Methodologies for the Study of Ocular Surface Disease
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Abbreviations are printed in boldface where they first appear with their definitions.


ABSTRACT

The ability to obtain reliable results from clinical trials of therapies for ocular allergic disease and dry eye disease is often limited because of inadequate control of variables, such as environment, patient lifestyle, compliance, and individual fluctuations that occur from one assessment visit to another. The controlled allergen challenge (CAC) model of allergic conjunctivitis allows signs and symptoms of the disease to be elicited in a physiologically accurate and reproducible manner. The rigid criteria for subject selection, the controlled allergic reaction, and the standardized and quantified grading systems allow for a reproducible baseline from which statistically and clinically significant differences between
formulations can be assessed. Similarly, the controlled adverse environment (CAE) model for dry eye mimics the environmental stimuli that lead to ocular surface drying. Preselected subjects have a reproducible, homogeneous baseline reaction from which the effects of various treatments can be significantly evaluated and compared. CAC and CAE provide accurate means to study highly variable and individual ocular surface disease.

**Keywords** allergy, dry eye disease, challenge models, clinical trials, controlled adverse environment model, conjunctivitis, controlled allergen challenge model, keratoconjunctivitis, ocular allergy

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**I. INTRODUCTION**
Allergic conjunctivitides and dry eye diseases and conditions are prominent examples of highly variable ocular surface disorders. The unpredictability of each disorder lies in its pathogenesis, as the clinical manifestations can be dramatically modified by external stimuli. Allergic conjunctivitis (AC) is brought on by ocular exposure to airborne allergens in IgE sensitized individuals. The specific type of allergen sensitivity, the exposure level, and the degree of sensitivity to that allergen are the most important variables; yet other factors can further influence these parameters, creating an uncontrollable setting for precise scientific study of a highly heterogeneous population. Dry eye disease and conditions can arise from multiple etiologies that result in decreased tear production. Both the external environment (humidity, wind, sun exposure) and certain visual tasks can facilitate ocular drying and exacerbate signs and symptoms when an underlying pathology is present.

The concept of disease models has a long history, and such models have been used to study diseases in vitro, in animals, and in humans. The relatively benign and self-limiting nature of allergic conjunctivitis and mild dry eye in particular has made them ideal for human clinical study. This review will discuss the use of human models of these diseases, appropriately described as "challenge" models, and how they have increased our knowledge of the pathogenesis and treatment of both seasonal allergic conjunctivitis and dry eye disease.

II. OCULAR ALLERGIC DISEASES

Ocular allergy can be broadly classified into four disease subcategories: seasonal allergic conjunctivitis (SAC), perennial allergic conjunctivitis (PAC), vernal keratoconjunctivitis (VKC), and atopic keratoconjunctivitis (AKC). It is estimated that over 20% of the general population suffers from some form of ocular allergy. Of those, over 90% have SAC, PAC, or both. The etiology of these more common forms of allergic conjunctivitis is predominantly a mast cell-located IgE antigen-antibody reaction, the clinical manifestation of which is limited to the conjunctiva and lids. PAC and SAC are differentiated only by the type of allergen sensitization: PAC is brought on by perennially available allergens, such as dust mites, molds, and animal dander, whereas SAC is more strictly related to airborne pollens in a given season. Common spring allergens in the northeastern United States include trees and grasses (graminaceae) which are joined by ragweed and other compositae in the summer and fall. The seasonality of allergens varies by region and climate, and, therefore, levels of certain seasonal allergens may peak in spring, summer, fall, or even winter, depending upon the geographic location.

A. Perennial and Seasonal Allergic Conjunctivitis

The hallmark symptom of PAC and SAC is ocular itching. The conjunctiva becomes hyperemic, with a very thin, tear-like discharge, and at times there is swelling of the lids and chemosis of the conjunctiva. Frequent attacks can lead to chronically swollen, teary, red, itchy eyes. However, it is more common to have episodes during a given season that completely resolve with the disappearance of the offending antigen. SAC is frequently associated with allergic rhinitis; thus, the patient presenting to the ophthalmologist may already have been evaluated with allergy skin tests or measurement of serum IgE levels. A thorough medical history is imperative, especially if the patient is free of allergic symptoms at
Laboratory tests are usually adjunctive and, with AC, are mainly for academic use. A conjunctival scraping for various allergic mediators such as tryptase or eosinophils is easily performed, but is only useful for well-established cases.\(^2\) However, tear IgE is often above normal and can correlate with increased serum IgE levels.\(^3,4\)

The most reliable clue to the diagnosis of SAC/PAC is the presence of itching, and this symptom can be used to differentially diagnose allergy from dry eye. The absence of a thick, ropey and/or purulent discharge should exclude infectious and vernal conjunctivitis, and the absence of corneal involvement can rule out AKC. SAC and PAC can be defined as a series of allergen challenges to the eye with varying frequency (i.e., acute, seasonal, and perennial, pertaining to the frequency of these attacks).

The pathogenesis of SAC involves mast cell activation and degranulation, with release of pre-formed mediators and initiation of the arachidonic acid cascade of prostaglandin synthesis. After years of increasingly sophisticated research in allergy, histamine is still considered the most important mediator responsible for the signs and symptoms of SAC and PAC.\(^5,6\) Late phase-related eosinophil activation is typically not involved, since toxic corneal effects and neutrophil infiltration are not manifested in this disease.\(^7\)

**B. Vernal and Atopic Keratoconjunctivitis**

In contrast to SAC and PAC, VKC and AKC are very rare, severe allergic diseases, defined by their chronicity and visually threatening corneal complications. AKC is an ocular component of a multi-system allergic and immunological disease, whereas VKC is often the sole allergic manifestation in affected patients.

