Evaluating the protective effect of ophthalmic lubricants from drying and hyperosmolar stress in an *in vitro* dry eye model

Amanda Davidson [1], Edel Duffy[2], Howard Ketelson[3]

[1] Dolphins Optometrists & Opticians, Dolphin House, 33 Muster Green S, Haywards Heath RH16 4AL, UK • [2] SCOPE Ophthalmics Ltd., Suite 5 Westland House, Westland Park, Willow Rd., Dublin 12, D12Y012, Ireland • [3] SCOPE Health Inc., 79 Madison Ave 8FL, New York, New York

Background

Ophthalmic lubricants are the cornerstone of dry eye management and have been designed to mimic the tear film components.[1] Many formulations contain two or more 'active' ingredients which play distinct and important roles to protect the ocular surface. It is noted that it is the overall formulation and composition and the way in which the components interact that impact the performance of an ophthalmic lubricant.[1]

Sodium hyaluronate eye drops are widely used in the management of dry eye disease with high molecular weight sodium hyaluronate being clinically superior to lower molecular weight versions for hydration.[2-4] Other ingredients used alongside sodium hyaluronate in eye drops for specific functions include, dexpanthenol for improved corneal healing,[5] and trehalose and ectoine for osmoprotection.[6-8]

It is recognised that there is a need to better understand the specific physico-chemical and biological roles of each ingredient composing the different formulations.[9] Human corneal cellular *in vitro* models can be leveraged to directly compare the cell viability and protection potential of eye drops with combinations of ingredients.

Purpose

The purpose of this study was to evaluate the protective effect of four ophthalmic lubricants in an in vitro model that stimulates a desiccated and hyperosmolar ocular surface.

Methods

The eyedrops were evaluated using HCEC's to assess cell viability and thereby protection under desiccation and hyperosmolar stress conditions. Four topical ophthalmic lubricants with different active ingredient combinations were included: Hylo Dual Intense® (hyaluronic acid + ectoine); Eye Doctor Advanced Triple Action Drops (hyaluronic acid + sacha inchi seed oil + treaholse); Thealoz® Duo (hyaluronic acid + trehalose) and Visupharma Trehapan® (Hyaluronic acid + trehalose + D-Panthenol).

The *in vitro* cell model consisted of immortalized human corneal epithelial cells (HCECs) cultured in DMEM/F12 media with 1% FBS and 1% penicillin/streptomycin. HCECs were seeded into 96-well plates at a density of 15,000 cells/well and incubated for 16 hr at 37°C and 5% CO2. Once confluent, media was aspirated, and HCECs were treated with the formulas for 15 min, then rinsed once with PBS to simulate removal of the formulation from the ocular surface by tear dilution before being subjected to desiccation or hyperosmolar stress. For desiccation conditions, HCECs were placed in an environmental chamber (37°C, 5% CO_2, 45% humidity). For hyperosmotic conditions, HCEC's were exposed to ~ 500 mOsM NaCl for 16-24 hours (37°C, 5% CO_2, 95% humidity). Cell viability was subsequently measured using the alamarBlue™ assay to assess metabolic activity.

Results

The level of cell viability and protection offered by the formulates varied significantly in both the desiccation and hyperosmotic stress models.

In both the desiccation and hyperosmolar models Hylo Dual Intense showed significantly better cell viability versus the Dry Eye Disease control arm. No other ophthalmic lubricant was significantly different to the negative DED control arm (Figure 1).

In the desiccation model the lowest level of protection was provided by Eye Doctor Advanced Triple Action Drops (5.27% + 22.91) with Thealoz® Duo (78.69% + 34.62) and Visupharma Trehapan® (67.26 + 9.33) providing moderate protection. Hylo Dual Intense ® provided the highest protection (109.56 + 49.67) that was significantly higher than Eye Doctor Advanced Triple Action Drops ($p<0.05$) (Table 1 & Figure 2).

