

## A SODIUM HYALURONATE OPHTHALMIC SOLUTION FOR REDUCING DRY EYE AND ENHANCING CORNEAL WOUND HEALING AFTER PHOTOREFRACTIVE KERATECTOMY

NGAMJIT KASETSUWAN<sup>1\*</sup>, USANEE REINPRAYOON<sup>1,2</sup>

<sup>1</sup>Department of Ophthalmology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. <sup>2</sup>Department of Ophthalmology, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. Email: ngamjtk@gmail.com

Received: 20 November 2014, Revised and Accepted: 02 January 2015

### ABSTRACT

**Objective:** The objective of this work was to evaluate the efficacy and safety of 0.3% sodium hyaluronate (SH) versus hydroxypropyl-methylcellulose (HPMC)/dextran in reducing dry eye symptoms, recovering tear film function and enhancing corneal wound healing in patients who underwent photorefractive keratectomy (PRK) surgery.

**Methods:** This was a randomized, masked-assessor, controlled, parallel-group and Phase II trial. 24 patients received 1 drop of 0.3% SH or HPMC/dextran into each eye every 2 hrs during the first month after surgery, then as needed until the day (D) 168. Efficacy and safety criteria were assessed during six follow-up visits (D1, D3, D7, D28, D84 and D168).

**Results:** About 0.3% SH caused a greater improvement than control in half of the efficacy criteria, including symptoms intensity and frequency, comfort of the eye drops, corneal haze, Schirmer I test, fluorescein and rose Bengal staining. At D28 and D84, a statistically significant difference favoring 0.3% SH was demonstrated for fluorescein corneal staining ( $p=0.0114$  and  $p=0.0308$ , respectively). At D3, completed re-epithelization of the cornea was observed, 98% and 96% in 0.3% SH and control group respectively. The overall results for each of the measures used to assess the safety indicated that 0.3% SH had an excellent safety profile over 168 days of treatment.

**Conclusion:** About 0.3% SH is a safe and effective treatment in reducing dry eye and enhancing corneal epithelial wound healing after PRK surgery.

**Keywords:** Dry eye, Artificial tears, Sodium hyaluronate, Ocular lubricants, Refractive surgery, Photorefractive keratectomy.

### INTRODUCTION

Nowadays, photorefractive keratectomy (PRK) surgery is wide spread for correction of various degrees of refractive errors, such as myopia and hyperopia [1,2]. However, one of the major disadvantages encountered after this procedure is a decreased corneal sensitivity, resulting from the reduction in sensitive corneal receptors due to the cornea cutting. This leads to post-operative reduced tear secretion and increased osmolarity of the tear film [3], tear film instability [4] and consequently dry eye symptoms [5,6], which is the most common complications after refractive surgery [7]. In some cases, epithelial wound healing is also hindered [8]. As a consequence, most surgeons prescribe non-preserved artificial tears for reducing dry eye symptoms and increasing tear film stability following PRK surgery.

The recent exploitation of polymers with novel rheological properties, such as hyaluronic acid (HA), led to the formulation of artificial tears with beneficial effects on the relief of dry eye [9-13]. The most important property of sodium hyaluronate (SH) solutions is their viscoelasticity. This property allows such solutions, when instilled into the eye, to behave differently during and between blinks [14]. During blinks, shear stress causes the molecules of SH in a solution to align with one another. As a result, the solution becomes elastic and relatively non-viscous, and spreads easily over the surface of the cornea. Between blinks, the molecules of SH form a tangled meshwork, and the solution becomes less elastic and more viscous. Consequently, the pre-corneal tear film is stabilized and the residence time of the solution on the surface is maximized. SH is also highly effective in entrapping water. Therefore, evaporation of water from SH solutions is slow, and the beneficial effects of such solutions are prolonged [15]. Finally, SH solutions adhere well to the mucins of the ocular surface, forming long-lasting coatings [16].

The patented formulation of 0.3% SH contains ions naturally present in the tear fluid to maintain the physiology of the cornea, namely calcium,

magnesium, potassium, sodium and chloride. The SH in the present formulation has several specific features that help to maximize the efficacy and tolerability of this product in patients with dry eye. It is obtained by bacterial fermentation and is, therefore, free from potentially allergenic animal proteins, and it contains a specific fraction of SH with a high degree of purity. These eye drops also have a pH of 7.2-7.4, similar to that of natural tears, and are free from preservative. Furthermore, they have been formulated to be hypotonic, in order to compensate the hypertonicity of tears in patients experiencing ocular dryness [17]. The investigated product, containing 0.3% of SH (i.e., 3 times the concentration of 0.1% that is the minimum necessary for efficacy in the treatment of dry eye [18]) could be a promising ophthalmic lubricant in post PRK surgery. It has been shown to be efficient in significantly reducing dry eye symptoms [19], enhancing pre-corneal tear film stability and uniformity [20], relieving sensation of dryness and improving contact lens wear comfort [18].

The aim of this study was to assess the efficacy and safety of 0.3% SH in reducing dry eye symptoms, recovering tear film function and enhancing corneal wound healing in patients who have undergone PRK surgery. The comparative product was hydroxypropyl-methylcellulose (HPMC)/dextran as a standard treatment of dry eye.

