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Original Article

Disease aetiology-based design of multifunctional microemulsion eye drops for moderate or severe dry eye: a randomized, quadruple-masked and active-controlled clinical trial

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ABSTRACT.

Purpose: **To assess the safety and efficacy of multi-ingredient sacha inchi microemulsion (SIME) eye drops designed to target (1) tear film instability, (2) tear hyperosmolarity, and (3) ocular surface damage and inflammation in moderate or severe dry eye.**

Methods: **This randomized, quadruple-masked, active-controlled parallel study in 64 adult patients comprised three parts. Part 1 (n = 3): one eye was treated with SIME for one day. Part 2 (n = 9): randomized eyes were treated with SIME and 0.2% hyaluronic acid (HA) control eye drops 3 times a day for 10 days. Part 3 (n = 26 + 26): randomized treatment was applied on both eyes 3 times a day for 30 days. OSDI change was tested for superiority of SIME over HA. Ocular assessments were performed at baseline and after the last dose.**

Results: **Both treatments were well tolerated without adverse device effects. Tear film break-up time (p = 0.0025) and ocular protection index (p = 0.0026; change vs. HA, p = 0.047) increased significantly with SIME after 30 days. Tear osmolarity decreased more in SIME than in the HA group and significantly with both eye drops in hyperosmolar subgroups. Corneal (p = 0.014) and nasal conjunctival staining (p = 0.043) were reduced with SIME in per-protocol patients (n = 24). Conjunctival (p = 0.001) and lid redness (p = 0.012) decreased with SIME in all patients (n = 26). Symptoms decreased by about 25 OSDI units with both treatments (p < 0.0001) and with nonsignificant difference between treatments.**

Conclusions: **Sacha inchi microemulsion (SIME) proved safe and efficacious in improving each aetiologic factor for dry eye as revealed through objective tests. Hyperosmolar stress dominating blink cycles must be disrupted by biophysical protection of the ocular surface to facilitate resolution of cellular damage and inflammation, and relief of ocular symptoms.**

Key words: fatty acids, omega-3 – hyaluronic acid – lubricant eye drops – ophthalmic emulsion – osmolar concentration – protective agents – tears – trehalose

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Introduction

An increasingly common condition of the ocular surface, dry eye continues to be a major reason for seeking medical eye care, causing a significant impact on public economy, work productivity and quality of life (McDonald et al. 2016; Stapleton et al. 2017). The evolving comprehension of mechanisms underlying the onset and progression, and of the causative factors, leads the way for designing safe remedies against those mechanisms of the complicated disease. While the 1995 NEI/Industry Workshop (Lemp 1995) names tear deficiency and tear evaporation, the main causes of the 'disorder', the 2007 definition by the Tear Film and Ocular Surface Society's International Dry Eye Workshop (TFOS DEWS) (WorkSho 2007), were 'a multifactorial disease' involving tear film instability, osmolarity, and ocular surface inflammation and damage. The recent dry eye classification by TFOS DEWS II (Nelson et al. 2017) formulates 'tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities' to play aetiologic roles; 'neurosensory abnormalities' are explained to contribute to the common mismatch between signs and symptoms exhibited in a proportion of patients (Jones et al. 2017).

The pathophysiology described by TFOS DEWS II (Bron et al. 2017; Nelson et al. 2017) may be outlined to comprise essentially a sequence of three

main *aetiologic* factors, namely (1) tear film instability, causing and accompanied by (2) localized hyperosmolarity, leading to (3) epithelial damage and inflammation. The effects of a wide variety of endogenous, external and iatrogenic *causative* factors (Bron et al. 2017; Gomes et al. 2017) are eventually conveyed to the common aetiologic factor pathway (Supporting Information, Fig. S1). It is notable, firstly, that all aetiologic factors can be translated into measurable parameters usable as independent end-points in clinical trials. Secondly, the factors may be understood to generate and feed each other in a temporal sequence of a self-perpetuating 'vicious circle' (Nelson et al. 2017). Thirdly, starting from this scheme, it should be possible to design a locally acting treatment modality which attenuates all aetiologic factors simultaneously, as targeting one aetiologic segment alone might make it less likely 'to prevent a return to the vicious circle' (Jones et al. 2017). The present paper reports results of a clinical trial in dry eye patients using a novel emulsion eye drop formulation with sacha inchi seed oil 'multitargeting' the three aetiologic factors. Discussed is the importance of biophysical protection of the ocular surface for recovery from damage, inflammation and symptoms.

Materials and Methods

Subjects

The study followed the tenets of the Declaration of Helsinki, GCP principles of EN ISO 14155, and national laws and regulations. Recruitment of subjects started after obtaining a positive statement from The Research Ethics Committee of the Northern Savo Hospital District (Kuopio, Finland). Participants were outpatients previously diagnosed with dry eye at Kuopio University Hospital (Kuopio, Finland). The prospective study subject received written and oral information of the purpose and course of the study and of management of personal information. Written informed consent was obtained before any screening procedure if the subject decided to participate.

Inclusion criteria: 18–80 years of age; either (1) ocular surface disease index (OSDI) ≥20 and tear film break-up time (TBUT) <10 seconds, or (2) OSDI ≥20 and positive ocular (corneal and conjunctival) staining pattern by Oxford grading; body weight ≥45 kg; under stable topical and/or systemic therapy for ≥4 weeks before study procedures and ability to abstain from other therapies during study; and ability and willingness to self-administer eye drops (Bron et al. 2017; WorkSho CS of the IDE 2007). Exclusion criteria: medical history of ocular surgery, trauma or refractive laser vision correction procedure <3 months earlier; corneal/conjunctival infection; Sjögren's syndrome; contact lens use during study and <1 week earlier; ocular allergic symptoms; known allergy to eye drop constituents; current or planned pregnancy or nursing during study. Decision on eligibility and to which one of the three parts the subject would participate was made by the investigator.

Study treatments

The investigational medical device was sacha inchi microemulsion (SIME; Finnsusp, Lieto, Finland), an eye drop formulation with 0.1% sacha inchi (*Plukenetia volubilis*) seed oil, polysorbate 80, sorbitan monooleate, 0.2% high-molecular-weight (1.0–1.5 MDa) hyaluronic acid (HA), 2% trehalose dihydrate and glycerol in trometamol/citrate buffer. The mildly hypotonic formulation utilizing a proprietary emulsion technology (Finnsusp) has low surface tension and the potassium concentration in the physiological range of healthy tears (Willcox et al. 2017). The control device was 0.2% medium-molecular-weight (0.7–0.9 MDa) HA in isotonic phosphate saline buffer (Finnsusp). Both formulations were optically clear, colourless, odourless, preservative-free, stabile, pH neutral and sterile solutions filled in visually similar translucent 10-ml multidose eye drop containers (Aptar Pharma, Radolfzell, Germany, and Röchling Medical, Neuhaus am Rennweg, Germany), allowing complete masking of product identity from participants, healthcare providers, data collectors and statisticians. The bottles were labelled with unique code numbers and stored in a locked cabin under monitored temperature and humidity conditions in the examination room.

