Combining Sodium Hyaluronate and Polyvinylpyrrolidone Therapies for the Rabbit Cornea: A New Approach to Relief of the Human Dry Eye Syndrome

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ABSTRACT

Purpose of the study: The novel combination of 0.1% sodium hyaluronate (HA) and 5.0% polyvinylpyrrolidone (PVP) into one eyedrop was investigated to test the hypothesis of its increased relief of dry eye syndrome (DES).

Materials and methods: We evaluated HA and PVP, either alone, or in combination, by utilizing 16 rabbits, where their right eyes received one or two different eyedrops, and their left eyes, as controls, received none. The DES replica in rabbits was induced by 0.1% benzalkonium chloride (BAC) eyedrops. BAC was instilled into the right eyes of all rabbits, which were divided into four groups of four. In Group 1 M, the rabbits received only BAC. A second eyedrop given to the right eyes of Group 2 M was HA, of Group 3 M was PVP, and of Group 4 M was the combination of both HA and PVP. All eyes were followed clinically for 14 d, and thereafter, examined histopathologically.

Results: Clinically, the HA+PVP combination yielded the least perilimbal conjunctival erythema (p<0.05), and the least corneal epithelial fluorescein staining (p<0.001) compared to each treatment alone. Histopathologically, all four rabbits’ right eyes in the combination group 4 M displayed the greatest preservation of the corneal epithelium (p<0.001) and of the perilimbal conjunctival goblet cell density (p<0.001).

Conclusions: This unique combination of both HA and PVP into one eyedrop, was more potent than either treatment alone in protecting the ocular surface. A preparation, containing both HA and PVP may become useful for DES patients.

Keywords: Conjunctiva, cornea, dry eyes, goblet cells, tears

INTRODUCTION

Dry eye is a multifactorial disease of the ocular tear film and the integrated ocular surface/lacrimal gland reflex unit,¹² affecting a significant percentage of the world population, especially those older than 40 years of age.³ Dry eye syndrome (DES) can be associated with ocular discomfort, blurred vision, and in severe cases, irreversible damage to the cornea. Sodium hyaluronate (HA)⁴⁵ and polyvinylpyrrolidone (PVP)⁷ eyedrops have been individually demonstrated to each effectively treat the symptoms and signs of DES without adverse effects.⁶ However, some patients require four to six daily topical ophthalmic applications of either of these eyedrops, in order to obtain symptomatic relief from DES.⁵

The novel ophthalmic preparation of a combination of HA and PVP into one eyedrop mixture was intended to improve the efficiency and compliance of dry eye therapy by formulating a more potent ocular surface lubricant. Previous approaches for the treatment of DES have utilized the limited efficacy of
the application of a single pharmaceutical agent. In the present study, we tested the hypothesis which stated that by combining HA and PVP, the efficacy of DES therapy would be enhanced. We believe that this may be the result of PVP’s inherent ability to form stable molecular complexes with large anionic polymers such as sodium hyaluronate.

The replication of the human DES in experimental animals has had limited success. Previous animal dry eye simulations have included mechanical inhibition of blinking with a blepharostat, surgical removal of the lacrimal gland, lacrimal gland inflammation, androgen deficiency, pharmacologic blockade in the lacrimal gland and desiccating environments. However, none of these replicas seem to simulate accurately the complexity and chronicity of this common and debilitating disorder. An alternative rabbit dry eye replica was established and described by Xiong and associates in 2008. In addition, this preservative (benzalkonium chloride; BAC)-induced dry eye simulation has some of the anterior segment ocular surface abnormalities which are similar to the clinical signs of human DES. Furthermore, Yu and colleagues in 2013 also described the use of preservatives (BAC) inducing ocular surface toxicity, and protection of the ocular surface by utilizing HA.