### METHODS

The final protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. The study was consistent with an international conference on harmonization of technical requirements for registration of pharmaceuticals for human use, good clinical practice and the applicable regulatory requirements including the declaration of Helsinki.

### Study design

This was a masked-assessor, randomized, controlled, parallel-group and Phase II trial in patients who have undergone PRK surgery.

The study was carried out in one center in Thailand. A total of 24 patients were randomized to receive 1 drop of 0.3% SH (n=12) or HPMC/dextran (n=12) every 2 hrs until the D28 visit, then every 2 hrs as needed when having dry eye symptoms until D168. The study consisted of eight visits, namely D-30 to D-2 (screening, pre-operative assessments and baseline), D0 (PRK surgery and randomization), and D1, D3, D7, D28, D84 and D168 as follow-up visits.

At the screening visit, patients were checked for compliance with the inclusion and exclusion criteria (pre-PRK assessments) and were instructed not to use any concomitant in-eye medication until the end of the study.

At D0, eligible patients underwent primary PRK surgery in one or both eyes. Post-PRK assessments were performed and treatment, 0.3% SH or HPMC/dextran, was provided in accordance with the randomization list on the separate sealed envelope, by an independent research assistant so as to maintain the assessor blind to the treatment received. In the case of bilateral surgery, the patient was assigned to the same treatment in both eyes.

At each follow-up visit, patients returned to the clinical site for efficacy and safety evaluations, which were performed in both eyes. Patients were asked not to use the test products for at least 2 hrs before assessments and measurements at each follow-up visit. Efficacy assessments performed included average number of instillations, symptom intensity and frequency, repercussion of symptoms on daily life activities and comfort of the eye drops, refraction, corneal haze, Schirmer I test, corneal topography and central corneal pachymetry, corneal sensitivity, tear film break-up time (TBUT), corneal staining with fluorescein, conjunctival staining with rose Bengal, phenol red thread, corneal mapping, tolerability assessments (slit lamp examination and examination of ocular adnexa). Safety criteria consisted in visual acuity measurements and collection of adverse events (AEs) (Table 1).

#### Study materials

Solution of 0.3% SH was supplied in its commercially available packaging (each containing 20 monodoses) by the manufacturer (Vismed® Gel, Holopack Verpackungstechnik GmbH, Abtsgmünd-Untergröningen, Germany) in heat-sealed monodoses containing 0.45 ml of sterile solution. One drop of 50 µl contains 150 µg of SH (0.3% w/v).

A commercially HPMC/dextran product (Tears Naturale Free®, Laboratoires Alcon, France) was used in its original monodoses, corresponding to 0.9 ml of sterile, clear and colorless solution made of 0.3% HPMC and 0.1% dextran 70.

#### Study population

Male and female patients aged between 15 and 60 years, with myopia (-0.50 to -12.00D) and astigmatism (up to -4.00D) and with stable refraction (change of refraction <0.50D over past year), were enrolled in the study.

Main exclusion criteria were dry eye, defined as follows: Two or more dry eye symptoms (among soreness, itchiness, dryness, grittiness and burning) or, Schirmer I test of <10 mm wetting/5 minutes, TBUT of <7 seconds, total score of corneal staining with fluorescein more than 3. Furthermore, patients with previous PRK surgery, corneal sensitivity <40 mm, central pachymetry <450 µm or any other ocular surgery or trauma within the last 4 months prior to study inclusion.

#### Statistics

For each parameter, the mean of both eyes was used if surgery was done on both eyes bilaterally and the value of the operated eye if surgery was done on one eye. For continuous parameters or scores, the Student's t-test or the Wilcoxon test (in-case of non-normality of the distribution in at least one treatment group) were applied. For binary parameters, the Chi-square test was used (Fisher exact test in-case of low estimated figures). For ordinal parameters, the Mantel-Haenszel Chi-square test

was used. The parametric two-sided 95% confidence interval for the differences between the two treatment groups was issued.

Clinical objectives were the comparison between the two treatment groups at each time point, and the baseline value was defined as the value at the screening visit.

Although no determination of sample size was carried out since this was an exploratory study, the sample size rationale was based on two considerations. First by estimating treatment effects (and their associated standard deviations) and second, by providing initial data in case a relatively large improvement could be seen with respect to the comparison against control at least on an exploratory basis. The sample size was planned for 24 per protocol evaluable patients per study group.

For inferential purposes of exploratory statistical tests in quantitative variables it should be noted that this sample size had a power of 80% at the two sided 5% significance level (t-test or U-test methodology) to detect critical differences in averages of about one standard deviation.

Categorical variables could have been assessed by means of e.g. 2 by 3 contingency tables with the Chi-square test. Under the assumption of the same error probabilities quoted above, a maximum critical difference around some 50%-points can be detected. The analysis was performed using SAS statistical software.

The following data sets were defined. The intent-to-treat (ITT) data set was defined as patients who had at least one administration of the allocated product and a value at baseline for any efficacy endpoint. The full analysis set (FAS) was defined as patients who had at least one administration of the allocated product and a non-missing value for any efficacy endpoint at baseline and at least one time after instillation. The per-protocol (PP) dataset included all patients of the FAS data set without major deviation of the protocol.

