Efficacy and safety of 0.18% sodium hyaluronate in patients with moderate dry eye syndrome and superficial keratitis

Abstract Background: Sodium hyaluronate (SH) is used in patients with dry eye. We evaluated the efficacy and safety of SH and carboxymethylcellulose (CMC) in the treatment of dry eye syndrome with superficial keratitis. Methods: A total of 22 patients with moderate dry eye and superficial keratitis were enrolled in a prospective, randomised, masked-observer, parallel-group, single-centre study. Patients were randomly assigned to a 0.18% SH or 1% CMC solution for a 2-month period. In addition to the commonly assessed parameters in patients with dry eye (among others symptoms and corneal staining with fluorescein), flow cytometry analysis of CD44, HLA DR expressions in impression cytology was investigated as a potential efficacy parameter. Results: Both treatments improved the symptoms and ocular surface and were well tolerated. SH significantly (p<0.05) decreased CD44 values compared with CMC. Comfort was significantly (P<0.05) better in the SH group than in the CMC group throughout the study. Recovery in keratitis (type, extent and depth) and symptoms were faster in the SH group than in the CMC group. Blurred vision was reported by patients in the CMC group only. Conclusions: SH was well tolerated and tended to show a faster efficacy than did the CMC-based formulation in patients with moderate dry eye and superficial keratitis. SH could therefore advantageously be prescribed from the early stages of dry eye disease. This study also showed that flow cytometry in impression cytology specimens is a reliable tool for exploring the ocular surface at the epithelial level and that CD44, in addition to HLA DR, could be an interesting endpoint for future trials in dry eye syndrome with products based on SH.

Keywords Dry eye · Sodium hyaluronate · CD44

Introduction

Dry eye is a disorder of the tear film due to tear deficiency or excessive tear evaporisation and is associated with symptoms of ocular discomfort, dryness, scratchiness, burning, soreness and grittiness [2]. This pathology also includes objective signs of ocular surface damage (i.e. keratitis), tear film instability and tear hyperosmolarity [15].

Several authors obtained excellent results in treating this pathology with solutions containing sodium hyaluronate (SH) [5, 6, 9, 12, 17, 27, 33]. In brief, when these solutions were given at regular intervals, clinical signs were alleviated and tear film stability was improved, with better results compared to a control solution (the vehicle or saline). Hyaluronic acid is a biopolymer that occurs naturally in all vertebrates, for example in the vitreous body of the eye, the extracellular matrix of the skin and in the synovial
fluid. This glycosaminoglycan consists of alternating sequences of N-acetyl-glucosamine and glucuronic acid in linear chains with varying molecular weights [22].

The objective of this exploratory trial was to compare the performance profiles of SH and carboxymethylcellulose (CMC) in patients with moderate dry eye syndrome and superficial keratitis due to Sjögren syndrome (SS) or diagnosed as primary dry eye syndrome. In addition to the commonly assessed parameters in patients with dry eye (among others symptoms and corneal staining with fluorescein), a technique of flow cytometry in impression cytology was investigated as a potential efficacy parameter.

**Patients and methods**

Overall study design and plan

This was a randomised, masked-observer, parallel-group, single-centre trial. Before initiation of the study, the informed consent form and protocol were approved both by TRB Chemedica and the ethics committee (CCPRB) of Amboise Paré, APHP, Boulogne/Seine, University of Versailles, France. The study was conducted in accordance with the Good Clinical Practice guidelines for the evaluation of medicinal products, the Declaration of Helsinki and the French Regulations for the “Protection of persons undergoing biomedical research” and “Data Protection Act”.

At the day 0 visit, patients were checked for inclusion and exclusion criteria and baseline assessments for parameters were performed. There was a 48-h washout period without treatment before the baseline visit. Thereafter, treatment with SH or CMC was given to recruited patients according to the randomisation list. Three follow-up visits were scheduled, namely days 7, 28 and 56.