The rationale for the application of the dry eye rabbit simulation described by Xiong and associates was that we wanted to use a dry eye replica that was easily reproducible, that avoided errors caused by individual ocular variation and measurement protocols, and minimized confounding. This BAC-induced rabbit eye simulation devised by Xiong and associates fulfilled these criteria. Specifically, this design provided ocular surface manifestations that were similar to human dry eye syndrome, including tear deficiency, lower conjunctival goblet cell density, and decreased mucin secretion. In Xiong’s rabbit dry eye simulation, as performed in our study, the rabbit eye examination intervals, after BAC instillation was initiated, were on Days 3, 5, 7 and 14. Furthermore, there was already another statistically significant sign of another indicator of Dry Eye Syndrome, namely, corneal fluorescein staining, that occurred by Day 5 in Xiong’s studies. Finally, in Xiong’s investigation, the presence of statistically significant rose bengal staining changes seen by Day 3, and the appearance of statistically significant corneal fluorescein changes visualized by Day 5, resembled clinical human dry eye syndrome, where rose bengal changes typically precede corneal fluorescein changes. For these reasons, we concluded that Xiong’s example was the most appropriate for this study.

In the present study, we utilized the general approach of Xiong and associates. Specifically, we tested the hypothesis which stated that by combining HA and PVP into one eyedrop, we would obtain a more effective treatment regimen than utilizing either HA or PVP alone, in the protection of the ocular surface. The potency of this type of artificial tear preparation might also translate into a new formulation that could provide further alleviation of the human DES.

**METHODS**

This study was based on the Organization for Economic Cooperation and Development principles of Good Laboratory Practice. Animal handling was performed according to the guidelines of the National Institutes of Health and the Association for Assessment and Accreditation of Laboratory Animal Care. The study was performed after approval by ‘The Israel Board for Animal Experiments’ and in compliance with ‘The Israel Animal Welfare Act,’ Ethics Approval Number IL-13-01-007. The study plan was reviewed by the Israeli National Ethics Committee to ensure that (a) the number of animals was fit to reach a valid conclusion and (b) the number of animals did not exceed that requirement. As such, we also confirmed adherence to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

A total of 16 New-Zealand White male rabbits, age 15 to 16 weeks at study initiation (average weight of 3.2 kg) were used. Four rabbits were assigned to each group according to random numbers. The animals were kept in 60 (L) × 60 (W) × 40 (H) cm cages, were acclimatized to the lab for one week following arrival, and housed in a normal environment of relative humidity 50–70% and temperature of 20–22 °C. Animals were provided with a commercial rabbit diet (Harlan Teklad 70785 Rabbit Diet), and allowed free access to drinking water, supplied to each cage via polyethylene bottles with stainless steel sipper tubes.

A simulation of DES in rabbits was induced by the method described by Xiong and associates utilizing topical 0.1% BAC ophthalmic solution. Topical BAC was applied to the right eyes of all 16 rabbits twice on Days 0, 3, 4, 5, 6, 7, 10, 11, 12, 13 and 14. The left eyes served as the control eyes and received no eyedrops at all.

The rabbits were allocated into four equal groups according to the eyedrops received, according to the treatment schedule described above. Group 1M only received BAC in their right eyes. In Group 2M, at each interval, the rabbit’s right eye received topical 0.1% sodium hyaluronate (HA), at least five minutes after topical BAC was instilled into the same eye. In Group 3M, the second eyedrop that followed BAC was topical 5.0% polyvinylpyrrolidone (PVP). In Group 4M, the second eyedrop after BAC was the preparation containing both HA and PVP. This unique HA+PVP combination was composed of sodium hyaluronate, and polyvinylpyrrolidone 5%, the exact
concentrations of the respective active ingredient, when each one was administered alone. We placed a citrate buffer of trisodium citrate and citric acid, which adjusted the pH, and also contained sodium chloride. These components kept the osmolarity of the combination product of HA + PVP, at a value of 300 mOsm./kg. The osmolarity was found to be constant throughout six months of incubation at 40 °C. The molecular weight of the specific hyaluronate used was 1.8 million Daltons. The viscosity of the HA was 6.5 centipoise, of the PVP was 6.9 centipoise, and of the HA+PVP mixture was 7.2 centipoise. PVP 5% was utilized because that is the most common concentration available for public use in Europe and Asia. Ophthalmic slit-lamp examinations of the anterior segments including cobalt-blue corneal fluorescein staining testing were performed on Day 0, as well as Days 3, 5, 7 and 14, after the second set of daily eyedrops were administered. The ophthalmologic examinations included a scoring system for the severity of perilimbal and circumferential conjunctival injection (Table 1) and a grading system, similar to that described by Xiong et al.,19 for the extent of corneal epithelial staining with a single sterile sodium fluorescein 0.6 mg ophthalmic strip uniformly and gently applied in the center of the inferior cul-de-sac of each rabbit eye (Table 2).

