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CLINICAL STUDY

A randomised, crossover, multicentre study to compare the performance of 0.1% (w/v) sodium hyaluronate with 1.4% (w/v) polyvinyl alcohol in the alleviation of symptoms associated with dry eye syndrome

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Abstract

Aims **To assess the safety and performance of a 0.1% (w/v) solution of sodium hyaluronate (HA, Fermavisc®, in the alleviation of symptoms of severe dry eye in comparison with a 1.4% (w/v) solution of polyvinyl alcohol.**

Methods **A randomised, crossover, multicentre study carried out at eight centres in the UK. Eligible patients giving written informed consent were randomised to the order in which they would receive the two study products. Each treatment period lasted for 4 weeks, then the patient crossed over to the other study product. Symptoms of burning and grittiness were assessed by visual analogue scale (VAS) at each study visit and other objective clinical assessments of ocular structure and function were carried out at baseline and the end of each treatment period.**

Results **Thirty-nine patients were entered into the study and 32 completed both treatment periods and were included in the statistical analyses. A significant improvement in the patients' VAS assessment of burning was seen after treatment with HA ($P = 0.03$, 95% Confidence Interval: −23.5 to −1.1). This treatment also resulted in a significantly lower rose bengal staining score ($P = 0.04$, 95% Confidence Interval: −1.62 to −0.05 for the right eye).**

Conclusion **The results show a significant clinical benefit in terms of relief of the symptom of burning when HA is applied topically to the eye three or four times per day or as required. HA also appears to have a protective effect on the corneal epithelium, as shown by a reduction in the level of staining of corneal epithelial cells by rose bengal. This study confirms that Fermavisc® is a safe and effective product for use in the alleviation of symptoms of severe dry eye syndrome.**

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Introduction

Sodium hyaluronate (HA, hyaluronic acid, hyaluronan) is a linear polymer composed of long chains of repeating disaccharide units of N-acetylglucosamine and glucuronic acid. It is

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a naturally occurring substance found in nearly all connective tissue matrices of vertebrate organisms and is the most characteristic component of synovial fluid. In the eye, HA is found in the vitreous and also, in a much lower concentration, in the aqueous humour and in the connective tissues of the drainage angle.[1] HA is a natural component of tears with a proportion of that on the ocular surface being synthesised by corneal epithelial cells.[2] Dry eye results from a diminished supply of tears to the eye or a change in the tear composition. Age-related atrophy reduces the formation of tears and some destructive processes also affect the lacrimal gland and cause dry eye. In keratoconjunctivitis sicca (KCS) lacrimal gland secretion is decreased, the precorneal tear film is hypertonic and the tears have a reduced lysozyme content. Sjögren's syndrome is a chronic autoimmune disease characterised by lymphoid cell infiltration of the lacrimal and salivary glands with acinar and ductal cell destruction leading to diminished glandular secretions.

Topical application of HA, which is a viscous, physiological tear substitute, has been shown to confer both subjective and objective symptomatic relief in patients with dry eye syndrome resulting from KCS or Sjögren's syndrome.[3–6] HA has also been reported to protect the corneal epithelium.[7] A randomised, crossover study has shown that a preservative-free, 0.1% (w/v) solution of sodium hyaluronate, manufactured by a process of continuous fermentation (Vitrolife UK Ltd) performs significantly better than saline in both subjective and objective tests in patients with severe dry eye.[8] The purpose of the current investigation was to further assess the safety and performance of Fermavisc® in dry eye syndrome in a randomised, multicentre, crossover study in which it is compared with a product marketed for relief of the symptoms of dry eye syndrome (Refresh™).

Materials and methods

Study population

Subjects were to be drawn from eight participating hospitals in the following locations in the UK: Liverpool, Wolverhampton, Cambridge, Sheffield, London, Newcastle, Bristol and Aberdeen. They included male and female adults with proven severe dry eye syndrome, as shown by a tear function index (TFI) of $\leq$50, associated with primary or secondary Sjögren's syndrome, meeting four of the six criteria of the European Classification of Sjögren's syndrome.[9] The TFI is calculated as the ratio of the Schirmer's score to the tear clearance rate (TCR). This parameter has been shown to be more sensitive and specific in the diagnosis of dry eye than either of the two tests independently.[10] The effects of tear drainage are reflected in both the Schirmer's score and the TCR and expressing the TFI as a ratio of the two eliminates the forces of tear drainage and provides a measure of secretory ability, this being the principal influence on tear dynamics which discriminates between normal patients and those with dry eye.

