dry eye signs and symptoms in keratoconjunctivitis sicca (KCS), for example in Sjögren's syndrome.

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OCULAR THERAPY IN KERATOCONJUNCTIVITIS SICCA USING TOPICALLY APPLIED ANDROGENS OR TGF-β

RELATED APPLICATIONS

This application is a continuation-in-part of Sullivan, U.S. Patent Application No. 08/971,768, filed November 17, 1997, which was a continuation-in-part of Sullivan, U.S. Patent Application No. 08/477,301, filed June 7, 1995, now U.S. Patent No. 5,688,765, which was a continuation-in-part of Sullivan, U.S. Patent Application No. 08/124,842, filed September 21, 1993, now U.S. Patent No. 5,620,921 (under reexamination as control no. 90/004,056), which was a continuation under 37 CFR 1.62 of U.S. Patent Application No. 07/871,657, filed April 21, 1992, the whole of which are hereby incorporated by reference herein.

GOVERNMENT RIGHTS

Part of the work leading to this invention was made with United States Government funds under Grant No. EY05612 from the National Institutes of Health. Therefore, the U.S. Government has certain rights in this invention.

FIELD OF THE INVENTION

This invention relates to treating keratoconjunctivitis sicca (KCS), especially as manifested in Sjögren's syndrome.

BACKGROUND OF THE INVENTION

The preocular tear film plays an essential role in the maintenance of corneal integrity, the protection against microbial challenge and the preservation of visual acuity (1). These functions, in turn, are critically dependent upon the stability, tonicity and/or composition of the tear film structure, which includes an underlying mucin foundation (derived from conjunctival

goblet cells and conjunctival and corneal epithelial cells), a substantial, middle aqueous component (originating primarily from lacrimal gland acinar and ductal epithelial cells) and an overlying lipid layer (secreted by the meibomian glands) (1,2). Alteration, deficiency or absence of the tear film may lead to intractable desiccation of the corneal epithelium, ulceration and perforation of the cornea, an increased incidence of infectious disease, and ultimately, severe visual impairment and blindness (2,3).

Throughout the world, countless individuals suffer from tear film dysfunctions, which are collectively diagnosed as keratoconjunctivitis sicca (KCS) or, simply, dry eye (1,2). These lacrimal abnormalities may be subdivided into four general categories: (a) aqueous tear deficiencies, which are most frequently responsible for dry eye states, originate from lacrimal gland disorders and include autoimmune disease, congenital alacrima, paralytic hyposecretion or excretory duct obstruction; (b) mucin deficiency, which is observed in various conjunctival cicatrization conditions, such as Stevens-Johnson syndrome, trachoma, pemphigoid, thermal and chemical burns, as well as hypovitaminosis A; (c) lipid abnormalities, which may occur during meibomian gland dysfunction (e.g., posterior blepharitis); and (d) diminished eyelid function (1).

By far, the greatest single cause of KCS worldwide, excluding those countries wherein trachoma remains epidemic, is Sjögren's syndrome (2). This syndrome, which is the second most common autoimmune disease (7,14), occurs almost exclusively in females and is characterized by inadequate mucin production, meibomian gland dysfunction, and an insidious and progressive lymphocytic infiltration into the main and accessory lacrimal glands, an immune-mediated, extensive destruction of lacrimal acinar and ductal tissues and the

consequent development of persistent KCS (7-10). In primary Sjögren's syndrome, which afflicts about 50% of the patient population, the disease is also associated with an immunological disruption of the salivary gland and pronounced xerostomia. In secondary Sjögren's, the disorder is accompanied by another disease, which is most often rheumatoid arthritis and less frequently systemic lupus erythematosus (SLE), scleroderma, polymyositis, polyarteritis nodosa, Hashimoto's thyroiditis, chronic hepatobiliary disease, chronic pulmonary fibrosis, purpura hyperglobulinemia or Raynaud's phenomenon (2,11). During the course of Sjögren's syndrome, autoimmune sequelae may also encompass focal lymphocytic adenitis of eccrine and mucosal glands, biliary cirrhosis, sclerosing cholangitis, pancreatitis, atrophic gastritis, interstitial nephritis and pneumonitis, peripheral vasculitis, B cell lymphoma and a diverse array of central and peripheral nervous system and skeletal muscle complications (12,13).

The etiology of Sjögren's syndrome may be due to the interaction of numerous factors, including those of genetic, endocrine, neural, viral and environmental origin (15,16). However, a potential cause may relate to primary infection by, and reactivation of, Epstein-Barr virus (EBV) and/or cytomegalovirus (CMV) (17-20). These herpes viruses are present in lacrimal and salivary glands of Sjögren's patients (17-20) and may induce the inappropriate HLA-DR expression, T helper/inducer cell activation, B cell hyperactivity and autoantibody production evident in these affected tissues (8). However, whether herpes, or even retroviral (21,22), action represents a cause of, or merely an epiphenomenon in, Sjögren's syndrome remains to be determined (23-25).

At present, a perception is that Sjögren's syndrome may be clinically irreversible (7), an autoimmune disease to be controlled, yet not cured (10). In the scientific

literature, reports have suggested that systemic or topical administration of estrogens (4), cyclosporine A (6) or glucocorticoids (26) might alleviate the ocular manifestations of this disorder. However, other studies indicate that such pharmaceutical exposures are ineffective (27-29) and, in fact, may accelerate and/or amplify the disease (28,30). Indeed, estrogen action may be involved in the etiology of Sjögren's syndrome (30,31).

Others have suggested that tear stimulants, such as bromhexine (32) or isobutylmethylxanthine (33), might improve ocular symptoms. These drug effects, though, may be subjective (34), susceptible to tachyphylaxis (4) and/or limited by the requirement for functional and responsive lacrimal tissue (4,35).

It has also been proposed that systemic androgen treatment might provide a potential therapy for Sjögren's syndrome and its associated defects. This proposal is based upon the finding that autoimmune disorders commonly display a sexual dichotomy, with estrogens increasing disease severity in females and androgens suppressing autoimmune sequelae in males (15,16,36-38). In fact, systemic androgen therapy has been utilized to effectively diminish autoimmune expression in animals models of SLE, thyroiditis, polyarthritis and myasthenia gravis (15,38-43), as well as the human condition of idiopathic thrombocytopenic purpura (44). However, research has also demonstrated that the systemic administration of androgens to patients with primary or secondary Sjögren's syndrome or SLE is apparently unable to correct various peripheral manifestations of these disorders (49,54,55,62). In addition, systemic androgen treatment of female patients with Sjögren's syndrome exposes these individuals to possible undesirable side effects, including virilization, menstrual irregularities (e.g., amenorrhea), hepatic dysfunction, edema,

hematologic abnormalities, behavioral changes and metabolic alterations. Similarly, chronic treatment of males with systemic androgens has been characterized as dangerous (63), because of the numerous potential side effects. For these reasons, a recent report has indicated that systemic androgen therapy is inappropriate for the treatment of the multiple immune dysfunctions in Sjögren's syndrome (63).

Others have suggested that anti-viral compounds may represent a new therapeutic approach for ocular disease in Sjögren's syndrome. Researchers have speculated that such compounds may be effective in counteracting the viral (e.g., EBV- and/or CMV)-induced infection in lacrimal tissue, that may possibly precipitate the gland's immune-associated dysfunction (17,19,20). The potential efficacy of this strategy, though, is highly speculative: current scientific information does not show definitively that these viruses are directly involved in the pathogenesis or progression of Sjögren's syndrome (23-25).

