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Influence of Perfluorohexyloctane Eye Drops on Tear Film Thickness in Patients with Mild to Moderate Dry Eye Disease: A Randomized Controlled Clinical Trial

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Abstract

Purpose: The aim of this mechanistic clinical study was to explore the effect of water-free perfluorohexyloctane eye drops on tear film thickness (TFT) in patients with dry eye disease (DED).
Methods: Forty-eight patients with mild to moderate DED participated in this randomized, single-masked, observer-blinded parallel group study in a 1:1 ratio to receive either perfluorohexyloctane or unpreserved 0.9% saline solution (Hydrabak®, Thea, France) eye drops 4 times daily in both eyes for 4 weeks. A custom-built ultrahigh-resolution optical coherence tomography system was used to measure TFT. Furthermore, evaluation of lipid layer thickness (LLT) and noninvasive tear film breakup time, as well as standard clinical tests for signs and symptoms of DED were performed.
Results: Mean TFT and LLT at baseline were comparable between the 2 treatment groups. After a single drop instillation, perfluorohexyloctane eye drops temporarily increased TFT immediately. After multiple dosing, perfluorohexyloctane eye drops gradually increased TFT over time with a maximum effect at the end of the study (least square mean difference: 6.42%; $P = 0.0142$ at week 4). LLT values measured before drop instillation showed a more prominent increase in LLT for perfluorohexyloctane eye drops ($13.36\% \pm 26.33\%$ vs. $3.21\% \pm 28.65\%$). All other parameters got better in both treatment groups with no statistical difference between groups.
Conclusions: These results demonstrate that perfluorohexyloctane eye drops increase TFT as well as LLT over time. These tear film reestablishing attributes are in line with the mode of action of perfluorohexyloctane eye drops to avoid evaporation through stabilization of the lipid layer.

Keywords: perfluorohexyloctane, dry eye disease, tear film, lipid layer, randomized controlled study

Introduction

Dry eye disease (DED) is a multifactorial disorder affecting the ocular surface. It comes with a loss of tear film homeostasis, and is accompanied by ocular and visual function symptoms. Tear film instability and hyperosmolarity, ocular surface inflammation and damage, as well as neurosensory abnormalities all contribute to the disease process.[1] In clinical practice, classification of DED into either aqueous-deficient or evaporative DED may facilitate the understanding of the mechanisms that underlie development and relationship of the disease. Epidemiological and clinical evidence suggest that the majority of DED is predominantly evaporative in nature.[1] The most common cause of evaporative DED is a dysfunction of the Meibomian glands. Meibomian glands, found in the upper and lower eyelids,

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secrete lipids onto the ocular surface, which form the outer layer of the tear that stabilizes the tear film, protecting tears from evaporation.

Although multiple treatment practices for DED exist, evaporative DED type of patients lack specific and effective therapies.

Recently, novel eye drops, consisting of 100% perfluorohexyloctane (NovaTears®, Novaliq, Germany), have been introduced for the treatment of DED in Europe, Australia, and New Zealand. The new component offers several advantages over traditionally used demulcents or emollient in ophthalmic (drug) products, in particular, for evaporative dry eye patients. Due to its low surface tension,[2] the perfluorohexyloctane eye drop rapidly spreads across the ocular surface forming a long-lasting protective layer at the tear film-air interface that prevents evaporation of the aqueous phase of the tear film and reduces the shearing forces of the eyelid during blinking. As perfluorohexyloctane eye drops are a 1-component and completely nonaqueous liquid, no bacterial growth is possible in the formulation, and therefore, it does not contain any preservatives. In 2 observational studies in patients with DED, treatment with perfluorohexyloctane significantly improved subjective and objective symptoms and signs of the disease.[3,4] Perfluorohexyloctane is currently also developed under the development code NOV03 under IND for the U.S. market.