After acquisition of baseline data, in the case of missing values, the last observation carried forward (LOCF) method was applied using at each visit the last observation available for analysis on FAS and with LOCF baseline value included for analysis on ITT population.

## RESULTS

### Patient disposition

A total of 24 patients were screened and randomized, i.e. 12 in each treatment group. To avoid bias, the study was stopped before the recruitment of the 48 patients planned because the equipment used for surgery has been changed by the institution (King Chulalongkorn Memorial Hospital) after 24 patients. Two patients in the control group were prematurely discontinued. In both patients, the reported reason for premature discontinuation was "patient migrated to abroad," occurring after D84. The ITT, FAS, and PP data sets consisted of 24, 24 and 23 patients, respectively.

### Demographic data

There were no statistically significant differences between the groups for demographic and baseline characteristics (data not shown). Patients were predominantly female (66.7%) and had a mean±standard deviation (SD) age of 30.3 (5.0) years (minute: 21.0; max: 45.0; median: 30.0 years). Ophthalmic history, mainly dry eye, was reported by 25.0% of the patients. They were included because their dry eye symptoms had been recovered before recruitment and they fulfilled inclusion and exclusion criteria. A total of 16.7% of patients had a non-ophthalmic history, including skin and subcutaneous tissue disorders (8.3%), psychiatric disorders (4.2%) and respiratory, thoracic and mediastinal disorders (4.2%).

### Efficacy endpoint

Results for the efficacy endpoints for the ITT population are summarized in Table 2.

Table 1: Schedule of procedures and assessments

Procedures and assessments	V1	V2	V3	V4	V5	V6	V7	V8
	D-30 to D-2 screening pre-operative assessments	D0 surgery randomisation	D1±0 D	D3±0 D	D7±2 D	D28±3 D	D84±3 D	D168±3 D
Demographic data	X							
Eye selection for PRK surgery	X							
Medical history	X							
Concomitant diseases and medications	X	X	X	X	X	X	X	X
Verification of inclusion and exclusion criteria	X	X						
Informed consent	X	X						
PRK surgery		X						
Allocation of eye to treatment by research assistant (after surgery)		X						
First administration of the assigned treatment in the presence of the research assistant (after surgery)		X						
Drug accountability by research assistant			X	X	X	X	X	X
Visual acuity	X**					X	X	X
Refraction (manifest and/or cycloplegic)	X				X***	X	X	X
Corneal haze						X	X	X
Examination of ocular adnexae	X		X	X	X	X	X	X
Symptom intensity on VAS	X		X	X	X	X	X	X
Symptom frequency	X		X	X	X	X	X	X
Repercussion of symptoms on activities of daily life	X		X	X	X	X	X	X
Comfort of the eye drops			X	X	X	X	X	X
Slit lamp examination	X		X	X	X	X	X	X
Corneal sensitivity	X				X*	X	X	X
Schirmer I test	X					X	X	X
Phenol red thread	X				X*	X	X	X
TBUT	X				X*	X	X	X
Corneal staining with fluorescein	X				X*	X	X	X
Conjunctival staining with rose Bengal	X				X*	X	X	X
Corneal mapping				X	X			
Corneal topography	X					X	X	X
Central corneal pachymetry	X					X	X	X
AE report	X	X	X	X	X	X	X	X

\*Was assessed in case the epithelium was healed, \*\*Counting finger, \*\*\*After contact lens removal. VAS: Visual analogue scale, TBUT: Tear film break-up time, AE: Adverse events, PRK: Photorefractive keratectomy

For fluorescein staining, 0.3% SH caused a larger decrease in mean±SD corneal staining scores of fluorescein compared with control. At D28 and D84, this decrease was statistically significant ( $p$  [Wilcoxon]=0.0114 and 0.0308, respectively) in favor of 0.3% SH (Fig. 1).

Regarding corneal mapping, completed re-epithelization of the cornea was observed (98% in 0.3% SH group at D3). The comparison of mean±SD values of transitional and healed zone at D3 and D7 showed no statistically significant differences between the two treatment groups. In the 0.3% SH group, corneal haze was reported in none of the patients at D168, whereas opacities were still reported in 3 out of 12 patients in the control group (Fig. 2).

There was a trend for 0.3% SH to cause a larger improvement than control in conjunctival staining with rose Bengal. There was a greater increase in mean±SD values of tear volume in the 0.3% SH group compared with the control group. However, no statistically significant differences were observed between the two groups for Schirmer I test. For phenol red thread and TBUT, no statistically significant differences in mean±SD values were found between the two groups.

Regarding symptom intensity and frequency, 0.3% SH caused a faster decrease than control but no statistically significant differences between the two groups were observed at any time point. There was no statistically significant difference in the repercussion of symptoms on daily life activities between the two groups.

Interestingly, 0.3% SH showed better appreciation by the patients for the comfort of the eye drops immediately after instillation at D1, D3 and D184.

#### Safety criteria

One AE was reported in the control group. This AE was serious, of mild intensity, occurred accidentally and was considered by the investigator as not related to the study treatment. This AE was resolved after treatment and its duration was 30 days.

