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ORIGINAL ARTICLE

The Effect of Tear Supplementation with 0.15% Preservative-Free Zinc-Hyaluronate on Ocular Surface Sensations in Patients with Dry Eye

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Abstract

Purpose: To evaluate the effect of tear supplementation with preservative free 0.15% zinc-hyaluronate on ocular surface sensations and corneal sensitivity in dry eye patients.
Methods: Ocular surface sensations were assessed using the ocular surface disease index (OSDI) questionnaire and by recording ocular sensations during forced blinking in parallel with noninvasive tear film breakup time measurement in 20 eyes of 20 dry eye patients. Corneal sensitivity thresholds to selective stimulation of corneal mechano-, thermal- and chemical receptors were measured using the Belmonte gas esthesiometer. All baseline measurements were repeated after 1 month of treatment with 0.15% zinc-hyaluronate.
Results: After 1 month, a significant decrease in mean OSDI score (from 35.66 ± 12.36 to 15.03 ± 11.22; $P < 0.001$) and a significant improvement in tear film breakup time (from 3.83 ± 0.80 to 8.67 ± 4.50 s; $P < 0.001$) was observed compared to baseline. Sensory responses during the interblink period also significantly decreased after 1 month ($P < 0.004$). Corneal sensitivity thresholds to mechanical stimulation (90.61 ± 20.35 vs. 103.92 ± 17.97 mL/min; $P < 0.025$) and chemical stimulation (33.21 ± 0.51 vs. $33.58\% \pm 0.44\%$ CO_2; $P < 0.025$) significantly increased after 1 month, however sensitivity thresholds to thermal stimulation remained unchanged compared to baseline ($P > 0.05$).
Conclusion: Prolonged use of 0.15% zinc-hyaluronate results in an improvement of tear film stability and a decrease of dry eye complaints. The decrease in corneal mechano-and polymodal receptor excitability suggests that zinc-hyaluronate helps to recover normal corneal sensitivity, and thus might have a beneficial additional effect on reducing ocular surface complaints in dry eye patients.

Keywords: dry eye, tear supplementation, hyaluronate

Introduction

Ocular discomfort related to ocular surface dryness is one of the most commonly reported complaints in ophthalmology with a reported prevalence of 5%–35%.[1] The characteristic symptoms, such as ocular dryness, foreign body sensation, burning, redness and photophobia reported by patients are often accompanied by the classic clinical signs, such as decreased tear film breakup time and ocular surface staining due to corneal and conjunctival epithelial cell damage.[2] As the cornea has abundant innervation with sensory receptors, even minor disturbances in the protective tear film layer can lead to the development of abnormal ocular surface sensations.[3]

Recent studies proved that corneal nerve endings do not only react to different types of external stimuli but can also develop abnormal activity due to ocular surface desiccation and increased tear film osmolarity, leading to the onset of the unpleasant sensations that accompany dry eye.[4–7] The most common cause of ocular surface desiccation is increased tear film evaporation due to disruption of the outermost lipid or the innermost mucous layer.[8,9]

Current therapy of dry eye is based on tear supplementation to improve tear film stability and to decrease evaporation, thus alleviating symptoms of irritation.[10–18] Hyaluronic acid, a natural polymer, helps to maintain ocular surface hydration and can already be found in several artificial tears

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recommended to alleviate dry eye symptoms. A recent hyaluronate modification involves zinc-hyaluronate complex formation by adding zinc-chloride to an aqueous sodium-hyaluronate resulting in a very stable molecular structure, which functions as both a mechanical barrier and a biocompatible film on the ocular surface. Apart from its beneficial effect on tear film stability due to its elastoviscous characteristics, previous results indicate that hyaluronate can also reduce the excitability of the peripheral nociceptor endings underlying pain.[19] The aim of this study was to investigate the characteristics of ocular surface sensations and corneal sensitivity in dry eye patients before and after tear supplementation with 0.15% zinc-hyaluronate.