This mechanistic study was designed to further characterize the short- and long-time effects of perfluorohexyloctane on the tear film itself, the most critical component in DED, and to gain a better understanding of perfluorohexyloctane's mode of action. For this purpose, patients with mild to moderate DED were randomized to receive either perfluorohexyloctane or sodium chloride 0.9% eye drops (NaCl, Hydrabak®, Thea, France) as control for a period of 4 weeks. Tear film thickness (TFT) was measured using a custom build ultrahigh-resolution optical coherence tomography (OCT) system[5] multiple times after single instillation and after a treatment period of 15 and 30 days, respectively. The course of change in TFT after single instillation was intended to provide information about the corneal residence time of perfluorohexyloctane. The assessment after 15 and 30 days of multiple dosing was intended to collect information whether perfluorohexyloctane eye drops have a sustained effect on TFT. This instrument is suitable to detect small changes in TFT with high reproducibility and sensitivity. As such, it might present an interesting diagnostic tool for mechanistic trials in ocular surface diseases, given that currently available outcome measures like tear film break up time (TFBUT) are variable, invasive, and not well standardized.[6] Furthermore, precorneal lipid layer thickness (LLT) was measured at the same time points using white light interferometry. In addition, other clinical signs and symptoms for DED were assessed.

Methods

Patients

Forty-eight patients with mild to moderate DED were included in this randomized, single-masked, observer-blinded parallel group study. The study protocol was approved by the Ethics Committee of the Medical University of Vienna and the study was performed in adherence to the guidelines of the Declaration of Helsinki as well as Good Clinical Practice guidelines. Patients were selected by the Department of Clinical Pharmacology of the Medical University of Vienna and written informed consent was obtained from all study participants. The study was registered at clinicaltrials.gov (NCT03048526) and released on February 9, 2017. Recruitment started in December 2016 and the study was completed in April 2017.

All patients passed a screening examination 14 days before inclusion. Patients were asked to abstain from instillation of topical lubricants for 24 h before the screening visit. Screening included a physical examination and 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 visual analogue scales (VAS) for various symptoms. In addition, an ophthalmic examination was performed, including best-corrected visual acuity using the Early Treatment Diabetic Retinopathy Study (ETDRS) acuity charts, slit-lamp biomicroscopy, and indirect fundoscopy, measurement of tear film breakup time (TFBUT), Schirmer I test, corneal fluorescein and conjunctival lissamine green staining, and measurement of intraocular pressure (IOP).

To be included, patients had to be at least 18 years if age and had to have normal ophthalmic findings, except history of DED for at least 3 months, TFBUT ≤10 s, and Schirmer I test ≥5 and ≤15 mm, which reflects mild to moderate disease stage according to the Dry Eye Workshop (DEWS).[7] Further inclusion criteria were presence of at least 1 symptom of DED as sticky feeling, burning/stinging, foreign body sensation, itching, blurred vision, sensitivity to light, pain, and a score between 20 and 70 on the VAS for symptoms of DED.

The study eye was defined as the eye with the lower TFBUT at the screening visit and was chosen for analysis. If TFBUT was identical for both eyes, the eye with the higher corneal staining score at the screening visit was the study eye and chosen for analysis. If these were also identical for both eyes, the right eye was the study eye and chosen for analysis. Measurements of TFT, lipid layer thickness (LLT), and noninvasive TFBUT (NITFBUT) was performed in the study eye only.

Patients were excluded if they fulfilled at least 1 of the following criteria: clinically significant slit-lamp findings at screening visit, abnormal lid anatomy, DED secondary to scarring, participation in a clinical trial in the 4 weeks preceding the screening visit, symptoms of a clinically relevant illness in the 3 weeks before the screening visit, presence or history of a severe medical or surgical condition as judged by the clinical investigator, intake of parasympathomimetic or antipsychotic drugs, wearing of contact lenses, glaucoma in the medical history, treatment with corticosteroids in the 4 weeks preceding the study, topical treatment with any ophthalmic drug, except topical lubricants, in the 4 weeks preceding the study, ocular infection or clinically significant inflammation, ocular surgery in the 3 months preceding the study, presence of punctal plugs, Sjögren's syndrome, Stevens-Johnson syndrome, active ocular allergies, pregnancy, planned pregnancy or lactating, and known hypersensitivity to any component of study medication.

Eye drops

On the first study day, patients were randomized in a 1:1 ratio to receive either perfluorohexyloctane eye drops

(NovaTears) consisting of 100% perfluorohexyloctane or sodium chloride (0.9%) eye drops (NaCl, Hydrabak eye drops) as a comparator.

Patients were instructed to instill 1 drop of perfluorohexyloctane or NaCl eye drops in each eye 4 times daily for 30 days. The eye drops were instilled once by authorized personnel on the first study day (visit 1) and thereafter by the patients themselves.