In this hyperosmotic model Thealoz® Duo (-16.4% + 16.13) did not demonstrate protection against hyperosmotic stress. The Eye Doctor Advanced Triple Action Drops (12.97 + 9.48) and Visupharma Trehapan® (8.49 + 20.28) provided low protection. Hylo Dual Intense® provided the highest level of protection against hyperosmotic stress, which was significantly higher than Thealoz® Duo ($p<0.05$). (Table 1 & Figure 2).

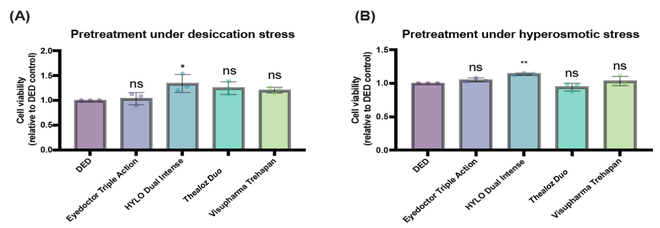

FIGURE 1. Cell viability of HCECs pretreated with different eyedrops under desiccation stress or hyperosmotic stress. Human corneal epithelial cells (HCECs) were pretreated with either 50 µL of PBS, Eyedoctor Triple Action, HYLO Dual Intense, Thealoz Duo or Visupharma Trehapan. After 15 minutes, cells were then rinsed once with PBS before being subjected to (A) desiccation stress (45% humidity at 37°C for 5 minutes) or (B) hyperomsotic stress for 24 hours. Cell viability was subsequently measured using the alamarBlue™ assay to assess metabolic activity. n = 3. Data are normalized to the DED model pretreated with PBS and presented as mean ± SD. ns = not significant ($p > 0.05$); *$p < 0.05$ vs. DED group (one-way ANOVA).

Table 1. Protection of different eyedrops in HCECs under desiccation stress or hyperosmotic stress

	Desiccation Stress	Hyperosmolar Stress
Hylo Dual Intense	109.56 + 49.67	40.88 + 1.26
Eye Doctor Triple Action	5.27 + 22.91	12.97 + 9.48
Thealoz Duo	78.69 + 34.62	-16.4 + 16.13
Visupharma Trehapan	67.26 + 9.33	8.49 + 20.28

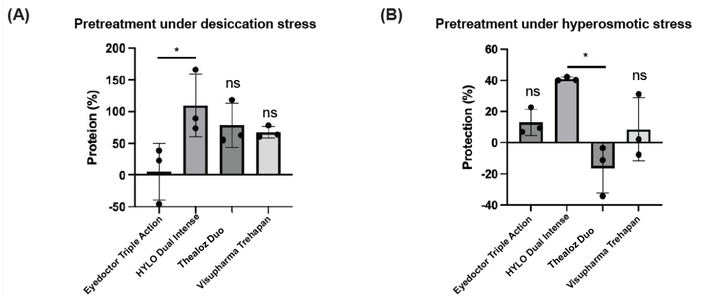

FIGURE 2. Protection of different eyedrops in HCECs under desiccation stress or hyperosmotic stress. Human corneal epithelial cells (HCECs) were pretreated with either 50 µL of PBS, Eyedoctor Triple Action, HYLO Dual Intense, Thealoz Duo or Visupharma Trehapan. After 15 minutes, cells were then rinsed once with PBS before being subjected to **(A)** desiccation stress (45% humidity at 37°C for 5 minutes) or **(B)** hyperomsotic stress for 24 hours. n = 3. **(C)** Data are normalized to the DED model pretreated with PBS and presented as mean ± SD. ns = not significant ($p > 0.05$); *$p < 0.05$ vs. Hylo Dual Intense (one-way ANOVA).