The semiquantitative observational grading that was used in the present study is common in numerous ophthalmologic studies; see for example Papas.21 Even regarding corneal and conjunctival staining, Bron and colleagues22 have concluded that “the monitoring and assessment of corneal and conjunctival staining can be greatly enhanced by the use of a grading scale”.

On day 14, following ophthalmologic examination, the rabbits were anesthetized and then euthanized with intravenous injection of sodium phenobarbital (200 mg/kg), and both eyes were enucleated and fixed in 10% neutral buffered formalin (approximately 4% formaldehyde solution). All rabbit eyes underwent uniform, sagittal and vertical sectioning, 3 mm from the temporal corneal edge, that included segments of uninterrupted, 4 mm of interpalpebral, superior and inferior perilimbal, and adjoining bulbar conjunctiva, as well as the full-thickness cornea in the center of the continuous section. The specimens were trimmed, embedded in paraffin, cross-sectioned, and stained with hematoxylin and eosin (H&E) or periodic-acid-Schiff (PAS) reagents. The H&E slides of the cornea were histologically evaluated, with uniformity in the central cornea of each rabbit eye, to measure the thickness of the corneal epithelium, and to grade the severity of the reduction of layers (Table 3) of the corneal epithelial cells. Regularity and smoothness of the corneal epithelial surface was evaluated as well. The PAS stained slides of the analogously located conjunctiva in all eyes allowed a standardized, comparative, and objective evaluation. As a result, there was impartial grading of the conjunctival goblet cell depletion compared to the untreated control eye (Table 4). All of these goblet cell counts were performed within the same 4 mm of bulbar interpalpebral and perilimbal conjunctiva, in each eye, measured from the superior and inferior termini of Descemet’s membrane, in every eye. One slide per rabbit eye was used for the PAS staining. Both sides of every 4 mm vertical section of each rabbit eye were analyzed and counted, and then the superior-half and inferior-half perilimbal goblet cell counts for each sample were added together. The ophthalmic

## TABLE 1 Grading of perilimbal conjunctival vascular congestion.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Intensity</th>
<th>Extent of perilimbal conjunctival erythema</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>All clear</td>
</tr>
<tr>
<td>0.5</td>
<td>Trace</td>
<td>1–90° Arc of Limbus</td>
</tr>
<tr>
<td>1.0</td>
<td>Mild</td>
<td>91–180° Arc of Limbus</td>
</tr>
<tr>
<td>1.5</td>
<td>Moderate</td>
<td>181–270° Arc of Limbus</td>
</tr>
<tr>
<td>2.0</td>
<td>Severe</td>
<td>271–360° Arc of Limbus</td>
</tr>
</tbody>
</table>

## TABLE 2 Grading of corneal epithelial fluorescein staining.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Intensity</th>
<th>Area of corneal surface changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>100%: Smooth and unstained</td>
</tr>
<tr>
<td>0.5</td>
<td>Trace</td>
<td>1–25%: Corneal epithelial irregularity</td>
</tr>
<tr>
<td>1.0</td>
<td>Mild</td>
<td>26–50%: Corneal epithelial irregularity</td>
</tr>
<tr>
<td>1.5</td>
<td>Moderate</td>
<td>51–75%: Corneal epithelial irregularity</td>
</tr>
<tr>
<td>2.0</td>
<td>Severe</td>
<td>76–100%: Corneal epithelial irregularity</td>
</tr>
</tbody>
</table>