Therefore, the currently prescribed, therapeutic approach for the management of KCS in Sjögren's syndrome is the frequent application of artificial tear substitutes, which permit lubrication of the eye's anterior surface (3,4,5,9,10). Unfortunately, this therapy does not represent a cure and does not ameliorate the inherent, ocular immunopathology and resulting KCS associated with this chronic, extremely uncomfortable and vision-threatening disease (3).

SUMMARY OF THE INVENTION

The invention generally features a new approach to the management of KCS, especially as manifested in Sjögren's syndrome, the topical application to the eye of a preparation containing a therapeutic amount of an androgen or androgen analogue, at a dose rate of less

than 1 mg/day, or a therapeutic amount of TGF-β. This method of treatment can alleviate the ocular manifestations of Sjögren's syndrome (e.g., as due to lacrimal and meibomian gland dysfunction), the special symptoms that cause great distress, while not exposing the patient to the possible undesirable side effects of systemic treatment.

In one aspect, the invention features a method for treating keratoconjunctivitis sicca (KCS) that includes providing a therapeutic agent including a therapeutically effective amount of an androgen or androgen analogue in a pharmaceutically acceptable substance, and administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient.

Preferably, the substance is phosphate buffered saline or a carrier substance such as hyaluronate and the androgen or androgen compound has unusual structural features; or the compound is a testosterone, 4,5α-dihydrotestosterone, 17β-hydroxy-5α-androstane, or 19-nortestosterone derivative; or the compound is a nitrogen-substituted androgen.

In another aspect, the invention features a similar treatment method wherein the therapeutic agent includes TGF-β.

The invention also features measurement of the increased tear levels of TGF-β to provide a diagnostic test to monitor the therapeutic effect of topical treatment with androgen analogues.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof and from the claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Various androgen compounds, as herein discussed, significantly reduce the magnitude of lymphocyte infiltration in lacrimal tissue of animal models of

Sjögren's syndrome. Androgens also stimulate the functional activity of both the lacrimal and meibomian glands. The nature of androgen action on autoimmune expression in lacrimal tissue appears to be unique and lacrimal gland-specific. It also appears that this hormone effect is not linked to a generalized, systemic anti-inflammatory function. Building on these new discoveries, the method of the invention involves a rejection of the classical therapeutic approach to treatment for Sjögren's syndrome, a belief that any administered therapeutic agent must be able to control all aspects of the disease. Because steroid hormones (e.g., glucocorticoids), with solubility characteristics analogous to those of androgens, rapidly gain access to adjacent ocular tissues after topical application (78), it is proposed, instead, that topical application of a therapeutic amount of an androgen or androgen analogue to the eye be used to suppress lacrimal gland inflammation and to increase the functional activity of the lacrimal and meibomian glands, and thus treat the debilitating ocular manifestations of this disease. Topical application of a therapeutic androgen can provide for symptomatic relief of the worst ocular symptoms of Sjögren's syndrome without the chance of the patient experiencing the undesirable side effects of systemic administration. Furthermore, since the androgen-induced suppression of lacrimal gland inflammation could be mediated through the induction of transforming growth factor-β (TGF-β), a potent immunosuppressive compound, local application of TGF-β should also have the same effect.

During the past decade, it has become increasingly recognized that the endocrine system exerts a tremendous, regulatory impact on immunological expression (15,16,36,37,45,64-76). The precise nature of this endocrine control, though, appears to be both cell-

and tissue-specific (45). Thus, depending upon the target, the consequence of hormone action may be stimulation, antagonism or inhibition of immune function. Moreover, individual hormone effects on the immune system are often not generalized. Rather, endocrine influence may actually strengthen, diminish or elicit no effect on immunological activity in different tissues (45). Given this background, it is not surprising that the systemic administration of selected hormones (e.g., androgens) is unable to correct all immune defects in multidimensional, autoimmune disorders, such as Sjögren's syndrome or SLE.

Yet, if appropriate endocrine therapy could be targeted to specific, responsive tissues, hormone action could safely and effectively ameliorate an immunopathology located in those particular tissues. To relieve the symptoms that cause the most ocular distress in Sjögren's syndrome, those targeted, responsive tissues are the lacrimal and meibomian glands. According to the invention, lipophilic, regulatory hormones applied locally on or adjacent to the ocular surface can act directly on accessory and main lacrimal tissues and meibomian glands of Sjögren's syndrome patients and suppress the disease-related glandular inflammation and dysfunction in these tissues. This effect is completely independent of systemic hormone activity. The aim of this immunoendocrine interaction is to: (a) reduce lymphocyte infiltration in adjacent lacrimal tissue and thereby alleviate immune-mediated destruction, and lymphocyte compression, of acinar and ductal cells; (b) permit accessory and/or palpebral lacrimal glands to secrete basal tear volumes; (c) to enhance the function of meibomian glands and thereby promote increased stability and decreased evaporation of the tear film; and (d) avoid the side effects that parallel systemic exposure to these hormones. In effect, topical androgen

treatment can generate functional regions of lacrimal tissue and augment meibomian gland activity, thereby enhancing tear output and maintenance and correcting the dry eye problem.

This pharmaceutical strategy has not been proposed previously. Most probably, this is because the mechanism of androgen action on immune function has been thought to be mediated through, or assisted by, factors from the thymus and hypothalamic-pituitary axis, or else involve direct effects on lymphocytes (37,65,68,77). Specifically, androgens have been thought to suppress immune processes indirectly by first acting, for example, on the thymus, hypothalamic-pituitary axis, bone marrow or spleen, which tissues would then release appropriate factors or cells to mediate immunosuppression. Consequently, given these postulated mechanisms of androgen action, hormone administration to the surface of the eye, prior to the method of the invention, would have been believed to be entirely ineffective: the quantity of steroid delivered from the eye to the blood stream, and then to the thymus, hypothalamus, pituitary, bone marrow or spleen, would have been believed to be insufficient to induce the generation of enough immunosuppressive agents to ameliorate lacrimal inflammation.

Topical ocular application of androgens can relieve the immunopathology of Sjögren's syndrome

A critical requirement for the justification of topical ocular application therapy is to demonstrate that androgens suppress lacrimal gland immunopathology in Sjögren's syndrome. In addition, it is important to show that this androgen action is targeted to lacrimal tissue, and independent of generalized, systemic effects. In the examples given below it is shown that all three of these criteria are met, i.e., that androgens do suppress

lacrimal gland immunopathology in Sjögren's syndrome, that androgen action is targeted to lacrimal tissue, and that androgen action is independent of generalized, systemic effects.

EXAMPLE I

Androgen influence on lacrimal gland immunopathology in the MRL/Mp-lpr/lpr mouse model of Sjögren's syndrome (47)

The purpose of the following study was to determine whether androgen therapy might inhibit the progression of, or reverse, autoimmune disease in the lacrimal gland after the onset of Sjögren's syndrome. Towards that end, the study utilized adult, female MRL/Mp-lpr/lpr (MRL/lpr) mice, which are an animal model for both Sjögren's syndrome (50,51) and SLE (79,80). Lacrimal tissues of these mice, as in humans, contain multifocal and extensive lymphocytic infiltrates in perivascular and periductal areas, significant glandular disruption and marked fibrosis (50,51).

Physiological or supraphysiological levels of testosterone were administered systemically, and not topically, because the location of the lacrimal gland in mice is inaccessible from the ocular surface. The results demonstrated that androgens exert a significant impact on autoimmune expression in lacrimal glands of MRL/lpr female mice. Administration of testosterone for 17 or 34 days dramatically reduced the extent of lymphocyte infiltration in lacrimal tissue: this hormone action was time-dependent and involved marked diminutions in both infiltrate size and area. Moreover, hormone therapy appeared to reverse the inflammation-induced disruption of acinar and ductal epithelium. Of interest, there was no significant difference in experimental results between the physiological and supraphysiological

doses of testosterone. In contrast, the magnitude of lymphocyte infiltration progressively increased in lacrimal glands of placebo-treated mice during the experimental time course. Testosterone therapy also significantly diminished immunopathology in the submandibular gland, but the extent of this effect was less than found in lacrimal tissue.