The pathogenesis of VKC involves an IgE- and Th2 lymphocyte-mediated allergic reaction with additional hypersensitivity responses that are often ill-defined. Multiple factors can contribute to the etiology of VKC, including environmental allergens, climate, and genetic predisposition. Its predominance in male children and its resolution with puberty also suggest a hormonal component. Mast cells, eosinophils, and other mediators play major roles in its clinical manifestation. Tissue remodeling reactions, such as giant papillae formation, extensive fibrovascular proliferation invading the peripheral cornea, and various degrees of superficial corneal opacification, are further sequellae of the chronic inflammation associated with VKC. Many elements contribute to this dramatic response, including epithelial changes, connective tissue deposition, edema, inflammatory cell infiltration, and glandular hypertrophy.\(^8\)

AKC is associated with increases in mast cells, eosinophils, lymphocytes, and basophils in the conjunctiva. Symptoms may include corneal neovascularization, anterior polar cataracts, blepharitis and loss of lashes. The corneal damage commonly observed may be attributed to eosinophil activation and release of epithelial-toxic mediators. Elevated levels of tear and serum IgE are found during acute
phases, while serum IgE tends to drop when signs and symptoms decline. Sustained IgE-stimulated mast cell activation leads to immunoglobulin switching, and Th2-derived cytokines direct further production of IgE and continued mast cell activation.9

The greater complexity of VKC and AKC, in regard to both their pathogenesis and clinical spectrum, make them inappropriate candidates for study with the conjunctival allergen challenge (CAC) model. Conversely, PAC and SAC, which represent the majority of allergy cases, are strictly mast cell IgE-mediated diseases, the clinical manifestation of which can be recreated by instillation of allergen into the eyes of sensitized individuals. Thus, research on the pathogenesis and treatment of these diseases is ideally suited to the CAC model.

C. Allergic Challenge

1. History

Challenge of the human eye with solutions of allergen was first attempted by Blackley in the 1870s to test certain pollens, which were later used as a parameter to measure "resistance during experiments to desensitize patients."10 In the first part of the twentieth century, human ocular challenge was revived as a non-quantitative method of testing sensitization when skin tests were negative.11,12

Abram performed allergen challenge by instilling pollen grains into the conjunctival sac, noting that eyes often reacted positively when skin tests were negative.13 Tuft and associates, in the 1960s, performed more than 7000 challenges with powdered inhalant allergens, such as dust, feathers, and animal dander.14,15 These early studies used ocular challenge as a means of diagnosing versus studying allergy. Stegman and Miller, in 1975, challenged patients with timothy pollen and measured the increased protein content in tears.16

In the 1980s, Mikuni used Japanese cedar pollen to demonstrate that pretreatment with cromolyn could decrease the signs and symptoms of ocular allergy.17 Since that time, challenge studies have explored various aspects of the ocular allergic reaction, including early- and latephase responses,18 various tear mediator levels,19,20 treatment effects on stabilizing mast cells,21 and even ocular treatment effects on nasal allergic symptoms.22

2. Role of Histamine

The role of histamine in allergic disease has been recognized for many years. Its role in the wheal-and-flare skin reaction was established in the early 1900s.23 In the immediate allergic reaction, histamine binds to H1 receptors on nociceptive type-C nerves extensively branched in the mucosa and activates pain centers in the brain that are responsible for the itching and congestion, and systemic reflexes, such as tearing.24
The importance of histamine in ocular allergy was determined through a series of experiments, beginning with the isolation of histamine from VKC patients' tears. Research revealed that tissue from these patients had a significantly higher percentage of mast cell degranulation than normal tissue. Additionally, VKC is typically characterized by a relative lack of functional histaminase, the histaminedegrading enzyme. These aspects of the disease indicated that it would be possible to isolate histamine from tear collection from VKC patients. The subsequent collection of histamine from SAC/PAC eyes was found to be possible only with the use of histaminase-blockers.

Once histamine was isolated, its pivotal role was confirmed through human ocular challenge with use of topical application of histamine. Further research established the ability of histamine to induce itching on the ocular surface through H1 receptors and redness through both H1 and H2 receptors. When histamine is instilled into the eye, it dose-dependently reproduces the signs and symptoms of allergic conjunctivitis, and, as expected, when its effects are inhibited with antagonists, the signs and symptoms of the disease disappear or diminish.

3. Role of Other Mediators

Ocular challenge with allergens has been used to study many of the mediators present during an allergic reaction, including tear inflammatory mediators, tissue adhesion protein expression, and cellular infiltration. Tryptase and eosinophil cationic protein (ECP) are examples of the myriad molecules evident in the ocular allergic reaction that can be studied with use of conjunctival allergen challenge. These studies have revealed that the early peaks of tear histamine and tryptase levels indicate the mast cell's responsibility for the allergic reaction. Additionally, the release of ECP from eosinophils has been shown to occur in the inflammatory phase of more severe allergic responses.

However, these other mediators suspected to have a role in ocular allergy have been instilled in the eyes of allergic and nonallergic patients with mixed results. Prostaglandin D2 is found in tears after allergen challenge, and when eyes are challenged with this mediator, signs of ocular allergy result, such as redness and conjunctival chemosis.

Platelet activating factor (PAF) also reproduces some signs and symptoms of ocular allergy, particularly inflammation, when instilled in the eye, and is, therefore, probably involved in the immediate reaction. A role for the leukotrienes in allergic conjunctivitis is less clear. These mediators do not produce signs and symptoms of the disease when administered to the eye. LTB4 elicits a profound neutrophil infiltration approximately 6 hours after challenge, an event dissimilar to those that occur in these diseases. Nevertheless, an important role of the leukotrienes in the late phase of allergy has not been excluded. None of the other mediators used in topical ocular challenge have reproduced the signs and symptoms of allergy as well as histamine.