Study design

This was a prospective, single-site, randomized, active-controlled, parallel, quadruple-masked study to evaluate the safety, ocular tolerability and efficacy of SIME for up to 30 days in adult and elderly patients with moderate or severe dry eye. The study comprised three sequential parts (Fig. 1) with scheduled visits to the study centre in the beginning (all parts) and on the end-of-study date (Parts 2 and 3). Proceeding to the next part was possible in the absence of significant adverse device effects (i.e. treatment-emergent adverse events). Parts 1–3 involved different participants. One of the authors (KK) was responsible for conducting the study with all assessments at the Department of Ophthalmology, Kuopio University Hospital (study site).

Part 1. At study site, one drop of SIME was dosed four times on the worst eye of three subjects at 2-hour intervals, the fellow eye remaining untreated.

Part 2. Nine subjects self-administered SIME and HA drops on randomized eyes three times a day for 10 (±1) days, the first drop given under control of the study personnel. On Day 10, no morning dose was administered, assessments were performed at the study site, and a study diary and eye drop bottles were collected.

Part 3. Fifty-two subjects, allocated into randomized SIME or control groups (n = 26 for both), self-administered the corresponding eye drops on both eyes three times a day for 30 (±2) days. On Day 30, with no morning dose, assessments were performed, the diary with an additional questionnaire was returned, and the eye drop bottle collected.

Randomization lists for Parts 2 and 3 were prepared by a randomization expert (4Pharma, Turku, Finland) not involved in other study procedures. The sealed code envelope was to be opened on the investigator's resolution in cases of emergency only. Another code envelope was used according to a specific protocol if an eye drop bottle had to be replaced without jeopardizing masking or randomization.

Adherence to dosing schedule was analysed in study diaries and/or case report forms and by measuring the weight reduction in collected eye drop bottles.

Assessments

All study assessments were performed at baseline and after the last dose in an examination room monitored for temperature and relative humidity (RH).

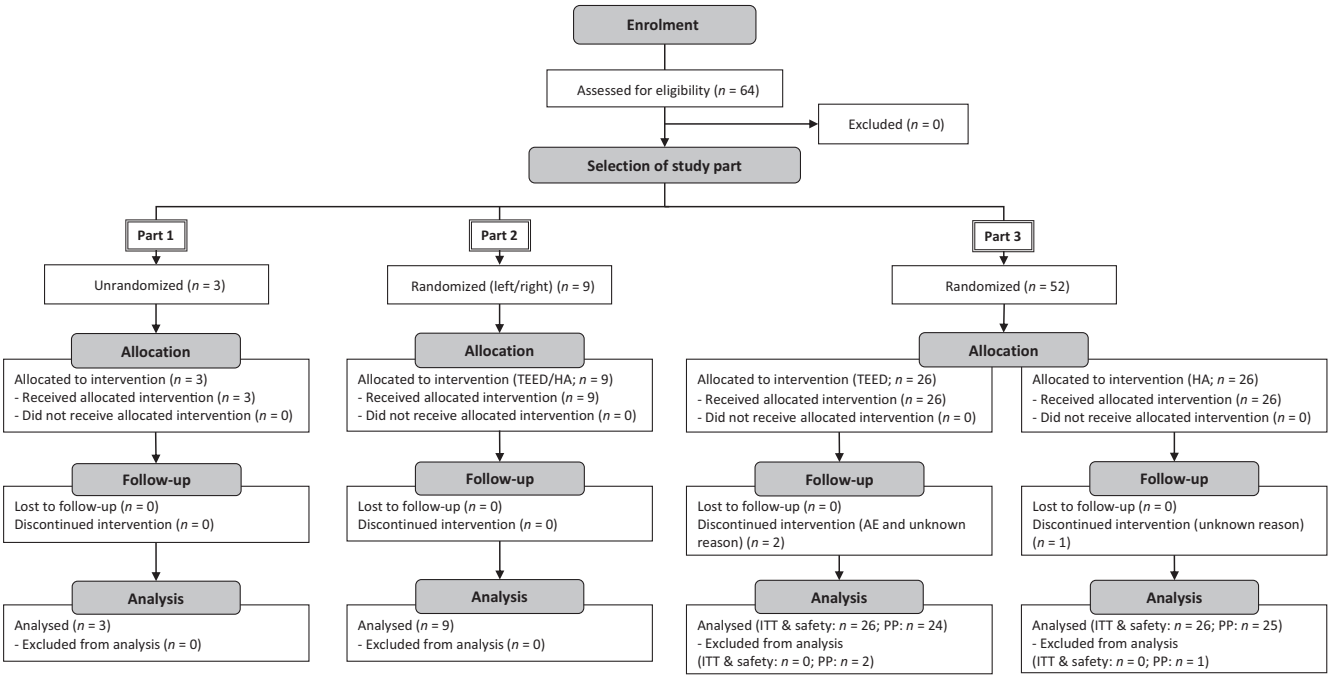

Fig. 1. Flow diagram of the study.

Safety and ocular tolerability, evaluated as the primary objective, included best-corrected visual acuity (ETDRS charts 1 & 2, 2000 series), conjunctival (bulbar) and lid redness (IER grading for both; 0 = not existing, 1 = very slight, 2 = slight, 3 = moderate, 4 = severe), intraocular pressure (IOP; Goldmann tonometer), digital photography of the anterior eye and visual assessment of ocular reactions. Ocular biomarkers used as primary efficacy end-points in Parts 2 and 3 included tear osmolarity (I-PEN® Tear Osmolarity System, I-MED Pharma, Dollard-des-Ormeaux, QC, Canada) (Chan et al. 2018) and fluorescein TBUT. For secondary efficacy objectives, blink rate and corneal and conjunctival staining (Oxford grading 0–5 for both; Bron et al. 2003) were assessed; blink rate (min^{-1}) was converted to interblink interval (IBI; s) to calculate the ocular protection index (OPI=TBUT/IBI) (Ousler et al. 2008). Ocular surface disease index (OSDI) was used for both safety and efficacy. Adverse events (AEs) were collected throughout the study during visits to study site, in study diaries, and from direct contacts to the study personnel between visits.

Statistical methods and determination of sample size

A total number of 64 patients (both males and females) were to be included in the study. In Part 1, preliminary acute safety and ocular tolerability information was to be obtained from all three completing subjects. In Part 2, nine subjects were recruited to obtain data from at least eight completing subjects. In Part 3, 52 subjects were planned to be needed to obtain information on the safety, tolerability and efficacy of daily treatments from at least 44 (83%) completing subjects allocated in two randomized treatment groups. Sample size calculation was conducted for Part 3 only, using OSDI as the parameter of interest. The evaluation of the study was to be based on the total evidence collected. The null-hypothesis to be tested was

H0: There is no difference between study products in OSDI scores.

