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**Effect of single instillation of two hyaluronic acid based topical lubricants on tear film
thickness in patients with dry eye syndrome**

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Structured Abstract

Purpose: The present study investigated the effect of single administration of two different hyaluronic acid based topical lubricants on tear film thickness (TFT) in patients with moderate to severe dry eye disease (DED).

Methods: Sixty patients were randomized to either receive eye drops containing unpreserved sodium hyaluronate, triglycerides and phospholipids (HTP), unpreserved sodium hyaluronate alone (HA) or unpreserved sodium chloride (NaCl). TFT and lipid layer thickness (LLT) was assessed before and at defined time points after single instillation with a custom built ultrahigh-resolution optical coherence tomography (UHR-OCT) system and a white light interferometer. Standard tests for DED such as a visual analogue scale (VAS), corneal fluorescein staining (CFS), Schirmer I test and tear break up time (BUT) were performed.

Results: The time course in TFT was significantly different between the three administered agents ($p < 0.001$). Single administration of HTP significantly increased TFT over a period of 40 minutes, an increase in TFT over 20 minutes was observed after instillation of HA while NaCl had no effect. No significant difference in the change in BUT, CFS or LLT was observed between the three groups ($p = 0.57$, 0.97 and 0.86 respectively). A significant improvement in subjective symptoms (VAS) was found after single instillation of HTP ($p = 0.03$ vs. baseline) and HA, but not after administration of NaCl ($p = 0.03$, $p < 0.01$ and $p = 0.08$ vs. baseline, respectively, $p = 0.57$ between groups).

Conclusions: Single administration of HTP and HA significantly increased TFT showing a longer residence time and improved subjective symptoms, while instillation of NaCl had no effect. Long-term studies with multiple instillations will be necessary to further investigate the effect of the tested products on the clinical outcome.

Introduction

The prevalence of dry eye disease (DED) is constantly increasing and currently is reported to be up to 50% ¹. Since the disease is multifactorial, several risk factors, such as female sex, older age or concomitant diseases and therapies, as well as environmental conditions, have been identified ^{2,3}. Regardless of the underlying pathogenesis, all forms of DED are associated with disturbances in the tear film ⁴.

Thus, the mainstay of therapy - in particular for mild to moderate cases - is administration of topical lubricants, which are designed to moisture the ocular surface. This is achieved with complex formulations including a large variety of surfactants, emulsifiers and electrolytes, which considerably differ between products. Besides lubrication of the ocular surface, additional beneficial mechanisms of topical lubricants include reduction of friction between the eye lids and the ocular surface, tear film stabilization and tear volume replenishment ⁵.

Although the physical and chemical properties differ between commercially available products, studies assessing and comparing their in-vivo effects on the ocular surface are sparse. This is also related to the fact that the standard clinical diagnostic methods, such as assessment of tear break up time (BUT), Schirmer test or fluorescein staining only give an indirect measure of the integrity of the tear film and suffer from limited reproducibility.⁶

Recently introduced techniques now allow for the direct visualization and the quantification of the most critical component in DED, the tear film itself. These techniques include for example ultra-high resolution optical coherence tomography (UHR-OCT) for the measurement of tear film thickness (TFT) or white light interferometry for the quantification of lipid layer thickness (LLT) of the precorneal tear film ^{4,7}. We have previously reported that using UHR-OCT is

capable to measure TFT with high reproducibility^{7,8}. Further, previous data indicate that UHR-OCT is capable to assess the residence time of topically administered agents^{9,10}.

The main objective of the present study therefore was to investigate the effects of single administration of two different hyaluronic acid based topical lubricants on TFT in patients with moderate to severe DED and compare it to topically administered physiological saline solution. This allowed us to draw conclusions on the tear film stabilizing properties and the differences in ocular residence time. In addition, other clinical standard parameters for DED, as well as subjective symptoms after single instillation, were assessed.

Material and Methods

Subjects

The present study was performed in accordance with the Declaration of Helsinki and the Good Clinical Practice (GCP) guidelines of the European Union. The protocol of the study was approved by the Ethics Committee of the Medical University of Vienna. Sixty male and female patients with DED, that had given informed consent, were included in the present study.