A computer-generated randomization list was provided by the sponsor and treatment numbers were allocated in ascending order using the next available consecutive number by an investigator not involved in the study procedures. The investigators performing the measurements were blinded to the treatment assignment. In addition, patients were blinded to the treatment assignment until the end of the first study day (visit 1).

Description of the study days

For included patients, 3 study days were scheduled. Measurements were performed in the following order on all study days, as described in detail below:

On the first study day (visit 1), OSDI patient questionnaire, VAS questionnaire, slit-lamp biomicroscopy, corneal fluorescein staining, measurement of TFBUT, conjunctival lissamine green staining, and Schirmer I test were performed. After a break of 60 min, baseline assessment of TFT, LLT, and NITFBUT was conducted. Then, study medication was applied by trained study personnel. After application of the study medication, TFT, LLT, and NITFBUT were performed at the predetermined time points (TFT and LLT: 10, 20, 40, 60, 120, and 240 min after instillation and NITFBUT: 30, 60, 120, 240 min after instillation). During the breaks between the measurements, patients had to remain at the study site to guarantee comparable environmental conditions. After completion of all measurements, patients were asked to continue instillation of the allocated eye drops 4 times daily in both eyes until the next study visit.

The second study day (visit 2, 2 weeks after start of study) and the third study day (visit 3, 4 weeks after start of study) were performed as described for visit 1, except that at visit 2 and 3, TFT and LLT were only measured twice (10 and 20 min) after instillation and NITFBUT was only measured once (30 min) after instillation. Table 1 gives an overview of the procedures for each study day.

Measurement techniques

Intraocular pressure. IOP was measured after topical anesthesia with oxybuprocainhydrochloride combined with sodium fluorescein with a slit-lamp mounted Goldmann applanation tonometer.

Ocular surface disease index. Symptoms of dry eye were assessed using the OSDI, which was developed by the Outcomes Research Group at Allergan, Inc. (Irvine, CA).[8] The questionnaire that underlies the OSDI is specifically designed for patients with DED and asks patients about the frequency of specific symptoms and their impact on vision-related tasks of daily life.

Dry eye VAS. Subjects were asked to rate their ocular symptoms (both eyes simultaneously) by placing a vertical

Table 1. Schedule of Assessments

Procedure	*Visit 0; Day −14±3*	*Visit 1; Day 0*	*Visit 2; Day 15±3*	*Visit 3; Day 30±3*
Informed consent	X			
Physical examination and medical history	X			
Height and weight	X			
Blood pressure and heart rate	X			
Pregnancy test	X			X
OSDI questionnaire	X	X	X	X
VAS questionnaires	X	X	X	X
Visual acuity (logMAR)	X			X
Fundoscopy	X			
Slit-lamp biomicroscopy	X	X	X	X
IOP measurement	X			X
TFBUT	X	X	X	X
Corneal fluorescein staining	X	X	X	X
Conjunctival lissamine staining	X	X	X	X
Schirmer I test	X	X	X	X
NITFBUT (5 time points)		X		
NITFBUT (2 time points)			X	X
LipiView assessment (7 time points)		X		
LipiView assessment (3 time points)			X	X
TFT assessment (7 time points)		X		
TFT assessment (3 time points)			X	X
AE query		X	X	X
Randomization		X		
Diary dispensation		X		
Medication dispensation		X		
Return of medication and diary				X

AE, adverse event; IOP, intraocular pressure; NITFBUT, noninvasive tear film break up time; OSDI, ocular surface disease index; TFBUT, tear film breakup time; TFT, tear film thickness; VAS, visual analogue scales.

mark on a horizontal line to indicate the level of discomfort (0 corresponding to ''no symptoms'' and 100% corresponding to ''maximal symptoms''). Patients were asked to rate the severity of the dry eye symptoms dryness, sticky feeling, burning/stinging, foreign body sensation, itching, blurred vision, sensitivity to light, pain, and frequency of dryness. The assessment line length of the scale was 100 mm.

Visual acuity. Visual acuity was measured using the standard ETDRS acuity chart and reported in logMAR units.

Tear film breakup time. TFBUT was measured following the guidelines published in the Report of the International Dry Eye WorkShop (DEWS 2007) using Minims-Fluorescein Sodium 2.0% eye drops.[9] Measurements were repeated 3 times and the mean value was used.