The comparison of mean±SD un-corrected visual acuity values showed no statistically significant ( $p$  [Student or Wilcoxon]≥0.2255) differences between the two treatment groups in the safety population.

At all-time points, eye lids and periocular adnexae were rated by the investigator as "normal" in all the patients of the two groups for the safety population.

There were no statistically significant ( $p$  [Wilcoxon]≥0.1286) differences between the two treatment groups of mean±SD scores of the slit lamp examination at any time point for the safety population.

#### DISCUSSION

Nowadays, more attention is given towards comfort of patients after corneal refractive surgery. The appearance of signs and symptoms of dry eye in the days following surgery is frequently observed and the search for a better handling of such patients is requested [19,21].

**Table 2: The efficacy endpoints (symptom intensity, frequency and their impact on daily life activities, comfort of the eye drops, corneal haze, Schirmer I test, corneal sensitivity, TBUT, staining with fluorescein and rose Bengal, and phenol red thread) in the ITT population**

	Time point (D=Day)	HPMC/dextran (n=12)	0.3% SH (n=12)	p value	Confidence interval (95%)
Symptom intensity (difference from baseline, mean±SD, summed visual analogue scales, from 0 to 500)	D1-D0	128.75±104.83	133.67±93.50	0.9046	-89.01, 79.18
	D3-D0	70.38±61.75	43.38±66.07	0.3123	-27.14, 81.14
	D7-D0	20.71±58.66	18.71±31.19	0.9179	-37.77, 41.77
	D28-D0	2.88±48.58	0.54±21.95	0.8809	-29.58, 34.25
	D84-D0	-21.21±33.80	-4.58±28.13	0.2880	-42.95, 9.70
	D168-D0	-22.13±32.99	-16.38±26.55	0.8774	-31.10, 19.60
Symptom frequency (difference from baseline, mean±SD, summed scores, from 0 to 15)	D1-D0	5.75±4.54	4.92±3.23	0.6094	-2.50, 4.17
	D3-D0	3.08±2.15	2.17±2.66	0.3631	-1.13, 2.96
	D7-D0	1.58±2.50	1.75±2.05	0.6119	-2.10, 1.77
	D28-D0	0.75±1.71	0.25±1.54	0.7568	-0.88, 1.88
	D84-D0	-0.42±1.00	-0.08±1.16	0.4484	-1.25, 0.58
	D168-D0	-0.50±0.90	-0.58±0.90	0.6319	-0.68, 0.85
Repercussion of symptoms on daily life activities (difference from baseline, mean±SD, score, from 0 to 3)	D1-D0	1.00±1.13	1.08±1.00	0.9519	-0.98, 0.82
	D3-D0	0.50±1.00	0.67±1.15	0.7091	-1.08, 0.75
	D7-D0	-0.17±0.58	0.17±0.58	0.1771	-0.82, 0.16
	D28-D0	0.00±0.85	0.00±0.74	0.6293	-0.68, 0.68
	D84-D0	-0.25±0.45	-0.08±0.67	0.2377	-0.65, 0.32
	D168-D0	-0.25±0.45	-0.17±0.58	0.373	-0.52, 0.36
Comfort of the eye drops (score, from 0 to 2)	D1	0.75±0.45	0.67±0.49	0.6870	NA
	D3	0.33±0.49	0.17±0.39	0.3764	
	D7	0.17±0.39	0.25±0.62	0.9645	
	D28	0.17±0.39	0.25±0.45	0.6520	
	D84	0.33±0.49	0.17±0.39	0.3764	
	D168	0.08±0.29	0.17±0.39	0.5797	
Corneal haze (score, from 0 to 4)	D28	0.08±0.16	0.10±0.20	0.9393	NA
	D84	0.10±0.29	0.13±0.25	0.6843	"
	D168	0.13±0.25	0.00±0.00	0.0788	"
Schirmer I test (difference from baseline, mean±SD, mm wetting/5 minutes)	D28-D0	0.08±8.40	5.46±10.88	0.1892	-13.60, 2.85
	D84-D0	0.17±9.18	1.13±10.39	0.8171	-9.26, 7.34
	D168-D0	0.08±10.76	4.33±11.22	0.3538	-13.56, 5.06
Corneal sensitivity (difference from baseline, mean±SD, score, from 0 to 3)	D7-D0	-2.50±8.66	-3.96±8.62	0.3740	-5.86, 8.78
	D28-D0	-2.08±5.31	-0.83±1.95	0.8939	-4.64, 2.14
	D84-D0	-0.83±1.95	0.00±0.00	0.1658	-2.00, 0.33
	D168-D0	0.00±0.00	-0.21±0.72	0.3593	-0.22, 0.64
TBUT (difference from baseline, mean±SD, seconds)	D7-D0	-3.13±2.89	-3.78±3.99	0.9770	-2.30, 3.60
	D28-D0	-2.35±3.44	-2.60±3.02	0.8517	-2.49, 2.99
	D84-D0	-1.38±3.87	-2.54±4.22	0.4877	-2.26, 4.59
	D168-D0	-1.71±2.44	-1.25±3.96	0.7361	-3.24, 2.33
Staining with fluorescein (difference from baseline, mean±SD, summed scores, from 0 to 12)	D7-D0	2.04±1.56	1.75±2.16	0.7466	-1.30, 1.89
	D28-D0	1.17±1.39	-0.92±1.77	0.0114*	0.74, 3.43
	D84-D0	0.21±1.45	-0.88±1.21	0.0308*	-0.05, 2.21
	D168-D0	0.04±1.29	-0.38±1.37	0.5353	-0.71, 1.54
Staining with rose Bengal (difference from baseline, mean±SD, global score of type and extent, from 0 to 3)	D7-D0	-0.33±1.11	-0.08±1.41	0.6349	-1.33, 0.83
	D28-D0	0.21±1.60	0.17±1.29	0.9446	-1.19, 1.27
	D84-D0	0.67±1.78	-0.04±1.27	0.2730	-0.60, 2.01
	D168-D0	0.13±1.73	-0.25±1.14	0.5375	-0.87, 1.62
Phenol red thread (difference from baseline, mean±SD, mm wetting/15 seconds)	D7-D0	-1.38±9.84	-2.75±8.65	0.7196	-6.47, 9.22
	D28-D0	3.21±10.10	-2.79±6.38	0.0958	-1.15, 13.15
	D84-D0	-0.88±8.81	-3.00±9.91	0.5844	-5.81, 10.06
	D168-D0	-1.79±5.97	1.58±8.59	0.2758	-9.64, 2.89