A screening examination was carried out in each patient within 14 days before the study day. Patients were asked to abstain from instillation of topical lubricants for 24 hours before the visit. Screening included medical history, a pregnancy test in women of childbearing potential, subjective assessment of symptoms of dry eye syndrome using the Ocular Surface Disease Index (OSDI) questionnaire and a visual analogue scale (VAS). In addition, an ophthalmic examination was performed, including visual acuity using the ETDRS acuity charts, slit lamp biomicroscopy and indirect funduscopy, measurement of break up time (BUT), Schirmer I Test, corneal fluorescein staining and measurement of intraocular pressure (IOP).

In order to be included, patients had to be aged at least 18 years and to have normal ophthalmic findings except history of DED for at least 3 months, BUT ≤ 10 seconds or Schirmer I test ≤ 10 mm and an OSDI score ≥ 23 points. The eye with the lower BUT was chosen for analysis. If BUT was identical for both eyes, the eye with the lower Schirmer I test was chosen. If Schirmer I test results were also identical for both eyes, the right eye was used for analysis. Measurements of TFT and LLT were only performed in the study eye.

Patients were excluded if they fulfilled at least one of the following criteria: participation in a clinical trial within 3 weeks prior to the study, symptoms of a clinically relevant illness within 3 weeks prior to the first study day, presence or history of a severe medical condition as judged by the clinical investigator, intake of parasympathomimetic or anti-psychotic drugs, wearing of contact lenses, glaucoma in the medical history, treatment with corticosteroids within 4 weeks prior to the study, topical treatment with any ophthalmic drug except topical lubricants within 4 weeks prior to the study, ocular infection or clinically significant inflammation, ocular surgery within 3 months prior to the study, Sjögren's syndrome, Stevens-Johnson syndrome, history of allergic conjunctivitis, pregnancy, planned pregnancy or lactating.

Topical lubricants

The following three different topical agents were tested, of which the first two were based on hyaluronic acid. The first tested agent (HTP, Laboratoires Horus Pharma, France) comprised sodium hyaluronate (0.18%), triglycerides and phospholipids, lipoic acid, hydroxypropyl methylcellulose, sodium citrate, sodium hydroxide, citric acid, sodium chloride and water for injections. The second agent (HA, Visméd Multi® Eye Drops Laboratoires Horus Pharma, France) consisted of sodium hyaluronate (0.18%), sodium chloride, potassium chloride, disodium phosphate, sodium citrate, magnesium chloride, calcium chloride and water for

injections. Physiological saline solution 0.9% (NaCl, Hydrabak® Eye Drops, Laboratoires Thea, France) was used as control. All of the three agents were preservative free.

Study Design

The present study was performed in a randomized, single masked, observer blinded, active-controlled, parallel group design. Patients were randomized to one of the three study groups. To obtain comparable baseline conditions, patients were asked to refrain from administration of topical lubricants in the 24 hours before the study day.

On the study day, after baseline measurement of TFT and LLT, one drop equaling 35µl of one of the three different agents was administered. Measurements of TFT and LLT were repeated 10, 20, 40, 60, 120, and 240 minutes after instillation. After the last measurement, the following procedures were performed: subjective assessment using a VAS, visual acuity, BUT, corneal fluorescein staining, Schirmer I test and assessment of adverse events. During the breaks between the measurements, patients remained in the hospital to guarantee comparable environmental conditions.

Methods

Tear Film Thickness (TFT)

An ultrahigh resolution spectral domain OCT system for imaging of the anterior chamber was employed in the present study. The device comprises a broad band Ti:Sapphire laser operating at 800 nm with (spectral bandwidth 170 nm). The theoretical axial resolution in tissue is about 1.3 µm, while the lateral resolution at the front surface of the cornea is 21 µm.