Corneal fluorescein staining. Minims-Fluorescein Sodium 2.0% eye drops were used to detect corneal epithelial defects. Grading was performed according to the NEI/Industry Workshop guidelines.[10] Briefly, the cornea was divided into 5 sectors (central, superior, inferior, nasal, and temporal), each of which was scored on a scale of 0–3, where 0 means no staining and 3 means maximum staining, with a maximal score of 15.

Conjunctival staining with lissamine green. Lissamine green (LG, EasyOpht, Italy) was used to detect conjunctival defects. Before placing the strip in the lower fornix of the eye, a drop of sterile saline was added to the strip. Grading was performed according to the Oxford Scale.[11]

TFT as measured with OCT. An ultrahigh-resolution spectral domain OCT system for imaging of the anterior chamber was employed in this study.[5] The device comprises a broad band Ti: Sapphire laser operating at 800 nm (with spectral bandwidth 170 nm) and a high-speed CCD camera with a maximum readout rate of 70 kHz. The 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 $4 \times 4 \times 1$ mm (horizontal × vertical × depth), each containing $512 \times 128 \times 1{,}024$ 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). Patients' heads were stabilized on a modified slit-lamp head rest. Patients were asked to look straight onto an internal fixation target and blink normally. Data acquisition started immediately after opening of the eye. During the measurement procedure, patients were instructed not to blink. Three 3D volumes were recorded within 3 s 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 to obtain geometrical distances.

Schirmer I test. Schirmer I test (without anesthesia) was performed following the guidelines published in the Report of the International Dry Eye WorkShop.[9] Briefly, 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 min, the wetting of the Schirmer paper was measured. For this study, Schirmer plus® strips (Gecis, France) were used.

Noninvasive TFBUT. NITFBUT was measured at specified time points using the Bon Antares tear film topographer (Bon Optic GmbH, Lübeck, Germany). The placid disc-based topography system is equipped with specific software for advanced tear film analysis with white light (Tearscope). Patients were instructed to look straight onto an internal fixation target and blink normally. Data acquisition started after opening of the eye and NITFBUT was measured automatically. Three consecutive measurements were performed and the mean value was calculated.

LipiView ocular surface interferometer. LLT was measured using the LipiView Ocular Surface Interferometer (Tear Science, Inc., NC). Patients were asked to look into a special camera that records a 20-s video of the interference pattern of the tear film, which was then analyzed by the software of the device.

Statistical analysis

The primary outcome variable was the relative change from baseline in TFT measured with ultrahigh-resolution OCT. To detect differences between the 2 treatment groups, a mixed model repeated measurement (MMRM) analysis (2 sided, significance level $\alpha = 0.05$) with comparison of least square (LS) means with 95% confidence intervals (CI) was used. The covariates were the TFT at baseline (measurement before drop instillation) and duration between baseline and visit (including time point). Absolute and relative change from baseline for each treatment were calculated and summarized by visit and time point and within the same visit (before and after instillation). A 2-sample *t*-test was used to assess differences between treatment groups.

Secondary outcome variables (LLT and NITFBUT) were analyzed in the same way as the primary outcome variable to describe differences between the 2 treatment groups (MMRM, 2 sided, significance level $\alpha = 0.05$). For other continuous secondary outcome variables (blink frequency, Schirmer I test, TFBUT, mean VAS, OSDI, and total score of corneal and conjunctival staining), absolute and relative changes from baseline were calculated and summarized by visit and by treatment. A 2-sample *t*-test was used to assess differences between treatment groups. All patients who entered the study were included in the statistical analysis set.

Results

Demographics

A total of 50 subjects were screened, of which 48 met the inclusion and exclusion criteria and entered the study. Patients were on average 37.5 ± 12.5 years old, 36/48 (75.0%) were female, and 12/48 (25.0%) were male. Heart rate and blood pressure were in the normal range at baseline (data not shown). The 2 treatment groups were overall balanced with regard to demographic data. However, the control group included 9 male patients (37.5%), whereas in the

perfluorohexyloctane group, only 3 male patients (12.5%) were included. Two patients prematurely discontinued in the perfluorohexyloctane group.

Outcome variables

The mean TFT at baseline was comparable in the 2 treatment groups, with 4.21 ± 0.57 µm in the perfluorohexyloctane and 4.15 ± 0.56 µm in the NaCl group. As the primary outcome analysis, the MMRM analysis demonstrated that the relative increase (%) in TFT from baseline was significantly higher with perfluorohexyloctane eye drops than with NaCl, with an estimated LS mean difference of 3.33% (95% CI 0.44–6.23; $P = 0.025$; Fig. 1).