EXAMPLE II

Androgen impact on lacrimal gland immunopathology in the NZB/NZW F1 mouse model of Sjögren's syndrome (48)

The objective of this investigation was to assess the efficacy of androgen treatment for lacrimal disease by utilizing another autoimmune, animal model (NZB/NZW F1 [F1] mouse) of Sjögren's syndrome (52,59). As in humans, lacrimal glands of this mouse strain, which displays a fundamental B cell defect, harbor dense, lymphocytic aggregates (50,52), which contain a prevalence of B and helper T cells (58). Moreover, this murine disease is accompanied by focal destruction of acinar and ductal tissue and apparent ocular surface dryness (50,52). In contrast, immune dysfunction in the MLR/lpr model appears to have a different etiology and involves a basic, immunoregulatory disorder of T cells (47).

Autoimmune, female F1 mice were treated systemically with vehicle or varying concentrations of testosterone for 0, 17, 34 or 51 days after the onset of disease; again, the systemic route for hormone treatment was utilized because lacrimal tissue in F1 mice may not be accessed from the ocular surface. Results showed that the extent of lymphocyte infiltration increased dramatically in control mice during the experimental time course. However, testosterone administration induced a significant, time-dependent decrease in lymphocytic accumulation in the lacrimal gland. Following 34 to

51 days of androgen therapy, the magnitude of lymphocyte infiltration had been suppressed 22- to 46-fold, compared to that in placebo-treated tissue. This hormone effect was associated with significant reductions in the number of focal infiltrates, the area of individual foci and the total quantity of lymphocyte infiltration per lacrimal section. In certain groups, testosterone exposure also stimulated a rise in tear volumes, relative to those measured in the same mice prior to treatment. With few exceptions, the impact of physiological and supraphysiological testosterone treatment on lacrimal autoimmune expression in F1 mice was essentially identical, the suppression of autoimmune disease.

EXAMPLE III

Effect of androgen therapy in Sjögren's syndrome: hormonal influence on lymphocyte populations and Ia expressionin lacrimal glands of MRL/lpr mice

Previous research demonstrated that androgen treatment dramatically curtails lymphocyte infiltration in lacrimal glands of mouse models of Sjögren's syndrome. The purpose of this study was to determine whether this androgen action involves the selective suppression of specific lymphocyte populations or Class II antigen (i.e., Ia) expression in lacrimal tissue. Towards this end, autoimmune female MRL/Mp-lpr/lpr mice were administered placebo- or testosterone-containing compounds systemically for 0, 17 or 34 days after the onset of disease. Results showed that androgen exposure exerts both a quantitative and a qualitative influence on inflammatory cell populations in the lacrimal gland of MRL/lpr mice. Thus, testosterone, but not placebo, treatment induced a precipitous decrease in the total number of T cells, helper T cells, suppressor/cytotoxic T cells, Ia-positive lymphocytes and B cells. Androgen

administration also significantly diminished the lacrimal density, as well as the frequency, of B220+ (i.e., possibly immature T) cells.

These findings, when compared with other observations (45,47,48,56), suggest that testosterone's anti-inflammatory activity may be unique and lacrimal gland-specific. First, the androgen-induced immunosuppression in lacrimal tissue does not extend to peripheral lymph nodes (56,57), indicating that this steroid hormone does not cause a generalized depression in lymphocyte migration to, or proliferation in, systemic or mucosal sites. Second, testosterone exposure reduces the magnitude of lymphocytic infiltration in submandibular glands of MRL/lpr mice (47), but the nature of this hormonal influence may be unlike that found in lacrimal tissue, and the overall susceptibility of salivary focal infiltrates to androgens and pharmacological agents appears quite different from that found in lacrimal tissue (47). Third, androgens exert significant control over immunological functions in lacrimal glands, but not necessarily those of salivary or systemic tissues (45).

EXAMPLE IV

Impact of steroids and immunosuppressive agents on lacrimal autoimmune disease in the MRL/lpr mouse model of Sjögren's syndrome

The objective of the following experiments was to determine whether other steroid hormones or immunosuppressive agents might duplicate the effect of testosterone on lacrimal gland autoimmunity. Female MRL/lpr mice were treated with systemic vehicle, steroids or immunosuppressive compounds for 21 days after disease onset. The pharmaceutical agents evaluated in this study included: (a) *testosterone*, which, has been shown to

significantly reduce lacrimal gland inflammation; (b) *19-nortestosterone*, an anabolic androgen with attenuated virilizing activity; (c) *danazol*, a synthetic steroid, which is utilized in the treatment of certain immune diseases in humans (81) and is known to diminish specific, peripheral immune defects in MRL/lpr mice (60); (d) *17β-estradiol*, a female sex steroid, which has been hypothesized as a potential treatment for ocular disease in Sjögren's syndrome (4); (e) *a synthetic, non-androgenic steroid*, which apparently suppresses lymphocyte infiltration in salivary glands of F1 mice and corrects other systemic autoimmune defects; (f) *cyclosporine A*, an anti-inflammatory agent, which ameliorates specific, peripheral immune dysfunctions in MRL/lpr mice (61) and has been proposed as an effective therapeutic agent for lacrimal disease and KCS in Sjögren's syndrome (6); (g) *dexamethasone*, a potent anti-inflammatory glucocorticoid, that has been suggested as a possible therapeutic agent for lacrimal immunopathology in Sjögren's syndrome (26); and (h) *cyclophosphamide*, an immunosuppressive agent, that decreases various autoimmune sequelae in systemic (83-85) and salivary (59,82) sites in MRL/lpr mice. The comparative results demonstrated that the suppressive influence of testosterone on focal infiltrate area, number of foci and percentage lymphocyte infiltration in lacrimal tissue was duplicated by the administration of the anabolic androgen, 19-nortestosterone, or cyclophosphamide, but not by therapy with estradiol, danazol, the synthetic non-androgenic steroid, cyclosporine A, or dexamethasone. In addition, testosterone, 19-nortestosterone and cyclophosphamide, as well as dexamethasone, reduced lymphocyte infiltration in the submandibular gland. However, neither androgen interfered with the pronounced inflammation of lymphatic

tissues, including the spleen, and superior cervical and mesenteric lymph nodes. Androgen treatment alone also stimulated an increase in the lacrimal gland output of total protein and IgA antibodies into tears; these antibodies, which protect the ocular surface against bacterial colonization, viral attachment, parasitic infestation and fungal- or toxin-induced impairment (46), are typically diminished in mucosal sites in Sjögren's syndrome (53).

Overall, these combined findings demonstrate that androgens, or their anabolic analogues, suppress autoimmune expression in, and enhance tissue function of, lacrimal glands of animal models of Sjögren's syndrome. Androgen action also appears to represent a tissue-specific response independent of generalized, systemic effects, thus justifying topical ocular therapeutic application. Cyclophosphamide, the only non-androgen to reduce lymphocyte infiltration in lacrimal tissue upon systemic administration, is not believed to be appropriate for topical therapy in humans because of its mode of action. This alkylating agent, which is thought to suppress autoimmune function by a direct modification of cellular DNA, must first be metabolized by the liver before becoming active. Therefore, cyclophosphamide would not be capable of local action upon topical application.