Subjects receiving topical beta-blockers or topical or systemic carbonic anhydrase inhibitors were excluded from the study as these medications are likely to interfere with the assessment of dry eye. Decongestants and systemic beta-blockers may also affect tear production and subjects receiving these medications at the start of the study were asked to continue them unchanged during the course of the study. Subjects with active blepharitis were excluded but could be reassessed for eligibility after treatment of this condition. Previous anterior segment inflammation or trauma, whether surgical or otherwise, excluded a patient but punctal occlusion or lateral tarsorrhaphy did not. Contact lens users were excluded, as were subjects who had received an experimental drug or device within the previous 6 weeks or had a known hypersensitivity to either of the study products. Pregnant or lactating females and those of childbearing potential in whom the possibility of pregnancy could not be excluded were not considered for entry. All subjects were required to give written informed consent to entry before any study specific procedures were undertaken.

Study design

The study was a randomised, comparative, double-blind two-period crossover multicentre study. Ethics approval was obtained from the North West Multicentre Research Ethics Committee and the Local Research Ethics Committees of the eight participating centres before recruitment commenced. All study procedures were conducted according to the principles of the Declaration of Helsinki (Republic of South Africa, 1996), the ICH Tripartite Guideline for Good Clinical Practice (July 1996) and the European Standard for the Clinical Investigation of Medical Devices (EN 540, 1993).

The schedule of study visits is shown in Figure 1. At baseline (Visit 1) the visual analogue scale (VAS) score of the patients' symptoms of burning and grittiness were recorded and a number of objective clinical assessments were performed. Eligible patients were randomised to receive either Fermavisc® (0.1% (w/v) sodium hyaluronate in phosphate-buffered saline) or

Assessments/Activity	Visit 1 Day 0	Visit 2 Day 14	Visit 3 Day 28	Visit 4 Day 42	Visit 5 Day 56
Informed consent	✔				
Symptoms (VAS)	✔	✔	✔	✔	✔
Tear Meniscus	✔		✔		✔
Tear Film Break-up	✔		✔		✔
Rose Bengal	✔		✔		✔
Tear Function Index	✔		✔		✔
Randomisation	✔				
Adverse Event Check		✔	✔	✔	✔
Dispense Product 1	✔	✔			
Dispense Product 2			✔	✔	

Figure 1 Schedule of study visits.

Refresh (1.4% (w/v) polyvinyl alcohol, 6% povidone) for a period of 4 weeks. Both products were administered as 1–2 drops topically to the eye three or four times a day or as required. The subjective VAS measurements were repeated 2 weeks after the start of treatment (Visit 2) and all clinical assessments were repeated at 4 weeks (Visit 3) at which time patients were crossed over to the other study product. The subjective assessments were repeated 2 weeks into this second treatment period (Visit 4) and all the clinical assessments were repeated at the final study visit, after 4 weeks of treatment with the second study product (Visit 5). There was no washout period between the two treatments, as there is no carry-over effect associated with the use of these products. There was no run-in period prior to randomisation.

Clinical assessments

Patients were asked to rate their symptoms of burning and grittiness in the eye on vertical 10 cm visual analogue scales (VAS). These scales have been shown to be sensitive indicators of subjective symptom relief.[11] A number of objective tests of ocular structure and function were also carried out. Firstly, the height of the inferior and superior tear menisci were recorded, using the 0.2 mm beam of the slit lamp, as $<$0.2 mm, 0.2–0.4 mm and $>$0.4 mm. A solution of 5 drops of 1% rose bengal, 1 drop of 0.5% benoxinate and 5 drops 2% fluorescein was then prepared in a sterile gallipot, using an empty minim to mix by aspiration. Three microlitres of the freshly prepared solution were instilled into the lower fornix in order to perform the remaining tests. The staining of the bulbar (medial, superior, temporal, inferior) conjunctiva and cornea by rose bengal was scored, each section to a maximum of three points. The minimum total score was 0 and the maximum 15. The tear film break-up time (time between last blink and first disturbance of the corneal tear film, BUT) was assessed as a measure of the stability of the preocular tear film.