## TABLE 3 Grading of severity of loss of corneal epithelial cell layers.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Intensity</th>
<th>Layers of keratinocytes remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Normal at 5 layers remaining</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>4 Epithelial layers remaining</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>3 Epithelial layers remaining</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>2 Epithelial layers remaining</td>
</tr>
<tr>
<td>4</td>
<td>Maximum</td>
<td>0–1 Epithelial layers remaining</td>
</tr>
</tbody>
</table>

## TABLE 4 Grading of PAS positive conjunctival goblet cell loss.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Intensity</th>
<th>Depletion of goblet cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal</td>
<td>Normal quantity of goblet cells</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>1–25% of Goblet cells missing</td>
</tr>
<tr>
<td>2</td>
<td>Moderate</td>
<td>26–50% of Goblet cells missing</td>
</tr>
<tr>
<td>3</td>
<td>Severe</td>
<td>51–75% of Goblet cells missing</td>
</tr>
<tr>
<td>4</td>
<td>Maximum</td>
<td>76–100% of Goblet cells missing</td>
</tr>
</tbody>
</table>
pathologist was blinded to the study. The absolute goblet cell count was computed from identical 4 mm cross-sectional areas, and compared to the control eyes.

All clinical and histopathologic statistical correlations compared the results of Groups 2 M (BAC + HA), 3 M (BAC + PVP), and 4 M (BAC + [HA+PVP]), with Group 1 M (only BAC). Statistical analysis was carried out using GraphPad Prism 5 software (Ver. 5.02, La Jolla, CA). Each set of data was subjected to two-way ANOVA statistical analysis (group versus days) followed by Bonferroni post-hoc multiple comparisons of all the groups. The treatments that exhibited statistical significance \( p \leq 0.05 \) compared to the untreated controls were listed in the figures. Higher efficacy was shown, among others, in lower statistical \( p \) values.

## RESULTS

### Clinical Results

The direct anterior segment slit-lamp examinations revealed that there was significant conjunctival erythema in at least 180° arc of the perilimbal region (Figure 1), in all four rabbit right eyes of Group 1 M, that came as early as 3 d after the topical BAC was initiated. In this same group, the conjunctival vascular congestion (Figure 2, Left) progressed in severity to include at least 270° of arc of the perilimbal conjunctiva (Figure 1) by Day 14. In Group 2 M, HA was able to reduce the intensity of the conjunctival vascular reaction to 180° arc of the perilimbal conjunctiva of the rabbits’ right eyes by Day 14 (Figure 1). In Group 3 M, the introduction of PVP produced slightly greater relief than HA in the reduction of conjunctival injection in the earlier part of the study, but was equivalent to HA when Day 14 was reached (Figure 1). However, the HA+PVP formulation in Group 4 M produced a statistically significant effective and steady inhibition of conjunctival vascular congestion (Figure 2, Right), that never involved more than 90° arc of the perilimbal conjunctival circumference (\( p < 0.001 \)) (Figure 1). All 16 left rabbit eyes, that received no BAC or other eyedrops, displayed the absence of perilimbal conjunctival vascular congestion throughout the experiment intervals that were examined.

The cobalt-blue fluorescein test for corneal epithelial injury was most marked in Group 1 M, with the persistence of at least 75% of the corneal surface area with corneal epithelial staining leading up to Day 14 (Figure 3). HA in Group 2 M and PVP in Group 3 M were individually able to limit the corneal epithelial trauma from BAC to 50% of the corneal surface area by Day 14. By contradistinction, the formulation of the HA+PVP eyedrop in Group 4 M gave the highest level of protection against corneal epithelial injury, by continuously having less than 25% of the corneal

![Figure 1](image1.png)

**Figure 1** Histogram of clinical perilimbal conjunctival erythema. The data revealed that the HA+PVP (BAC+HA+PVP) combined formulation exhibited a more significant effect (\( p < 0.05 \)), on reduction of clinical perilimbal conjunctival erythema, throughout the study, on all tested days, than the application of HA (BAC+HA) or PVP (BAC+PVP) applied alone. *When all four values are identical – no standard error is shown. When all four values are zero – no column is shown. BAC, benzalkonium chloride; HA, sodium hyaluronate; PVP, polyvinylpyrrolidone.
surface area with corneal epithelial staining, even up to Day 14 of this study ($p < 0.001$) (Figure 3). All 16 left rabbit eyes, that received no BAC or other eyedrops, had no significant corneal epithelial staining with fluorescein, throughout the experiment intervals that were examined.