4. Allergy Models
The weakness of histamine or other mediator challenge as an accurate model for ocular allergic disease lies in the absence of IgE- mast cell stimulation. The great majority of antiallergic agents used today work on "stabilizing" the mast cell (i.e., preventing it from degranulating or releasing its intracellular contents), after the IgE antibody-antigen reaction has been triggered on the mast cell surface. An allergy model was needed that not only reproduced the signs and symptoms, but the pathogenic factor as well. Similarly, instillation of secretagogues, such as compound 48/80, induces degranulation of mast cells and, thus, the release of histamine in both animal and human eyes. These secretagogues are nonspecific, acting on mast cell membranes without the initial IgE antibody-antigen reaction. However, it is of note that conjunctival mast cells are 48/80 responsive, while some other types of mast cells are not.

The heterogeneity of mast cells, according to both tissue and species, has implications for preclinical evaluation of mast cell stabilization agents. Although a drug may be efficacious in one type of mast cell, this may differ from tissue to tissue. Some ophthalmic antiallergics, such as olopatadine, have been screened using the appropriate tissue, human conjunctival mast cells, while others have used conjunctival tissue from other species, such as rat, or human tissue from umbilical cord.

Problems of reproducibility, grading, and standardization of ocular allergen challenge were recognized long ago as limitations to its use in research. Moller attempted to address some of these issues in 1984 in researching the reliability of tests to objectively confirm cases of allergy, and was influential in reintroducing this model as a potential clinical research tool. In the 1970s, motivated by frustrating seasonal allergic conjunctivitis trials, and the near impossibility of proving the effectiveness of even the most potent of drugs because of the vast array of uncontrollable variables, we directed our work toward refining ocular allergen challenge to establish it as the most standardized method for testing and comparing the efficacy of new antiallergic agents.

a. Limitations of Environmental AC Drug Trials

The following discussion illustrates the shortcomings of a clinical environmental allergic conjunctivitis drug trial and the ways in which the CAC model can significantly improve pharmacological testing.

A clinical environmental trial of a new antiallergic drug in SAC patients often fails due to intra- and inter-patient variability. The patient is sensitized to one or more seasonal airborne pollens with greatly varying degrees of sensitivity/response (inter-subject variability). The individual then, within the active pollen season, alters his/her exposure with daily activities, such as working outside or in air conditioning, driving in a convertible or in a closed car, doing outside or inside weekend activities, or traveling into areas of greater or lesser pollen concentrations. These diurnal and day-to-day fluctuations occur during the intervals between office visits, with no control of allergen exposure possible (intra-subject variability). The patient is typically seen weekly or biweekly during a seasonal study and has completed a daily diary on signs, symptoms and activities. In a disease that is defined as a series of discrete, self-limiting challenges, the chance of finding a patient in the same state of allergen exposure from one visit to the next is extremely low. Due to this variability, it is difficult to accurately assess
clinically significant differences between active and placebo treatment groups.

Inconsistent conditions also prevail across subjects, creating still more inter-subject variability. Allergen challenge resolves the problem of variability by predefining for each subject the exact dose of allergen that elicits a bilaterally homogeneous moderate reaction. Additionally, challenge is performed out of season when the subject is not otherwise exposed to allergen. Any sign of allergic reaction at baseline excludes the subjects from the study. These measures assure that all subjects have the same moderate allergic reaction, meaning a homogeneous baseline against which drugs can be accurately evaluated.

Evaluating drug and placebo or two active drugs in the same patient, one eye versus the contralateral eye, also controls intersubject variability. An additional obstacle in environmental studies is presented by placebo treatment. Placebo can act as a surrogate tear substitute, sometimes allowing a patient considerable relief from allergic signs, as the drop can wash away allergens and lubricate a red, irritated eye. Previous studies have shown placebo to have as much as 70% drug effect in an environmental study. Complicating this is the limited duration of an allergy season. In the month or six weeks of a seasonal allergy study, the active pollen season gradually diminishes, and the patient improves over time, regardless of therapy. Thus, a seasonal study does not provide a suitable baseline from which changes over time can be accurately attributed to drug activity.

Another limitation of the environmental design regards compliance, which can be unpredictable, and which is known to decrease over time, regardless of diary input and queries at office visits. A challenge study is usually limited to one drop of drug administered in an identical fashion during an in-office visit, minimizing compliance problems.

Another factor in environmental studies is patient avoidance. If study subjects do not expose themselves to allergens, there is no challenge to the drug to test its level of efficacy. Further, the variability of individual exposure can also complicate the situation. Some subjects may have high exposure (i.e., being outdoors all day), while others may have minimal to no exposure.

The Vienna Chamber and the "Cat Room" are two similar examples of models to study ocular allergy. These models have essentially taken the environmental model and condensed it into a specific location. Rather than allowing patients free movement within their normal environments, they are contained in a room that circulates air containing airborne pollens, such as ragweed (the Vienna Chamber), or a room in which cats are present (the "Cat Room" model). Although some Cat Room studies have been plagued by other study design issues, such as lack of baseline evaluations and confirmation visits, the overall shortcoming of these models lies in their inherent similarity to the environmental model, albeit on a smaller scale. Within the study chamber, pockets of high and low allergen concentration can be present, which potentially creates inconsistent allergen exposure and, thus, variable allergic reactions. In addition, a significant placebo effect has been observed in studies using these models.