The Schirmer's strip was placed in the lateral part of the inferior fornix of the eye for 5 min and the Schirmer's score, which is a measure of natural tear production, obtained by measuring the length of the wet portion, was noted. The staining intensity was then matched with laminated dilution standards to determine the TCR. The standards were prepared by staining a Schirmer's test strip with the following serial dilutions of the test mixture: 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64. The TFI was obtained by dividing the Schirmer's score by the TCR.

Study materials

Fermavisc® is a 0.1% (w/v) preservative-free solution of sodium hyaluronate in phosphate buffered saline, manufactured by a process of continuous fermentation from *Streptococcus equi* (Vitrolife UK Ltd). The comparator medication (Refresh™ Allergan) is a preservative-free 1.4% (w/v) solution of polyvinyl alcohol in 0.6% povidone, which is available over the counter and widely used for the relief of symptoms of dry eye. Both materials were supplied as 0.4 ml sterile, preservative-free solutions in a plastic ampoule for single application. Although the ampoules for the two products differed, the ampoule was over-labelled to blind its identity and the external packaging was identical for the two materials. During each of the treatment periods, the products were to be administered as 1 or 2 drops, three or four times per day or as required. One hundred single-use ampoules were issued at each study visit, scheduled at intervals of 14 days, providing up to seven applications per day. In order to ensure that the patient had a continuous supply of product, the interval between the study visits could be reduced. Where the time between visits extended beyond the agreed study visit windows, the investigator was asked to confirm that the patient was still using the study product supplied at the time of their assessment.

Performance and safety variables

The primary variable for the assessment of performance of the study treatments was the subjective VAS assessment by the patient of the symptoms of burning and grittiness in the eye. The objective tests of ocular structure and function (Figure 1) and the recording of adverse events were a means of assessing the safety of the investigational product.

Statistical considerations

As there was no prior knowledge of the variability of the VAS scores of burning and grittiness, which was the primary outcome variable, statements of power are made in terms of the unknown residual standard deviation, σ. A sample size of 64 evaluable patients has an 80% power to show a statistically significant difference between the two study products on the primary outcome variable at the 5% level, if the true mean between treatment difference equals 0.5σ.

A single randomisation scheme, using a block size of four within centres, was produced for the study. The study products were packaged identically in order to maintain the study blind and packs were labelled with a unique study number. Patients were randomised by selecting the lowest study number available.

A blind review of the VAS values indicated that the distribution was appropriate for an analysis using parametric methods. Analysis was by the method for crossover studies described by Armitage and Berry[12] in which a standard two-sample *t*-test is used to compare the mean responses in the two periods for the HA→Comparator and Comparator→HA groups, in order to test for a treatment effect (Arcus Quickstat Biomedical, version 1.1, Research Solutions). Two-tailed tests of significance were applied throughout. The same method of analysis was used for the objective measurements. No formal analysis of adverse events was undertaken. All patients completing both treatment periods were included in the analysis.

Results

Patient disposition

A total of 39 subjects were randomised into the study from five of the eight participating centres. Of these, seven subjects withdrew before completing both phases of the study (three adverse events, three lack of performance and one withdrawn consent) and were not included in the statistical analyses. Four of the 32 evaluable patients did not have confirmation of the diagnosis of Sjögrens syndrome and three of these had a baseline TFI of > 50. The demographics and baseline characteristics of the 32 evaluable subjects are shown in Table 1. Thirteen of the patients had undergone bilateral (12) or unilateral (1) punctal occlusion. No patients had undergone lateral tarsorrhaphy. All patients were affected by bilateral dry eye syndrome.

Performance

The VAS scores of burning and grittiness, which are the primary performance variables in this study, are shown at baseline (Visit 1) and at the end of each of the two treatment periods (Visit 3, Visit 5) in Tables 2 and 3. Analysis of variance for a two-period crossover study shows a significant treatment effect in favour of HA (Effect magnitude = −12.3, 95% CI: −23.5 to −1.1, $P = 0.03$) in the analysis of burning sensation. There was no statistically significant treatment effect for the measurement of grittiness (Effect magnitude = −7.2, 95% CI: −20.1 to 5.6, $P = 0.26$).

