

CLINICAL SCIENCE

Long term treatment with sodium hyaluronate-containing artificial tears reduces ocular surface damage in patients with dry eye

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Background/aims: Several studies have reported that sodium hyaluronate is able to improve both symptoms and signs in patients with dry eye but none have demonstrated an improvement of conjunctival epithelial cell abnormalities of the ocular surface. The aim of this study was to explore the effect of sodium hyaluronate-containing eye drops on the ocular surface of patients with dry eye during long term treatment.

Methods: A randomised double blind study was undertaken in 86 patients with medium to severe dry eye (that is, rose bengal and/or fluorescein test score of at least 3, tear film break up time <10 seconds, or Schirmer's test <5.5 mm). Patients were treated with either preservative-free sodium hyaluronate or saline for 3 months at a dose of one drop 4-8 times a day. Bulbar impression cytology, slit lamp examinations, and subjective symptoms were evaluated after 1, 2, and 3 months. Impression cytology was considered the primary efficacy parameter of the study.

Results: The efficacy analysis was performed on a total of 44 patients who were able to fully adhere to the protocol. After 3 months of treatment sodium hyaluronate improved impression cytology score ($p = 0.024$ v baseline). At the same time also the difference with respect to placebo was statistically significant ($p = 0.036$). Study medication was well tolerated and no treatment related adverse events occurred during the study.

Conclusions: Sodium hyaluronate may effectively improve ocular surface damage associated with dry eye syndrome.

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The eyes of patients suffering from severe dry eye syndrome are characterised by a deterioration of the corneal epithelium with a development of punctate erosions and increased permeability. Compared with normal eyes the conjunctival epithelium of such patients presents squamous metaplasia with decreased or abnormal goblet cells.¹

Artificial tears are, to date, the mainstay of the therapy for dry eye syndrome. They have been designed with a focus on physical properties relating to wetting of the ocular surface and usually contain hydrophilic polymers, which lubricate the eye during blinking.² The ideal tear replacement should have a composition which is compatible with the maintenance of a normal ocular surface epithelium. When damage exists, the artificial tear solution should provide an environment in which the epithelium can recover the normal structure and function.

A topical application of sodium hyaluronate has been shown to confer both subjective and objective improvement in patients with dry eye syndrome or keratoconjunctivitis sicca (KCS).³⁻⁵ Conflicting results, however, have been obtained regarding the efficacy of sodium hyaluronate on ocular surface damage. Condon *et al* have recently reported a reduction in cell degeneration as assessed by rose bengal.⁶ Accordingly, Wynsbeek *et al* indicated that hyaluronate is able to protect the corneal epithelium.¹⁰ On the other hand, Nelson and Farris have published a report stating that sodium hyaluronate did not change significantly the degree of squamous metaplasia of the bulbar conjunctival surface, as shown by impression cytology during a short term treatment period.¹¹

The aim of the present study was to explore the long term effect of sodium hyaluronate eye drops on the ocular surface of patients with moderate to severe dry eye.

PATIENTS AND METHODS

Study population

A total of 86 patients with medium to severe dry eye syndrome were enrolled in the study. Diagnosis was based on stringent inclusion criteria. Patients with the following characteristics were included in the study: (1) history of dry eye for at least 2 months¹²; (2) tear film abnormalities (that is, tear break up time <10 seconds, and/or anaesthetised Schirmer's test <5.0 mm in one or both eyes); (3) ocular surface damage (rose bengal and/or fluorescein test score ≥ 3) in at least one eye.¹³ Patients with external ocular disease, glaucoma, wearing of contact lenses, or using systemic or topical medication, such as sleeping tablets, tranquillisers, antidepressants, monoamine oxidase inhibitors, dopaminergics, neuroleptics, benzodiazepines, antiserotonergic, β blocking, and antiemetic agents, were excluded from the study.