Although these models employ the appropriate train of thought in narrowing the environment to contain
patients in one enclosed area, they do not take this concept to the level needed to obtain repeatable, precise measurements. The ultimate localization of allergen challenge is to instill patient-specific allergen directly onto the ocular surface. This is the concept forming the foundation of the CAC model, which has supplanted the now obsolete Vienna Chamber and Cat Room models for ocular allergy.

**b. Conjunctival Allergen Challenge Model**

The study methodology that has evolved to evaluate drug efficacy in the CAC model strives, above all, to create the most accurate and homogenous baseline for a given population. Subjects are skin-tested to determine which seasonal allergen elicits an allergic reaction. This allergen is then instilled into the eye in increasing concentrations until an appropriate allergic response is established. At the second visit, the ocular allergic response is confirmed. Then subjects are randomized into treatment groups for drug evaluation.

Drug can be administered in both eyes, placebo can be administered in both eyes, or drug can be administered in one eye and placebo in the contralateral eye, depending on the study design. The comfort of a drug or its vehicle may be evaluated at this time by having the subjects complete a standardized comfort evaluation query. Allergen challenge is then performed bilaterally with the predefined dose, and the signs and symptoms of allergy are recorded at set time points previously established as being of maximum effect on the allergen response curve.

Itching has been identified to peak early, after 3 to 5 minutes, redness after 10-12 minutes, and chemosis and eyelid swelling after 20 and 30 minutes, respectively. Standardized grading systems have been created for evaluating these signs and symptoms, including photographic representations of each half grade for redness, and quantified scanning for lid swelling. Subjects grade their own itching according to a standardized five-point scale.

Variations can be added to the CAC protocol to evaluate the duration of action of a particular agent. Following treatment instillation, subjects may be instructed to return after 4, 6, 8, 12 or even 24 hours for the allergen challenge. Statistically and clinically significant differences between treatment and placebo establish the duration of efficacy of a drug. The onset of drug activity can also be evaluated by shortening the time from drug administration to allergen challenge.

Parallel groups can also be compared in some cases, with instillation of drug or placebo in both eyes of one subject every day for a specified loading period, after which allergen challenge is performed. While the data may not be as tightly controlled, as the intrasubject comparison is lost, statistically and clinically significant data have been accumulated with this variation when it is suspected that a drug requires a loading period to reach maximal efficacy.

The CAC can also be employed for drug evaluation following high dose allergen challenge, provoking the latephase allergic response and an increase in the level of cellular infiltrates in certain patients. Although the challengeinduced severe allergic reaction is variable among SAC/ PAC patients, it
indicates the ability of the CAC model to be used not only in evaluation of agents at the clinical level (i.e., itching, redness, swelling indices), but also at the mediator level. This allows the underlying effects of a drug to be studied beyond its clinical efficacy. For example, a recent high-dose allergen challenge study evaluated the ability of a topical antiallergic agent (Patanol, Alcon) to block the release of mediators from mast cells in the human eye. The study revealed that a reduction in allergic signs and symptoms correlated with the reduction in the mediators responsible for the immediate reaction and the later inflammatory reaction. This included reductions in tear histamine levels, eosinophils, neutrophils, and leukocytes, as well as reduction of adhesion molecule expression.

The CAC model can be used to assess the clinical signs and symptoms of AC as well as allergic mediator levels in a standardized, reproducible fashion. It is also a well-controlled method for accurately measuring the onset, duration, and potency of any ocular anti-allergic drug. However, caution should be taken when working with allergen challenge, and it should be recognized that allergens can also induce other allergic conditions, such as rhinitis symptoms or breathing difficulties. Therefore, the CAC model should always be employed with a "crash cart" available containing the equipment necessary to treat cardiac arrest, and physicians/nurses trained in cardiac pulmonary resuscitation should be present. Subjects should also have the option of an allergy relief drop post-assessment.

The limitations of the CAC model are those features that are considered strengths of the environmental model. If the goal of a study is to assess the "real world" scenario, in which patients may undergo highly variable pollen exposures and in which allergen exposure varies drastically depending on the ambient environment of the subject, the environmental model may be preferable. However, assessing quantitative, exact differences in drug performance is much more difficult to measure in such environmental scenarios.

Often, a combination of both a CAC model study and an environmental study is beneficial for fully evaluating the safety and efficacy of an anti-allergic drug. The major advantages of the CAC model (controlled, accurate measure of drug onset, duration, and efficacy) combined with those of the environmental model (long-term safety evaluation) allow for thorough treatment assessment.

In an environmental-CAC study, the variability of environmental pollen counts from day to day is incorporated into the analysis of signs and symptoms, thereby giving a more accurate picture of actual drug efficacy. This method of analysis has been used in recent studies and has shown that, while a placebo eye drop and an active drug may appear to perform similarly with low-level pollen exposure (i.e., when there is a relative lack of challenge to the eyes), as pollen levels increase, the active drug holds to this level of performance, managing the signs and symptoms of ocular allergy. However, with a placebo or a relatively ineffective drug, the signs and symptoms increase as a function of pollen levels.

c. Conclusion

The CAC model of allergic conjunctivitis is the ideal method for testing the activity and duration of anti-
allergic pharmacological agents. It elicits the signs and symptoms of the disease in a physiologically accurate and reproducible manner. The rigid criteria for subject selection, the controlled allergic reaction, and the standardized and quantified grading systems allow for a reproducible baseline from which statistically and clinically significant differences between active and control or two active formulations can be tested. The CAC model has been used to evaluate every anti-allergic agent available today for the treatment of allergic conjunctivitis, and for the majority of these, CAC model studies were pivotal for FDA approval of the drug.