H1: There is a significant difference in OSDI scores in favour of the investigational product.

Data management and statistical analyses were carried out by 4Pharma Ltd (Turku, Finland) according to statistical analysis plan defining the analyses before the database was locked and the randomization code opened. Analysis of variance model was used for endpoints with a continuous scale to estimate within-group changes from baseline and between-group differences in changes. For ordinary-scale variables, Wilcoxon signed-rank test was used for within-group changes and Wilcoxon rank-sum test for between-group comparisons. Neither imputations for missing observations nor interim analyses were planned or performed. A two-sided p-value < 0.05 was considered statistically significant.

Statistical analyses were performed primarily on the intention-to-treat (ITT) population of Part 3 comprising all randomized subjects who received a study treatment at least once and from whom subsequent efficacy measurements were available. The safety population included subjects with at least one safety measurement obtained after randomization. The per-protocol (PP) population which excluded participants with significant protocol deviations was used as a secondary data set.

Results

Study subjects, compliance and environmental conditions

The planned total of 64 eligible study subjects was included, and 61 of them completed the study per protocol (Fig. 1, Table 1). Three patients discontinued in Part 3, one (SIME) after having discarded the emptied eye drop bottle after 25 days without attempting to receive a replacement, another (SIME) discontinuing on investigator's resolution at 25 days due to an AE unrelated to treatment (see Safety

Table 1. Subject demographics and disposition to data sets.

Patient demographics	Part 1	Part 2	Part 3	Total
Age, years* (range)	62.3 ± 19.3 (40–74)	59.0 ± 12.3 (37–76)	53.3 ± 12.6 (26–78)	54.5 ± 12.9 (26–78)
Sex, *n* (%)	2 (66.7%) females	7 (77.8%) females	36 (69.2%) females	45 (70.3%) females
	1 (33.3%) male	2 (22.2%) males	16 (30.8%) males	19 (29.7%) males
Weight, kg* (range)	72.7 ± 6.4 (69–80)	78.2 ± 10.3 (62–92)	76.0 ± 13.8 (49–110)	76.1 ± 13.0 (49–110)
OSDI sum score* (range)	na.†	51.5 ± 9.5 (42–68)	46.9 ± 11.0 (29–83)	-
TBUT, s* (range)	na.	2.3 ± 0.97 (1–4)	3.4 ± 2.6 (1–10)	-
ITT and safety data sets (*n*)	3	9	52	64
PP data set (*n*)	3	9	49	61

* Mean ± SD.
† na., not analysed as a group.

and ocular tolerability below) and one (HA) having discontinued for an unknown reason after 18 days. However, no subjects were lost to follow-up (Fig. 1), and ITT and safety data from all included participants were analysed (Table 1). There were no other major protocol deviations affecting the primary study outcomes. Concomitant topical medications are listed in Supporting Information, Table S1.

In Part 1, the study nurse administered all doses, ensuring 100% compliance.

In Parts 2 and 3, adherence of the PP subjects to dosing schedule was close to 100%, with a mean of 2.95–2.98 daily doses reported (Table S2); deficient dosing compliance was not considered a protocol deviation. The subjects administered the last dose 10.8 ± 3.3 hours (mean ± SD; Part 2) and 11.2 ± 1.9 (range 8–16 hours; Part 3) before the start of study assessments.

The ambient temperature in the examination room on visit days was 23.7 ± 0.5°C (range 22.8–24.4°C) with 55 ± 7% (range 43–68%) RH, demonstrating acceptable environmental conditions and stability for all assessments.

Safety and ocular tolerability

Both eye drops were well tolerated without AEs. A summary of changes in safety parameters is provided in Table S3. In Part 3, conjunctival and lid redness were reduced significantly with SIME (see Ocular surface damage and inflammation). One subject using SIME in Part 3 discontinued on investigator's resolution after 25 days due to appearance of small cutaneous papules all over the body, judged by the investigator to be unrelated to study treatment.

Tear film stability

Part 2. Mean TBUT increased by 134% in eyes with SIME and by 105% with HA at Day 10 (Fig. 2A). Blink rate decreased from 19.2 ± 7.4 to 12.9 ± 4.4 min^{-1}; no differences between treatment eyes were expected due to simultaneous blinking of eyes. Ocular protection index (OPI) increased from 0.75 ± 0.47 in both eyes by 59% with SIME and by 41% with HA, just exceeding the value 1.0 in both eyes (Fig. 3A).

Part 3. TBUT increased significantly by about 50% in subjects using SIME and nonsignificantly by 25% in controls (Fig. 2B, Table 2). Blink rates did not change considerably (Table 2). OPI improved significantly by 57% to about 1.5 OPI units with SIME ($p = 0.0026$) and by 4% with HA; treatment difference in changes from baseline was statistically significant for ITT population ($p = 0.047$) and marginally significant for PP population ($p = 0.071$) in favour of SIME (Fig. 3B, Table 2).

Tear osmolarity

Part 2. Tear osmolarity showed a mean decrease of 12 mOsm/l with both treatments to approximately 300 mOsm/l at Day 10 (Fig. 4A). An *ad hoc* analysis in patients with hyperosmolar tears at baseline (mean of eyes ≥308 mOsm/l) revealed a mean reduction by 19 mOsm/l in eyes treated with SIME ($n = 5$) and by 34 mOsm/l in HA control eyes ($n = 3$) to a mean level <308 mOsm/l (Fig. 4B).

Part 3. In both treatment groups, mean tear osmolarity was initially <308 mOsm/l and showed a small decrease by Day 30 (Fig. 4C, Table 2). In hyperosmolar ITT and PP data sets, tear osmolarities reduced significantly to about 300 mOsm/l with both treatments (Fig. 4D, Table 2).

Ocular surface damage and inflammation

Part 2. No marked changes in corneal and conjunctival staining or in conjunctival and lid redness were observed (Table S3).

Part 3. The effect of SIME on all staining scores assessed after 30 days was greater than that of control eye drops, although without statistical difference between treatments (Table 2). Mean ocular staining scores were of absent-to-mild intensity at baseline (0–2 score units on Oxford scale 0–5). Corneal staining decreased significantly with SIME in PP data set (−27%, $p = 0.014$, Wilcoxon signed-rank test) and marginally significantly in ITT subjects (−20%, $p = 0.077$) (Fig. 5A, Table 2). Similarly, SIME reduced conjunctival staining with statistical significance in the nasal conjunctiva of PP subjects (−22%, $p = 0.043$) and with marginal significance in ITT subjects (−19%, $p = 0.059$) (Fig. 5B and C, Table 2). The effects of HA eye drops were smaller and nonsignificant (Fig. 5, Table 2). Changes between staining classes are presented in Table S3.