Local ocular application of TGF-β can relieve the immunopathology of Sjögren's syndrome

The androgen-induced suppression of immunopathological lesions in autoimmune lacrimal tissue, and the parallel improvement in glandular function, could be mediated through an androgen interaction with epithelial cells, which would then cause the altered expression and/or activity of epithelial cytokines in the

lacrimal gland. If correct, this hypothesis would predict that: (a) epithelial cells are the target cells for androgen action in the lacrimal gland, and that androgen receptors are located within epithelial, but not other (e.g., lymphocytic), cells in lacrimal tissue; and (b) androgens increase the expression of anti-inflammatory cytokines, or decrease the activity of pro-inflammatory peptides, in the lacrimal gland. Therefore, to address this hypothesis, experiments were conducted to identify the presence, location and/or cellular distribution of androgen receptor protein and mRNA in lacrimal glands of various species and autoimmune, female MRL/lpr mice, as well as examine the impact of androgens on cytokine levels in lacrimal tissue.

EXAMPLE V

Presence, location and hormonal regulation of androgen receptors in lacrimal tissue

The mechanism(s) by which androgens regulate the immune expression in the lacrimal gland undoubtedly involves an initial hormone association with specific androgen receptors. Androgen receptors appear to mediate almost all known activities of androgens and are members of the steroid/thyroid hormone/retinoic acid family of ligand-activated transcription factors (90-96). The location of androgen receptors in other tissues is predominantly intranuclear (97-99), due to the presence of a nuclear targeting signal, homologous to that of the SV 40 large T antigen, which occurs in the receptor hinge region immediately following the DNA-binding domain (93). Following androgen binding to the receptor, the monomeric, activated hormone-receptor complex associates with an androgen response element in the control region of specific target genes, typically dimerizes with another androgen-bound complex and, in combination with

appropriate silencers, tissue-specific and basal promoter elements, regulates gene transcription (90,91,93,94,96). This androgen activity results in the alteration of mRNA production and ultimately protein synthesis in a variety of tissues (100-103); such regulation of protein elaboration appears to be the primary action of androgens (90,104).

A critical prerequisite, though, for the androgen-induced regulation of target gene transcription is the presence and location of androgen receptors in a given tissue (90). In fact, considerable research has demonstrated that a tissue's physiological responsiveness to steroids (e.g., androgens) is most often directly proportional to the concentration of that steroid's receptor protein, as well as to the amount of its associated mRNA (90,105,106).

Therefore, to determine whether high affinity and specific androgen receptors are present in the lacrimal gland, equilibrium binding methods were employed with various tritiated steroids and with lacrimal tissue cytosol from young adult, orchiectomized or ovariectomized rats. Analysis revealed that a single class of saturable, high-affinity and specific binding sites exist for androgens in lacrimal tissues of both male and female rats. To extend these findings, experiments were also performed to evaluate whether high-affinity androgen binding sites are located specifically within acinar epithelial cells of the rat lacrimal gland. Towards that end, acinar epithelial cells were isolated from lacrimal tissues of orchiectomized rats, processed for the preparation of cytosol and examined for tritiated dihydro-testosterone (DHT) binding sites. This analysis identified the existence of high-affinity androgen receptors within these cells.

To determine whether androgen receptor mRNA is present in lacrimal tissues of various species, the following studies were conducted. RNA was isolated from lacrimal glands of male and/or female mice, rats, hamsters, guinea pig, rabbits and human, as well as from rat spleen (negative control), rat prostate and human prostate LNCaP cells (positive controls), then reverse transcribed into cDNA, amplified with specific primers by polymerase chain reaction (PCR) and processed for Southern blot hybridization with a 32P-labeled fragment of rat androgen receptor cDNA. The size of amplified cDNA products was calculated by comparison to a series of molecular weight standards run in adjacent lanes in the 1.5% agarose gel. The results showed that lacrimal glands from all tested species contained a single, ~1,273 bp band of androgen receptor mRNA, which was identical to that observed in rat prostatic tissue and human prostatic LNCaP cells. In addition, the same RT-PCR results were found if "human-specific" oligomeric primers were utilized to amplify mRNA from human lacrimal tissue, LNCaP cells and rat prostate.

For comparative purposes, studies were also conducted to examine human and/or rat lacrimal glands for the existence of androgen receptor mRNA by the use of ribonuclease protection assays, as well as Northern blot techniques. In the former experiments, total cellular RNA was isolated from human lacrimal gland autopsy specimens (n = 5 males, 1 female), LNCaP cells, as well as rat lacrimal, prostatic and splenic tissues, then hybridized to 32P-labeled, human or rat androgen receptor riboprobes. Findings showed that androgen receptor mRNA occurred in both human and rat lacrimal glands, and that the size was equivalent to that of the prostatic androgen receptor mRNA. In the ribonuclease protection assays with rat tissues, no androgen receptor mRNA was detected in the spleen, whereas G-3-PDH mRNA was evident in all

rat lacrimal, prostatic and splenic samples. For comparison, it has also been demonstrated that androgen receptor mRNA occurs in rat lacrimal tissue, and that the molecular size (~10 kb; by Northern blots) is identical to that observed in the prostate, an androgen target organ (107).

To explore the endocrine basis for the androgen regulation of both the structure and function of the lacrimal gland in a variety of species, studies were also designed to: (a) determine the cellular distribution of androgen receptors in the lacrimal gland; and (b) examine the influence of gender and the endocrine environment on the glandular content of these binding sites. Lacrimal glands were obtained from intact, castrated, or sham-operated male or female adult rats, mice or hamsters, as well as from orchiectomized rats exposed to placebo compounds or physiological levels of testosterone. The cellular location of androgen receptors was evaluated by utilizing an immunoperoxidase protocol, in which a purified rabbit polyclonal antibody to the rat androgen receptor was used as the first antibody. Findings with lacrimal glands showed that: (a) androgen receptors are located almost exclusively in nuclei of epithelial cells; (b) the cellular distribution or intranuclear density of these binding sites is far more extensive in glands of males, as compared to females; (c) orchiectomy, but not sham-surgery, leads to a dramatic reduction in the immunocytochemical expression of androgen receptors; and (d) testosterone administration to orchiectomized rats induces a marked increase in androgen receptor content, relative to that in placebo-exposed glands. Overall, these findings demonstrate that gender and the endocrine system may significantly influence the distribution of androgen binding sites in rat lacrimal tissue. Moreover, these

results show that androgens up-regulate their own lacrimal gland receptors.

To assess whether the androgen control of, and gender-related differences in, androgen receptor protein in the lacrimal gland are associated with variations in the expression of androgen receptor mRNA, the following study was performed. Orchiectomized and ovariectomized Sprague-Dawley rats were administered subcutaneous implants of placebo- or testosterone-containing pellets for 7 days. Lacrimal glands were obtained from these animals, as well as intact male, female and sham-operated rats, and then processed for the measurement of androgen receptor mRNA by semi-quantitative reverse transcription (RT)-PCR. All androgen receptor mRNA determinations were standardized to the β-actin content in the same RNA sample. The results of this study showed that the levels of androgen receptor mRNA are significantly higher in lacrimal tissues of females, as compared to males. In addition, the findings demonstrated that orchiectomy increases, and androgen treatment reduces, androgen receptor mRNA content in lacrimal tissue. Thus, the effects of gender and androgen exposure on AR mRNA expression in the lacrimal gland are the opposite to those observed with AR protein. This type of AR autoregulation has also been found by other investigators in certain reproductive tissues.