For measurement of TFT, 3-dimensional volumes centered on the apex of the cornea and with a size of 4 mm x 4 mm x 1 mm (horizontal x vertical x depth), each containing 512 x 128 x 1024 pixels were acquired. Central TFT was evaluated from the tomograms around the central reflex of the probe at the apex (indicated by saturation of the spectrometers CCD camera). System parameters, such as focus of the probe beam and exposure time of the CCD camera were set to allow for optimal tear film imaging. The patients' head was stabilized on a modified slit lamp head rest. Subjects were asked to look straightforward onto an internal fixation target and blink normally. Data acquisition started after opening of the eye. Three 3D volumes were recorded within 3 seconds, and TFT was gained from the second and third volume. All axial distance values for the TFT obtained with OCT were divided by the average group refractive index for the tear film of 1.339 in order to obtain geometrical distances.

It has been shown that the time course of TFT (in μm) is a measure for ocular residence time of topical lubricants.^{11, 12}

Lipid Layer Thickness (LLT)

Lipid layer thickness was measured using the LipiViewII Ocular Surface Interferometer (Tear Science Inc, NC, USA). Patients were asked to look into a camera that recorded a 20-second video of the interference pattern of the tear film which was then analyzed by the software of the device¹³.

Break Up Time

Tear film break up time (BUT) was measured following the guidelines published in the Report of the International Dry Eye WorkShop (DEWS) 2007¹⁴. Briefly, Minims-Fluorescein Sodium 2.0% eye drops were applied in the conjunctival sac of the eye. The patient was instructed to blink naturally without squeezing several times to distribute the fluorescein. Within 10 - 30

seconds after fluorescein instillation, the patient was asked to stare straight ahead without blinking, until told otherwise. By the means of a stopwatch the time between last complete blink and first appearance of a dry spot was recorded. Once breakup of the tear film was observed, the patient was instructed to blink freely.

Corneal fluorescein staining

Minims-Fluorescein Sodium 2.0% eye drops were used to detect corneal epithelial defects. As grading scale for corneal damage, the NEI/Industry Workshop guidelines were used¹⁵. The cornea was divided into five sectors (central, superior, inferior, nasal and temporal), each of which was scored on a scale of 0–3, whereas 0 means no staining and 3 means maximum staining, with a maximal score of 15.

Schirmer I test

Schirmer I test (without anesthesia) was performed following the guidelines published in the Report of the International Dry Eye WorkShop (DEWS) 2007¹⁴. Briefly, the Schirmer test was performed as follows: Schirmer paper strips were inserted in the unanesthetized eye over the lower lid margin, midway between the middle and outer third. The patient was then asked to close the eye. After a time of 5 minutes the wetting of the Schirmer paper was measured. For the present study, Schirmer plus® strips (Gecis, France) were used.

Ocular Surface Disease Index (OSDI)

Symptoms of dry eye were assessed using the Ocular Surface Disease Index© (OSDI©) which was developed by the Outcomes Research Group at Allergan Inc (Irvine, Calif)¹⁶. The questionnaire that underlies the OSDI© is specifically designed for patients with dry eye

syndrome and asks patients about the frequency of specific symptoms and their impact on vision-related functioning.

Dry Eye Visual Analogue Scales (VAS)

Scores were determined using a 100 mm VAS on which 0 means no symptoms and 100 means the worst possible discomfort. The following parameters were assessed using VAS: foreign body sensation, pain, itching, stinging, epiphora, photophobia, blurred vision.

Intraocular pressure (IOP)

IOP was measured with a slit-lamp mounted Goldmann applanation tonometer. Before each measurement one drop of oxybuprocainhydrochloride combined with sodium fluorescein was used for local anesthesia of the cornea.

Sample size justification and data analysis

A sample size calculation based on previously published data⁷ regarding the reproducibility of the UHR-OCT system was performed prior to study start. With a total sample size of 60 patients, the study was powered to detect difference between groups of 15% or less with a power of 90% and a two-sided alpha-level of 0.05. Statistical analysis was done as a “per protocol” analysis. The main outcome variable was TFT. To detect differences between treatments, a three-way repeated measures ANOVA model was applied for analysis of all outcome parameters. Planned comparisons within the ANOVA model were performed to assess differences in the outcome parameters between time points and groups. A p-level of 0.05 or less was considered significant. All statistical analyses were carried out using CSS Statistica (Version 6.0, Tulsa, Oklahoma, US).