Methods

Patients who had been diagnosed as having dry eye symptoms for at least 3 months, with a tear film breakup time of <5 s and an ocular surface disease index (OSDI) score of ≥13 evaluated by the OSDI questionnaire[20] have been enrolled in this study at the Department of Ophthalmology, Semmelweis University. Patients with significant corneal staining (>Grade 2, Oxford Scale)[21] were excluded because corneal epitheliopathy can affect ocular surface sensitivity.[22–24] Subjects with a history of ocular pathologies other than dry eye or a systemic disease known to be associated with dry eye as well as contact lens wearers were excluded. Patients with a history of allergic, toxic or infectious conjunctivitis within 6 months before the enrollment were also excluded from the study.

During forced blinking, tear film quality was evaluated by measuring the noninvasive tear film breakup time (NI-BUT) with concomitant recordings of ocular surface sensations. The Belmonte gas esthesiometer was used to measure corneal sensitivity thresholds to selective stimulation of corneal mechano-, thermal-, and chemical receptors. All measurements were made in the morning hours by the same physician in 1 eye of each subject. In each patient, only the right eye was used for data collection and the fellow eye was closed with a patch. All procedures were repeated 5 min after the instillation of 1 drop of 0.15% preservative-free zinc-hyaluronate eye drop (Ophylosa®; Richter Gedeon Ltd., Hungary), as well as 1 month after daily (4/day) use of 0.15% preservative-free zinc-hyaluronate drops. At 1 month, none of the subjects received any drops at least 12 h before the measurements.

The study was conducted in compliance with the Declaration of Helsinki, and was approved by local Ethics Committee and the Institutional Review Board. All patients gave written consent before enrollment. The study was registered in the ClinicalTrials.gov database with the identification number NCT02951910 after completion of the study since trial database submission is not compulsory in Hungary before starting such a single center study involving only a small number of patients.

Measurement of NI-BUT

The NI-BUT was measured using the Keeler Tearscope Plus (Keeler, Windsor, United Kingdom) immediately after a complete blink. During forced blinking, tear film was recorded by a digital camera and the captured videos were analyzed by a masked observer. NI-BUT was defined as the time from the last blink when visible deterioration of the projected rings was detectable at any point over the corneal surface. In each patient, 3 measurements were made in sequence and the NI-BUT was averaged from the 3 measurements.

Measurement of ocular surface sensations

Continuous data of ocular surface sensations during forced blinking was collected using a rotary potentiometer, as it was described previously.[7,9] Briefly, after training participants were asked to continuously rate ocular surface sensations with the potentiometer forcing the eye to remain open.[7,9] Subjects were asked to adjust the potentiometer between no rotation (no sensation) and full rotation (maximum intensity of sensation). A specific MatLab program (The MathWorks, Natick, MA) was written to convert data from the potentiometer collected at 0.2-s intervals into numeric values on a 10 unit scale.

Measurment of corneal sensitivity thresholds

Corneal sensitivity thresholds to selective mechanical, chemical, and thermal stimuli applied on the central cornea was measured using the Belmonte gas esthesiometer with 3-s air pulses of adjustable flow rate, composition (CO_2%) and temperature. Mechanical threshold levels were determined by using variable flows of medicinal air (50–200 mL/min).[25] To prevent a change in corneal temperature air was heated to reach the ocular surface at 34°C.[7] For thermal stimulation, the air was heated or cooled to produce the required changes in corneal temperature with a 10 mL/min flow below mechanical threshold. For chemical stimulation, a mixture of medicinal air with different concentrations of CO_2 was used with a flow rate of 10 mL/min below mechanical threshold.

Statistical analysis

Statistical analysis was performed with the SPSS software (version 21.0, IBM Corp. Armonk, NY). Sample size was determined by statistical power calculation (power 0.90; $P = 0.05$) using data from previous studies at our institution. Since Shapiro-Wilk test indicated non-normality of data, the Wilcoxon signed-rank test was applied to compare repeated measurements on a single subject with the Bonferroni adjusted p set to 0.025.

Results

This study included 20 eyes of 20 subjects (12 men, 8 women) with a mean age of 62.89 ± 13.32 years. At baseline the mean OSDI score was 35.66 ± 12.36, the mean NI-BUT was 3.83 ± 0.80 s. As a result of tear supplementation with 0.15% zinc-hyaluronate, tear film breakup time significantly increased both after 5 min (9.74 ± 2.85 s; $P = 0.01$; Fig. 1A) and after 1 month of treatment (8.67 ± 4.50 s; $P < 0.001$; Fig. 1A). Tear supplementation with 0.15% zinc-hyaluronate for 1 month resulted in a significant decrease in the mean OSDI score compared to baseline (15.03 ± 11.22; $P < 0.001$; Fig. 1B).