Clinical assessments

Clinical assessments included corneal staining with fluorescein, staining with Lissamine green, break-up time (BUT), corneal topography, tear prism height, subjective dry eye symptoms and comfort of the eye drops in the eye (Table 1).

Staining of the cornea was assessed with a 0.5% fluorescein solution using a score rating scale for type (0 = no staining, 1 = micropunctate, 2 = macropunctate, 3 = coalescent macropunctate, 4 = patch), extent (0 = 0%, 1 = 0–15%, 2 = 16–30%, 3 = 31–45%, 4 = >45%) and depth based on penetration of fluorescein and slit lamp optic section (0 = no staining, 1 = superficial epithelium, 2 = deep epithelium, delayed stromal glow, 3 = immediate localised stromal glow, 4 = immediate diffuse stromal glow). Staining with Lissamine green was assessed using a grading scale (0 = none to 4 = >45%), on the cornea, the temporal conjunctiva and the nasal conjunctiva. Scores were summed up (maximum 12) and total scores were obtained for staining with fluorescein and Lissamine green.

<table>
<thead>
<tr>
<th>Table 1 Overview of the study visits</th>
<th>D0</th>
<th>D7</th>
<th>D28</th>
<th>D56</th>
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</thead>
<tbody>
<tr>
<td>Relevant previous and concomitant medications</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<tr>
<td>General and ophthalmic examinations</td>
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<td>Flow cytometry</td>
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<td>Staining with fluorescein and Lissamine green</td>
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<tr>
<td>BUT</td>
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<td>×</td>
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<td>×</td>
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<tr>
<td>Tear prism height</td>
<td>×</td>
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<td>×</td>
<td>×</td>
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<tr>
<td>Corneal topography</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<tr>
<td>Tear volume (Schirmer)</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Symptoms intensity on VAS</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<tr>
<td>Comfort of the eye drops</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<tr>
<td>Corrected visual acuity</td>
<td>×</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Slit lamp examination</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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<tr>
<td>Adverse events report</td>
<td>×</td>
<td>×</td>
<td>×</td>
<td>×</td>
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</tbody>
</table>

*In the target eye only. The target eye was defined as the most severely affected eye as assessed by fluorescein staining at the day 0 visit (highest global score). In case there was no difference in the two eyes for that parameter, the eye in which the tear volume (Schirmer test) was the lowest was chosen. If there was no difference in that parameter either, the left eye was arbitrarily chosen.

Conjunctival impression cytology

**Collection of specimens**

Details of the collection of conjunctival cytology specimens and immunostaining procedure used have been published elsewhere [3, 4]. Briefly, specimens were collected more than 15 min after the last dye test. After application of 1 drop of a contact anaesthetic, two 0.20-μm polyester sulfone filter membranes (Supor Membranes, Gelman Sciences) were applied successively onto the su-
perior and superotemporal bulbar conjunctiva in two different but neighbouring areas without exerting any pressure. Care was taken to collect specimens only in non-exposed regions of the conjunctiva. Membranes were immediately removed after contact. Membranes were put into cold fixative provided by the Immuno-Haematology Laboratory at the Ambroise Paré Hospital.