Results

A total of 60 patients aged between 20 and 57 years with DES were included and completed the study according to the protocol (age: 38.1 ± 11.6 years, mean $\pm$ SD). Of those participants, 17 patients were male and 43 patients were female. All included patients completed the screening visit and the study day within two weeks. The mean duration of DED in the whole study group was 7.0 ± 5.1 years. Most frequent findings in the medical history except DED were arterial hypertension and hypercholesterolemia. Baseline characteristics of the three study groups are shown in Table 1.

At baseline, TFT was significantly higher in the NaCl group, while no statistically significant difference between HTP and HA was observed. Time course of TFT was significantly different between the three groups ($p < 0.001$). Administration of NaCl did not increase TFT at any time point, while TFT significantly increased 10 minutes after administration of HTP and HA ($p < 0.001$ vs. baseline each). Twenty minutes after administration, TFT remained increased in the HTP and HA group ($p < 0.001$ vs. baseline each). After 40 minutes, TFT was still significantly elevated in the HTP group ($p < 0.001$ vs. baseline) but not in the HA group ($p = 0.11$ vs. baseline). No significant changes vs. baseline were observed at the other time points. Relative changes in TFT over time are shown in Figure 1. As shown in Table 1, no difference in LLT was observed between the three groups at baseline. The time course of LLT was comparable between the three groups ($p = 0.86$, Figure 2).

Changes in the other clinical and subjective parameters that were assessed are presented in Table 2. BUT increased in the HTP ($p = 0.05$ vs. baseline) and in the NaCl group ($p = 0.02$ vs. baseline), but not in the HA multi group ($p = 0.31$). Administration of HA induced a significant increase in Schirmer I test ($p = 0.01$), while this was not observed after administration of HTP

($p = 1.0$ vs. baseline) or NaCl ($p = 0.42$ vs. baseline). Corneal fluorescein staining significantly decreased in all 3 groups. Subjective symptoms significantly improved after administration of HTP and HA. This is reflected in the VAS score, which significantly decreased in the HTP ($p = 0.03$ vs. baseline) and in the HA ($p < 0.01$ vs. baseline), but not in the NaCl group ($p = 0.08$). No adverse events and no changes in visual acuity occurred during the course of the study.

Discussion

The aim of the present study was to investigate the effect of two different hyaluronic acid based eye drops on TFT in patients with moderate to severe DED and thus determine the ocular residence time of the two formulations. Our data shows that both preservative free solutions of hyaluronic acid 0.18% lead to a quick and pronounced increase of TFT after single drop administration, whereas topical administration of saline solution had no significant influence on precorneal TFT. Furthermore, TFT data shows that the hyaluronic acid formulation additionally containing triglycerides and phospholipids shows a longer residence time on the ocular surface compared to hyaluronic acid alone.

It has been recognized that besides of its moistening action, hyaluronic acid provides a biological signal to corneal epithelial cells accelerating the migration of the corneal epithelium and therefore promotes corneal wound healing¹⁷⁻¹⁹. Besides these beneficial biological actions, one of the major advantages of hyaluronic acid is its long stay on the ocular surface²⁰ mainly caused by its in-vivo viscoelastic properties.

In the current study, we used UHR-OCT to determine TFT after single administration of different hyaluronic acid based lubricant formulations. As described in the methods section of the manuscript, the UHR-OCT system provides a theoretical axial resolution of 1.2 μm in

tissue. We have previously investigated the reproducibility of the instrument in humans⁷ and showed a very high reproducibility with an intraclass correlation coefficient of 0.97. In addition, OCT is capable of revealing small changes in TFT after administration of topical lubricants that are much smaller than the axial resolution^{9, 10, 21, 22}, depending on the signal-to-noise ratio of the signal⁸. Thus, based on the decay of TFT over time, conclusions can be drawn regarding the residence time of topically administered agents based on non-invasive, easy to perform measurements.^{9, 21}

The data of the current study show that administration of hyaluronic acid containing eye drops, as well as eye drops containing a combination of hyaluronic acid and lipids, led to a significant increase in TFT after single drop administration, whereas administration of saline eye drops had no effect on TFT. In particular, HA eye drops showed an increase of TFT for 20 minutes after topical administration, which is comparable to previous studies investigating the effect of hyaluronic acid 0.15% eye drops on TFT ²¹.