III. DRY EYE SYNDROME AND CONTROLLED ADVERSE ENVIRONMENT (CAE) CHALLENGE

A. Diseases Associated with Dry Eye

Dry eye syndrome is defined as any disturbance in tear film physiology that leads to a clinically evident drying of the ocular surface. Although an actual diagnosis of primary dry eye may be less common, mild to moderate dry eye is thought to occur in 11-22% of the general population. It is more prevalent in people over 55 years of age, especially women. Dry eye can be caused by a deficiency in the aqueous, lipid, and/or mucin layer of the tear film.

The most well-known disease of aqueous layer deficiency is Sjogren syndrome, the pathogenesis of which involves autoimmune-initiated lacrimal gland damage. This can be a primary, local, ocular disease, or it may be secondary to other connective tissue diseases, such as rheumatoid arthritis, lupus erythematosis, systemic sclerosis, and other autoimmune diseases. Non-Sjogren dry eye can involve primary (congenital or acquired lacrimal gland dysfunction) and secondary pathologies of the lacrimal gland (sarcoidosis, HIV, graft vs. host disease, xerophthalmia, surgical lacrimal gland removal), obstruction of the lacrimal ducts (trachoma, pemphigoid, erythema multiforme), or reflex hyposcretion (neuroparalytic keratitis, contact lens wear, seventh nerve paralysis).

Dysfunction of the lipid layer of the tear film leads to hypervaporation of tears in the presence of a normally functioning lacrimal gland. Squamous lid margin metaplasia, keratinization and breakdown of the lid margin epithelium and lid lines impair lipid secretion onto the ocular surface. This condition leads to decreased oil release and hypervaporation.

Primary lipid layer pathologies leading to decreased oil release and hypervaporation include aplasia of the meibomian glands, and distichiasis. Secondary pathologies that lead to hypervaporation are posterior or anterior blepharitis and obstructive meibomian gland disease. Hypervaporation can also be associated with lid (ectropion, entropion, irregular lid margins) or ocular surface (exophthalmos, scarring or conjunctival irregularities) abnormalities, and contact lens wear. A mucin layer deficiency occurs when there is loss of goblet cell function caused by chronic inflammation, vitamin A deficiency, cicatrizing conjunctival disorders, chemical burns, or chemical preservatives that may precipitate mucous layer impairment.
Even individuals with normal ocular health can experience dry eye symptoms in adverse conditions, such as in airplanes or in arid, windy environments, or while performing visual tasks. Dry eye symptoms can also be a normal part of aging, particularly in post-menopausal women in whom an endocrine-immune directed reduction in mucosal secretions occurs.

The ultimate clinical manifestation of any of the above pathologies is tear film instability, tear hyperosmolarity, and subsequent ocular surface damage. Clinical signs of dry eye include conjunctival hyperemia and various corneal pathologies (keratitis, ulcers); alterations in biomicroscopy of the conjunctiva, tear film and meniscus; and histological abnormalities. Symptoms include burning and stinging, foreign body sensation, sense of dryness and difficulty in opening eyes in the morning, visual disturbances, photophobia and reflex tearing, and painful, heavy or tired eyes.45,47

B. Therapy for Dry Eye

Treatment options for dry eye syndrome are currently mainly palliative and not curative. Artificial tears are commonly used to substitute for the deficient natural tears. These can be classified according to their characteristics and principle ingredients intended to dilute hypertonic tears, increase tear volume, stabilize or correct the tear film imbalance, and/or provide nutrients to the tear film. These overthe-counter artificial tears are typically considered useful in their reduction of the symptoms (e.g. burning, discomfort) of dry eye. However, some of the currently available artificial tears have demonstrated improvements in the signs of dry eye, such as tear film break-up time (TBUT), keratitis, and conjunctival staining.48 Several of the more recently developed artificial tears incorporate mechanisms of action beyond temporary lubrication of the eye, such as cross linkage and gellation of the solution upon instillation on the ocular surface (Systane, Alcon), or replenishment of the lipid layer of the tear film (Soothe, Alimera Sciences).

In 1964, the FDA monograph classified "active" ingredients in tear substitutes in the following manner:

1) Astringents: locally acting pharmacological agents that, by precipitating protein, help to clear mucus from the outer surface of the eye;

2) Demulcents: usually water-soluble polymers applied topically to the eye to protect and lubricate mucous membrane surfaces and relieve dryness and irritation; and

3) Emollients: usually fats or oils applied locally to eyelids to protect or soften tissues and to prevent drying and cracking.47

Any product or products that can be classified in the above categories can be used in dry eye formulations within specified concentration ranges.

There is currently one prescription agent available for the treatment of dry eye, cyclosporine ophthalmic emulsion 0.05% (Restasis™, Allergan). The mechanism of this agent, though not fully elucidated, is
believed to involve limitation of T lymphocyte activation via inhibition of HLA-DR expression, as well as other cellular mediators that may promote T lymphocyte activation, allowing for the modulation of inflammation.\textsuperscript{49} This Cyclosporine A emulsion is indicated to increase tear production in patients whose tear production is presumed to be suppressed due to ocular inflammation associated with keratoconjunctivitis sicca. After six months of b.i.d. dosing, subjects in clinical studies demonstrated significant increases over vehicle in Schirmer strip wetting of 10mm\textsuperscript{2}.\textsuperscript{50} Commonly, an artificial tear is suggested to patients for concomitant administration with Restasis; this alleviates the burning and stinging of dry eye before the drug attains its maximal efficacy, and also alleviates the discomfort some patients experience with use of Restasis.\textsuperscript{51}

Present and future studies of tear substitution will strive to identify new agents that reduce ocular discomfort more effectively and for a longer duration, promote healing, and/ or prevent damage by correcting the specific deficiency in the tear film. Classes such as mucomimetics, anti-evaporative agents, anti-inflammatory drugs, secretagogues and even, simply, improved polymers are under investigation. An oral vitamin supplement specifically designed to treat dry eye is also currently in development.\textsuperscript{52} Evaluation of these new products and their effects on the ocular surface must be carried out in the most precise, standardized, and reproducible manner in order to demonstrate statistically and clinically significant results.