Patients were randomly treated for 3 months with either preservative-free 0.15% sodium hyaluronate ($n = 41$) or preservative-free saline/0.9% sodium chloride ($n = 45$). As

Table 1 Disposition of patients

	Na-Ha	Placebo	Total
Randomised	41	45	86
Excluded	22	20	42
Adverse events	6	5	11
Poor tolerance	4	8	12
Failure to adhere to protocol	12	7	19
Included (PP set)	19	25	44

Table 2 Inclusion characteristics of the "per protocol" set of patients evaluated for the efficacy analysis. Values for each patient were assessed before randomisation and are expressed as mean (SD) of the right and left eye

Demographic variable	Sodium hyaluronate group	Placebo (saline) group
Number of patients	19	25
Age	50.2 (15.1)	50.7 (15) [†]
Sex (M/F)	4/15	5/20
Symptom score	16.2 (2.4)	16.8 (2.7)
Anaesthetised Schirmer's test (mm)	7.1 (4.8)	6.0 (3.8)
Break up time (seconds)	6.4 (2.6)	5.1 (2.3)
Rose bengal stain (AU)	4.0 (2.4)	6.0 (2.9)
Fluorescein stain (AU)	3.2 (2.1)	3.6 (3.3)

Symptom scores were calculated according to McMonnies and Ho.¹²
AU= arbitrary units.¹³

shown in Table 1, almost 50 % of the randomised patients (42/86) were excluded from the study because of adverse events, poor tolerance, or failure to fully adhere to the protocol. Thus the "per protocol set" used for efficacy analysis consisted of a total of 44 patients (19 in the sodium hyaluronate group and 25 in the saline group). Table 2 shows the main characteristics of these 44 patients obtained during the baseline visit (visit 1).

Study design

Randomised, double blind, parallel groups at six centres located in Germany were involved in the trial.

Approval by the local ethics committee was obtained for all participating centres before starting the recruitment. The study was conducted according to the principles contained in the Declaration of Helsinki and good clinical practice.

Tests performed

All tests were performed before randomisation (visit 1 = baseline) and after 28 (SD 7) days (visit 2), 58 (8) days (visit 3), and 92 (10) days (visit 4) of treatment and included an evaluation of symptoms by a VARS (visual analogue rating scale), a complete ophthalmological examination, and impression cytology.

Subjective symptoms (foreign body sensation, burning, heaviness of the lids, photophobia, and stinging) were evaluated by using a VARS overall efficacy score (ratio between the sum of all VARS scores and the maximum obtainable score). A value of 100% equals no ocular symptoms—that is, maximum efficacy; a value of 0% equals maximum intensity of all symptoms assessed in both eyes—that is, no efficacy.

The ophthalmological examination included, in the following order, the assessment of tear film break up time (BUT), fluorescein staining, rose bengal staining, impression cytology, and anaesthetised Schirmer's test.¹³ This sequence of tests was performed in order to minimise interference of one test on the next.

For the impression cytology, cellulose acetate filter strips were pressed against the temporal interpalpebral bulbar conjunctiva after application of topical anaesthesia. Specimens were then stained with the PAS-Papanicolaou method^{7, 14, 15} and graded according to Nelson¹ by one operator in a blind manner. Impression cytology scores were obtained as the mean result from both eyes.

Study materials

Hyaluronan, as sodium hyaluronate of molecular weight approximately 3×10^6 D (BTG, Israel), was manufactured by a process of continuous fermentation from streptococci and formulated to a 0.15% solution in phosphate buffered saline at pH of 7.3 and osmolarity of 0.285 Osm/kg. The dynamic viscosity of the solution was 30 cP. The reference or control medication was a physiological sodium chloride solution 0.9% (w/v).

Both medications were supplied as 0.3 ml solution in sterile, unidose, preservative-free containers. An enrolment log of more than 1 year was planned because of the stringent inclusion/exclusion criteria of the protocol. Patients were prescribed to use one drop 4–8 times a day. This wide range was chosen because of the expected variability of both environmental conditions and eye drop needs during the study period. The number of eye drops used was evaluated by a diary card. In addition, used and unused unit doses were returned to the investigator and counted at the end of the study.