\*p<0.05, NA: Not applicable, SD: Standard deviation, TBUT: Tear film break-up time, ITT: Intent-to-treat, HPMC: Hydroxypropyl-methylcellulose, SH: Sodium hyaluronate

The present study showed that both artificial tears, 0.3% SH and HPMC/dextran, alleviate the signs and symptoms of dry eye disease, broken out in patients who have undergone PRK surgery. The HA based eye drops improved in a larger extent, than the comparative product, the following parameters: Symptom intensity and frequency, corneal haze, Schirmer I test, TBUT, corneal and conjunctival staining as long as the comfort of the eye drops.

The present data showed a decrease in tear film stability of about 25% after 4 weeks, when considering the mean of both groups. This is a distinct improvement in comparison with the 50% of decrease in tear film stability, usually reported at 6 weeks after PRK surgery [22,23]. The beneficial effect of artificial tears on the TBUT is here demonstrated and this parameter improved all along the trial with variation relatively to baseline.

A similar observation can be done for tear flow with a trend in favor of 0.3% SH compared to HPMC/dextran. The literature shows a deficient decrease of Schirmer I test in post PRK of about 50% at 6 weeks [23]. Communications between the ocular surface and lacrimal glands occurs through a sensory automatic neural reflex loop. The sensory nerves innervating the ocular surface connect with efferent autonomic nerves in the brain stem that stimulate secretion of tear fluid and proteins by the lacrimal glands [7]. In PRK, corneal sensitivity is indisputably altered due to the damage to the sensory nerve endings terminating corneal epithelium, which is removed by mechanical scraping during the procedure [19]. This unavoidable consequence of such a surgery compromises therefore the protective blink reflex and delay epithelial wound healing [24,25]. Both investigated products faced against this loss of sensitivity and the return to baseline values is observed after 3 months of treatment in case of 0.3% SH, and 6 months after HPMC/dextran.



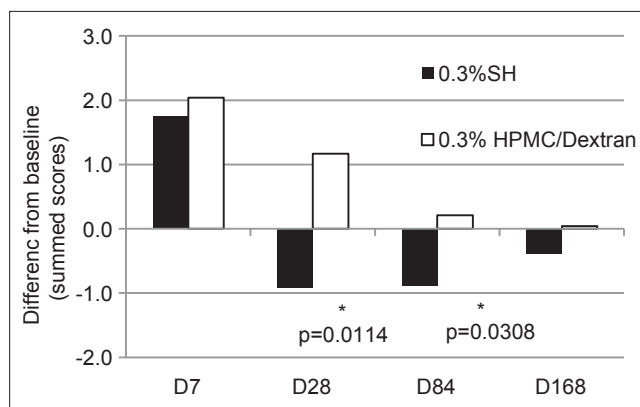
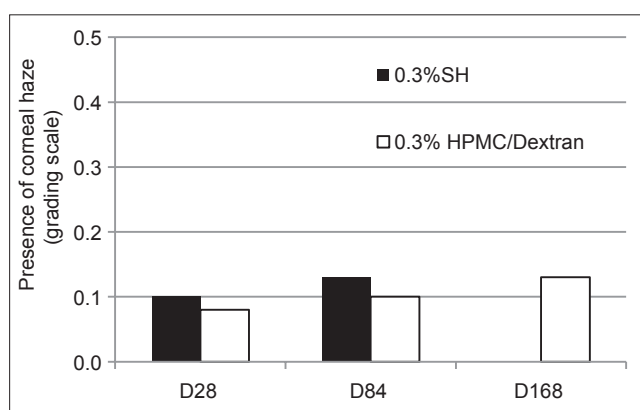


Fig. 1: Difference from baseline of the corneal fluorescein staining



In terms of fluorescein staining, the difference was statistically significant ( $p < 0.05$ ) after 1 and 3 months. At 6 months, there is still a better improvement due to the SH-based product, but the difference with the HPMC/dextran formulation is no more statistically significant ( $p = 0.5353$ ). This observation could be explained by the clinical status for this criteria that return to baseline level as post-surgical healing took place. Patients did not suffer from dry eye at the inclusion, therefore this is not surprising to not observe any statistically significant difference after a certain period of time. As reported earlier, fluorescein staining data in favor of the SH-based product, indicates its beneficial effect on the integrity of the ocular surface [15]. On a more general view point, numerous reports evidenced that SH has improved signs and symptoms of dry eye disease [15-20].