Significantly reduced mean conjunctival (−23%, $p = 0.001$) and lid redness (−29%, $p = 0.012$) scores were observed with SIME in the safety/ITT data set, while changes in the control group remained milder (ca. −15%) and nonsignificant (Fig. 6, Table 2).

Symptoms

Part 2. All nine patients presented initially severe dry eye symptoms with a mean OSDI sum score >50 units

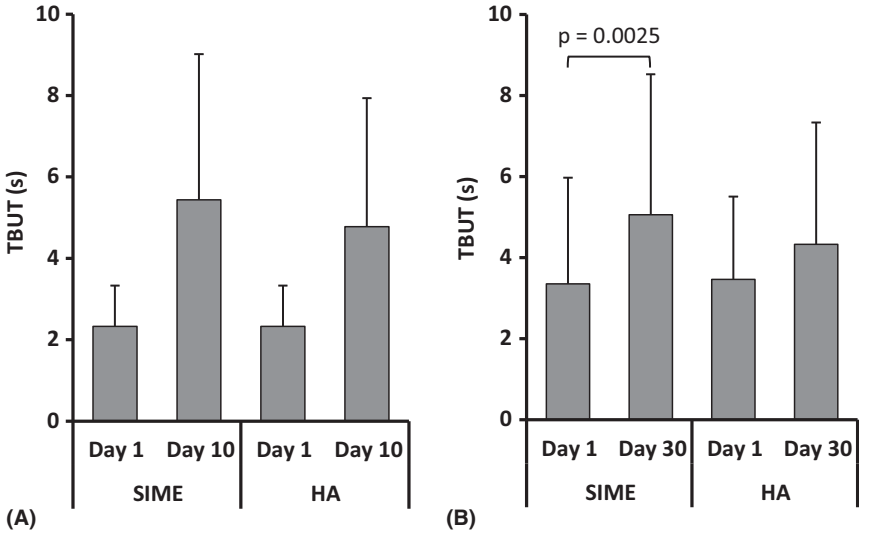

Fig. 2. Tear film break-up time (TBUT). A, Part 2 ($n = 9$); B, Part 3 ($n = 26$). Mean ± SD from ITT population. SIME, sacha inchi microemulsion eye drops; HA, hyaluronic acid control eye drops.

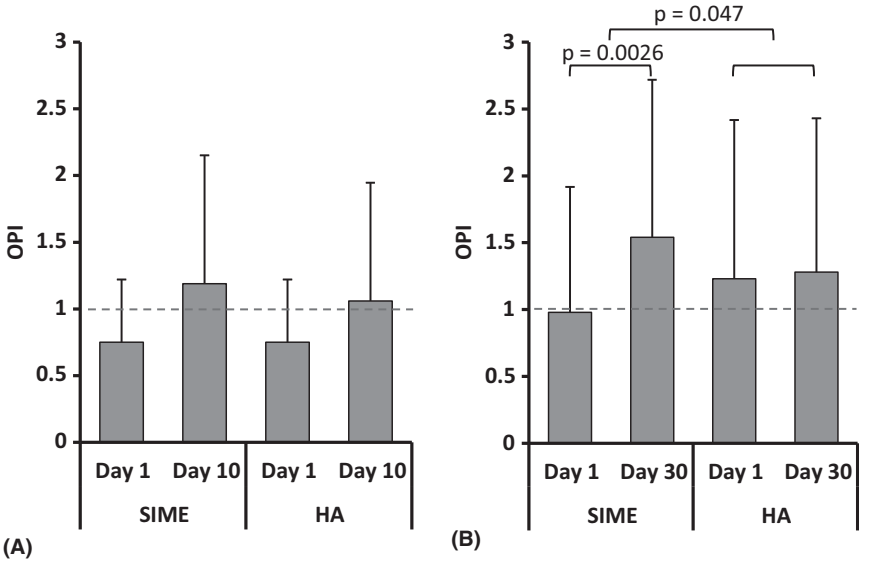

Fig. 3. Ocular protection index (OPI). A, Part 2 ($n = 9$); B, Part 3 ($n = 26$). Mean ± SD from ITT population. *Dash line* marks OPI = 1.

(Table 1) which decreased by 23% and 32% after 10 days with SIME and HA, respectively, when evaluated separately for the randomized treatment eyes. OSDI subscales for 'ocular symptoms' (three questions), 'vision-related functions' (six questions) and 'environmental triggers' (three questions) showed approximately similar degree of reduction at Day 10 for both eye drops (data not shown).

Part 3. Almost all patients reported severe symptoms at baseline (Table 1). Mean OSDI sum score decreased with both SIME (−51%) and HA control eye drops (−58%) from baseline (ITT data set), most patients reporting mild or nonexistent residual symptoms on Day 30 (medians 17 and 14, respectively); both of these within-group changes were highly significant ($p < 0.0001$) for PP and ITT populations but lacking significance between treatments (Fig. 7A, Table 2). Similarly, a highly significant reduction was seen in all three OSDI subscales ($p < 0.0001$) (Fig. 7B–D). Of interest, the median of 'vision-related functions' subscale decreased from 38 score units in both groups to ≤10 units (Fig. 7C), suggesting that the majority of subjects were effectively without vision-related functional symptoms after 30 days.

A summary of changes observed in the data sets of Part 3 is presented in Table 2.

The participants were asked to fill in an additional questionnaire about the investigational eye drops in the end of Part 3. A descriptive summary of the results is presented in Table S4 (ITT data set; one subject in SIME group did not respond). There were no drastic differences between treatments, except that the HA control eye drops were considered more comfortable in the eye (96% versus 72% with SIME) and caused slightly less stinging or burning sensations (31% versus 48% with SIME). About 75% of the participants considered the instructed dosing frequency sufficient, and about 70% of users in both groups would recommend the eye drops to others.

Discussion

The results of the present study suggest that moderate-to-severe dry eye can be managed by providing support to a sequence of events that appears to be required for the resolution of both signs and symptoms (Fig. S1). The core idea was to investigate a topical treatment modality designed to pose as much resisting pressure as possibly feasible against the three aetiologic factors for dry eye (Bron et al. 2017; Nelson et al. 2017), with nonpharmacologic modes of action and safety considerations. The data indicate significant and meaningful improvements in objective signs of each aetiologic category with emulsion eye drops at least after a 1-month daily regimen, whereas only one aetiologic category was significantly influenced with HA-containing eye drops.

The central outcome of the study is the understanding that (1) tear film instability and (2) tear hyperosmolarity need to be corrected to facilitate resolution of (3) cellular damage and inflammation. This temporal sequence of events complies with the aetiologic factor pathway presented by TFOS DEWS II (Bron et al. 2017; Nelson et al. 2017) and contemplated by others (Ousler et al. 2007; Pflugfelder & de Paiva 2017) (Fig. S1). All phases of the pathway should and can be targeted simultaneously for meaningful

Table 2. Summary of results in Part 3 categorized by aetiology.