Efficacy and safety variables

The primary efficacy variable used in this study was the ocular surface damage score as evaluated by impression cytology. The end point of the study was at visit 4 (3 months of treatment). Safety was assessed by monitoring all adverse events throughout the course of the study.

Statistics¹⁶

The statistical analysis was done by means of the software sas (version 8.1). A mean value of left and right eye was calculated for each variable.

For the evaluation of impression cytology results, the raw values of visits 2, 3, and 4 were compared using the Mantel-Haenszel test to detect any significant differences between the two treatment groups. A within group comparison of scores at each visit was also performed. Paired *t* test were used as the statistical test.

For all the other variables an analysis of variance (ANOVA) was used to detect difference between the two treatment groups. The efficacy analysis was performed on the "per protocol set" defined as the subset of subjects fully compliant with the protocol.

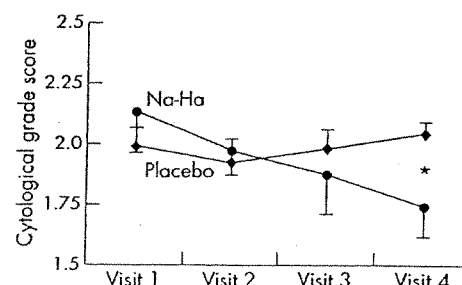


Figure 1 Impression cytology scores after treatment with sodium hyaluronate (Na-Ha) or saline (placebo). Data are expressed as mean values (SEM) of left and right eye scores obtained at each day visit (V1 = baseline; V2 = 1 month; V3 = 2 months; V4 = 3 months). Between groups comparison [Na-Ha v saline]: V1: $p = 0.115$; V2: $p = 0.720$; V3: $p = 0.479$; V4: $p = 0.036$. Within groups comparison: saline: V2 v V1: $p = 0.776$; V3 v V1: $p = 0.752$; V4 v V1: $p = 0.259$. Na-Ha: V2 v V1: $p = 0.083$; V3 v V1: $p = 0.158$; V4 v V1: $p = 0.024$.