The above findings demonstrate that androgen receptor protein and mRNA are present in lacrimal tissues of a number of species and that these binding sites possess high affinity, are specific for androgens, and are located almost entirely within nuclei of epithelial cells. To extend these results further, additional studies were performed to identify the cellular target(s) within autoimmune lacrimal tissue that may mediate the immunosuppressive effect of androgens. In addition, the endocrine regulation of androgen receptors in these

autoimmune lacrimal glands was explored. Adult, female MRL/lpr mice were exposed systemically to vehicle, steroid hormones or immunosuppressive agents for varying time intervals after the onset of disease. Immediately before or after treatment, lacrimal glands were obtained and processed to determine the cellular distribution and nuclear density of androgen receptors by immunoperoxidase and image analysis techniques, and to assess the levels of androgen receptor mRNA. The findings demonstrated that: (a) androgen receptors exist almost exclusively within nuclei of acinar and ductal epithelial cells in lacrimal tissue of MRL/lpr mice; (b) androgen receptors are not detectable in the extensive lymphocytic populations that infiltrate the gland; (c) testosterone administration induces a significant increase in the number of androgen receptor-containing cells in, as well as the density of androgen receptors in epithelial cell nuclei of, lacrimal tissue; (d) hormone action is steroid-specific: administration of androgen analogues, but not estrogens, glucocorticoids or cyclophosphamide, stimulate the accumulation of androgen receptors; (e) androgens autoregulate the amount of their receptor mRNA; and (f) androgen receptor density is significantly reduced following the withdrawal of androgen therapy. These results show that epithelial cells, but not lymphocytes, are the androgen target cells in lacrimal tissue, and appear to mediate the androgen-related immunosuppression and functional enhancement in lacrimal glands of autoimmune female mice. These findings also demonstrate that androgens increase the expression of their own receptor protein, and decrease the content of their receptor mRNA, in MRL/lpr lacrimal tissue.

In summary, these results show that epithelial cells, but not lymphocytes, are the androgen target cells in autoimmune lacrimal tissues, and that androgens up-regulate the expression of their own receptor protein

in these cells. In addition, it has been shown that: (a) specific, high-affinity and saturable androgen binding sites exist in rat lacrimal tissue; (b) the location of these receptors is almost exclusively within epithelial cells of lacrimal glands in non-autoimmune mice, rats and hamsters; (c) androgen receptor mRNA is present in lacrimal tissues of humans and numerous other species; (d) the appearance of androgen receptor protein and mRNA in the lacrimal gland is significantly influenced by gender and androgens; and (e) androgen act directly on epithelial cells of the rat lacrimal gland and these hormone actions may be inhibited by cellular exposure to androgen receptor, transcription and translation antagonists (108,109).

EXAMPLE VI

Role of epithelial cell TGF-β in lacrimal gland autoimmune disease

Autoimmune diseases invariably involve a deficiency in self tolerance, the generation of autoreactive immune cells, the activation of proto-oncogenes and the expression of immune response genes to the detriment of the host (110-112). However, an additional and extremely important feature of autoimmune disorders is the inappropriate secretion of cytokines (111,112). These peptides, which are produced by a wide variety of cells, e.g., immune, epithelial, endothelial, and neural (112-116), have been termed the "hormones of the immune system" (117) and normally play an integral role in immunological defense (112,118). An imbalanced production and release of cytokines, though, may lead to the subversion of tolerance to specific antigens, activation of effector functions of T and B cells, stimulation of proto-oncogene, Class II antigen and intercellular adhesion molecule expression, promotion of

the inflammatory process and destruction of target cells, e.g., epithelial (110,112,117-120). Given these actions, cytokines have also been implicated as the "mediators of autoimmune disease" (121). At present, numerous cytokines appear to be involved in autoimmune disorders (111,112,117-119,122) and to contribute significantly to the etiology and/or generation of inflammatory eye diseases (123,124).

Recently, studies have also implicated cytokines in the development and perpetuation of severe immunopathological lesions in exocrine tissues of Sjögren's syndrome patients (125), as well as in the striking decrease in glandular secretion that occurs in this disorder (126). Thus, salivary glands of individuals with Sjögren's syndrome show a tremendous increase in the interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α (TNF-α) mRNA levels in acinar epithelial cells, a substantial rise in IL-2, IL-10 and IFN- mRNA content in CD4 T cells, and a significant elevation in IL-1, IL-6, TNF-α, IL-10 and IFN- concentrations in saliva, relative to those amounts in healthy controls (127). Analyses of total salivary gland biopsies have also documented alterations in the mRNA expression of these cytokines, as well as of IL-1β and TGF-β mRNA (128-130). With regard to lacrimal glands, a limited evaluation of biopsies from Sjögren's syndrome patients by RT-PCR has identified elevated amounts of IL-1β, IL-6 and IFN- mRNA (128,131). Of interest, these changes in cytokine circuitry might explain not only the inflammatory progression of this disease, but also the associated xerophthalmia and xerostomia in Sjögren's syndrome. Thus, cytokine-induced lymphocyte infiltration may result in a significant decline in the neural innervation of inflamed tissue (132). Moreover, certain cytokines (e.g., IL-1β) may directly suppress transmitter release by adrenergic and cholinergic nerves (133),

thereby interfering with the neural control of epithelial cell fluid secretion (126).

This aberrant expression of cytokines in Sjögren's syndrome may well be due, in part, to the influence of sex steroids. In support of this hypothesis, estrogens, which may be involved in the pathogenesis, acceleration and amplification of Sjögren's syndrome (30,31), are known to enhance the production of pro-inflammatory cytokines, e.g., IL-1 and TNF-α (134). Conversely, androgens, which decrease the manifestations of many autoimmune disorders (16,37,135), may elicit the generation of immunosuppressive cytokines (136). In fact, the sex steroid regulation of cytokine synthesis is believed to account for the distinct sexual dimorphism found in the incidence of autoimmune disease (122).

Indeed, the principle mechanism by which androgens suppress autoimmune disease in lacrimal glands of female mouse models of Sjögren's syndrome may well be through the control of epithelial cell cytokine production. In support of this hypothesis, as has been described above, the anti-inflammatory action of androgens in lacrimal tissue appears to be mediated not through lymphocytes, but rather through epithelial cells. Moreover, epithelial cells in other tissues are known to secrete numerous cytokines, e.g., TGF-β (137), and also serve in exocrine sites as active cellular participants in the glandular inflammation in Sjögren's syndrome (138). In addition, as will be described below, androgens increase the mRNA and protein levels of the immunosuppressive cytokine, TGF-β1, in the lacrimal gland. This cytokine is thought to play a protective role in Sjögren's syndrome, and increased expression of TGF-β mRNA has been correlated with reduced inflammation in salivary glands of Sjögren's syndrome patients (129). In contrast, the absence of TGF-β1 leads to a pronounced lymphocytic

infiltration into both lacrimal and salivary glands (139).

Therefore, to test this hypothesis, studies were performed identify epithelial cell cytokines that may mediate, or be involved, in the androgen-induced suppression of lacrimal gland inflammation in Sjögren's syndrome. An initial inquiry was whether lacrimal gland epithelial cells express cytokines, that might be involved in the endocrine regulation of immune function in this tissue. These studies, which were conducted with high stringency, RT-PCR procedures, demonstrated that TGF-β1, TGF-β2, TGF-β3, IL-6, TNF-α and IL-1α mRNA may be detected consistently in lacrimal glands, as well as in isolated lacrimal acinar epithelial cells, of male and female rats. As a corollary to these studies, whether lacrimal glands of autoimmune mice contain mRNAs for anti-inflammatory, as well as pro-inflammatory, cytokines was also examined. This research, which was performed with high stringency RT-PCR techniques, showed that mRNA for IL-1α, IL-1β, IL-2 receptor, IL-6, TGF-β and TNF-α may be detected consistently in lacrimal glands of autoimmune female MRL/lpr mice. The identity of these amplified products was verified by agarose gel electrophoresis, molecular weight determinations and comparison to several positive controls (e.g., cDNA from MRL/lpr splenic tissue, P388D1 macrophages, and Clontech kit controls).