Discussion

- Two principal mechanisms in dry eye disease are ocular surface desiccation and tear film hyperosmolarity.[10] Hence *in vitro* desiccation and hyperosmolar stress models were selected to assess the efficacy of four ophthalmic lubricants in this study.
- The ophthalmic drop products selected were 'combination' based that contained a 'lubricant' and an 'osmoprotectant'.
- Lubricants such as high molecular weight sodium hyaluronate mitigate desiccation by ocular protection and increase ocular surface hydration. As a result, lubricants such as high molecular weight sodium hyaluronate prolong tear film retention, whilst osmoprotectants counteract hyperosmolar stress on the ocular surface.
- The product that combined high molecular weight hyaluronic acid and ectoine (Hylo Dual Intense®) was the only drop that provided protection against both desiccation and hyperosmotic stress in these *in vitro* stress models.
- This aligns with the mechanistic actions of each of the ingredients in the formula. High molecular weight hyaluronic is recognised as a highly effective lubricating and hydrating ingredient whilst ectoine is an osmoprotectant that helps stabilize cell membranes and reduces inflammation; with clinical and preclinical studies showing it improves tear film stability, protects against corneal damage, and enhances mucin production.[7,8]
- This study highlights that specific combinations of active ingredients in ophthalmic lubricants contribute to the performance of a drop and that difference combinations lead to different outcomes.

Conclusion

This study utilized two different *in vitro* cell models under dry eye stress conditions to simulate ocular surface protection. Differences between the commercially available lubricant drops were observed indicating that drops perform differently based on ingredient combinations. The human corneal cellular models provide insights into how these eyedrops may perform in dry eye patients, although full understanding of this would warrant clinical investigation.

References

1. Jones, L., Downie, L.E., Korb, D., Benitez-del-Castillo, J.M., Dana, R., Deng, S.X., Dong, P.N., Geerling, G., Hida, R.Y., Liu, Y. and Seo, K.Y., 2017. TFOS DEWS II management and therapy report. *The ocular surface*, *15*(3), pp.575-628.
2. Aragona, P., Simmons, P.A., Wang, H. and Wang, T., 2019. Physicochemical properties of hyaluronic acid–based lubricant eye drops. *Translational Vision Science & Technology*, *8*(6), pp.2-2.
3. Müller-Lierheim, W.G., 2020. Why chain length of hyaluronan in eye drops matters. *Diagnostics*, *10*(8), p.511.
4. Kojima, T., Nagata, T., Kudo, H., Müller-Lierheim, W.G., van Setten, G.B., Dogru, M. and Tsubota, K., 2020. The effects of high molecular weight hyaluronic acid eye drop application in environmental dry eye stress model mice. *International journal of molecular sciences*, *21*(10), p.3516.
5. 5. Bucher, F., B. Bachmann, F. Bock, D. Gross, C. Cursiefen, and F. E. Kruse. "Impact of hyaluronic acid, panthenol and its combination on epithelial wound healing in murine corneas." Investigative Ophthalmology & Visual Science 50, no. 13 (2009): 6285-6285.
6. Jones, L., Downie, L.E., Korb, D., Benitez-del-Castillo, J.M., Dana, R., Deng, S.X., Dong, P.N., Geerling, G., Hida, R.Y., Liu, Y. and Seo, K.Y., 2017. TFOS DEWS II management and therapy report. *The ocular surface*, *15*(3), pp.575-628.
7. Bilstein A, Heinrich A, Rybachuk A, Mosges R. Ectoine in the Treatment of Irritations and Inflammations of the Eye Surface. Biomed Res Int. 2021;2021:8885032. doi:10.1155/ 2021/8885032.
8. Lin N, Chen X, Liu H, et al. Ectoine enhances mucin production via restoring IL-13/IFN-gamma balance in a murine dry eye model. Invest Ophthalmol Vis Sci. 2024;65(6):39. doi:10.1167/iovs.65.6.39
9. Labetoulle, M., Benitez-del-Castillo, J.M., Barabino, S., Herrero Vanrell, R., Daull, P., Garrigue, J.S. and Rolando, M., 2022. Artificial tears: biological role of their ingredients in the management of dry eye disease. *International Journal of Molecular Sciences*, *23*(5), p.2434.
10. Baudouin, C., 2001. The pathology of dry eye. *Survey of ophthalmology*, *45*, pp.S211-S220.

Disclosures and Acknowledgments

This study was sponsored by SCOPE OPHTHALMICS Ltd (Dublin, Ireland). Amanda Davidson, Edel Duffy and Howard Ketelson are employees of SCOPE.

Correspondence: edelduffy@scopeeyecare.com