Regarding corneal haze which is a specific concern after refractive surgery, a trend in favor of the SH-based eye drops compared with the HPMC/dextran ones can be highlighted at d168. Such opacity within the cornea could be due to inflammation as long as the presence of cellular residues occurring after surgery [26]. It is therefore reasonable to hypothesize that the HA would bring an anti-inflammatory effect facilitating corneal haze diminution. The clinical anti-inflammatory properties of HA have been alluded in the light of recent studies demonstrating that HA acts as a biological inhibitor of inflammation [27,28]. In fact, it has been shown to suppress interleukin-1 $\beta$ -induced matrix metalloproteinase-1 (MMP-1) and MMP-3 expression [29]. However, the mechanism(s) involved remain unknown.

Another consequence of surgery is the release within the tears of inflammatory mediators and growth factors involved in the natural healing process of the cornea [30,31]. The delicate balance between the necessary inflammatory mediators and an overproduction shows

itself to be often disturbed after PRK intervention. Furthermore, local inflammation after PRK increases discomfort and healing time. Pro-inflammatory cytokines produced in response to the epithelial insult penetrate into the stroma, where they stimulate inflammatory factors. Such a response may induce pain and prolong the healing process [26,32]. Significant positive correlation has been observed between the levels of inflammatory cytokines in the conjunctival epithelium and the severity of ocular irritation [33]. The anti-inflammatory effect of the HA could be one of the main asset of the 0.3% SH formulation to support efficiently the corneal healing and the corneal haze disappearance.

HA has also been shown to have wound healing properties and to promote epithelial migration [34,35]. The stable protective coating that HA forms over the cornea prevents further damage and allows natural healing to take place more rapidly. Usually, a cornea epithelial healing is observed not later than 3 to 5 days after PRK surgery [36,37]. This is in agreement with our corneal mapping results. At D3, more than 96% of the patients completed their healing, with a trend in favor of SH. In that light, treatment with 0.3% SH appeared as the most efficient in terms of rapidity of onset. Numerous randomized clinical trials showed that investigated SH-based eye drops were well tolerated and caused a better improvement of dry eye disease than comparative products [38-40]. Specifically, the SH has been compared to HPMC/dextran and demonstrated a greater improvement in tear film stability than to the use of SH [41,42].

The physical similarity between the tear film and certain SH solutions is believed to contribute to the efficacy and tolerability of these solutions when they are used as lubricant eye drops [43,44]. One could hypothesize that an hypotonic tear substitute such as the 0.3% SH investigated in this study, would be able to restore the ocular surface homeostasis, improving therefore the lacrimal secretion that has been affected by the section of anterior stromal corneal nerves [45,46].

The present randomized and controlled trial demonstrates the benefit of artificial tears in reducing dry eye disease, restoring tear film function while enhancing corneal wound healing. The weakness of this study is the limitation of number of the patients. A larger or multicenter clinical trial may confirm the result. Furthermore, a study including LASIK patients would explore the added value provided by the use of the present 0.3% SH formulation in post-refractive surgery, whatever the surgical technique performed, and knowing that the corneal barrier function recovery is more delayed after LASIK than after PRK [47,48].

## CONCLUSION

In conclusion, both products were efficient in relieving objective signs and subjective symptoms of dry eye following PRK surgery. 0.3% SH caused a greater improvement than control in half of the efficacy criteria, including symptom intensity, symptom frequency, comfort of the eye drops, corneal haze, Schirmer I test, corneal staining with fluorescein and conjunctival staining with rose Bengal. A statistically significant difference favoring 0.3% SH was shown for corneal staining with fluorescein for the ITT population.

The overall results for each of the measures used to assess safety (visual acuity, general external ophthalmic examination and slit lamp examination) indicate that 0.3% SH had a similar profile to those of HPMC/dextran over 168 days of treatment. 0.3% SH is a safe and effective treatment in subjects with dry eye syndrome occurring after PRK surgery.

## ACKNOWLEDGMENTS

The authors thank Jean-Claude Peyrieux for statistical advice and Dr Marie Gaumet for medical writing.