Given these findings, the next set of studies was designed to determine whether the mRNA and protein levels of the immunoinhibitory cytokine, TGF-β1, in the lacrimal gland might be controlled by androgens, and influenced by gender. In the first series of experiments, total RNA was isolated from lacrimal glands of intact, castrated or sham operated male and female rats, as well as placebo- or testosterone-treated orchiectomized rats. Following this isolation, TGF-β1 and β-actin mRNA were analyzed by

agarose gel electrophoresis, RT-PCR, Southern hybridization, autoradiography and densitometry. For control purposes, all TGF-β1 measurements were standardized to those of β-actin. The results demonstrated that: (a) levels of TGF-β1 mRNA were significantly higher in lacrimal tissues of intact male, as compared to those of female, rats; (b) orchiectomy either decreased, or had no impact, on TGF-β1 mRNA content in lacrimal glands, whereas ovariectomy consistently had no effect on TGF-β1 mRNA amounts; and (c) androgen treatment significantly increased TGF-β1 mRNA expression in lacrimal tissues of orchiectomized rats, relative to that in glands of placebo-treated controls. These data demonstrated that gender influences, and androgens regulate, TGF-β1 mRNA levels in the lacrimal gland.

A second series of studies examined whether androgen exposure might significantly increase the content of TGF-β1 protein in lacrimal tissues of autoimmune mice. Accordingly, lacrimal tissues were obtained from female MRL/lpr mice after the onset of disease and following placebo, testosterone or cyclophosphamide treatment. Glands were then processed for the acid extraction of proteins and the analysis of TGF-β1 levels by use of a commercial assay. The results showed that androgen, but not cyclophosphamide, administration stimulated a significant rise in the total amount of TGF-β1 protein in lacrimal tissues of female MRL/lpr mice, as compared to levels in tissues of placebo controls.

Overall, these findings show that the mRNAs for cytokines believed to play a major role in exocrine tissue inflammation in Sjögren's syndrome are present in lacrimal tissues of normal rats and autoimmune mice. In addition, these results demonstrate that androgens stimulate the accumulation of TGF-β1 mRNA and protein in the lacrimal gland. TGF-β1, in turn, is known to exert

profound immunosuppressive activity, including the inhibition of T and B cell proliferation, cytotoxic T cell generation, natural and lymphokine-activated killing, T cell adhesion to the endothelium, macrophage function and IL-1, TNF and IFN-γ production, and is believed to down regulate inflammation in exocrine glands in Sjögren's syndrome (129,139,140). Consequently, the androgen-induced increase in TGF-β1 could act to suppress lymphocytic infiltration and to attenuate IL-1 and TNF-α production in the lacrimal gland. These hormonal effects would then provide a mechanistic explanation for the androgen-related suppression of autoimmune disease in lacrimal tissue during Sjögren's syndrome.

As an additional consideration, it is important to note that the androgen-induced rise in TGF-β content in the lacrimal gland will undoubtedly lead to enhanced secretion of TGF-β by lacrimal tissue and to an increased concentration of TGF-β in tears. Given that TGF-β (e.g., TGF-β1, TGF-β2) has been detected in tears, measurement of this latter androgen-associated effect in tears, by utilization of commercial or conventionally-prepared assay kits, would serve as an ideal diagnostic test to monitor the efficacy of topically applied androgens for the treatment of dry eye.

Androgen control of the meibomian gland

Of particular importance, the potential benefit of topical androgen therapy extends beyond the treatment of aqueous-deficient dry eye syndromes, as occur during lacrimal gland inflammation in Sjögren's syndrome. Topical administration of androgens can also serve as a safe and effective treatment for evaporative dry eye disorders due to meibomian gland dysfunction.

The meibomian gland is a large sebaceous (i.e., oil-producing) gland (141,142), and androgens are known to control the development, differentiation and function of

sebaceous glands in non-ocular sites (141). More specifically, androgens appear to act predominantly on acinar epithelial cells in sebaceous glands, and these cells contain both androgen receptor mRNA and protein (in their nuclei). These acinar cells respond to androgens by increasing both the production and secretion of lipids. In addition, androgen action in many sebaceous glands is augmented by, or dependent upon, the presence of an enzyme called 5α-reductase. This enzyme, which has two distinct isozymes (Types 1 and 2) encoded by different genes, converts testosterone and dehydroepiandrosterone (native and sulfated forms) into 5α-dihydrotestosterone, a very potent metabolite (141,143).

Given this background, it was proposed that androgens may also regulate meibomian gland function, enhance the quality and quantity of lipids produced by this tissue and stimulate the formation of the tear film's lipid layer. In addition, it is proposed that androgen deficiency, as occurs during menopause (decrease in ovarian androgen and adrenal androgen precursor secretion), aging in both sexes (decline in testicular androgen output in elderly males), autoimmune disease (e.g., Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis) and the use of anti-androgen medications (e.g., for prostatic hypertrophy or cancer) may lead to meibomian gland dysfunction and consequent evaporative dry eye.

If androgens do regulate meibomian tissue in a manner analogous to that of skin sebaceous glands, then meibomian glands should contain androgen receptor mRNA, androgen receptor protein and 5α-reductase. In addition, androgens should be able to control the synthesis and/or secretion of meibomian gland lipids, and androgen deficiency should be linked to meibomian gland

dysfunction and functional dry eye. These hypotheses are shown to be correct in the Example given below.

EXAMPLE VII

Effect of androgens on meibomian gland function

The first set of experiments to test these hypotheses was designed to determine whether the meibomian gland is an androgen target organ. Tissues were obtained from rats, rabbits and/or humans and analyzed for the presence of androgen receptor mRNA, androgen receptor protein and 5α-reductase mRNA. The results showed that: (a) meibomian glands from male and female rabbits and humans contain androgen receptor mRNA; (b) meibomian glands of rats and humans contain androgen receptor protein within the nuclei of their acinar epithelial cells; and (c) human meibomian glands contain mRNA for both Types 1 and 2 5α-reductase.

The next series of studies demonstrated that androgens influence the lipid profile within the rabbit meibomian gland. Orchiectomized animals (n = 5/treatment group) were treated with topical vehicle or 19-nortestosterone for 14 days, and meibomian glands were then processed for lipid evaluation. For comparative purposes, lipid content was also characterized in meibomian tissues of intact, untreated male controls. The results indicated that androgens exert a significant impact on the lipid pattern of the rabbit meibomian gland. Orchiectomy was associated with a loss of long-chain fatty acids (FA) in the diglyceride and/or triglyceride fraction of meibomian gland lipids, whereas the topical administration of 19-nortestosterone, but not placebo compounds, restored the FA profile towards that of intact animals. Androgen treatment of orchiectomized rabbits also induced the appearance of specific α-hydroxy FA and aliphatic alcohols and increased the percentage of

long-chain FA in the total lipid fraction of meibomian glands.

To determine whether androgen deficiency might be linked to meibomian gland dysfunction and evaporative dry eye, ophthalmic exams were performed and meibomian gland secretions were collected from urological patients (n = 15) taking anti-androgen medications as well as from age-matched (n = 6) and younger (n = 4) controls. The experimental results demonstrated that androgen deficiency appears to be associated with meibomian gland dysfunction, an altered lipid profile in meibomian gland secretions, a decreased tear film stability and functional dry eye. In brief, the clinical and biochemical results showed that patients, as compared to controls, had: (a) a higher frequency of light sensitivity, painful eyes and blurred vision; (b) a decrease in the tear film break up time, a higher frequency of orifice metaplasia, a poorer quality of meibomian gland secretions and severe meibomian gland disease; and (c) an attenuation in the amounts of cholesterol esters and wax esters, relative to those of cholesterol, as well as a decreased expression of specific molecular species in the diglyceride fraction of meibomian gland secretions.