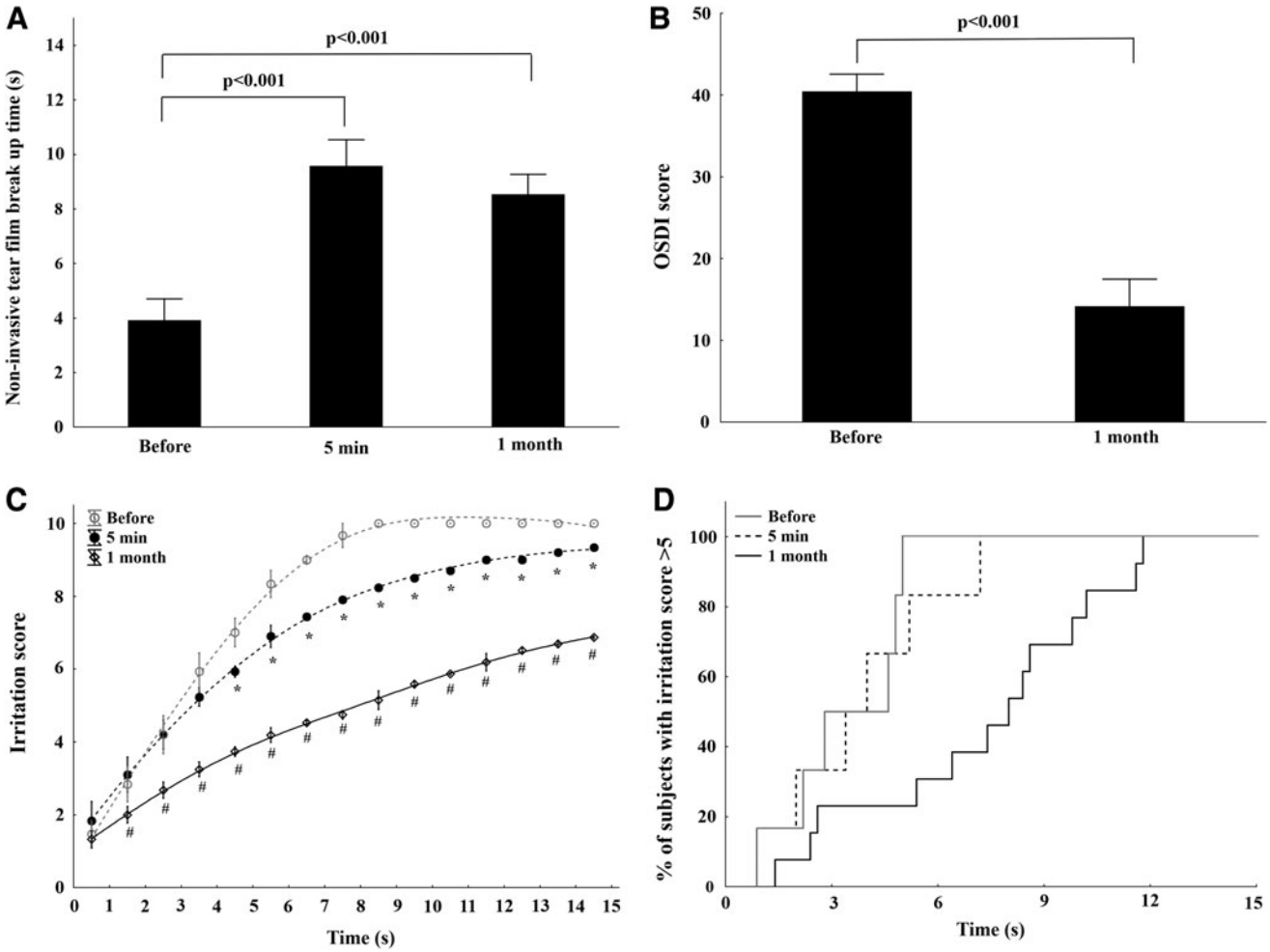

FIG. 1. The effect of tear supplementation with 0.15% zinc-hyaluronate on noninvasive tear film break up time **(A)**; on ocular surface complaints measured with the ocular surface disease index (OSDI) scores **(B)**; on irritation curves during the interblink period **(C)**; and on cumulative distribution of patients with moderate or high (>5) ocular surface irritation scores during the interblink period **(D)**.