C. Pitfalls in the Clinical Evaluation of Dry Eye Therapy

Dry eye treatments are traditionally evaluated in a clinical setting in a group of dry eye subjects who test the comfort of a proposed therapy with daily use and who return to the ophthalmologist for regular appointments to evaluate parameters of ocular surface health. As in the environmental model of allergy study, the subjects' daily activities and environmental conditions can greatly influence the signs and symptoms of dry eye. Variables such as humidity, air movement, and temperature can cause significant fluctuations in the integrity of the ocular surface and ocular discomfort. Furthermore, factors such as extended visual tasking (reading and PC use) that reduce blink rate and expose the ocular surface, the use of systemic medications that cause ocular drying, dehydration, and nutritional status can also exacerbate dry eye.

The variable nature of the signs and symptoms of dry eye create a difficult scenario for the evaluation of therapeutic agents. The "background noise" associated with the daily environmental and behavioral differences among study subjects creates a challenge in establishing a valid baseline and for measuring treatment effect. These many factors can complicate an environmental study design, often requiring large patient populations and resulting in increased variability.

Additionally, the parameters studied to test the effects of therapy must be selected very carefully and are in need of greater standardization. Variables in dry eye trials include the environment, patient population, and clinical endpoints. The duration of the study and the mechanism of action of the proposed therapy can also greatly influence the outcome of a study. It is important to keep in mind that evidence does not suggest the correlation of subjective symptoms and objective signs of dry eye.\textsuperscript{53}
D. Controlled Adverse Environment (CAE) Challenge

To perform evaluations of future dry eye therapies of differing mechanisms, a more precise, standardized model of evaluation is necessary. The CAE is a model that standardizes the study of dry eye in a manner analogous to the CAC model for ocular allergy. This model provides a controlled, reproducible environment that challenges the eyes of all patients equally and for the same amount of time. Clinical end points may be evaluated before and after exposure to the adverse environment. This model, in which variables are minimized, has been developed for use in evaluation of dry eye therapeutic agents. The CAE model can be adapted to match the mode of action of each compound, and to precisely evaluate and compare pharmaceutical agents intended for the treatment of dry eye.

The CAE is an environmental chamber that exacerbates the signs and symptoms of dry eye by regulating humidity (< 10%), temperature (76 ± 6ºF), airflow (constant, nonturbulent), lighting conditions (adequate to illuminate the environment without causing photosensitivity or minimizing the interpalpebral fissure), and visual tasking (television or PC use). By integrating specific diagnostic equipment, the CAE is able to measure both objective and subjective parameters according to standardized scales. A variety of clinical signs and symptoms can be evaluated before, during, and after CAE exposure.\(^4\)

This model allows researchers to evaluate the effects of dry eye therapies and provides a standardized method to study the underlying mechanisms of dry eye. Subjects are qualified based on a diagnosis of dry eye and a positive reaction to the CAE. This approach provides a preselected group of subjects who demonstrate a reproducible, homogeneous baseline reaction from which the effects of various treatments can be significantly evaluated under adverse and standard environmental conditions.

1. Study Design: Considerations

The proper selection of subjects is critical to the success of any study. In the evaluation of a potential dry eye therapy, its mechanism of action and scientific rationale must be taken into account when the test population is selected. An aqueous-based therapy should ideally be tested in patients with an aqueous-deficient tear film. These patients may have little need for a mucosimimetic component in their tear substitute, as their mucin layer is normal. Tear aqueous deficits are seen in Sjogren and non- Sjogren diseases of lacrimal gland dysfunction, or in lacrimal duct blockage.

Conversely, increasing the aqueous tear volume with a secretagogue in a patient with meibomian gland obstruction, squamous lid margin metaplasia, or blepharitis will produce little benefit if the primary pathology is not treated and the lipid layer is not augmented. These potential design flaws are prevented by enrolling subjects into dry eye trials according to the underlying mechanisms of their pathology, i.e., their specific tear deficiency (lipid, aqueous, mucous, and combinations). To accomplish this, methods for properly screening, characterizing, and categorizing patients must be identified and implemented.
The duration of the clinical trial ideally should also reflect the mechanism of action of the tear substitute or dry eye therapy. The efficacy of tear substitutes can be evaluated in short-term study because of their acute effects. Conversely, an anti-inflammatory agent aimed at inhibiting the inflammation at the origin of the tear deficit requires time, possibly weeks or months, to act and improve ocular surface parameters.

It is also important to establish the dry eye subject’s baseline response to the CAE challenge, assuring a symmetrical and reproducible reaction to adverse stimuli. Some patients, although diagnosed with the disease, may have anomalous reactions to the CAE challenge that render them unsuitable for clinical evaluations. For example, patients exposed to a CAE may experience significant reflex tearing, creating a period of temporary relief. This phenomenon has been termed "natural compensation," and it has been shown to inversely correlate with the severity of dry eye.55 Natural compensation is greater in both normal subjects (occurs in 10 minutes) and mild-to-moderate dry eye patients (occurs in 20 minutes) compared to those with moderate-to-severe dry eye (> 40 minutes or not at all).55 Enrolling a patient who is able to compensate for their ocular discomfort without treatment would adversely influence the clinical evaluation of drug efficacy.

