Method Optimization to Quantify Four Neuropeptides in the Human Tear Film

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Introduction

- Dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by tear film instability and hyperosmolarity, ocular surface inflammation and damage.[1] Recent work has shown that dry eye may be characterised using various biomarkers present in ocular surface tissue. [2,3]
- The ocular surface is a complex unit that is innervated by sensory and autonomic nerve fibers. The nervous and immune system "cross-communicate" with each other through the release and binding of cytokines and neuromediators.[4] This crosstalk is bidirectional and regulates ocular surface homeostasis.
- The ocular surface epithelial cells, lacrimal gland and nerve endings at inflammatory sites release neuropeptides such as Substance P (SP), Calcitonin Gene Related Peptide (CGRP), neuropeptide Y (NPY) and Vasoactive Intestinal Peptide (VIP). These neuropeptides play a key role in modulating the infiltration and activation of the immune cells and trigger the reflex tearing mechanism and ocular discomfort. These neuropeptides have been shown to help in the early phases of wound healing and help maintain corneal sensitivity in dry eyes. [5]

Purpose

- The purpose of this study was to optimize methods to quantify the amount of CGRP, SP, NPY and VIP found in the human tear film.

Methods

- 8 healthy non-contact lens wearers (6 female, 2 male) aged 18 to 35 years were recruited.
- Tears were collected using two differing tear collection techniques:
  - 5 µl of tears were collected using a sterile glass capillary tube from the temporal canthus of the right eye for "basal tear collection".
  - 20 µl of saline was instilled into the left eye with a sterile pipette and the subject was asked to blink once, then 5 µl of tears were collected using a glass capillary tube for "flush tear collection".

Methods continued…..

- Tears were collected within 5 minutes for each eye in individual subjects.
- All tears were stored at -80◦C until analyses took place.
- 2 µl of tears each were used to analyze these neuropeptides.
- Neuropeptide ELISA from Phoenix Pharmaceuticals with the range of detection from 1-10,000 pg/ml were used to measure the amount of CGRP, SP, NPY and VIP in basal and flush tears.

Results

- Statistical analysis was conducted using a repeated measures ANOVA through SPSS.
- **CGRP**: No difference was found between basal and flush tears for day 1 (p = 1.00) or day 2 (p = 0.77). No significant difference was found between basal tears for days 1 and 2 (p = 1.00) and flush tears for days 1 and 2 (p = 0.46).
- **SP**: A significant difference was found for levels of SP between basal and flush tears for day 1 (p = 0.037), but not between basal and flush tears for day 2 (p = 1.00). No difference occurred between basal tears for days 1 and 2 (p = 1.00), but there was a difference between flush tears for days 1 and 2 (p = 0.018).
- **NPY**: No significant difference was shown between basal and flush tears for day 1 (p = 0.31) or day 2 (p = 0.53). No significant difference was shown between basal tears for days 1 and 2 (p = 1.00) and flush tears for days 1 and 2 (p = 0.24).
- **VIP**: There was no significant difference between basal and flush tears for day 1 (p = 1.00) or day 2 (p = 0.29). No significant difference was also shown between basal tears for days 1 and 2 (p = 1.00) and flush tears for days 1 and 2 (p = 1.00).

Results continued…..

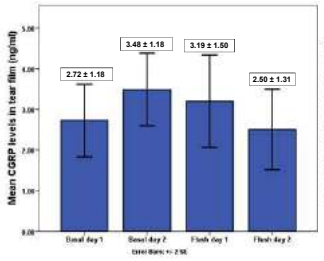

Figure 1: Histogram showing the mean CGRP levels in basal and flush tears for days 1 and 2

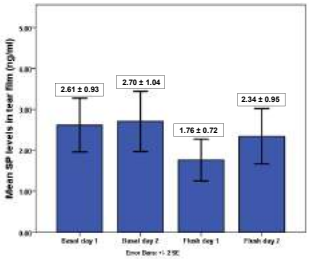

Figure 2: Histogram showing the mean SP levels in basal and flush tears for days 1 and 2

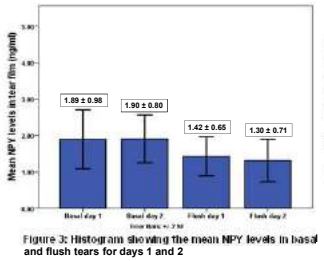

Figure 3: Histogram showing the mean NPY levels in basal and flush tears for days 1 and 2

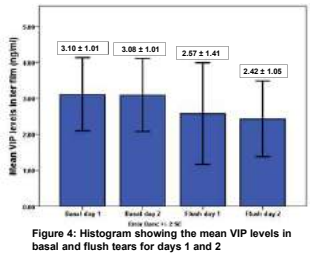

Figure 4: Histogram showing the mean VIP levels in basal and flush tears for days 1 and 2

Conclusions

- The results suggest that basal tears are appropriate for measuring these neuropeptides. Although there was no significant difference between two tear collection methods, basal tear collection is simpler and the flush tear collection could be used when a greater volume of tears are required.
- In future studies, the method described can be used to identify the differences in the levels of these 4 neuropeptides in patients with contact lens discomfort and varying degrees or types of dry eye.

References

1. Craig *et al.* TFOS DEWS II Definition and Classification Report. Ocul Surf. 2017;15 (3):276-283.
2. Willcox *et.al.* TFOS DEWS II Tear Film Report. Ocul Surf. 2017;15 (3):366-403.
3. Sabatino, F *et al.* The Intriguing Role of Neuropeptides at the Ocular Surface. Ocul Surf, 2017;15 (1):2-14.
4. Lemp *et al.* The definition and classification of dry eye disease: Report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). Ocul Surf 2007;5:75-92
5. Lambiase, A *et al.* Alterations of tear neuromediators in dry eye disease. Arch Ophthalmol, 2011; 129 (8):981-6.

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