Ocular surface sensations after tear supplementation

At baseline, the intensity of ocular surface sensations rapidly increased during forced blinking, while 1 drop of 0.15% zinc-hyaluronate significantly reduced sensory responses 5 min after its application ($P < 0.004$, Fig. 1C). After 1 month, 4×/day use of 0.15% zinc-hyaluronate resulted also in a significant decrease in ocular surface sensations compared to baseline during the interblink period ($P < 0.004$, Fig. 1C) even 12 h after the last application of the eye drop. At 1 month, sensory responses were significantly lower at every 5 s time periods during the interblink interval compared to data obtained at 5 min ($P < 0.004$, Fig. 1C).

The time to intense (score >5) ocular surface irritation significantly increased after 1 month of treatment compared to baseline ($P < 0.025$) and to data obtained 5 min after 1 drop of 0.15% zinc-hyaluronate ($P < 0.025$, Fig. 1D).

Corneal sensitivity after tear supplementation

Mechanical stimulation was defined by subjects as irritating or stinging, while CO_2 stimulation as irritating, with predominantly burning or pricking components. The irritation after cold stimulation was reported as mildly irritating, occasionally with cooling components.

Five minutes after instillation of 0.15% zinc-hyaluronate drop, corneal sensitivity thresholds to mechanical and chemical stimulation increased significantly ($P < 0.025$), however sensitivity thresholds to thermal stimuli remained unchanged (Table 1). Similarly, continuous treatment with 0.15% zinc-hyaluronate drop for 1 month resulted in a significant increase of mechanical and chemical sensitivity thresholds ($P < 0.025$; Table 1) even 12 h after the last application of eye drops. However, sensitivity thresholds to heat and cold stimuli remained unchanged ($P > 0.05$).

Discussion

The primary aim of the management of dry eye complaints is to improve tear film dynamics and thus alleviate patient's ocular discomfort.[26–28] Several clinical trials reported an improvement in both subjective symptoms and objective parameters (tear film stability, ocular surface staining) after tear supplementation,[14–18,27] although, in most cases frequent instillation of eye drops is required to maintain symptom remission. Treatment failure might be the result of the

Table 1. Sensitivity Thresholds to Selective Stimulation Before and After Tear Supplementation

	Baseline	*5 min*	*1 month*
Mechanical (mL/min)	90.61 ± 20.35	103.23 ± 16.11[a]	103.92 ± 17.97[a]
Cold (Δ°C)	–0.14 ± 0.11	–0.19 ± 0.13	–0.18 ± 0.14
Heat (Δ°C)	+0.33 ± 0.18	+0.38 ± 0.11	+0.37 ± 0.15
Chemical (%CO_2)	33.21 ± 0.51	33.51 ± 0.40[a]	33.58 ± 0.44[a]

[a]$P < 0.025$ compared to baseline.

unchanged abnormal ocular surface sensitivity in dry eye patients as not only tear film dynamics but the characteristics of ocular surface sensations are also significantly different in this population compared to normal subjects.[7]

Here we describe for the first time, that in dry eye patients unpleasant ocular surface sensations significantly decrease after 0.15% zinc-hyaluronate in parallel with a significant decrease in corneal sensitivity to mechanical and chemical stimuli. In a previous study we have shown, that HP-Guar significantly decreased ocular surface sensations shortly after its instillation, however this effect was attributed solely to an improvement in the mechanical barrier of the protective tear film layer.[7] Here we demonstrate that, compared to its short term effect, prolonged tear supplementation with 0.15% zinc-hyaluronate results in a further decrease of ocular surface irritation responses. Our results suggest that the beneficial effect of long term treatment might be the consequence of an improvement in tear film dynamics and also the result of a significant decrease in corneal sensory nerve excitability. The concept of a decrease in corneal nerve excitability is supported by the significant drop of symptom intensity curves and by the significantly increased sensitivity thresholds compared to baseline. It has to be emphasized, that the reduction in ocular surface irritation was measured 12 h after the last eye drop and tear film quality at this time point was comparable to that was 5 min after tear substitution suggesting a similar protection of the cornea by the tear film layer. The downward shift of the irritation curve as well as the increased sensitivity thresholds to mechanical and chemical stimuli suggest a decrease in the excitability of the corneal sensory receptors after prolonged treatment with 0.15% zinc-hyaluronate.