**Histopathologic Results**

Light microscopy of the H&E slides showed an irregular and rough corneal epithelium in Group 1M eyes that received only BAC (Figure 4a) which was not substantially avoided with the individual use of HA in Group 2M (Figure 4c), or the use of only PVP in Group 3M (Figure 4e). However, the formulation of the HA+PVP eyedrop in Group 4M provided a more regular and smooth corneal epithelial contour (Figure 4g) that was very similar to the normal control eyes (Figure 4i). Furthermore, the corneal epithelial thickness was markedly decreased to a mean of 0.03425 mm in the BAC-only treated eyes of Group 1M. The addition of HA in Group 2M improved the mean corneal epithelial thickness to 0.04675 mm.

**FIGURE 2 Day 14 after respective therapy given to the right rabbit eye.** (Left) Right eye of Group 1M rabbit that received only benzalkonium chloride (BAC). There is moderately severe conjunctival injection (arrow) in at least 270° arc of the limbal region. (Right) Right eye of Group 4M that received the sodium hyaluronate+polyvinylpyrrolidone (HA+PVP) formulation in addition to BAC. There is only a trace amount of conjunctival injection that never involved more than 90° arc of the limbal conjunctival circumference (arrow).

**FIGURE 3 Histogram of corneal epithelial fluorescein staining.** Histogram revealed clearly that the HA + PVP (BAC+HA+PVP) combination was more effective than either HA (BAC+HA) or PVP (BAC+PVP) alone, in reducing corneal epithelial fluorescein staining, throughout the study, on all days tested ($p < 0.001$).
FIGURE 4  Histopathologic uniform sections of central cornea (a, c, e, g and i), and perilimbal conjunctiva (b, d, f, h and j). In Group 1 M (a and b), that received only BAC to their right eyes: (a) The corneal epithelium is thinned and irregular (H&E $\times 200$). (b) The perilimbal conjunctiva shows a significant absence of goblet cells (arrows), consistent with 51% to 75% of the control amount missing (PAS, $\times 200$). In Group 2 M eyes (c and d) that received HA in addition to BAC to their right eyes. (c) Cornea with a slightly thicker, but irregular epithelium. (H&E, $\times 200$) (d) Conjunctiva has more goblet cells (arrows), but is still lacking 26% to 50% of the control population. (PAS, $\times 200$). In Group 3 M eyes (e and f) that received PVP in addition to BAC to their right eyes. (e) Cornea with thicker, but still irregular epithelium (H&E, $\times 200$). (f) Conjunctiva has a still higher density of goblet cells (arrows), with less than 26% depletion of the control quantity. (PAS, $\times 200$). In Group 4 M eyes (g and h), that received an HA+PVP mixture in addition to BAC to their right eyes: (g) The cornea displays a thicker and smoother epithelium (H&E, $\times 200$). (h) The perilimbal conjunctiva shows a much more potent preservation of goblet cell distribution (arrows), with no greater than 25% of the control amount missing (PAS, $\times 200$). In the control left eyes (i and j), that received no eyedrops: (i) The cornea has normal, thick and smooth epithelium containing five layers of keratinocytes (H&E, $\times 200$). (j) The perilimbal conjunctiva has a normal distribution of goblet cells (arrows) (PAS, $\times 200$). (Two right eyes, of the four right eyes in Group 4 M, were indistinguishable from these control left eyes in goblet cell density).
By contrast, PVP by itself in Group 3 M which had a mean corneal epithelial thickness of 0.05075 mm, and the formulation of the HA+PVP eyedrop in Group 4 M, which had a mean corneal epithelial thickness of 0.05000 mm approached, more closely, the normal corneal epithelial thickness of the controls of 0.05050 mm. Because of a “ceiling effect” of the treatments, all being close to the value of normal controls, the differences between them showed no statistical significance and, for that reason, the figure depicting them was not included. As another measure of corneal epithelial integrity, the severity of the reduction of corneal epithelial layers was highest (Figure 4a) at a mean grade of 2.50 with BAC in Group 1 M. The addition of HA preserved more layers at a mean grade of 1.25. Again, the individual addition of PVP, or the formulation of the HA+PVP eyedrop (Figure 4g) approached, more closely, the normal corneal epithelial layer quantity grade of 1.000, as displayed in the control eyes (Figure 4i). Further histopathologic analysis of the perilimbal interpalpebral conjunctival epithelium did not reveal the presence of any inflammatory cells. Finally, since there were no inflammatory cells seen in our perilimbal conjunctival sections with the H&E stain, we determined that immunoglobulin staining was not necessary. The light microscopic analysis of the conjunctival goblet cell density via the PAS stain technique is demonstrated in Figure 4(b, d, f, h, and j) and objectively calculated in Figure 5. In Figure 4(b), the perilimbal conjunctiva of a rabbit right eye of Group 1 M that received only BAC, definitively displays an extreme paucity of goblet cells, consistent with 51–75% of the control goblet cell count missing (Figure 5). In Group 2M, where only HA was added as an ocular lubricant, there were objectively more goblet cells present (Figure 4d), with a mean score of moderate depletion of 26% to 50% of the normal perilimbal conjunctival goblet cell distribution (Figure 5). The individual supplementation of PVP in Group 3 M showed a still higher density of conjunctival goblet cells (Figure 4f), with a mean score of only mild goblet cell depletion, approaching less than an absolute reduction of 26% of the control goblet cell amount (Figure 5). In marked contrast, the therapeutic combination of the HA+PVP eyedrop in Group 4 M, significantly enhanced the survival and stability of the perilimbal conjunctival goblet cell distribution, so that two out of the four rabbits in Group 4 M had a goblet cell appearance that was indistinguishable from a normal control rabbit eye (Figure 4j), and the remaining two eyes of this group had no greater than 25% of the goblet cells missing (Figure 4h and Figure 5). As such, the mean score of goblet cell depletion, which was 3.000 in the BAC group, became 0.500 with the HA+PVP formulation, which showed a statistically significant improvement in the preservation of goblet cell integrity (p < 0.001), compared to HA alone, which was only mildly improved at 2.250, and PVP, by itself, which was only slightly more improved at 1.500 (Figure 5).
DISCUSSION

The significantly enhanced effect of combining HA and PVP into one formulation, for possibly reducing ocular surface damage, was demonstrated in the present study by exhibiting, on slit-lamp biomicroscopy, its inhibition of perilimbal conjunctival vascular congestion and decreased corneal epithelial fluorescein staining, and histopathologically, its distinct preservation of conjunctival goblet cell density (all at p < 0.001). HA has been clearly shown to improve tear film stability, and to re-establish the health and stability of the ocular environment. PVP drops alone have been shown to lubricate the eye sufficiently to help maintain the integrity of the corneal and conjunctival epithelial cell-to-cell junction. However, PVP has also been characterized as having solubilizing, film-forming and thickening properties that can increase the bioavailability to the ocular surface of an accompanying active pharmaceutical compound. PVP has a high dipole and high polarity molecule, and interacts with the mucin surface by hydrogen or electrostatic bonding. The adherence to the mucin surface from one side and the water adherence on the other side, helps in maintaining a stable tear film. In addition, PVP has the ability to form complexes with large anionic polymers such as sodium hyaluronate. Although the HA+PVP mixture has already been successfully used as a mouth gel for treating oral mucositis, in our novel HA+PVP molecular complex for ophthalmic therapy, the ocular surface also benefits from the PVP amplifying the mucoadhesive property and water attraction of the sodium hyaluronate. As a result, this enhanced effect of the HA+PVP molecular complex may be more beneficial in the clinical therapy of DES. These mechanisms of action may account for the improved efficacy of the therapeutic formulation of the HA and PVP combination, in preserving the corneal and conjunctival epithelium, as well as the goblet cell density, in the rabbit ocular surface, of this study.