2. Parameters to be Used as Clinical End Points

An understanding of the patient population being studied, as well as the study treatment's mechanism of action, is essential in the selection of a study parameter. The end point must be clinically relevant, reproducible, and have an appropriate scale to detect changes elicited by the treatment. A study can be inconclusive or lack significance if physiologically irrelevant parameters are evaluated.

Ophthalmologists and researchers rely on various clinical and laboratory tests to diagnose dry eye and determine its underlying etiologies. The investigation of dry eye treatments is accomplished with conventional, as well as novel, diagnostic tools. Objective measures often include keratitis, conjunctival staining, TBUT, hyperemia, meniscus height, fluorophotometry, visual function, Schirmer test, impression cytology, flow cytology, osmolarity, evaporimetry, and meibometry. Generally accepted symptoms of dry eye include dryness, burning, stinging, foreign body sensation, grittiness, sensitivity to light, and blurriness.47

a. Ocular Surface Staining

Clinical end points have been refined over the years, improving their sensitivity capacity to yield reliable and reproducible results. The diagnosis of dry eye relies on dyes that help assess the integrity of the ocular surface. Epithelial damage due to this condition is most commonly measured by applying either fluorescein or rose bengal. Fluorescein is known to penetrate areas of the corneal epithelium and conjunctival epithelium where intercellular junctions are disrupted, whereas rose bengal selectively stains damaged areas of the ocular surface in which the tear film itself is discontinuous. The use of lissamine green has been proposed as an alternative dye with which to evaluate the ocular surface. It has been observed to identify degenerated cells and mucus. Studies have demonstrated that rose bengal and lissamine green exhibit similar staining patterns in individuals with dry eye, although lissamine green is
better tolerated by patients because it produces less irritation upon instillation. Therefore, lissamine green may be a more tolerable, yet comparable, alternative for clinical evaluation of ocular surface damage in the assessment of dry eye.\textsuperscript{56}

When ocular surface staining is evaluated, certain procedures should be considered to optimize observations. Researchers have shown that the volume of a diagnostic dye can influence the determination of ocular surface damage. For example, a large, uncontrolled amount of fluorescein can result in oversaturation of the epithelium, making it difficult to distinguish frank staining from fluorescein quenching. Five (5) $\mu$L has proven to be a sufficient amount of fluorescein to properly detect ocular surface damage. Staining can be further highlighted by using barrier filters such as a Wratten #12 or Tiffen #11 and standardizing the magnification to 16X.

The timing of observation and evaluation is also critical. Time to fluorescence can vary from one patient to the next, depending on tear volume and tear turn-over rate. Inadequate timing may result in weak or fading ocular surface damage that may be misdiagnosed. It is believed that approximately 5 minutes after fluorescein instillation is the ideal time to measure the presence of staining. When using lissamine green, a volume of 10 $\mu$L is recommended, and the evaluation should occur 1 to 4 minutes after instillation.

\textit{b. Tear Film Break-up Time (TBUT)}

One of the more exciting advancements in the standardization of objective tools involves a commonly used dry eye test known as TBUT. Traditionally, TBUT has been measured with large and varying amounts of sodium fluorescein (50 $\mu$l or more). With this technique, TBUT was determined to be greater than 10 seconds in normal eyes and fewer than 10 seconds in dry eye patients.

It has been shown that the accuracy and reproducibility of TBUT measurements depend on the amount of fluorescein instilled into the tear film. When amounts of sodium fluorescein that exceed the average tear volume (known to be 6 to 7 $\mu$L) are used, it is thought that the tear film stability is influenced, resulting in an artificially lengthened TBUT. To improve this technique, well-controlled, micro-quantities of fluorescein (1 to 5 $\mu$l) have been used to measure TBUT.\textsuperscript{57,58} Consequently, more reliable and reproducible reference values have been established. With this improved technique, TBUT was determined to be greater than 5 seconds in normals (mean = 7.1 ± 1.17 seconds) and less than 5 seconds in dry eye patients (mean = 2.2 ± 0.82).\textsuperscript{59}

Numerous mechanisms are responsible for maintaining the integrity of an ocular surface, one of which is blinking. It has been shown that the blink phenomenon facilitates the distribution and formation of the precorneal tear film across the cornea. Several factors have been shown to influence blink rate- \textit{environmental} (e.g., humidity, temperature, airflow, and lighting), \textit{activity-related} (e.g., personal computer use, reading, driving, and conversation), \textit{psychological} (e.g., emotional state, anxiety, guilt, and mental load), and physiological (e.g., gender, ocular surface conditions, corneal sensitivity, and muscular tension). A recent study showed that blink rate decreased from 17 to 4.5 blinks per minute.
while patients were reading. Any one of these factors can cause an individual to alter their blink frequency, resulting in an unprotected ocular surface and exacerbation of the signs and symptoms of dry eye.

To properly measure blink rate in patients, it is important that the technique is standardized, consistent and noninvasive. Researchers have developed a precise method for measuring blink rate that incorporates a digital microcamera and an infrared illuminator that tracks the diameter of a patient's pupil. During the blink rate evaluation, patients are isolated and are asked to complete a standard visual task. In the absence of this sophisticated equipment, blink rate may be counted manually while a patient is reading the ETDRS chart. This information is critical in helping the ophthalmologist better understand one of the many factors that can affect dry eye.