According to previous reports, 1 drop of 0.3% sodium hyaluronate increased tear film thickness by 30% for 23.5 min, and 0.15% sodium hyaluronate by 20% for 40 min.[15,29] In our study, at 1 month, measurements were made 12 h after the last application of the eye drop making the acute effect of 0.15% zinc-hyaluronate on tear film dynamics unlikely. To assess changes in corneal sensory nerve function, we measured the sensitivity thresholds of different types of corneal sensory nerve endings using a Belmonte gas esthesiometer. Our results showed that mechanical and chemical sensitivity threshold increased both 5 min after 1 drop, as well as after 1 month of tear supplementation, but thermal thresholds were not changed. Taken together these results it is reasonable to conclude, that prolonged treatment with 0.15% zinc-hyaluronate might lead to a normalization of abnormal corneal mechano- and polymodal receptor excitability. Abnormal corneal nerve sensitivity in dry eye patients has already been described, and mainly depend on the severity of dry eye.[6,30,31] However, the exact mechanism how 0.15% zinc-hyaluronate decreases the excitability of corneal sensory receptors remains to be elucidated. Previous results indicate, that the reducing effect on intra-articular nociceptor activity of hyaluronan solutions can be attributed to their rheological properties.[32] Moreover, it has already been demonstrated, that hyaluronate can reduce the excitability of the transient receptor potential vanilloid subtype 1 (TRPV1) channel on the short term, thereby lowering impulse activity in the peripheral nociceptor endings underlying pain.[19] Hyaluronate, a natural glycosaminoglycan is the main component of the extracellular matrix of the connective tissue and has a high concentration in ocular tissues such as the vitreous body. Hyaluronate is a biodegradable, biocompatible, nontoxic, nonimmunogenic and noninflammatory substance. The high capacity for binding and retention of water, as well as its characteristic viscoelastic properties has led to the use of this substance in ophthalmology in the last decades. It is widely used intraocularly during ocular surgery as ophthalmic viscosurgical devices,[33] as well as for ocular surface lubrication at different concentrations (0.1%–0.4%) to alleviate dry eye symptoms.[34] Hyaluronate forms a long-lasting protective coating on the surface of the eye helping to prevent tear film break-up and the development of dryness and irritation. Apart from its most common formulation as sodium-hyaluronate, another hyaluronate modification involves zinc-hyaluronate complex formation by adding zinc-chloride to aqueous sodium-hyaluronate. In addition to the typical hyaluronate characteristics, 0.15% zinc-hyaluronate has scavenger, bactericidal, bacteriostatic and fungicidal effects and these characteristics allows the omission of traditional preservatives from the formulation.[35]

To evaluate the relation of tear film quality and ocular surface sensitivity we applied a continuous rating of ocular surface complaints and measured corneal sensory nerve thresholds to selective stimuli in conjunction with the assessment of tear film quality by measuring tear film breakup time.[7,36–47] The Belmonte noncontact esthesiometer was used to assess the sensitivity of different types of corneal sensory fibers, such as mechanosensory fibers that activated by mechanical forces; polymodal nociceptive fibers that respond to mechanical and thermal stimuli, as well as to inflammatory mediators; and cold fibers that are activated by the decrease of temperature.[24,25] The high reproducibility of mechanical, thermal and chemical thresholds using the Belmonte gas esthesiometer has previously been reported.[31,48–50]

One might conclude from our results that the alleviation of ocular surface complaints after tear supplementation with zinc-hyaluronate might be the result of the stabilized tear film layer as well as of a decrease in corneal nerve excitability. In our opinion, the fact that prolonged tear supplementation increased sensitivity thersholds to mechanical and chemical stimulation even after 12 h of the application of the eye drop supports the assumption that 0.15% zinc-hyaluronate might have a direct effect on corneal nerve

sensitivity. Although we have demonstrated a positive effect of tear supplementation with 0.15% zinc-hyaluronate on corneal nerve function, changes in ocular surface sensitivity that occur during prolonged tear film substitution are not well understood. Changes in tear film osmolarity and alteration in the lipid layer and transmembrane mucin gel layer might be also responsible factors for the improvement in subjective symptoms.[4,51,52] Our future analyses aim to examine whether tear supplementation leads to the decrease of symptoms primarily as a result of better ocular surface protection or whether the decrease of abnormal ocular surface sensitivity is the main reason for clinical improvement.