Flow cytometry

Membranes were left in 2 ml of PBS containing 0.05% paraformaldehyde, gently agitated for 30 min before a mechanical cell detachment using a pipet tip; cells were then collected and centrifuged (200g, 5 min). They were processed for an indirect immunofluorescence procedure. They were first incubated at room temperature for 30 min with the following mouse monoclonal antibodies directed against (1) inflammatory markers: HLA DR (TAL.1B5 clone, Dakocytomation, Denmark), CD40 (member of TNF receptor family, MAB89 clone) and CD40L (CD154, TRAP-1 clone); (2) apoptosis-related markers: Fas (CD95, UB2 clone) and APO2.7 (2.7A6A3 clone); (3) some other markers according to their roles supposed to act in the ocular surface in a protective way: CD44 (known receptor for hyaluronic acid, J.173 clone), CD63 (or LAMP-3 lyposomal-membrane-associated glycoprotein, CLBGran/12 clone) acting as a protector of lyposomal membranes from digestion by hydrolytic enzymes [10] (localised intracellularly, it could be translocated to the cell plasma membrane upon cell activation [32]), P-glycoprotein (UIC2 clone; a membrane transporter acting to favour xenobiotic efflux from the cell [24]) and MUC5AC (a soluble mucin secreted by goblet cells) [28]. All those antibodies were purchased from Beckman-Immunotek (USA), except anti-MUC5AC products, which were kindly given by Dr Jacques Bara (Inserm U482, Saint Antoine Hospital, Paris). In addition, an isotype-matched control mouse IgG was used as a negative control. After two washings with PBS, cells were incubated with the secondary antibody, a fluorescein-conjugated goat anti-mouse immunoglobulin (Dako, Denmark) for 30 min and washed twice again before being analysed using a flow cytometer (Epic XL, Beckman, USA).

At least 1,000 cells were analysed for each antibody. Results were calculated as percentages of positive cells and as the level of expression after translating mean fluorescence intensities into arbitrary units of fluorescence (AUF) according to calibration curves established with calibrated beads (ImmunoBrite, Coulter, USA) using previously validated methods [7]. Final levels of expression were obtained after subtraction of non-specific levels of fluorescence calculated with the negative control antibodies.

Study materials

Commercially available Vismed (TRB Chemedica AG, Haar/München, Germany) eye drop solution was used in this study. The hyaluronic acid used in this formulation was obtained by bacterial fermentation from *Streptococcus* strains and was a specific fraction (Mw 1.2 million Da) with a high degree of purity. Vismed contains SH at a concentration of 0.18% and is a hypotonic (150 mOsml/l) solution containing calcium, magnesium and potassium ions at a physiological concentration. The comparative medication was Celluvisc (Allergan AG, Lachen, Switzerland), which consists of isotonic 1% CMC solution. Both products were supplied in their original sterile, single-use monodose container without any preservative. The recommended dosage for both products was 1 drop three times daily in each eye for 56 days.

Study population

Male and female patients 18 years of age and over were enrolled in the study. The main inclusion criterion was patients with documented moderate dry eye due to SS or diagnosed as primary dry eye syndrome. Written informed consent was obtained before the baseline measurements.

Other inclusion criteria were patients experiencing at least one of the dry eye symptoms (among soreness, scratchiness, dryness, grittiness and burning), occurring at least sometimes, Schirmer test of less than 10 mm wetting/5 min, BUT of less than 10 s and corneal staining with fluorescein with a total score (type + extent + depth) of 3 or more. Females with a reliable method of contraception or who were postmenopausal were included in the trial.

Patients with severe dry eye syndrome (i.e. corneal staining with fluorescein with a depth score of 3 or more and/or severe conjunctival hyperaemia and/or severe blepharitis) were excluded, as were those who had ocular surgery (whatever type) or ocular trauma within the last 4 months before inclusion, or a current history of disease that could interfere with the assessments in this study (e.g. glaucoma). No other in-eye solutions were permitted from the day 0 visit until the day 56 visit. Since the systemic medications could affect tear production, all patients were asked not to change their dose for the whole trial.

Statistics

The non-parametric Mann-Whitney *U* test was used for comparison between the two groups (type I error 5%, two-way test) in this exploratory trial.
Results

Patients

A total of 22 patients were included in the trial and randomised to treatment with either SH or CMC. One patient in the SH group dropped out of the study (lost to follow-up) and 21 patients completed the trial in accordance with the protocol.

The baseline characteristics of the study subjects included in the analysis are summarised in Table 2. There were no significant differences between the two groups for demographic and baseline characteristics. Three patients in the SH group and four in the CMC group had SS, while all the other patients had primary dry eye syndrome.