Overall, these results demonstrate that the meibomian gland is an androgen target organ, that topical androgens modulate lipid production within this tissue, and that androgen deficiency may cause meibomian gland disease. Moreover, these findings indicate that topical androgen application to the ocular surface can serve as a therapy for meibomian gland dysfunction and its associated evaporative dry eye syndromes, not only in Sjögren's syndrome, but also in other forms of KCS.

USE

Topical application of androgens or their analogues or of TGF-β to patients with KCS or other autoimmune diseases, especially as manifested in Sjögren's syndrome, can directly suppress the immunopathological defects in accessory lacrimal tissue and the main lacrimal gland's palpebral lobe, which is adjacent to the ocular surface. Furthermore, topical androgen treatment can increase both the production and secretion of lipids to reduce meibomian gland dysfunction. Selection of the most appropriate therapeutic compounds will depend upon a given hormone's immunological activity, potential side effects and form of administration. For example, topical testosterone may be quite effective in reducing lacrimal inflammation, and its methylated analogue appears to have no toxic side effects on parameters such as intraocular pressure (87). However, a variety of other modified and/or anabolic androgens (86,88) may be more effective than testosterone. In addition, with regards to administration, if the androgen is to be complexed to a carrier vehicle (e.g., hyaluronate), then a nitrogenated analogue might be indicated.

Therefore, the efficacy of a variety of modified and/or anabolic androgens in suppressing lacrimal gland autoimmune expression in female MRL/lpr mice was compared. Animals were administered vehicle or designated androgens systemically for 6 weeks after the onset of disease. The androgens examined in this test included: (a) *testosterone*; (b) *dihydrotestosterone* (also termed allodihydrotestosterone, androstanolone, stanolone, 5α-dihydrostestosterone); (c) *fluoxymesterone*; (d) *stanozolol*; (e) *nortestosterone propionate*; (f) *dehydroepiandrosterone* (an androgen precursor, also termed androstenolone, dehydroisoandro-sterone, DHEA, transdehydroandrosterone); (g) *oxandrolone*; (h) *methyldihydrotestosterone* (also termed

methylandrostanolone); (i) *oxymetholone;* (j) *5α-androstan-17β-ol-3-oxime;* (k) *5α-androstan-17α-ol-3-one-acetate;* (l) *2,(5α)-androsten-17β-ol;* (m) *5α-androstan-2α-methyl-17β-ol-3-one;* and (n) *methyltestosterone.*

The rationale for comparing the immunological activity of this specific array of androgenic compounds was multifold:

First, these hormones are representative of the major structural subclasses of androgens, as disclosed in Vida (88), hereby incorporated by reference. The subclasses include (a) androgenic compounds with unusual structural features (e.g., 17α-methyl-17β-hydroxy-2-oxa-5α-androstan-3-one, also termed oxandrolone); (b) testosterone derivatives (e.g., methyltestos-terone); (c) 4,5α-dihydrotestosterone derivatives (oxymetholone); (d) 17β-hydroxy-5α-androstane derivatives containing a ring A unsaturation, excluding testosterone derivatives (e.g., 2,(5α)-androsten-17β-ol); and (e) 19-nortestosterone derivatives (e.g., 19-nortestosterone propionate). It may be that certain structural features impart more optimal immunosuppressive characteristics, which would be of benefit in selecting specific androgens for human use.

Second, relative to standards (typically testosterone), these androgens include compounds displaying: (a) augmented androgenic (i.e., virilizing) activity coupled with an even larger increase in anabolic activity (e.g., fluoxymesterone); (b) enhanced anabolic action with unchanged androgenic effects (e.g., oxymetholone, dihydrotestosterone); (c) decreased androgenic ability with unchanged anabolic activity (e.g., 19-nortestosterone propionate); and (d) decreased androgenic capacity paralleled by increased anabolic activity (e.g., oxandrolone, stanozolol). Thus, the

analysis should identify an androgen with far more anabolic, than virilizing, activity to be utilized for the treatment of ocular manifestations of Sjögren's syndrome (e.g., oxandrolone possesses 322% of the anabolic and 24% of the androgenic activity of methyltestosterone (88)). Of course, it is possible that anabolic effects, per se, may not be involved in androgen suppression of lacrimal autoimmune symptoms. However, the results with 19-nortestosterone in MRL/lpr mice demonstrate that this anabolic androgen, which has significantly reduced androgenic activity in lacrimal tissue (89), was equally as effective as testosterone in abrogating lymphocyte infiltration in the lacrimal gland.

Third, these compounds contain a nitrogen-substituted androgen, 5α-androstan-17β-ol 3-oxime, which is created by the substitution of a nitrogen derivative for the 3-ketone function in dihydrotestosterone (very potent androgen) (88). This substitution does not inhibit androgen activity (88) and may permit steroid binding to hyaluronate for topical administration. Of interest, a variety of other nitrogenated androgens have been shown to express increased anabolic, but decreased androgenic, activity. These compounds typically contain 3-substitutions, but not nitrogen incorporation in the steroid ring structure, which appears to abolish androgen action (88).

The results of testing the effect of the representative compounds were that all androgen classes, whether parental, modified or anabolic analogue, were effective in suppressing lacrimal gland autoimmune expression, although to various degrees. With further routine additional testing, the most appropriate therapeutic compound for a specific condition can be determined. In addition, the therapeutic augmentation of basal tear secretion could allow the use of visual aids,

such as contact lenses, in the Sjögren's syndrome or other autoimmune patient populations.

Androgen therapy, which can be administered in the form of drops (e.g., free hormone, or complexed with carrier substances, such as hyaluronate) or ointment, should not require frequent applications, considering the mechanism and duration of androgen/cell interactions. The administration of a specific compound would be by routine methods in pharmaceutically acceptable substances, including buffer solutions (e.g., phosphate buffered saline) or inert carrier compounds, to the ocular surface or adjacent regions of the eye. Optimal dosage and modes of administration can readily be determined by conventional protocols. This treatment can: (a) decrease lymphocyte infiltration in adjacent lacrimal tissue and thereby alleviate immune-mediated destruction, and lymphocyte compression, of acinar and ductal cells; (b) permit accessory and/or palpebral lacrimal glands to secrete basal tear volumes; it is estimated that a tear secretion rate of only 0.1 μl/minute (i.e., one-tenth of normal) could maintain a stable tear film under favorable conditions (1); (c) make available regions of functional lacrimal tissue, that might respond to exogenous tear stimulants to enhance surface volume; and (d) enhance the function of meibomian glands and thereby promote increased stability and decreased evaporation of the tear film.

Topical administration of androgens would avoid the numerous side effects of parallel systemic exposure to these hormones, including virilization, menstrual irregularities (e.g., amenorrhea), hepatic dysfunction, edema, hematologic abnormalities, behavioral changes and metabolic alterations. As has been discussed above, androgens are *normally* made in the body of all individuals and serve an array of physiological functions. In males, the daily production rate of

testosterone equals 2.5 to 11 mg/day, whereas in females it is approximately 0.25 mg testosterone/day (this amount varies during the menstrual cycle) (86). When androgen replacement therapy is given to correct male hypogonadism (i.e., androgen deficiency), the typical *systemic* dosage is 10 to 20 mg androgen/day and is very frequently delivered by intramuscular injection; the absolute dosage and specific method of administration, though, depend upon the androgen.