When looking at visit 1 only, 10 min after drop instillation, administration of perfluorohexyloctane eye drops lead to a pronounced increase in TFT amounting to $13.39\% \pm 10.78\%$. In contrast, administration of NaCl led to a significantly lower increase in TFT by $6.98\% \pm 7.73\%$; $P = 0.02$ between groups at visit 1. However, at all subsequent time points at visit 1, no apparent differences between the 2 treatment groups were evident.

To address the potential sustained effect of perfluorohexyloctane after multiple dosing, the primary MMRM analysis was repeated to calculate the LS mean difference estimates for each visit separately. The LS mean difference estimates of the relative change from baseline in TFT showed more pronounced and gradual thickening of tear films with perfluorohexyloctane eye drops during the study, with 1.29% (95% CI –2.46 to 5.04; $P = 0.49$) at visit 1, 4.33% (95% CI –0.09 to 8.75; $P = 0.05$) at visit 2, and reached significance with 6.42% (95% CI 1.37–11.47; $P = 0.01$) at visit 3. This indicates that the effect on TFT builds up over time during repeated instillation.

The LLT at baseline was comparable in the 2 treatment groups, with 76.5 ± 15.7 nm in the perfluorohexyloctane and 71.4 ± 16.3 nm in the control group. Figure 2 shows the relative change in LLT values for both groups. As LLT values measured after instillation were difficult to interpret, MMRM analysis was performed additionally on values obtained at baseline, and before instillation at visits 2 and 3. The MMRM analysis on values recorded before instillation only demonstrated that the relative change (%) in LLT from baseline was significantly higher for perfluorohexyloctane eye drops than for NaCl; the estimated LS mean difference was 16.34% (95% CI 6.93–25.76; $P < 0.01$).

The mean NITFBUT at baseline was comparable in the 2 treatment groups, with 8.5 ± 4.4 s in the perfluorohexyloctane and 9.4 ± 5.4 s in the NaCl group. The NITFBUT tended to increase with both treatments over the course of the study at visit 3; however, as the variability in this parameter was high, these results have to be interpreted with caution (Fig. 3). Correspondingly, TFBUT increased in both treatment groups from 3.7 ± 1.7 s for perfluorohexyloctane and 3.8 ± 2.1 s for the control group at visit 1 to 4.7 ± 2.8

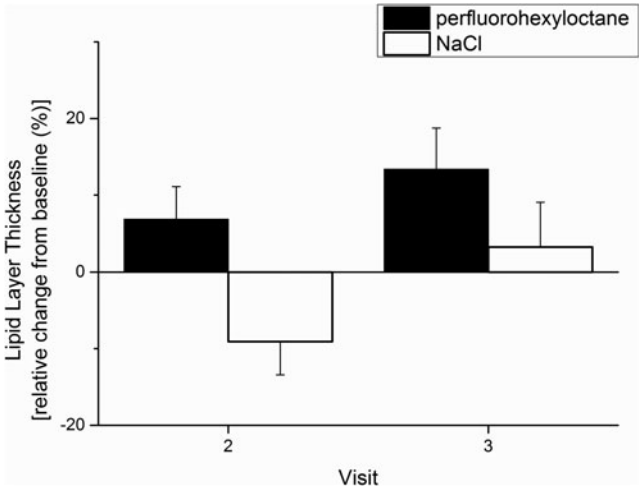

FIG. 2. Relative changes in LLT over time in the perfluorohexyloctane and 0.9% saline solution (NaCl) group on each study day before instillation ($n = 48$, mean ± SEM). LLT, lipid layer thickness.