The increased anterior segment protection displayed by this HA+PVP formulation in this study, suggests clinically, that this novel HA+PVP ophthalmic combination may have the potential advantage of having a lower effective dose frequency. Studies in oral medications, as well as in topical eyedrops, have clearly demonstrated that patient compliance is significantly improved by lowering the daily dose frequency of medications, providing the medicine has a sufficient duration of action.

Our histopathological method of preparation was uniform, by taking almost identical sagittal vertical sections, 3 mm from the temporal corneal edge of every eye. Subsequently, in those sections, the corneal epithelium’s integrity could be compared faithfully from group to group with regard to the effects of HA, PVP or the HA+PVP combination, on the BAC-induced simulated changes of the dry eye syndrome. Furthermore, the goblet cell counts could be considered accurate for objective analysis, since the comparable 4 mm of interpalpebral perilimbal bulbar conjunctiva, measured superiorly and inferiorly from the termini of Descemet’s membrane, was examined in every eye. Nelson and Wright have shown the difference of normal conjunctival goblet cell density between the interpalpebral bulbar and inferior palpebral ocular surface. Ralph has described how the goblet cell count increases as histologic examination is performed on bulbar conjunctival sections that are in a progressively greater centrifugal distance away from the cornea, in various meridians.

The mechanism of the BAC-induced corneal epithelial thinning occurs as the BAC exerts direct cell toxicity, damaging cell membranes and cytoplasmic organelles and impeding cellular metabolic function. As a result, BAC causes direct damage to the integrity of the corneal epithelium, and conjunctival goblet and accessory lacrimal gland cells, which eventually contributes to the reduction of corneal cell proliferation and viability, and impairs corneal healing. Furthermore, since PVP drops alone have been shown to lubricate the eye sufficiently to help maintain the integrity of the corneal and conjunctival epithelial cell-to-cell junction, and HA has been clearly shown to improve tear film stability, and to re-establish the health and stability of the corneal epithelium, we believe that the HA+PVP combined formulation helps to maintain a normal corneal epithelial thickness.

Pflugfelder has presented evidence that inflammation of the lacrimal gland and ocular surface, associated with cytokines and proteases in the tear fluid, and increased tear film osmolarity, has been identified in dry eye disease. Although our study did not examine the performance of the HA+PVP formulation in its effect on inflammation, which has been incorporated as a component of the definition of human dry eye disease, future studies should include evaluation of various inflammatory markers in the rabbit dry eye analogy and their implications regarding their response to specific therapeutic agents such as HA+PVP for the dry eye disorder.

On the basis of our experimental study, although it is limited to BAC-induced ocular surface damage, the novel formulation of an HA+PVP eyedrop has a pronounced tendency to protect the corneal epithelium from desiccation, and to maintain a normal conjunctival distribution of mucin-secreting goblet cells. This enhanced preservation of anterior segment cellular integrity may bring further relief to DES patients by reducing the dose frequency of topical lubricants, and thereby improving compliance.
Further clinical studies in patients with this HA+PVP formulation would help to elucidate these therapeutic benefits. The results of this investigation also highlight the interest in determining the temporal parameters governing the development of DES. As an example, the efficacy of this HA+PVP formulation, started on the same day when BAC was initiated, may not infer similar results for its clinical application to an already involved case of dry eye disease. As a result, one should consider the effect of the commencement of HA+PVP combination therapy, only from the third day following the initiation of the BAC eyedrops, in an experimental dry eye simulation. Future studies in our laboratory will be directed at addressing these issues.

In summary, the present study demonstrates the increased effectiveness of combining both HA and PVP into one eyedrop, in protecting the integrity of the ocular surface. Clinically, an artificial tear preparation that contains a combination of HA and PVP may provide dry eye patients with significant alleviation of their symptoms, and assist in maintaining the intactness of their anterior segment.

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