For *topical* androgen therapy, however, the androgen dosage per day would be expected to be far less than the amount normally produced by an adult female as seen in the following calculation: Lacrimal glands in experimental animals contain about 100 fmol cytosol androgen receptor/mg protein. If an equivalent concentration of receptor occurs in humans, and if one considers the weight of accessory and palpebral lacrimal tissue and level of protein per mg gland weight, then less than 1 ng of androgen would be required to saturate all androgen binding sites in human lacrimal tissue. This amount is over 250,000-fold less than the daily testosterone production rate in women.

However, it is understood that the entire dose of androgen would not be delivered solely to lacrimal tissues; much of the administered pharmaceutical would be cleared through the nasolacrimal duct. Therefore, an excess over the required dose must be administered. If the dosage were increased 100,000-fold to 0.1 mg, as suggested for the topical treatment of elevated intraocular pressure (144), then this dose would still be significantly less than the amount normally produced by adult *females*. Furthermore, if the topically applied androgen were testosterone or 19-nortestosterone, an anabolic androgen which has significantly reduced androgenic activity in lacrimal tissue as described above, and all of the steroid were to pass through the

nasolacrimal canal and to the intestine, then the effective dose would be approximately 0.01 mg (i.e., 10-fold less than the administered dose), due to hepatic metabolism.

To verify that topical androgen administration is inefficient for increasing serum androgen levels, an experiment was carried out in which castrated rabbits were treated for 14 days with either systemic topical 19-nortestosterone. The systemic exposure was achieved by the implantation of subcutaneous, slow release pellets into the subscapular region. These pellets, which contained 19-nortestosterone were designed to release physiological amounts of androgen over a period of 21 days. The topical exposure involved the application of a drop (40 drops ±one ml) of 19-nortestosterone (1 mg/ml) to both eyes of rabbits twice a day. Systemic androgen treatment was shown to elicit physiological concentrations of serum androgens. In contrast, topical exposure did not raise androgen concentrations in serum: no 19-nortestosterone could be detected in serum following the topical application of androgens to the ocular surface.

TGF-β therapy can be administered in the form of drops or locally by injection. The administration of a specific compound would be by routine methods in pharmaceutically acceptable substances including buffer solutions (e.g., phosphate buffered saline) or inert carrier compounds, to the ocular surface or adjacent regions of the eye. The dosage of TGF-β administered (preferably in the range of 10 pg to 10 mg, and more preferably 10 ng to 10 μg) can be optimized according to the formulation and method of delivery, and the mode of administration can be readily determined by conventional protocols. This TGF-β treatment should suppress lymphocyte infiltration in adjacent lacrimal tissue and

thereby ameliorate immune-mediated destruction of acinar and ductal epithelial cells.

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While the present invention has been described in conjunction with a preferred embodiment, one of ordinary skill, after reading the foregoing specification, will be able to effect various changes, substitutions of equivalents, and other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

CLAIMS

What is claimed is:

1. A method for treating keratoconjunctivitis sicca (KCS) due to androgen deficient disorders and not caused by estrogen deficiency, said method comprising

selecting a patient showing signs or symptoms of aqueous tear deficiency, lipid abnormality, Sjögren's syndrome, lacrimal gland dysfunction, lacrimal gland inflammation or meibomian gland dysfunction;

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance; and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of said patient.

2. The method of claim 1 wherein in said administering step, said therapeutic agent is applied to the ocular surface of the eye.

3. The method of claim 1 wherein in said administering step, said therapeutic agent is applied to a region of the eye adjacent the ocular surface.

4. The method of claim 1 wherein in said providing step, said pharmaceutically acceptable substance comprises hyaluronate.

5. The method of claim 1 wherein in said providing step, said pharmaceutically acceptable substance comprises phosphate buffered saline.

6. The method of claim 1 wherein said androgen or androgen analogue is from a structural subclass of androgens comprising androgenic compounds with unusual structural features.

7. The method of claim 6 wherein said androgen or androgen analogue is 17α-methyl-17β-hydroxy-2-oxa-5α-androstan-3-one.

8. The method of claim 1 wherein said androgen or androgen analogue is a testosterone derivative.

9. The method of claim 1 wherein said androgen or androgen analogue is a 4,5α-dihydrotestosterone derivative.

10. The method of claim 1 wherein said androgen or androgen analogue is a 17β-hydroxy-5α-androstane derivative containing a ring A unsaturation.

11. The method of claim 1 wherein said androgen or androgen analogue is a 19-nortestosterone derivative.

12. The method of claim 1 wherein said androgen or androgen analogue is a nitrogen-substituted androgen.

13. The method of claim 1, wherein said patient shows signs or symptoms of aqueous tear deficiency.

14. The method of claim 1, wherein said patient shows signs or symptoms of lipid abnormality.

15. The method of claim 1, wherein said patient shows signs or symptoms of Sjögren's syndrome.

16. The method of claim 1, wherein said patient shows signs or symptoms of lacrimal gland dysfunction.

17. The method of claim 1, wherein said patient shows signs or symptoms of lacrimal gland inflammation.

18. The method of claim 1, wherein said patient shows signs or symptoms of meibomian gland dysfunction.

19. A method for treating keratoconjunctivitis sicca (KCS) due to aqueous tear deficiency, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of aqueous tear deficiency.

20. A method for treating keratoconjunctivitis sicca (KCS) due to lipid abnormality, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lipid abnormality.

21. A method for treating keratoconjunctivitis sicca (KCS) due to Sjögren's syndrome, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of Sjögren's syndrome.

22. A method for treating keratoconjunctivitis sicca (KCS) due to lacrimal gland dysfunction, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lacrimal gland dysfunction.

23. A method for treating keratoconjunctivitis sicca (KCS) due to lacrimal gland inflammation, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lacrimal gland inflammation.

24. A method for treating keratoconjunctivitis sicca (KCS) due to meibomian gland dysfunction, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of meibomian gland dysfunction.

25. A method for treating tear film dysfunction due to aqueous tear deficiency, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of aqueous tear deficiency.

26. A method for treating treating tear film dysfunction due to lipid abnormality, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or

androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lipid abnormality.

27. A method for treating treating tear film dysfunction due to Sjögren's syndrome, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of Sjögren's syndrome.

28. A method for treating treating tear film dysfunction due to lacrimal gland dysfunction, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lacrimal gland dysfunction.

29. A method for treating treating tear film dysfunction due to lacrimal gland inflammation, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lacrimal gland inflammation.

30. A method for treating treating tear film dysfunction due to meibomian gland dysfunction, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of meibomian gland dysfunction.

31. A method for treating ocular surface disease due to aqueous tear deficiency, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a

patient showing signs or symptoms of aqueous tear deficiency.

32. A method for treating ocular surface disease due to lipid abnormality, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lipid abnormality.

33. A method for treating ocular surface disease due to Sjögren's syndrome, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of Sjögren's syndrome.

34. A method for treating ocular surface disease due to lacrimal gland dysfunction, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lacrimal gland dysfunction.

35. A method for treating ocular surface disease due to lacrimal gland inflammation, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of lacrimal gland inflammation.

36. A method for treating ocular surface disease due to meibomian gland dysfunction, said method comprising

providing a therapeutic agent comprising a therapeutically effective amount of an androgen or androgen analogue that has androgenic effectiveness and not estrogen effectiveness in topical application, said androgen or androgen analogue being in a pharmaceutically acceptable substance, and

administering said therapeutic agent topically to the ocular surface or immediate vicinity of an eye of a patient showing signs or symptoms of meibomian gland dysfunction.

37. The method of any one of claims 1 and 13-36 wherein said therapeutic agent is administered topically at a dose rate less than or equal to 0.1 mg/day.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/06787

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :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. : 514/178, 912

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,863,912 A (SOUTHREN et al.) 05 September 1989, see the entire document.	1-37

☐ 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 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		
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Date of the actual completion of the international search
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