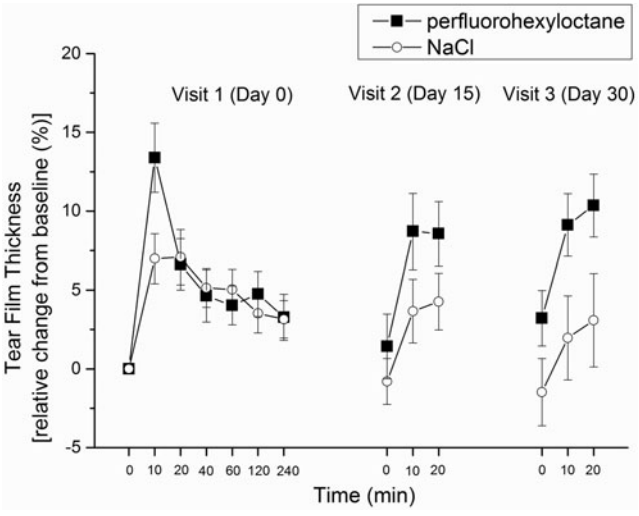

FIG. 1. Effect of perfluorohexyloctane eye drops and 0.9% saline solution (NaCl) on the relative change in tear film thickness from baseline at all different measurement time points ($n = 48$, mean ± SEM). SEM, standard error of the mean.

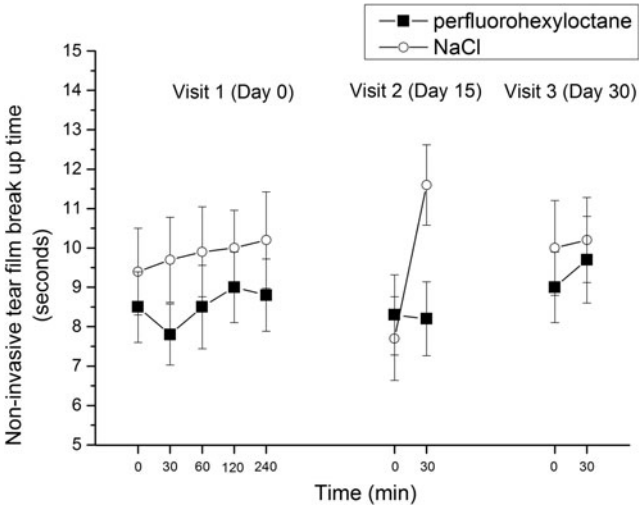

FIG. 3. Effect of perfluorohexyloctane eye drops and 0.9% saline solution (NaCl) on the absolute change in NITFBUT (s) at all different measurement time points ($n = 48$, mean ± SEM). NITFBUT, noninvasive tear film breakup time.

(perfluorohexyloctane), to 5.4 ± 3.8 (NaCl) at visit 2, and to 5.1 ± 2.7 s and to 5.7 ± 3.1 s at visit 3, respectively, with no apparent differences between groups ($P = 0.67$).

Corneal fluorescein staining decreased in both groups over time. The time course appeared to be different between the treatments. NaCl showed its maximum effect after 2 weeks, whereas perfluorohexyloctane showed a constant improvement that reached its maximum after 4 weeks (Fig. 4). By the end of treatment (visit 3), mean corneal fluorescein staining total score had decreased more with perfluorohexyloctane (by 32.13%) than with NaCl (by 17.79%); however, the difference between the groups did not reach statistical significance ($P = 0.2786$). No statistically significant changes in conjunctival lissamine green staining were observed with either treatment.

Mean Schirmer's test scores at baseline were 10.5 mm/5 min in the perfluorohexyloctane group and 9.1 mm/5 min in the NaCl group. At visit 3, they increased by 2.4 and 1.5 mm/5 min in the perfluorohexyloctane group and NaCl group, respectively. The difference was not statistically significant.

OSDI score at visit 1 was 44 ± 22 in the perfluorohexyloctane group and 40 ± 14 in the NaCl group. By the end of the treatment period (visit 3) the OSDI total score had decreased by 24% in both treatment groups with no significant differences between groups ($P = 0.96$).

The mean total score of ocular complaints as assessed with a VAS as mean of all questions was comparable between the 2 treatments at the screening visit, with 35 ± 15 mm in the perfluorohexyloctane and 32 ± 14 mm in the control group. At the final visit (visit 3), the mean total VAS score had decreased with perfluorohexyloctane treatment by 36.8% and in the control group by 36.9%, indicating an improvement of ocular symptoms over time with no apparent difference between groups. Absolute values for all secondary outcome parameters assessed can be found in Supplementary Table S1.

Safety evaluation

Overall, 9/48 patients (18.8%) experienced any Adverse event or Adverse device effect (AE/ADE) during the course of the study, 4 patients (16.7%) in the perfluorohexyloctane and 5 patients (20.8%) in the NaCl group. There were no reports of any serious AE/ADE and any device deficiency, and none of the patients reduced dosage or discontinued treatment or the study due to an AE/ADE. No changes in visual acuity or IOP occurred during the course of the study with any of the treatments (Supplementary Table S1).