A total of 14 patients (7 patients in each group) had used previous in-eye medication(s) for treatment of dry eye. At baseline, dryness, grittiness and burning were simultaneously reported by seven patients in the SH group and ten patients in the CMC group. All of the patients experienced at least one subjective symptom, occurring at least often.

Efficacy results

Flow cytometry

At baseline, there were no significant differences between the two groups for all the inflammatory, apoptotic and mucus-related parameters assessed. At day 56, there was a significant decrease ($P=0.031$) in CD44 (AU/FL) expression in the SH group as compared to the CMC treated eyes (Fig. 1). From baseline, there was a strong tendency in both groups for expression of MUC5AC, CD63 and UIC2 to increase (Fig. 2). However, this trend for overexpression of markers supposed to act as protective mediators or antigens was more marked in the SH group than in the CMC group. From baseline, there was a strong tendency in both groups for HLADR-positive cells to reduce, while CD40 expression decreased in the SH group and increased in the CMC group (Fig. 2). The expressions of CD40L-positive cells were only slightly modified in both groups. No significant difference for any parameter was found between the two products.

Concerning the apoptosis-related markers, there was a strong tendency for expression of Apo2.7 to decrease in the SH group, whereas values for this parameter increased in the CMC group (Fig. 2). Expression of Fas decreased in both groups from baseline, but this was more marked in the CMC group than in the SH group. No significant difference for any parameter was found between the two groups.

Objective clinical parameters

Both treatments tended to reduce the total corneal fluorescein staining scores (type, extent and depth) of keratitis (Table 3). During the first month of treatment, the decrease in mean total fluorescein scores tended to be more marked in the SH group as compared to that of CMC group, but no significant differences were found between the two groups. After 2 months of treatment, scores were similar in both groups. Results from categories (type, extent, depth) followed the same trend as did total scores. In both groups,

![Fig. 1](image)

**Flow cytometric cell values for CD4 (mean ± SD)**

Table 2 Demographic and other baseline characteristics (±SD) of patients. No significant difference was found between groups ($P=0.05$)

<table>
<thead>
<tr>
<th>Demographic parameter</th>
<th>SH</th>
<th>CMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57</td>
<td>69</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161</td>
<td>161</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>56</td>
<td>61</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>1/9</td>
<td>0/11</td>
</tr>
<tr>
<td>Living environment and hobbies</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>(number of factors experienced$^a$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous in-eye medication for dry eye</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>(number of patients)</td>
<td></td>
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<tr>
<td>Subjective symptoms (number of symptoms experienced$^b$)</td>
<td>3.7 (0.9)</td>
<td>4.3 (0.9)</td>
</tr>
<tr>
<td>Tear volume (mm wetting/5 min)</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

$^a$Among smokers, dusty work, air conditioning, extensive computer screenwork, swimming.
$^b$Among soreness, scratchiness, dryness, grittiness, burning.
results of staining with Lissamine green showed that scores tended to decrease at day 7 and day 28 and stabilise at day 56. BUT values tended to increase in both groups and no significant change was found in tear prism height. At day 56, SRI values of corneal topography tended to increase from baseline in the SH group and decrease in the CMC group (Table 3).

Subjective assessments

Comfort scores were higher at all the time points in the SH group as compared to those observed in the CMC group and the difference was significant (P=0.039) in favour of SH at day 7 (Fig. 3). Blurred vision was the most reported sensation of discomfort and was reported in 40–60% of patients in the CMC group at each visit, but not in the SH group. Duration of blurred vision ranged between 10 min and 2 h.