As a conclusion, in this study we have shown, that not only improved tear film quality, but also a decrease in the excitability of corneal sensory receptors might be responsible for the clinical improvement after prolonged use of 0.15% zinc-hyaluronate in dry eye patients. Based on these results we may conclude that the use of 0.15% zinc-hyaluronate eye drop is recommended for the relief of mild to moderate dry eye symptoms when abnormal ocular surface sensations are present despite treatment with other agents.

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Author Disclosure Statement

No competing financial interests exist.

References

1. International Dry Eye WorkShop. The epidemiology of dry eye disease: report of the Epidemiology Subcommittee of the International Dry Eye WorkShop. *Ocul. Surf.* 5:93–107, 2007.
2. Gifford, P., Evans, B.J., and Morris, J. A clinical evaluation of Systane. *Cont. Lens Anterior Eye.* 29:31–40, 2006.
3. Acosta, M.C., Belmonte, C., and Gallar, J. Sensory experiences in humans and single-unit activity in cats evoked by polymodal stimulation of the cornea. *J. Physiol.* 15:511–525, 2001.
4. Parra, A., Gonzalez-Gonzalez, O., Gallar, J., and Belmonte, C. Tear fluid hyperosmolality increases nerve impulse activity of cold thermoreceptor endings of the cornea. *Pain.* 155:1481–1491, 2014.
5. Belmonte, C., and Gallar, J. Cold thermoreceptors, unexpected players in tear production and ocular dryness sensations. *Invest. Ophthalmol. Vis. Sci.* 52:3888–3892, 2011.
6. Kovacs, I., Luna, C., Quirce, S., et al. Abnormal activity of corneal cold thermoreceptors underlies the unpleasant sensations in dry eye disease. *Pain.* 157:399–417, 2016.
7. Dienes, L., Kiss, J.H., Perényi, K., et al. The effect of tear supplementation on ocular surface sensations during the interblink interval in patients with dry eye. *PLoS One.* 10:e0135629, 2015.
8. Blackie, C.A., Solomon, J.D., Scaffidi, R.C., et al. The relationship between dry eye symptoms and lipid layer thickness. *Cornea.* 28:789–794, 2009.
9. Varikooty, J., and Simpson, T.L. The interblink interval I: the relationship between sensation intensity and tear film disruption. *Invest. Ophthalmol. Vis. Sci.* 50:1087–1092, 2009.
10. Ousler, G.W., Michaelson, C., and Christensen, M.T. An evaluation of tear film breakup time extension and ocular protection index scores among three marketed lubricant eye drops. *Cornea.* 26:949–952, 2007.
11. Benelli, U., Nardi, M., Posarelli, C., and Albert, T.G. Tear osmolarity measurement using the TearLab Osmolarity System in the assessment of dry eye treatment effectiveness. *Cont. Lens Anterior Eye.* 33:61–67, 2010.
12. Paugh, J.R., Nguyen, A.L., Huang, P., and Hwang, J.S. Retention and retention of effect of topical formulations in dry eye subjects. *Optom. Vis. Sci.* 85:873–879, 2008.
13. Sánchez, M.A., Arriola-Villalobos, P., Torralbo-Jiménez, P., et al. The effect of preservative-free HP-Guar on dry eye after phacoemulsification: a flow cytometric study. *Eye (Lond).* 24:1331–1337, 2010.