Although TBUT information is considered to be reliable and reproducible, a lack of consensus on the interpretation of TBUT and its clinical relevance still exists. When studying the relationship between TBUT and the inter-blink interval (IBI); or time between complete blinks), it may be suggested that their interaction assists in maintaining the integrity of an ocular surface. For example, a protected surface exists when the TBUT either matches or exceeds the IBI. In contrast, an unprotected ocular surface exists when TBUT is less than the IBI. This relationship can be clinically relevant, as repeated, intermittent exposures of a tear film deficient cornea leads to ocular discomfort, followed by clinical signs such as keratitis and redness. The discordance between the IBI and TBUT can be worsened by factors that shorten TBUT (e.g., keratoconjunctivitis sicca and systemic antihistamines) or by factors that lengthen the IBI (such as fatigue and visual tasking).

The Ocular Protection Index (OPI) has been developed to help quantify the interaction between the IBI and TBUT. The OPI is calculated by dividing TBUT by the IBI. If the OPI is < 1, a patient's cornea is at risk for exposure, and if the OPI is = 1, a patient's cornea is not. This approach to measuring clinically relevant alterations in TBUT has proven useful in assessing factors that cause dry eye and in evaluating therapeutic agents that improve tear film stability.

c. Tear Film Osmolarity/Fluorophotometry

Because of its sensitivity and specificity as a single test or in combination with other tests, tear film osmolarity may represent the "gold standard" for diagnosing dry eye. Although tear film osmolarity may be a more accurate tool, there are still significant obstacles associated with its measurement procedure. For example, the technique requires a large sample of basal tears to be collected while reflex tearing is avoided to ensure a valid analysis. This is a difficult feat to accomplish and should be performed by an experienced technician. Additionally, although commercial osmometers are available, there is a significant demand for an instrument that is more reliable, user-friendly and cost-effective.

The most effective and reproducible method of measuring tear production is fluorophotometry. A fluorophotometer determines tear turn-over rate, tear volume and tear flow by precisely measuring the decay of fluorescein in the tear film. Researchers have been able to demonstrate the ocular drying effects
of a systemic antihistamine by measuring tear production with fluorophotometry (FM-2 Fluorotron™ Master, OcuMetrics). After only 4 days of QD dosing, tear volume decreased from 8.61 to 5.12 µL and tear flow decreased from 1.65 to 0.86 µL / minute in normal eyes.

**d. Corneal Sensitivity/Symptoms**

The reduction of corneal sensitivity caused by neurotrophic changes has been well documented in patients with herpes simplex keratitis, diabetes, and those undergoing keratorefractive surgery such as radial keratotomy, photorefractive keratectomy (PRK) and laser assisted in-situ keratomileusis (LASIK). Studies have shown that tear film contributory structures, such as the lacrimal gland, meibomian glands, and goblet cells, are highly innervated and that decreased corneal sensitivity can lead to impaired tear secretion and pathological changes of the corneal epithelium. Consequently, the ability to monitor corneal sensitivity is critical to understanding the progression of the signs and symptoms of dry eye. An instrument known as the Luneau Cochet Bonnet aesthesiometer can be used to measure corneal sensitivity. By changing the length of a nylon filament and the extent of the force applied to the cornea, corneal sensation can be quantified. Researchers have shown that patients diagnosed with dry eye have decreased corneal sensitivity.

When investigating complaints of dry eye symptoms, the interviewer should withhold from patients a vocabulary to describe their discomfort. This approach allows the patient to honestly report his or her individual symptomatology so that a more exact diagnosis can be made. Since an important therapeutic goal is to improve the patient's symptoms, the proper evaluation of an individual's symptoms results in a better assessment of treatment efficacy.

Our improved understanding of the relationship between patient symptoms and TBUT suggests a potentially simpler noninvasive determinant of tear film instability. This test is known as the symptomatic tear film break-up time test (SBUT), in which the patient looks straight ahead while the time from their last complete blink to the moment they report ocular awareness is recorded. This time should be within approximately one second of the patient's TBUT. Not only can this test be performed by the ophthalmologist in the office, but it can also be performed by the patient at home. The symptomatic break-up test allows dry eye patients to independently monitor their condition under different circumstances and evaluate the ability of current available treatments to relieve their symptoms.

**e. Tear Film Break-up Pattern**

An additional type of assessment is the recently developed tear film break-up pattern (TFBUP) test in which patterns formed as the tear film integrity breaks during measure of tear film break up time can be evaluated. These patterns have been identified and categorized into five distinct groups. TFBUP appears to be a clinically relevant finding that can help further the understanding of tear film stability and how it is disrupted. Further examination of how various tear film deficiencies correspond to specific TFBUPs is currently under way.
3. Modifications of the CAE Model

As with the CAC model of allergic conjunctivitis, the CAE model allows the investigator to tailor the study design to define specific objectives unique to his/her interests. Even normal populations can be targeted, including contact lens wearers, in order to evaluate the effects of adverse environmental stimuli on contact lenses or solutions. Furthermore, a sign/symptom or quantifiable parameter can be targeted if the agent is suspected to act specifically or differentially on one or more. As mentioned previously, the population is also selected to reflect the treatment to be tested. For example, subjects with lacrimal gland dysfunction may be selected for the evaluation of a secretagogue rather than subjects with duct obstruction.

4. Conclusions

The CAE model for dry eye mimics the environmental stimuli that lead to ocular surface drying and clinical pathology in the appropriate population. It provides the investigator with a preselected group of subjects who have a reproducible, homogeneous baseline reaction from which the effects of various treatments can be significantly evaluated and compared—their onset and duration of action, as well as any necessary loading periods. CAE challenge standardizes the study of dry eye in a manner analogous to the CAC model of ocular allergy. With such accurate means to study highly variable and individualistic ocular surface diseases, ever-increasing knowledge of these conditions as well as new and effective treatments are within our grasp.

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