Although both formulations used in the currently study share the same concentration of sodium hyaluronate (0.18%), HTP eye drops induced a more pronounced effect on TFT at 40 minutes after administration. The exact reason for this difference is unclear but may be related to the differences between the two formulations. In contrast to HA, HTP eye drops contain a mixture of triglycerides, phospholipids, lipoic acid and hydroxypropyl methylcellulose in addition to hyaluronic acid, which may be responsible for the prolonged residency time on the ocular surface. As such, it has been shown that a combination of hyaluronic acid and hydroxypropyl methylcellulose exhibit low surface tension and improves the rheological characteristics, which in turn may be beneficial for patients with DED.²³ Further, it has been shown in previous experiments that lipid containing drops lead to improved tear film stability, most probably due

to a reduced evaporation of the pre-corneal tear film^{24, 25}. This may well contribute to a stabilization of the precorneal tear film and a longer residency time on the ocular surface. However, in the current study, measurement of the precorneal lipid film did not indicate a significant difference between the two groups. Thus, further studies are required to clarify which component is responsible for the differences between the two groups and what is the relation between TFT and lipid layer thickness.

Of note, baseline values of TFT were among $5.04 \pm 0.46 \mu\text{m}$ in the studied group of patients with DES. Although this was slightly higher than values observed in previous studies^{10, 22}, there was still a significant increase after administration of both formulations, indicating a strong capacity of these two eye drops to increase TFT. At one point a slight increase in TFT was seen in the saline group. This most likely is a chance finding related to the variability of measurements over time.

Although our data indicate a long residence time of both hyaluronic acid-based lubricants, the clinical relevance needs to be further investigated. Our study showed that HTP eye drops induced a significant increase in BUT and a significant improvement in corneal fluorescein staining scores after single administration, while HA led to an increase in Schirmer I test scores and corneal fluorescein staining scores. Notwithstanding, one has to be careful when interpreting these results: First, all agents were administered only once to assess TFT after single administration. Consequently, the conclusions that can be drawn on the clinical efficacy of the agents are limited and would require a study design with repeated instillation over a longer period of time. Furthermore, the sample size chosen was based on the primary outcome variable in order to detect changes in TFT. Thus, both the sample size and the length of the

observation may not be appropriate to assess differences in other clinical outcome variables such as BUT or Schirmer test.

No significant differences in the time course of LLT were observed between the three treatments. This is in contrast to previous experiments showing an increase in LLT after single administration of lipid containing drops ¹³. The reason for this discrepancy needs to be further investigated. In this context it needs also to be considered that although the LipiView instrument shows good reproducibility, ²⁶ the instrument has a cut-off at 100nm LLT, which might limit the detection of eye-drop induced increases in LLT. Furthermore, one could argue that effect of treatments on LLT builds up with time and regular instillations, so that a longer study period might be more suitable to detect differences between treatments. Finally, technical limitations may limit the ability to detect changes in LLT because we cannot exclude that the natural lipid layer and the lipids contained in the eye drop formulation have different refractive indices.

In terms of subjective symptoms our data showed that the administration of both HA and HTP led to a significant decrease in the VAS scale for symptoms of DED, in particular foreign body sensation, pain, itching, stinging, epiphora, photophobia and blurred vision whereas no change in VAS was observed after administration of NaCl. Although these effects were recorded after single instillation only, our results underline the good tolerability and safety profile of the hyaluronic acid-based lubricants.

In conclusion, single administration of HA and HTP eye drops significantly increased TFT, showing a longer residence time after instillation, and improved subjective symptoms in patients with moderate to severe DED, while instillation of NaCl had no effect on these

parameters. All study treatments were well tolerated and safe. Long-term studies with multiple instillations will be necessary to further investigate the long time effect of HA and HTP on TFT and on clinical outcome variables.

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