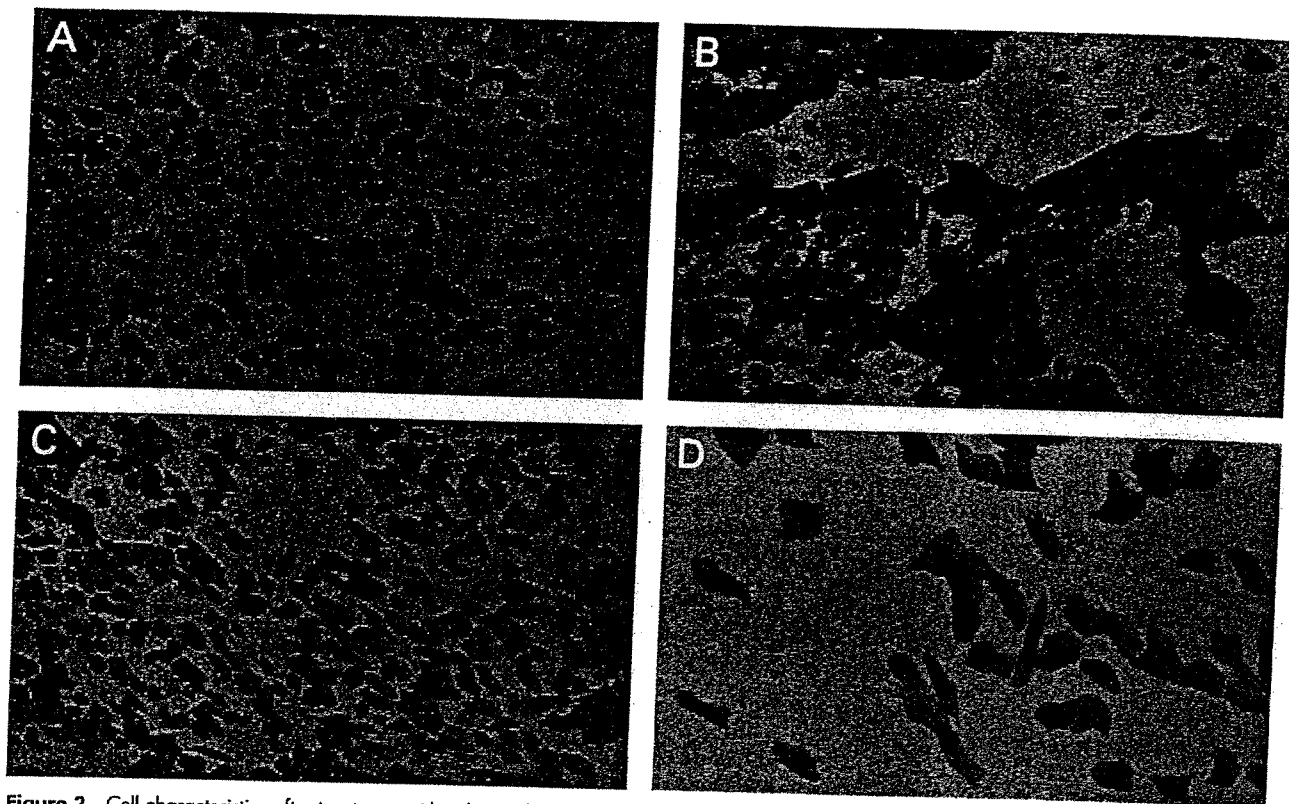


Figure 2 Cell characteristics after treatment with either sodium hyaluronate or saline. (A) Cytological characteristics before treatment (visit 1): presence of large epithelial cells, showing a polygonal shape and a variable staining cytoplasm and a nucleus/cytoplasm ratio of 1:3 – 1:4; goblet cells are absent. (B) Sodium hyaluronate group after 3 months of treatment (visit 4): presence of both smaller cells with polygonal and round shape and of goblet cells. (C and D) Saline group after 3 months of treatment (visit 4): presence of polygonal cells with keratinised, basophilic cytoplasm with markedly reduced nucleus/cytoplasm; goblet cells are absent.

A statistically significant difference at the 95% confidence level was assumed in the presence of a *p* value less than 0.05.

RESULTS

Impression cytology

Impression cytology grades obtained during the study are shown in Figure 1. No significant differences were observed between sodium hyaluronate and placebo groups at visits 2 and 3, whereas after 3 months of treatment (visit 4) scores obtained in patients treated with sodium hyaluronate were statistically significantly lower than those obtained in patients treated with saline ($p = 0.036$). These results may be explained as either a treatment related effect or a detrimental long term effect of saline on ocular surface. In order to clarify this issue a within group comparison was also performed. Interestingly, the impression cytology grade remained unchanged over time in the saline group, whereas it improved in the hyaluronate group. This positive effect over baseline became statistically significant after 3 months of treatment ($p = 0.024$).

Representative cytological pictures of the two treatment groups are shown in Figure 2. Baseline cytological characteristics (visit 1) were: presence of large epithelial cells with a polygonal shape, a nucleus/cytoplasm ratio of 1:3–1:4, and absence of goblet cells (Fig 2A). After 3 months of treatment with sodium hyaluronate the main cytological feature was the presence of smaller cells with both polygonal and round shape and the presence of goblet cells (Fig 2B). On the contrary, in the saline treated group the cytological features were presence of polygonal cells with keratinised, basophilic cytoplasm, markedly reduced nucleus/cytoplasm ratio, and absence of goblet cells (Fig 2C and D).

Other variables

In both treatment groups an improvement for all symptoms compared with baseline was observed. After 3 months of treatment the overall efficacy score was better in the hyaluronate group but this difference was not statistically significant (ANOVA, $p = 0.059$).

The mean number of eye drops used daily (sum of both eyes) was comparable in the two groups of treatment (hyaluronate group: mean 9.1 (SD 1.4); saline group: 9.9 (1.1)).

For the other tests considered (tear film break up time, fluorescein staining, rose bengal staining, and Schirmer's test) no statistically significant difference between treatment groups was observed. However, a more or less pronounced improvement over baseline was noticed for all variables in both treatment groups (data not shown).