Safety

Evaluation of safety included the following objective tests of ocular structure and function: Schirmer's score, tear clearance rate, tear function index, rose bengal staining, tear film break-up time and measurement of tear meniscus. All subjects had inferior and superior tear menisci graded as 1 (≤0.2 mm) at baseline and this was unchanged at all subsequent study assessments. The other study parameters were subjected to crossover analysis and the results are shown in Table 4. There were no significant differences during the two treatment periods for Schirmer's score, tear clearance rate, tear function index or tear film-break up time. However, the results for rose bengal staining (Tables 5 and 6) show a significantly lower staining score for patients receiving Fermavisc® compared with those receiving the comparator product, although this was only evident for the measurements in the right eye (Effect magnitude −0.84, 95% CI −1.62 to −0.05, $P = 0.04$).

A total of 30 adverse events (AE) occurred in 18 patients during the trial. Eighteen of these events were considered to be probably or possibly related to one of the study products or there was insufficient evidence to assess causality. Ten events occurred during use of the investigational product and eight occurred during use of the comparator product (Table 7).

The reported AEs included blepharitis, dull ache/pressure in the eyes, sticky eyes, corneal defect/scar, eye discomfort, ocular itchiness, ocular discharge and a worsening of the dry eye symptoms of burning and grittiness. In three cases (007, 012, 066), use of the study product was stopped. One adverse event (057, Visit 5, ocular discharge) required additional treatment with 0.5% G. chloramphenicol and was noted to be ongoing at the end of follow-up. In all other cases the adverse event resolved with no further action being taken.

Nine of the adverse event reports were suggestive of an increase in dry eye symptoms or problems with stickiness of the eyes in the morning, three while receiving HA and six while receiving the comparator treatment (Table 7). This may be partly attributable to

Table 1 Demographics of 32 evaluable subjects by randomised group

Variable	*HA→ Comparator*	*Comparator →HA*	*Total*
Age (SD):	56.4 (12.8)	61.6 (15.5)	58.8 (14.1)
Range:	27.9–75.8	23.0–77.3	23.0–77.3
Sex: M	2	0	2
F	15	15	30
Race: Caucasian	17	14	31
Asian	0	1	1
Sjögren's: primary	5	6	11
secondary	12	9	21
Smoker: Y	0	2	2
N	17	13	30

Patients in the HA→Comparator group received Fermavisc® from V1 to V3 and Refresh™ from V3 to V5. Patients in the Comparator→HA group received Refresh™ from V1 to V3 and Fermavisc® from V3 to V5.
Note: Four patients did not have confirmation of the diagnosis of Sjögren's syndrome specified by the study protocol but they have been included in the study.

Table 2 Mean VAS scores (SD) of burning at baseline (V1), V3 and V5

Visit No.	*HA→Comparator*	*Comparator→HA*
1	42.9 (29.2)	29.3 (29.8)
3	35.5 (31.4)	41.3 (33.9)
5	37.8 (31.6)	19.0 (15.9)

Table 3 Mean VAS scores (SD) of grittiness at baseline (V1), V3 and V5

Visit No.	*HA→Comparator*	*Comparator→HA*
1	49.1 (24.6)	53.6 (29.0)
3	50.6 (25.4)	49.9 (36.2)
5	46.7 (26.9)	31.5 (24.2)

Table 4 *P*-values and 95% confidence intervals for crossover analysis of safety parameters

Test	*Eye*	*Test for treatment effect*	
		P value	*95% CI*
Schirmer's score	Left	0.43	−2.2, 1.0
Schirmer's score	Right	1.00	−1.3,1.3
TCR	Left	0.86	−3.5, 2.9
TCR	Right	0.70	−2.9, 4.2
TFI	Left	0.40	−45.6, 18.9
TFI	Right	0.61	−28.7, 48.4
BUT	Left	0.40	−1.9, 0.8
BUT	Right	0.16	−2.0, 0.3
Rose bengal	Left	0.37	−1.0, 0.4
Rose bengal	Right	0.04	−1.6, −0.05

Table 5 Rose bengal staining scores (SD) for right eye at baseline (Visit 1), Visit 3 and Visit 5

Visit No.	*HA→Comparator*	*Comparator→HA*
1	6.76 (2.63)	6.20 (2.62)
3	5.24 (2.08)	6.33 (1.84)
5	5.18 (1.74)	4.60 (1.88)

Table 6 Rose bengal staining scores (SD) for right eye at baseline (Visit 1), Visit 3 and Visit 5

Visit No.	*HA→Comparator*	*Comparator→HA*
1	7.24 (2.05)	6.53 (2.64)
3	4.59 (1.94)	5.20 (2.04)
5	4.82 (2.40)	4.8 (1.9)

patients restricting themselves to use of the products three or four times a day, even although the recommended dosage regimen was 'as required'. The observed increase in symptoms may therefore be a reflection of a reduced frequency of application rather than a lack of performance of the study products. This being so, then the reduced frequency of these reports in patients receiving HA may indicate a longer duration of action of this treatment.