There was a trend for total symptoms to decrease faster in the SH group than in the CMC group (Fig. 4). In fact, at the day 7 and day 28 visits, 80% (8/10) and 45% (5/11) of patients showed an improvement in the total VAS score in the SH group and the CMC group, respectively. The decrease in symptoms tended to stabilise in the SH group, while values in the CMC group further decreased. At day 56, 90% of patients showed a decrease in total VAS scores in both groups. Each symptom separately (i.e. soreness, scratchiness, dryness, grittiness and burning) showed a similar profile of improvement to that of total symptoms. Dryness, as a dry eye symptom, showed the greatest improvement in both groups.
The benefit of SH could also be partially explained by the intrinsic wound healing properties of SH compared with CMC. Indeed, SH has been shown in vitro to accelerate elongation of the epithelial cells and in vivo corneal abrasion models have shown the dose-dependent ability of hyaluronan to reduce wound areas [25, 26].

Based on flow cytometric analysis of conjunctiva epithelial cells, this study also showed that CD44, a hyaluronan receptor that is known to be overexpressed in patients with dry eye syndrome, is expressed by 30–50% of cells and that SH markedly reduced the expression of this marker compared to CMC. This tended to prove that SH had a significant affinity for the CD44 hyaluronic acid receptors that are present on conjunctival cells. However, little is known about the effect of SH on its CD44 receptor in patients with dry eye syndrome. In women with primary SS, Levesque et al. [16] found serum levels of CD44 superior to those found in healthy women. The increase in the levels of in vitro expression of CD44 on lymphocytes from patients with primary SS reached statistical significance when compared to similarly cultured lymphocytes from controls [1]. Recently, the hyaluronan receptor CD44 was found to play a role in the corneal cell-cell and cell-matrix interactions. Zhu et al. [35] have suggested that its regulation was closely related to corneal inflammatory reactions where enhanced expression of CD44 was observed on the epithelium. Moreover, the induction of CD44 on corneal endothelium might play a potential role in compensatory processes when corneal endothelial cells are injured [35]. In another study [22], hyaluronate binding to CD44 has been shown to enhance the growth of the corneal epithelial cells, and more recently, Gomes et al. showed that SH promotes migration of human corneal epithelial cells in vitro [11]. This would therefore favour corneal wound healing. However, the authors did not find any regulatory effects of SH on CD44 expression. They used a technique of APAAP that only quantified the proportion of CD44 expressing cells. This study was done in vitro on primary cultures of human corneal cells, whereas our findings were observed in conjunctival cells taken from impression cytology specimens. Moreover, our technique of flow cytometry allowed precise quantification of levels of CD44 expression and is more sensitive to detect slight variations in antigen expressions. This could therefore explain this apparent discrepancy.

Despite the fact that there were no significant variations, evidence is that Fas, Apo2.7, HLA DR and CD40, the apoptosis-related and inflammatory markers tended to decrease and the potentially protective markers MUC5AC and CD63 conversely, tended to increase. CD44 mediates the uptake and clearance of hyaluronan, whose local turnover from the extracellular matrix occurs after ligation with CD44. The CD44-HA complex is internalised by invagination of the plasma membrane, and this endosome fuses with lysosomes to perform the hyaluronan degrada-
tion within lysosomes via a low pH active hyaluronidase. This could explain the increase of CD63, a lysosomal membrane protein, as a result of this fusion with some lysosomal membranes appearing at the cell plasma membrane.

Although the precise mechanism of action of this complex has not been completely elucidated so far, the affinity of hyaluronan to this receptor could be related to the reduction of the clinical signs and symptoms that we observed in these patients. CD44 receptor could therefore be a reliable monitoring parameter of the dry eye disease in future studies.

In conclusion, the results of this trial showed that 0.18% SH was well tolerated and provided better comfort than did 1% CMC. As a consequence, the SH solution tended to show a faster efficacy than the CMC-based formulation in patients with moderate dry eye syndrome and superficial keratitis. SH 0.18% could therefore be prescribed advantageously from the early stages of the dry eye disease. SH 0.18% was also more efficient than CMC in reducing CD44 expression in these patients after 2 months of treatment. Further studies with a larger number of patients are needed to confirm whether CD44 is a reliable parameter to assess the long-term benefits of dry eye products based on SH.

References