## REFERENCES

1. Machat JJ. Excimer Laser Refractive Surgery: Practice and Principles. Thorofare, NJ: Slack Inc.; 1996.

2. Tsi RJ. Laser *in situ* keratomileusis for myopia of -2 to -25 diopters. *J Refract Surg* 1997;13 Suppl 5:427-9.
3. Tomlinson A, Khanal S, Ramaesh K, Diaper C, McFadyen A. Tear film osmolarity: Determination of a referent for dry eye diagnosis. *Invest Ophthalmol Vis Sci* 2006;47(10):4309-15.
4. Goto E, Yagi Y, Matsumoto Y, Tsubota K. Impaired functional visual acuity of dry eye patients. *Am J Ophthalmol* 2002;133(2):181-6.
5. The definition and classification of dry eye disease: Report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf* 2007;5:75-92.
6. Begley CG, Chalmers RL, Abetz L, Venkataraman K, Mertzanis P, Caffery BA, *et al*. The relationship between habitual patient-reported symptoms and clinical signs among patients with dry eye of varying severity. *Invest Ophthalmol Vis Sci* 2003;44(11):4753-61.
7. Quinto GG, Camacho W, Behrens A. Postrefractive surgery dry eye. *Curr Opin Ophthalmol* 2008;19(4):335-41.
8. Krueger RR, Seiler T. *The Excimer Manual: PRK Postoperative and Complications Management*. New York: Little, Brown and Company; 1997. p. 121-70.
9. Mazzacane D, Braggio F. Randomized controlled trial of high-molecular-weight hyaluronic acid in dry eye syndromes. *Ann Ophthalmol Clin Ocul* 1993;119(1):1-15.
10. Taiti L, Scrivanti M, Mencucci R, Bardi L, Salvi G. A double-masked crossover comparison of a new artificial tear preparation containing hyaluronic acid (L02A) and a reference artificial tear preparation containing hydroxypropyl-methylcellulose in dry-eye syndrome treatment. *Ann Ophthalmol Clin Ocul* 1996;119(1):33-46.
11. Milazzo G, Papa V, Aragona P, Russo S, Russo P, Di Bella A. Efficacy of sodium hyaluronate eye drops of different osmolarities in the symptomatic treatment of dry eye patients. *Adv Exp Med Biol* 2002;506(Pt B):1233-5.
12. Aragona P, Papa V, Micali A, Santocono M, Milazzo G. Long term treatment with sodium hyaluronate-containing artificial tears reduces ocular surface damage in patients with dry eye. *Br J Ophthalmol* 2002;86(2):181-4.
13. Baeyens V, Bron A, Baudouin C, Vismed/Hylovis Study Group. Efficacy of 0.18% hypotonic sodium hyaluronate ophthalmic solution in the treatment of signs and symptoms of dry eye disease. *J Fr Ophtalmol* 2012;35(6):412-9.
14. Tiffany JM. Viscoelastic properties of human tears and polymer solutions. *Adv Exp Med Biol* 1994;350:267-70.
15. Nakamura M, Hikida M, Nakano T, Ito S, Hamano T, Kinoshita S. Characterization of water retentive properties of hyaluronan. *Cornea* 1993;12(5):433-6.
16. Saetone MF, Chetoni P, Torracca MT, Burgalassi S, Giannacini B. Evaluation of mucoadhesive properties and *in vivo* activity of ophthalmic vehicles based on hyaluronic acid. *Int J Pharm* 1989;51:203-12.
17. Gilbard JP, Carter JB, Sang DN, Refojo MF, Hanninen LA, Kenyon KR. Morphologic effect of hyperosmolarity on rabbit corneal epithelium. *Ophthalmology* 1984;91(10):1205-12.
18. Hamano T, Horimoto K, Lee M, Komemushi S. Sodium hyaluronate eyedrops enhance tear film stability. *Jpn J Ophthalmol* 1996;40(1):62-5.
19. Ang RT, Dartt DA, Tsubota K. Dry eye after refractive surgery. *Curr Opin Ophthalmol* 2001;12(4):318-22.
20. Aragona P, Di Stefano G, Ferreri F, Spinella R, Stilo A. Sodium hyaluronate eye drops of different osmolarity for the treatment of dry eye in Sjögren's syndrome patients. *Br J Ophthalmol* 2002;86(8):879-84.
21. Pisella PJ, Godon C, Auzeir O, Baudouin C. Influence of corneal refractive surgery on the lacrimal film. *J Fr Ophtalmol* 2002;25(4):416-22.
22. Hong JW, Kim HM. The changes of tear break up time after myopic excimer laser photorefractive keratectomy. *Korean J Ophthalmol* 1997;11(2):89-93.
23. Ozdamar A, Aras C, Karakas N, Sener B, Karacorlu M. Changes in tear flow and tear film stability after photorefractive keratectomy. *Cornea* 1999;18(4):437-9.
24. Pérez-Santonja JJ, Sakla HF, Cardona C, Chipont E, Alió JL. Corneal sensitivity after photorefractive keratectomy and laser *in situ* keratomileusis for low myopia. *Am J Ophthalmol* 1999;127(5):497-504.
25. Matsui H, Kumano Y, Zushi I, Yamada T, Matsui T, Nishida T. Corneal sensation after correction of myopia by photorefractive keratectomy and laser *in situ* keratomileusis. *J Cataract Refract Surg* 2001;27(3):370-3.