14. Robert, P.Y., Cochener, B., Amrane, M., et al. Efficacy and safety of a cationic emulsion in the treatment of moderate to severe dry eye disease: a randomized controlled study. *Eur. J. Ophthalmol.* 26:546–555, 2016.
15. Schmidl, D., Schmetterer, L., Witkowska, K.J., et al. Tear film thickness after treatment with artificial tears in patients with moderate dry eye disease. *Cornea.* 34:421–426, 2015.
16. Christensen, M.T., Cohen, S., Rinehart, J., et al. Clinical evaluation of an HP-guar gellable lubricant eye drop for the relief of dryness of the eye. *Curr. Eye. Res.* 28:55–62, 2004.
17. Jee, D., Park, S.H., Kim, M.S., and Kim, E.C. Antioxidant and inflammatory cytokine in tears of patients with dry eye syndrome treated with preservative-free versus preserved eye drops. *Invest. Ophthalmol. Vis. Sci.* 55:5081–5089, 2014.
18. Rolando, M., Autori, S., Badino, F., and Barabino, S. Protecting the ocular surface and improving the quality of life of dry eye patients: a study of the efficacy of an HP-guar containing ocular lubricant in a population of dry eye patients. *J. Ocul. Pharmacol. Ther.* 25:271–278, 2009.
19. Caires, R., Luis, E., Taberner, F.J., et al. Hyaluronan modulates TRPV1 channel opening, reducing peripheral nociceptor activity and pain. *Nat. Commun.* 6:8095, 2015.
20. Miller, K.L., Walt, J.G., Mink, D.R., et al. Minimal clinically important difference for the ocular surface disease index. *Arch. Ophthalmol.* 128:94–101, 2010.
21. Bron, A.J., Evans, V.E., and Smith, J.A. Grading of corneal and conjunctival staining in the context of other dry eye tests. *Cornea.* 22:640–650, 2003.
22. Belmonte, C., Acosta, M.C., and Gallar, J. Neural basis of sensation in intact and injured corneas. *Exp. Eye. Res.* 78:513–525, 2004.
23. Situ, P., Simpson, T.L., Fonn, D., and Jones, L.W. Conjunctival and corneal pneumatic sensitivity is associated with signs and symptoms of ocular dryness. *Invest. Ophthalmol. Vis. Sci.* 49:2971–2976, 2008.
24. De Paiva, C.S., and Pflugfelder, S.C. Corneal epitheliopathy of dry eye induces hyperesthesia to mechanical air jet stimulation. *Am. J. Ophthalmol.* 137:109–115, 2004.
25. Belmonte, C., Acosta, M.C., Schmelz, M., and Gallar, J. Measurement of corneal sensitivity to mechanical and chemical stimulation with a CO2 esthesiometer. *Invest. Ophthalmol. Vis. Sci.* 40:513–519, 1999.
26. Friedman, N.J. Impact of dry eye disease and treatment on quality of life. *Curr. Opin. Ophthalmol.* 21:310–316, 2010.
27. Dry Eye WorkShop. Management and therapy of dry eye disease: report of the Management and Therapy Subcommittee of the International Dry Eye WorkShop. *Ocul. Surf.* 5:163–178, 2007.
28. Schaumberg, D.A., Uchino, M., Christen, W.G., et al. Patient reported differences in dry eye disease between men and women: impact, management, and patient satisfaction. *PLoS One.* 8:e76121, 2013.