TFOS DEWS II aetiologic factor and symptoms	Measured variable (unit)	Study population	Mean ± SD change from baseline on Day 30 SIME ITT, *n* = 26; PP, *n* = 24	HA ITT, *n* = 26; PP, *n* = 25	Effect size; 95% CI†
1. Tear film instability	TBUT (s)	ITT	1.71 ± 3.42 (p = 0.0025)*	0.87 ± 1.81 (p = 0.11)	0.85; −0.68 to 2.37 (p = 0.27)
		PP	1.65 ± 3.48 (p = 0.0055)*	0.88 ± 1.84 (p = 0.12)	0.77; −0.83 to 2.36 (p = 0.34)
	Blink rate (min^{-1})	ITT	0.02 ± 8.72 (p = 0.99)	−2.15 ± 10.2 (p = 0.25)	2.17; −3.10 to 7.45 (p = 0. 41)
		PP	−0.19 ± 9.03 (p = 0.93)	−1.96 ± 10.3 (p = 0.32)	1.77; −3.81 to 7.36 (p = 0.53)
	OPI (ratio)	ITT	0.56 ± 1.08 (p = 0.0026)*	0.05 ± 0.68 (p = 0.78)	0.51; 0.0073 to 1.01 (p = 0.047)*
		PP	0.53 ± 1.11 (p = 0.0068)*	0.05 ± 0.69 (p = 0.80)	0.49; −0.043 to 1.02 (p = 0.071)
2. Tear film hyperosmolarity	Tear osmolarity, all (mOsm/l)	ITT	−4.75 ± 16.2 (p = 0.21)	−1.77 ± 21.5 (p = 0.64)	−2.98; −13.6 to 7.64 (p = 0.58)
		PP	−4.73 ± 14.6 (p = 0.22)	−2.36 ± 21.8 (p = 0.53)	−2.37; −13.1 to 8.32 (p = 0.65)
	Tear osmolarity, ≥308 (mOsm/l)	ITT	−17.1 ± 10.4 (p = 0.0038)*	−15.1 ± 16.6 (p = 0.0011)*	−1.99; −15.6 to 11.6 (p = 0.76)
		PP	−14.6 ± 8.5 (p = 0.015)*	−15.1 ± 16.6 (p = 0.0010)*	0.43; −13.7 to 14.5 (p = 0.95)
3A. Ocular surface damage	Corneal staining (Oxford scale 0–5)	ITT	−0.27 ± 0.78 (p = 0.077)	−0.21 ± 0.51 (p = 0.070)	−0.06 (p = 0.77)
		PP	−0.38 ± 0.70 (p = 0.014)*	−0.22 ± 0.52 (p = 0.070)	−0.16 (p = 0.45)
	Conjunctival staining, temporal (Oxford scale 0–5)	ITT	−0.17 ± 0.51 (p = 0.13)	−0.02 ± 0.79 (p = 0.90)	−0.15 (p = 0.53)
		PP	−0.19 ± 0.51 (p = 0.12)	−0.02 ± 0.81 (p = 0.90)	−0.17 (p = 0.52)
	Conjunctival staining, nasal (Oxford scale 0–5)	ITT	−0.23 ± 0.60 (p = 0.059)	−0.12 ± 0.65 (p = 0.44)	−0.11 (p = 0.73)
		PP	−0.27 ± 0.61 (p = 0.043)*	−0.12 ± 0.67 (p = 0.44)	−0.15 (p = 0.59)
3B. Ocular surface inflammation	Conjunctival redness (IER grading 0–4)	Safety/ITT	−0.46 ± 0.65 (p = 0.001)*	−0.27 ± 0.65 (p = 0.065)	−0.19 (p = 0.36)
	Lid redness (IER grading 0–4)	Safety/ITT	−0.37 ± 0.67 (p = 0.012)*	−0.19 ± 0.55 (p = 0.13)	−0.18 (p = 0.30)
Sum of planned primary and secondary end-points with significant improvement from baseline‡		Safety/ITT	4	1	1
		PP	4	1	0
Symptoms	OSDI sum score (scale 0–100)	ITT	−24.6 ± 16.2 (p < 0.0001)*	−26.5 ± 14.6 (p < 0.0001) *	1.9 (p = 0.66)
		PP	−25.0 ± 16.5 (p < 0.0001)*	−27.2 ± 14.5 (p < 0.0001)*	2.2 (p = 0.62)

† Estimate of SIME versus HA difference, 95% confidence interval for continuous variables, and between-group significance of change.
‡ Excluding *ad hoc* analyses.
* Statistically significant difference (p < 0.05).