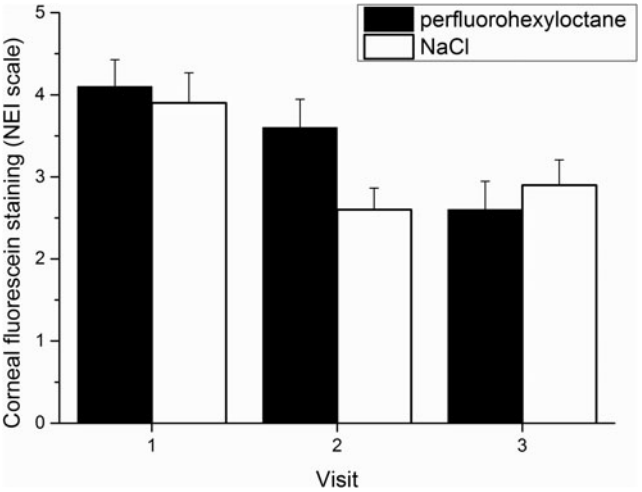

FIG. 4. Effect of perfluorohexyloctane eye drops and 0.9% saline solution (NaCl) on the absolute change in corneal fluorescein staining score according to the NEI scale ($n = 48$, mean ± SEM).

Discussion

This single-center, single-masked, observer-blinded, randomized postmarket study was designed to evaluate the effect of single and multiple instillation of perfluorohexyloctane water-free eye drops on TFT as measured with ultrahigh-resolution OCT in comparison to 0.9% saline solution administration in patients with mild to moderate DED. Our study met its preplanned primary endpoint by demonstrating that perfluorohexyloctane leads to a significantly higher increase in TFT after single administration compared to control. Furthermore, our data show, for the first time in a longitudinal design, that a 4-week administration of water-free eye drops leads to a gradual increase in TFT as measured with OCT, indicating stabilization of the precorneal tear film.

Artificial tears with a lipid component are thought to replenish the lipid layer and are particularly used in patients with evaporative forms of DED. To our knowledge, there is no other eye drop therapy addressing specifically evaporative DED associated with Meibomian gland dysfunction.[12] Perfluorohexyloctane eye drops are the first water-free eye drops and may be a promising alternative as they target directly the lipid layer of the tear film. The low surface tension[2] leads to fast spreading over the ocular surface without blinking. This is related to the amphiphilic nature and film-forming properties in the presence of lipids. Due to the small drop size (~10 μL), the eye is not overfilled after drop instillation, while residual time on the eye is increased without causing vision blurring, mainly due to a similar refractive index of perfluorohexyloctane compared to water. These properties of perfluorohexyloctane are particularly important for the treatment of evaporative DED likely resulting in a direct interaction with tear film lipids, leading to rearrangement and consecutive stabilization of the tear film.

The data of this study support this theoretical concept. In particular, our study results show that the administration of perfluorohexyloctane eye drops stabilizes the tear film after single and multiple administration. Interestingly, this TFT increasing effect is more pronounced after long-term treatment. In particular, the results of the study show that there is a gradual increase of TFT after multiple dosing of perfluorohexyloctane, with a maximum effect at the end of the investigated treatment period (week 4).

In this study, we have used a custom-built ultrahigh-resolution OCT system to visualize the precorneal tear film. We have previously shown that this instrument is suitable to detect subtle changes in TFT with high reproducibility and sensitivity, and that TFT values correlate with signs and symptoms of DED.[5,14,15] As such, previous experiments indicate that single instillation of water-based topical lubricants leads to a temporary increase in TFT.[16–19] However, this experiment is the first to show, in a longitudinal design, that a 4-week treatment can lead to a gradual increase in TFT over time, indicating a long-term restoration

of the precorneal tear film. Thus, ultrahigh-resolution OCT may, in the future, serve as a novel parameter to assess success of DED treatment in terms of normalization of ocular tear film.