29. Snibson, G.R., Greaves, J.L., Soper, N.D., et al. Precorneal residence times of sodium hyaluronate solutions studied by quantitative gamma scintigraphy. *Eye (Lond).* 4:594–602, 1990.
30. Tomlinson, A., Doane, M.G., and McFadyen, A. Inputs and outputs of the lacrimal system: review of production and evaporative loss. *Ocul Surf.* 7:186–198, 2009.
31. Bourcier, T., Acosta, M.C., Borderie, V., et al. Decreased corneal sensitivity in patients with dry eye. *Invest. Ophthalmol. Vis. Sci.* 46:2341–2345, 2005.
32. Gomis, A., Miralles, A., Schmidt, R.F., and Belmonte, C. Intra-articular injections of hyaluronan solutions of different elastoviscosity reduce nociceptive nerve activity in a model of osteoarthritic knee joint of the guinea pig. *Osteoarthritis Cartilage.* 17:798–804, 2009.
33. Balazs, E.A. Hyaluronan as an ophthalmic viscoelastic device. *Curr. Pharm. Biotechnol.* 9:236–238, 2008.
34. Kong, X., Yan, C., Ma, W., et al. Sodium hyaluronate's effect on xerophthalmia: a meta-analysis of randomized controlled trials. *Curr. Med. Res. Opin.* 32:477–484, 2016.
35. Illés, J., Jávor, A., and Szíjártó, E. Zinc-hyaluronate: an original organotherapeutic compound of Gedeon Richter Ltd. *Acta Pharm. Hung.* 72:15–24, 2002.
36. Jamison, R.N., Gracely, R.H., Raymond, S.A., et al. Comparative study of electronic vs. paper VAS ratings: a randomized, crossover trial using healthy volunteers. *Pain.* 99:341–347, 2002.
37. Begley, C., Simpson, T., Liu, H., et al. Quantitative analysis of tear film fluorescence and discomfort during tear film instability and thinning. *Invest. Ophthalmol. Vis. Sci.* 54:2645–2653, 2013.
38. King-Smith, P.E., Fink, B.A., Fogt, N., et al. The thickness of the human precorneal tear film: evidence from reflection spectra. *Invest. Ophthalmol. Vis. Sci.* 41:3348–3359, 2000.
39. Prydal, J.I., Artal, P., Woon, H., and Campbell, F.W. Study of human precorneal tear film thickness and structure using laser interferometry. *Invest. Ophthalmol. Vis. Sci.* 33:2006–2011, 1992.
40. Palakuru, J.R., Wang, J., and Aquavella, J.V. Effect of blinking on tear dynamics. *Invest. Ophthalmol. Vis. Sci.* 48:3032–3037, 2007.
41. Benedetto, D.A., Clinch, T.E., and Laibson, P.R. In vivo observation of tear dynamics using fluorophotometry. *Arch. Ophthalmol.* 102:410–412, 1984.
42. Montes-Mico, R., Alio, J.L., Munoz, G., and Charman, W.N. Temporal changes in optical quality of air-tear film interface at anterior cornea after blink. *Invest. Ophthalmol. Vis. Sci.* 45:1752–1757, 2004.
43. Nichols, J.J., Mitchell, G.L., and King-Smith, P.E. Thinning rate of the precorneal and prelens tear films. *Invest. Ophthalmol. Vis. Sci.* 46:2353–2361, 2005.
44. King-Smith, P.E., Nichols, J.J., Nichols, K.K., et al. Contributions of evaporation and other mechanisms to tear film thinning and break-up. *Optom. Vis. Sci.* 85:623–630, 2008.
45. Wang, J., Aquavella, J., Palakuru, J., and Chung, S. Repeated measurements of dynamic tear distribution on the ocular surface after instillation of artificial tears. *Invest. Ophthalmol. Vis. Sci.* 47:3325–3329, 2006.
46. Christensen, M.T., Blackie, C.A., Korb, D.R., et al. An evaluation of the performance of a novel lubricant eye drop. *Invest. Ophthalmol. Vis. Sci.* 51: E-Abstract 4141, 2010.
47. Sindt, C.W., and Foulks, G.N. Efficacy of an artificial tear emulsion in patients with dry eye associated with meibomian gland dysfunction. *Clin. Ophthalmol.* 7:1713–1722, 2013.
48. Vega, J.A., Simpson, T.L., and Fonn, D. A noncontact pneumatic esthesiometer for measurement of ocular sensitivity: a preliminary report. *Cornea.* 18:675–681, 1999.
49. Murphy, P.J., Lawrenson, J.G., Patel, S., and Marshall, J. Reliability of the non-contact corneal aesthesiometer and its comparison with the Cochet-Bonnet aesthesiometer. *Ophthalmic. Physiol. Opt.* 18:532–539, 1998.
50. Tesón, M., Calonge, M., Fernández, I., Stern, M.E., and González-García, M.J. Characterization by Belmonte's gas esthesiometer of mechanical, chemical, and thermal corneal sensitivity thresholds in a normal population. *Invest. Ophthalmol. Vis. Sci.* 53:3154–3160, 2012.
51. Sharma, A. Energetics of corneal epithelial cell-ocular mucus-tear film interactions: some surface-chemical pathways of corneal defense. *Biophys. Chem.* 47:87–99, 1993.
52. Argueso, P., and Gipson, I.K. Epithelial mucins of the ocular surface: structure, biosynthesis and function. *Exp. Eye. Res.* 73:281–289, 2001.

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