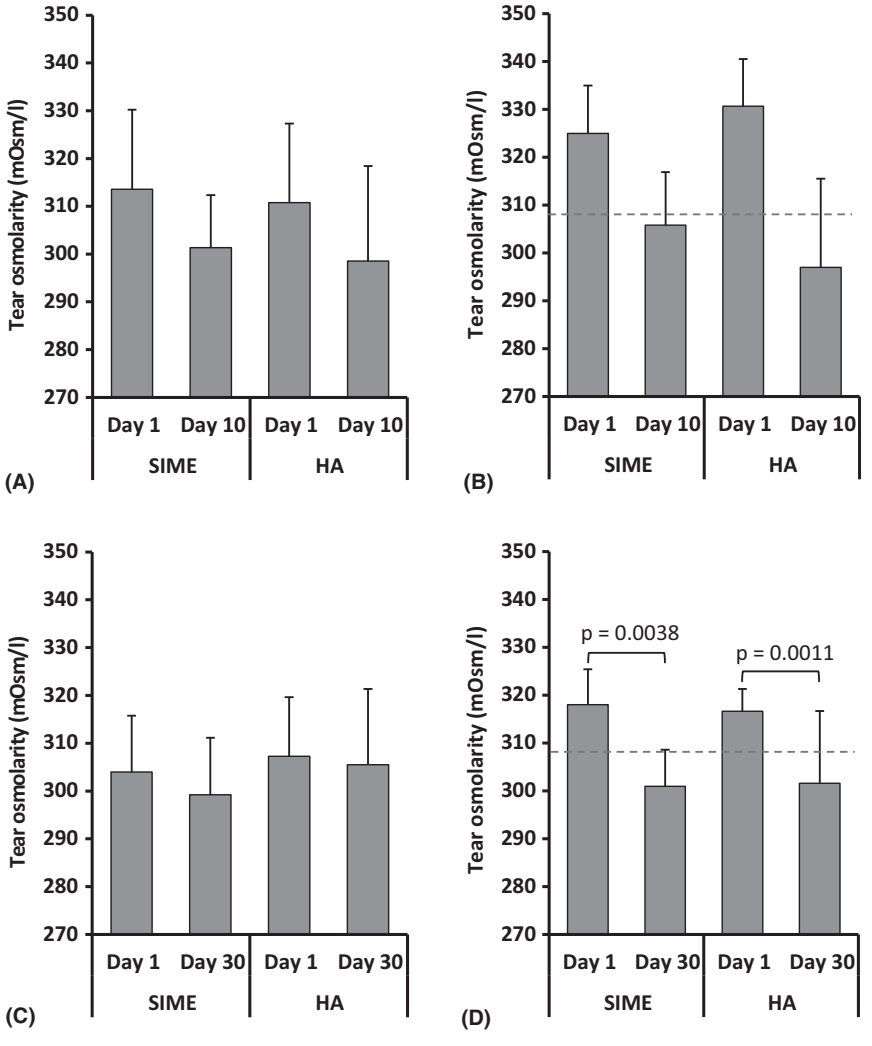

Fig. 4. Tear osmolarity. A, Part 2 (n = 9); B, Part 2 patients with tear hyperosmolarity (≥308 mOsm/l, *dash line*) at baseline (SIME, n = 5; HA, n = 3); C, Part 3 (n = 26); D, Part 3 patients with tear hyperosmolarity (SIME, n = 8; HA, n = 14). Mean ± SD from ITT population.

improvements and to minimize chances for a return to the 'vicious circle', defined as the goal of dry eye management (Jones et al. 2017). As the first step, hyperosmolar stress dominating each blink cycle in dry eye is disrupted by establishing continuous biophysical protection on the ocular surface through stabilizing the tear film and its lipid layer, and normalizing tear osmolarity. Determination of OPI and tear osmolarity proved reliable tools to identify these events. After this crucial turning point, recovery of the viable ocular epithelia will begin, if sufficiently supported, followed by improved symptoms.

Unstable tear film is considered the primary driver and an independent starting point for dry eye, exposing ocular mucosal epithelia to damaging desiccation and hyperosmolar stress, accompanied by wetting and friction defects (Bron et al. 2017). The importance of continuous protection of the ocular surface was discovered through the inclusion of OPI (Bron et al. 2017; Ousler et al. 2002, 2005, 2008; Work-Sho CS of the IDE 2007) as an efficacy parameter in the study. Although having been used in clinical trials in the past (Evangelista et al. 2011; Nebbioso et al. 2013; Ousler et al. 2007; Rolando et al. 2009; Simmons & Vehige 2007; Torkildsen et al. 2008), OPI would deserve more appreciation as a methodological tool that captures the functional relationship between eye blinking and tear film stability at the

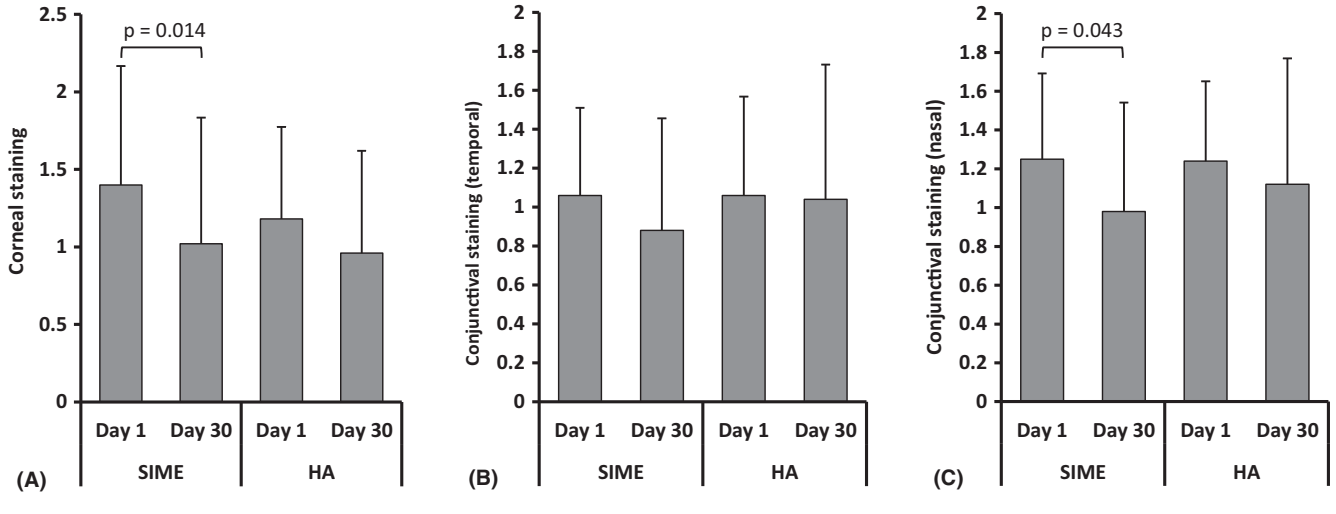

Fig. 5. Signs of ocular surface damage. A, Corneal staining; B, temporal conjunctival staining; C, nasal conjunctival staining. Mean ± SD of the mean values of eyes (Oxford scale 0–5) in PP population of Part 3 (SIME, n = 24; HA, n = 25).

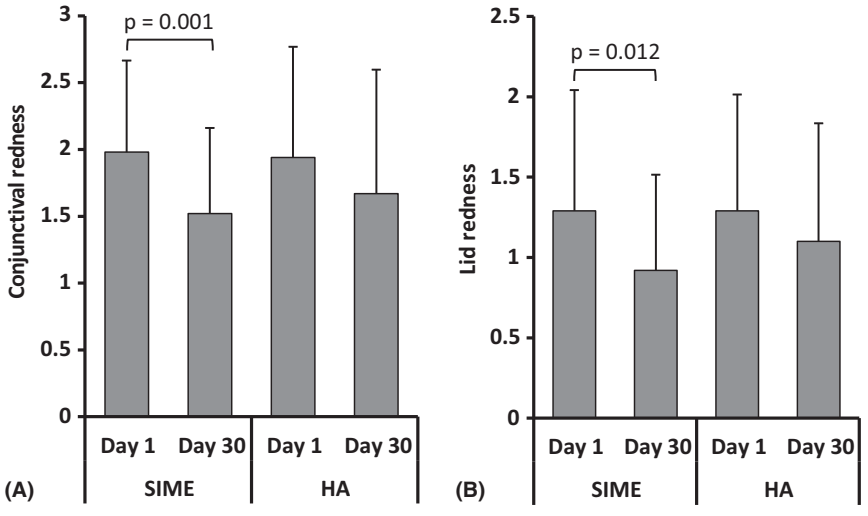

Fig. 6. Signs of ocular surface inflammation. A, Conjunctival redness, B, lid redness. Mean ± SD of the mean values of eyes (IER scale 0–4) in safety/ITT data set population of Part 3 (n = 26).