In this study, a numerical improvement of secondary sign endpoints, namely TFBUT, NITFBUT, and corneal fluorescein staining, and also improvements in symptoms (OSDI and VAS) were observed in both treatment groups. In principle, these findings are consistent with previous multicenter studies in patients with primarily hyperevaporative forms of DED showing a significant improvement of signs and symptoms after 6 weeks of treatment with perfluorohexyloctane eye drops. Those experiments show that clinical sign endpoints such as corneal fluorescein staining and TFBUT, as well as symptom endpoints (OSDI score and VAS) improved compared to baseline, indicating a good clinical efficacy of perfluorohexyloctane eye drops.[3,4] This is also reflected in the data of this trial and holds also true for the results of Schirmer I test, which increased after treatment with perfluorohexyloctane. However, although a positive treatment effect of perfluorohexyloctane eye drops was observed in the secondary endpoints, this effect failed to reach statistical significance compared to the control group.

When interpreting the above-mentioned results of the secondary outcome parameters, it needs to be considered that this study was designed and powered to detect changes in TFT between groups and not in clinical variables like fluorescein staining, TFBUT, or Schirmer test. This is important to note, since the standard clinical measures of DED show a large variability,[20,21] which is particularly true for the above-mentioned clinical signs.[22] Thus, considerably larger sample sizes will be necessary to finally investigate the effect of perfluorohexyloctane on clinical signs, while TFT measurement using OCT shows higher sensibility and lower variability to determine morphological changes of the tear film.

The latter considerations hold analogously true for the interpretation of the symptom scores OSDI and VAS. Although both groups showed a considerable amelioration in both OSDI and VAS, no statistically difference was detected between the treatment and the control group. Thus, to finally estimate the effect of perfluorohexyloctane on symptom scores in a placebo- or comparator-controlled study, larger trials are needed. Indeed, as shown in SEECASE-1 study with larger sample sizes and a longer treatment period of 8 weeks, differences in corneal fluorescein staining as well as in Dryness Score could be demonstrated.[13]

As a secondary, exploratory objective of the study, the lipid component of the precorneal tear film was assessed using white light interferometry. Our data show that immediately after instillation, LLT apparently increased after perfluorohexyloctane instillation, but after 20 min until 2 h after instillation, surprisingly dropped below the control group. Although the reason for this observation is not entirely understood, one could hypothesize that it may be related to the method itself. First, it needs to be considered that, although the LipiView instrument shows good reproducibility, measurements directly after instillation of eye drops might not be reliable because of technical reasons in line with the instructions for use, which ask for a waiting period of at least 4 h after any drop instillation.[23] As the measurement of the LLT is based on the detection of different refractive indices between the tear film and the lipid film, drop instillation of very lipophilic drops as, in this study, may blur the interface between the 2 layers and interfere with the measurement principle of the system. In addition, any type of lubricants may lead to a short-term change in the difference of the refractive index between the 2 layers. This is of special importance for perfluorohexyloctane eye drops as used in this study because they show a similar refractive index compared to water and may therefore complicate the detection of differences between layers, in particular, immediately after administration.[24] To overcome this potential limitation, we have analyzed the preinstillation values at the 2 and 4 weeks' time point. Indeed, the data of this analysis showed an increase of LLT in the perfluorohexyloctane group compared to the control group, indicating an increase of LLT after 4 weeks of instillation.

A limitation of the trial design is that the observation time was limited to 4 weeks. Thus, it cannot be excluded that with a longer treatment time, the effect of perfluorohexyloctane treatment on TFT would be even more pronounced. This is also supported by the fact that after administration of the control agent, the increase in TFT tended to level off, whereas in the perfluorohexyloctane group, the effect increased until the last measurement time. Consequently, a study with a longer treatment period is necessary to finally investigate when the maximum treatment effect is obtained. Furthermore, the number of subjects was limited to 48. Although this allows for the good estimation of the effects of topical treatment on TFT, final conclusions regarding clinical signs and symptoms will require larger samples sizes.

In summary, the study met its primary objective by demonstrating that perfluorohexyloctane increases TFT after single and repeated instillation. In addition, the TFT increasing effect built up over time and reached its apparent maximum at the end of the 4-week study treatment, which is in line with the mode of action of perfluorohexyloctane water-free eye drops preventing evaporation by stabilizing the lipid layer. Consistently, the observed increase of LLT in the perfluorohexyloctane group further substantiated the overall observed improvement of the tear film. Thus, treatment with perfluorohexyloctane water-free eye drops appears to be a safe and well-tolerated new therapeutic approach for patients with mild to moderate DED.

Author Disclosure statement

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Supplementary Material

Supplementary Table S1

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