26. Netto MV, Mohan RR, Ambrósio R Jr, Hutcheon AE, Zieske JD, Wilson SE. Wound healing in the cornea: A review of refractive surgery complications and new prospects for therapy. *Cornea* 2005;24(5):509-22.
27. Hirabara S, Kojima T, Takahashi N, Hanabayashi M, Ishiguro N. Hyaluronan inhibits TLR-4 dependent cathepsin K and matrix metalloproteinase 1 expression in human fibroblasts. *Biochem Biophys Res Commun* 2013;430:519-22.
28. Saito S, Momohara S, Taniguchi A, Yamanaka H. The intra-articular efficacy of hyaluronate injections in the treatment of rheumatoid arthritis. *Mod Rheumatol* 2009;19(6):643-51.
29. Hiramitsu T, Yasuda T, Ito H, Shimizu M, Julovi SM, Kakinuma T, *et al*. Intercellular adhesion molecule-1 mediates the inhibitory effects of hyaluronan on interleukin-1beta-induced matrix metalloproteinase production in rheumatoid synovial fibroblasts via down-regulation of NF-kappaB and p38. *Rheumatology (Oxford)* 2006;45(7):824-32.
30. Vesaluoma MH, Tervo TT. Tenascin and cytokines in tear fluid after photorefractive keratectomy. *J Refract Surg* 1998;14(4):447-54.
31. Tuominen I, Vesaluoma M, Teppo AM, Grönhagen-Riska C, Tervo T. Soluble Fas and Fas ligand in human tear fluid after photorefractive keratectomy. *Br J Ophthalmol* 1999;83:1360-3.
32. Ramirez-Florez S, Maurice DM. Inflammatory cells, refractive regression, and haze after excimer laser PRK. *J Refract Surg* 1996;12(3):370-81.
33. Solomon R, Donnenfeld ED, Perry HD. The effects of LASIK on the ocular surface. *Ocul Surf* 2004;2(1):34-44.
34. Nakamura M, Hikida M, Nakano T. Concentration and molecular weight dependency of rabbit corneal epithelial wound healing on hyaluronan. *Curr Eye Res* 1992;11(10):981-6.
35. Gomes JA, Amankwah R, Powell-Richards A, Dua HS. Sodium hyaluronate (hyaluronic acid) promotes migration of human corneal epithelial cells *in vitro*. *Br J Ophthalmol* 2004;88(6):821-5.
36. Tsiklis NS, Kymionis GD, Kounis GA, Pallikaris AI, Diakonis VF, Charisis S, *et al*. One-year results of photorefractive keratectomy and laser *in situ* keratomileusis for myopia using a 213 nm wavelength solid-state laser. *J Cataract Refract Surg* 2007;33(6):971-7.
37. Cherry PM, Tutton MK, Adhikary H, Banerjee D, Garston B, Hayward JM, *et al*. The treatment of pain following photorefractive keratectomy. *J Refract Corneal Surg* 1994;10 2 Suppl: S222-5.
38. Brignole F, Pisella PJ, Dupas B, Baeyens V, Baudouin C. Efficacy and safety of 0.18% sodium hyaluronate in patients with moderate dry eye syndrome and superficial keratitis. *Graefes Arch Clin Exp Ophthalmol* 2005;243(6):531-8.
39. Montés-Micó R, Cáliz A, Alió JL. Changes in ocular aberrations after instillation of artificial tears in dry-eye patients. *J Cataract Refract Surg* 2004;30:1649-52.
40. Vogel R, Crockett RS, Oden N, Laliberte TW, Molina L, Sodium Hyaluronate Ophthalmic Solution Study Group. Demonstration of efficacy in the treatment of dry eye disease with 0.18% sodium hyaluronate ophthalmic solution (vismed, rejena). *Am J Ophthalmol* 2010;149(4):594-601.
41. Prabhasawat P, Tesavibul N, Kasetsuwan N. Performance profile of sodium hyaluronate in patients with lipid tear deficiency: Randomised, double-blind, controlled, exploratory study. *Br J Ophthalmol* 2007;91(1):47-50.
42. McCann LC, Tomlinson A, Pearce EI, Papa V. Effectiveness of artificial tears in the management of evaporative dry eye. *Cornea* 2012;31(1):1-5.
43. Kaura R, Tiffany JM. The role of mucous glycoproteins in the tear film. In: Holly FJ, editor. *The Preocular Tear Film in Health, Disease and Contact Lens Wear*. Lubbock, Texas: Dry Eye Institute; 1986. p. 728-32.
44. Tiffany JM, Winter N, Bliss G. Tear film stability and tear surface tension. *Curr Eye Res* 1989;8(5):507-15.
45. Tuunanen TH, Tervo TM. Schirmer test values and the outcome of photorefractive keratectomy. *J Cataract Refract Surg* 1996;22(6):702-8.
46. Wilson SE. Laser *in situ* keratomileusis-induced (presumed) neurotrophic epitheliopathy. *Ophthalmology* 2001;108(6):1082-7.
47. Polunin GS, Kourenkov VV, Makarov IA, Polunina EG. The corneal barrier function in myopic eyes after laser *in situ* keratomileusis and after photorefractive keratectomy in eyes with haze formation. *J Refract Surg* 1999;15 2 Suppl: S221-4.
48. Nejima R, Miyata K, Tanabe T, Okamoto F, Hiraoka T, Kiuchi T, *et al*. Corneal barrier function, tear film stability, and corneal sensation after photorefractive keratectomy and laser *in situ* keratomileusis. *Am J Ophthalmol* 2005;139(1):64-71.