individual level. Measuring TBUT without IBI gives little information of the ocular protection status; even significant increases in TBUT may be insufficient for protection, if IBI is correspondingly increased (i.e. blink rate is significantly decreased), as evidenced to be possible (Stonecipher et al. 2016). Both parameters can be easily measured. In some of the previous studies, OPI was raised at least in a portion of subjects to just above 1.0 at 5 min (Ousler et al. 2007) to 60 min (Evangelista et al. 2011) after eye drop instillation and lasting up to 7 days of daily regimen (Rolando et al. 2009). In the present study, TBUT exceeded the unchanged IBI at 10 days (OPI > 1), continuing improvement to a level of 1.5 at 30 days with emulsion eye drops. Compared to fluorescein TBUT, a noninvasive technique could have produced still higher protection values (Wolffsohn et al. 2017). The gradual improvement of OPI during 30 days may reflect the fact that the recovering epithelial viability likely supports stabilization of the tear film and *vice versa*. It is notable that the improvement in OPI could not be an immediate treatment reaction, because instillation of the last dose took place in the evening at least 8 hours before assessments. A single instillation of a 50% higher concentration of trehalose was shown to increase tear film thickness in dry eye patients for a few hours compared to HA eye drops (Schmidl et al. 2015) with low molecular weight and viscosity (Salzillo et al. 2016); no significant effect on TBUT was seen (Schmidl et al. 2015). The observed improvement in OPI by the SIME formulation versus HA eye drops cannot, therefore, be attributed to the short-term action of trehalose, but rather to strengthening of the lipid layer and lowering of tear surface tension (Gokul et al. 2018) by the sacha inchi seed oil component (Fig. S1). The ocular surface was obviously tear film-protected in a more permanent way in most patients using SIME (median OPI = 1.4) after 30 days, signifying effectively no tear film break-ups within blink intervals. The presence of a protective biophysical microenvironment on the ocular surface can be substantiated by noting the greater effect size for SIME in tear film instability, osmolarity, and inflammation and damage parameters (Table 2).

Interestingly, tear osmolarities decreased with both treatments and seemed to stop approximately at the healthy level, that is around 300 mOsm/l (Wolffsohn et al. 2017), independent of the initial tear osmolarity. In hyperosmolar tears, a larger reduction was observed, but again, to no lower than the healthy tear level. The changes were consistent with the minimal clinically important difference (MCID) (Wolffsohn et al. 2017). Also, the changes were independent of whether hypotonic (SIME) or isotonic (HA) eye drops were used, suggesting that hypotonicity of eye drops may have little relevance in reducing tear hyperosmolarity to a clinically meaningful extent. Studies that link the ability of ocular lubricants to reduce tear film osmolarity and their impact on symptoms and signs of dry eye have been called for (Jones et al. 2017). As tear osmolarity is currently outlined to be under the influence of body hydration, tear film lipid layer characteristics, palpebral aperture, blink interval, tear film stability and environmental conditions (Wolffsohn et al. 2017), it seems evident that a change in the properties of the tear film itself is the only explanation for the decrease in tear osmolarity now observed; blink interval was not changed, and randomization should have eliminated the influence of the remaining factors. Therefore, correcting tear film instability – including its lipid layer – is a major determinant for managing tear hyperosmolarity as well.

Hyperosmolarity reinforces oxidative and inflammatory reactions causing ocular surface cell damage (Baudouin et al. 2013; Clouzeau et al. 2012; Marek et al. 2018). Corneal and conjunctival fluorescein staining marks the compromised integrity of tight junctions or the glycocalyx of viable ocular cells, whereas conjunctival redness indicates vascular dilatation as a sign of inflammation (Wolffsohn et al. 2017). The SIME regimen decreased these objective parameters significantly after 30 days – with no marked changes yet at 10 days – corroborating the fact that tissue damage and inflammatory activity are late-stage markers (Novack et al. 2017) which follow changes in tear film stability and osmolarity. It is possible that natural antioxidants, especially high concentration of γ-tocopherol in sacha inchi seed oil (Chirinos et al. 2015; Wang et al. 2018), provided cytoprotective and wound-healing activity by attenuating hyperosmolarity-associated oxidative stress. Also, trehalose (Čejková et al. 2012; Chiambaretta et al. 2017; Luyckx & Baudouin 2011; Schmidl et al. 2015), HA (Albano et al. 2016; Li et al. 2013; Pauloin et al. 2009) and omega-3 polyunsaturated fatty acids (Zárate et al. 2017) have been found effective in these processes.

Clinical symptoms of dry eye likely originate from the damaged ocular surface causing nociceptive or neuropathic pain (Aggarwal & Galor 2018). Hyperosmolarity, dryness and cooling by tear evaporation also induce the release of pro-inflammatory mediators