Safety

The safety was evaluated in all 86 randomised patients (safety subset) All study medications appeared to be safe and were generally well tolerated. No differences between treatment groups were observed with regard to the possibly treatment related adverse events. No treatment related serious adverse events occurred during the study.

DISCUSSION

It has been reported that patients with keratoconjunctivitis sicca show substantial abnormalities of the ocular surface, in particular of the epithelial morphology and goblet cells distribution with a shift toward squamous metaplasia. From the clinical point of view one of the most important clinical feature of dry eye syndrome is an alteration of the corneal and conjunctival epithelium, as demonstrated by fluorescein and

rose bengal vital stains. However, these methods do not seem to correlate with the degree of squamous metaplasia and goblet cell abnormalities found in patients with dry eye.¹¹

Sodium hyaluronate has been shown to improve both fluorescein and rose bengal stains in patients with dry eye,^{8,9} suggesting that the treatment with this agent promotes corneal and conjunctival epithelial healing. The efficacy of sodium hyaluronate to reduce histological changes of ocular surface, in patients with dry eye, was evaluated by Nelson¹¹ following a treatment period of 8 weeks. That study showed that although sodium hyaluronate improves rose bengal staining it did not change the degree of squamous metaplasia. It was concluded that significant changes of this parameter could take longer than 8 weeks of treatment. In the present study impression cytology scores were used as a direct indicator of ocular epithelial damage. We found an improvement of ocular surface features after treatment with sodium hyaluronate compared with saline. The differences between the two groups became statistically significant after 3 months of treatment. These results may be interpreted as a consequence of a worsening of the ocular surface damage produced by the long term treatment with saline instead of an improvement in the hyaluronate group. However, the impression cytology grade remained unchanged over time in the placebo group, whereas it improved in a statistically significant manner in the hyaluronate group, suggesting a direct treatment related effect. Interestingly, we have recently reported that sodium hyaluronate significantly improved signs and symptoms in patients with moderate to severe dry eye independently from the saline composition of the ophthalmic solutions, suggesting that a greater importance should be given to sodium hyaluronate rather than the physical/chemical properties, such as osmolarity or ions.⁸

How hyaluronate improves ocular surface is at present unknown. However, several mechanisms can be advocated.

Hyaluronan is a natural polymer and its concentration increases in response to ocular damage and during corneal wound healing.¹⁷ In vitro hyaluronate promotes cell migration and can stabilise ocular surface epithelial barrier^{18,19} suggesting that it may be directly involved in the process of epithelial repair by activation of the CD44 (the hyaluronate receptor). CD44 is expressed in corneal and conjunctival cells^{20,21} and its activation promotes the interaction with cytoskeletal proteins²² suggesting a role for hyaluronate in cell adhesion and motility. Moreover, binding of hyaluronate to CD44 may stimulate cell proliferation through a mechanism involving a kinase cascade.²³

Hyaluronate may play a part in the controlling the localised inflammation often present in patients with keratoconjunctivitis sicca.²⁴ Interestingly it has been recently reported that the expression of the CD44 is increased in patients with moderate dry eye and superficial keratitis and that sodium hyaluronate given for a 2 month period is associated with a decreased expression of this adhesion molecule.²¹

Hyaluronate increases the stability of the precorneal tear film, which protects the ocular surface from environmental agents²⁵ and it has water retentive properties, which improve ocular surface wettability.²⁵⁻²⁷ Therefore, hyaluronate may contribute to a favourable microenvironment during ocular repairing processes.

Finally hyaluronate has viscoelastic properties that can lubricate the ocular surface reducing friction during blinking and ocular movements.

In conclusion, this study suggests that sodium hyaluronate has a beneficial effect on the conjunctival epithelium, as demonstrated by impression cytology in a well defined and homogeneous population of patients with dry eye, selected by using

stringent inclusion criteria. Thus, sodium hyaluronate, with its reported efficacy on symptoms, the described properties on wound healing, and anti-inflammatory action, can be considered particularly useful for the treatment of dry eye.

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