Three serious adverse events (SAE) were reported during the study and all of these were classified as unrelated to the study products.

Discussion

The results of the study show a significant clinical benefit in terms of relief of the symptom of burning in those dry eye patients receiving 0.1% (w/v) sodium hyaluronate compared with those receiving the comparator drops (95% CI = −23.5 to −1.1, $P = 0.03$). This is in spite of the reduced power of the study resulting from early termination and only one half of the planned number of patients being recruited. The difficulty in recruiting patients for the study was due

Table 7 Adverse events with 'probable', 'possible' or 'insufficient evidence' relationship to study products

Subject	*Visit*	*Description of event*	*Severity*	*Related to product*	*Product No.**	*Action*	*Outcome*
001	4	Mild blepharitis	Mild	Possible	HA	None	Resolved
002	3	Bilateral blepharitis	Mild	Insufficient evidence	HA	None	Resolved
002	2	Dull ache/pressure both eyes	Mild	Possible	HA	None	Resolved
007	2	*Very dry eyes worse at night	Severe	Probable	HA	Treatment stopped	Resolved
009	4	*Lashes sticky in the morning	Mild	Probable	HA	None	Resolved
011	4	Superficial corneal scar	Mild	Possible	HA	None	Resolved with sequelae
011	5	L eye very uncomfortable	Moderate	Possible	HA	None	Resolved
012	3	R eye very red, sore, uncomfortable	Severe	Probable	HA	Treatment stopped	Resolved
016	4	*Itchiness both eyes	Moderate	Probable	HA	None	Resolved
057	3	Ocular discharge	Moderate	Insufficient evidence	HA	Other treatment	Continuing
004	3	*Sticky eyes early morning	Mild	Possible	C	None	Resolved
008	3	Bilateral red swollen eyes	Moderate	Possible	C	None	Resolved
008	3	*Gritty eyes worse whole day	Moderate	Possible	C	None	Resolved
010	4	*Severe/burning grittiness	Severe	Probable	C	None	Resolved
011	3	*Lashes stuck together (bilateral)	Mild	Probable	C	None	Resolved
011	3	L corneal epithelial defect centrally	Mild	Possible	C	None	Resolved
025	3	*Increased grittiness on 2nd week of box 2-R eye	Moderate	Insufficient evidence	C	None	Resolved
066	2	*Very gritty eyes with crusty deposits in the morning	Moderate	Possible	C	Treatment stopped	Resolved

* Adverse events which suggest an increase in dry eye symptoms.
HA = sodium hyaluronate; C = comparator.

to the stringent entry criteria, which were designed to ensure that only patients with severe symptoms of dry eye participated. There was no significant difference in the symptom of grittiness between the two study groups. Fermavisc® has previously been shown to confer subjective symptom relief when compared with saline[8] and these benefits are now confirmed in the current study in which the comparator is an established, marketed product widely used by patients with dry eye. A previous comparison of 0.1% sodium hyaluronate and 1.4% polyvinyl alcohol demonstrated an improvement in mean tear-film osmolality and the rose bengal staining score in both groups. However there was no significant difference in the performance of the two products and there was no subjective assessment of symptoms in the study.[13]

Safety in the current study was assessed by the occurrence of adverse events and objective measurements of ocular structure and function. The adverse event profile and results of the safety evaluation confirm that 0.1% (w/v) sodium hyaluronate is safe and well-tolerated when applied topically to the eye to alleviate the symptoms of dry eye syndrome. The reduction of rose bengal staining seen with Fermavisc® use has been reported previously[8] and has also been seen in other studies of hyaluronan use in KCS.[14] This protective effect may be attributable in part to a reduction in epithelial cell disruption by hyaluronan[15] and in part to the action of hyaluronan in maintaining the mucous layer of the preocular tear film, thus preventing the uptake of stain by healthy epithelial cells.[16]

This study clearly provides further evidence that the topical application of a 0.1% (w/v) sodium hyaluronate solution Fermavisc® is safe and provides a worthwhile clinical benefit in terms of symptom relief and protection of the corneal epithelium in patients with severe dry eye syndrome.

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