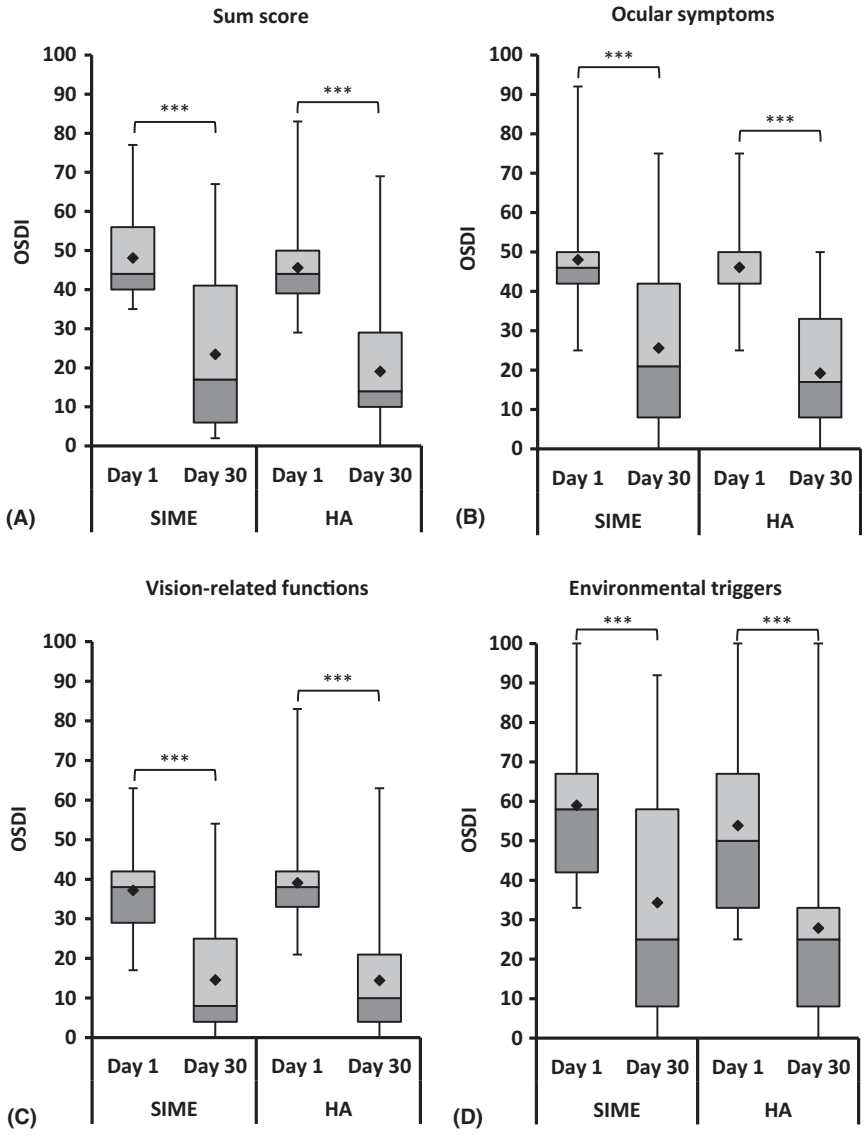

Fig. 7. Box–whisker plot of ocular surface disease index (OSDI). A, OSDI sum score; B–D, subscale scores of OSDI. The mean is marked with a diamond. Boxes represent interquartile ranges separated by the median; whiskers indicate the minimum and maximum values. All data are from ITT population of Part 3 ($n = 26$). ***$p < 0.0001$.

which aggravate neuronal activity, sensitivity and damage (Belmonte et al. 2017). Regenerating ocular mucosa with resolving inflammation can thus be expected to mitigate ocular symptoms. The symptoms experienced by the patients indeed decreased in a consistent, time-dependent, and clinically highly relevant and significant magnitude, again corroborating the expectation that counteracting the aetiologic factors will ultimately lead to improvement of symptoms.

The mean reduction in the OSDI sum score was −23% in 10 days and −53% in 30 days, clearly reflecting time dependence. Improvements by 25 OSDI units have usually not been achieved in randomized clinical trials within similar time frames (Essa et al. 2018; Fariselli et al. 2018; Kim et al. 2017). The observed change of 2.6 times the MCID (Miller et al. 2010; Wolffsohn et al. 2017) demonstrates the SIME and control formulations capable of reducing symptoms to a clinically meaningful extent that can be noticed by the patient. OSDI was also one of the primary end-points, selected to demonstrate superiority of SIME over HA control. This goal was not achieved due to the unexpectedly strong effect of the control eye drops on symptom scores. The OSDI results highlight the benefit from HA alone, provided a sufficiently high molecular weight is used, although HA did not significantly change all aetiologic factors for dry eye. A lipid component may prolong the retention of eye drops on the ocular surface (Maïssa et al. 2010) and provide a prophylactic effect against aggravation of symptoms in certain environmental conditions (Gokul et al. 2018). Improvement in dry eye symptoms has direct consequences in the quality of life of patients typically complaining of problems with visual function, such as glare and blurred or fogged vision (Novack et al. 2017). A clear difference should be made between dry eye-related and eye drop-induced blurring of vision; the former is a chronic trouble and a part of functional symptom questionnaires (such as OSDI question number 4), whereas the latter is an immediate and transient consequence of instilling liquid on the ocular surface. Blurring asked in the additional questionnaire (Table S4) is referring to instillation-induced blurring, mainly resulting from formulation characteristics such as volume, viscosity and surface tension. Furthermore, visually and cognitively demanding work reduces blink rate and OPI and may therefore be a prominent risk factor for dry eye and its symptoms (de Kluizenaar et al. 2016). Reduced symptoms may also contribute to improvement of certain neurological or psychological factors (Galor et al. 2015).

Weaknesses of the study include the sample size which, were it larger, could have revealed more parameters with statistically significant changes or differences between treatments, possibly without an MCID or significantly different changes in OSDI. Selecting an active control was considered ethically justified for patients with a life-restricting disease. Based on total evidence, the effect of SIME was consistently larger on all signs (Table 2), suggesting nonequivalence of interventions. A planned limitation was the comparison between contralateral eyes in Part 2, successfully used in dry eye studies (Larmo et al. 2018 and references therein), although potentially subject to intereye signalling (Novack et al. 2017); both eye drops contain 0.2% HA and showed all effects to the same direction, diminishing the likelihood of a blocking effect in the fellow eye. The safety results of Part 2 were also necessary to proceed to Part 3.

Furthermore, the baseline characteristics may indicate a bias in selecting participants with slightly more severe average signs and symptoms for Part 2 than for Part 3; however, assignment to study parts was based on subject availability and could not affect randomization within either part. As a final potential limitation, tear production was not measured to focus on DEWS TFOS II aetiologic factors (Bron et al. 2017; Nelson et al. 2017) which do not include deficient tear production.

Dry eye prevalence increases linearly with age and is associated with female sex, the two most important risk factors (Stapleton et al. 2017). The current data are believed to be obtained from a patient population representing these characteristics (Table 1), and thus, interpretation of the benefits of sacha inchi microemulsion and HA eye drops can be generalized for all patients with moderate or severe dry eye.

Conclusions

The results of this clinical trial suggest that disruption of hyperosmolar stress is indispensable for rescue processes on the ocular epithelia to begin. This can be achieved by establishing continuous biophysical protection for the ocular surface by supporting the stability of the tear film layers and by normalizing its osmolarity. OPI and tear osmolarity proved reliable analytical tools to verify the turning point. The novel microemulsion formulation including sacha inchi seed oil is presumably the first eye drop designed and tested to target (1) tear film instability, (2) hyperosmolarity, and (3) cell damage and inflammation on the ocular surface, the aetiologic factors for dry eye. Measurable, clinically meaningful and statistically significant improvements in each aetiologic category were observed within 1 month of daily treatment regimen, accompanied by significantly relieved subjective symptoms. Changes in signs exceeded the effects of a conventional control eye drop with a single active agent only. The lipid component with sacha inchi is a novel supplement which improves ocular surface protection and tear film characteristics in addition to HA, trehalose and glycerol. Patients with dry eye are expected to benefit from an effective, nonpharmacologic and topical management option also by easier product availability, reduced costs, minimal adverse effects and undesirable side-effects, and improved quality of life.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Scheme for disruption of the 'vicious circle' of dry eye by sacha inchi microemulsion eye drop components (A) and consequential changes observed in the clinical trial (B).
Table S1. Concomitant medications (number of users in ITT/safety population).
Table S2. Dosing compliance (PP population; mean ± SD).
Table S3. Safety and tolerability (ITT/safety population; mean ± SD).
Table S4. Descriptive statistics for additional user questionnaire used in Part 3 (ITT population).