CLINICAL STUDY

Improvement of the ocular surface using hypotonic 0.4% hyaluronic acid drops in keratoconjunctivitis sicca

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Abstract

Background **The ocular surface changes of keratoconjunctivitis sicca (KCS) could be the result of the effect of an altered tear film on the epithelial environment.**
Purpose **To evaluate the possibility of improving the environmental conditions of the ocular surface by lowering tear osmolarity, increasing tear film volume and stabilising the tear film. Also, to study the effect of such an improvement on the epithelial cells of the ocular surface.**
Methods **One hundred and thirty-five patients with a diagnosis of KCS were treated on a randomised basis with either unpreserved hypotonic 0.4% hyaluronic acid (HHA) eye drops or 0.3% hydroxypropylmethylcellulose plus 0.1% Dextran 70 (HPMC) eye drops 6 times a day for 60 or 90 days. In all patients a Schirmer I test, break-up time (BUT), ocular surface staining with 1% Bengal Rose, or 2% fluorescein, as well as subjective symptoms, were recorded before and 15, 30 and 60 days after the beginning of the study. Patients were divided into three subgroups and the effect of the treatment was studied using three different techniques: the tear ferning test, conjunctival impression cytology and tear osmolarity measurement.**
Results **Improvements in BUT, vital staining, Schirmer I and symptoms were recorded in both groups of treatment, with significant differences for patients treated with 0.4% HHA. On day 60, 30 min after installation: tear ferning patterns changed from 100% pathological (types III–IV) to 93% physiological (types I–II) in the 0.4% HHA group and from 100% pathological to 78% physiological in the 0.3% HPMC group ($p < 0.01$ between groups). Tear osmolarity shifted from 353 ± 23 to 305 ± 6 mosmol/l in the 0.4% HHA group and from 346 ± 15 to 336 ± 8 mosmol/l in the 0.3% HPMC group ($p < 0.001$ between groups). On day 90, the impression cytology score improved from 1.2 to 1.9 in the 0.4% HHA group while it did not change in the 0.3% HPMC group ($p < 0.05$ between groups).**
Conclusion **In KCS appropriate treatment with a hypotonic 0.4% HHA tear substitute can change the tear environment and results in improvement of the epithelial conditions of the ocular surface.**

Key words Conjunctival impression cytology test, Dry eye, Hydroxypropylmethylcellulose + dextran (HPMC), Hypotonic hyaluronic acid (HHA), Keratoconjunctivitis sicca, Tear ferning test, Tear osmolarity, Tear substitutes

Keratoconjunctivitis sicca (KCS) is an ocular surface disorder resulting from reduced tear film volume and stability and is clinically characterised by corneal and conjunctival changes and typical symptoms (burning, foreign body sensation, photophobia and pain) that greatly spoil the quality of life of patients with this disease. From a diagnostic point of view the disease can be recognised, staged and monitored on the basis of some objective signs, such as: tear production (measured by the Schirmer test), break-up time (BUT), staining of the ocular surface with 1% Bengal Rose or 2% fluorescein,[1] evaluation of tear ferning patterns[2] and impression cytology of the conjunctiva.[3] More recently, tear osmolarity has been suggested to be a main pathogenetic factor of the disease able to induce changes in ferning patterns of tear samples and to modify the cytological profile of the ocular surface such as goblet cell density and epithelial morphology.[4]

This study evaluated the possibility of improving the environmental condition of the ocular surface in KCS by lowering tear osmolarity, increasing tear film volume and stabilising the tear film, and the effect of such an improvement on ocular surface cell conditions.

Patients and methods

The study was a multicentre trial conducted in Italy by Fidia Oftal spa, Catania, Italy. The protocol and informed consent for the study was approved by the ethics committee at each of the participating centres. All patients were over 18 years old and all signed the informed consent prior to recruitment.

One hundred and thirty-five consecutive patients with a clinical diagnosis of moderate to severe KCS were enrolled in a controlled, phase III clinical trial. Inclusion criteria were: Schirmer I test < 5.5 mm/5 min, Bengal Rose staining positive and typical,[5] BUT < 7 s, typical KCS symptoms. Exclusion criteria were: infectious keratoconjunctivitis or inflammatory disease not related to dry eye, previous ocular surgery, concomitant ocular pathologies, eyelid or eyelashes abnormalities, nasolacrimal apparatus alteration, consumption of drugs affecting tearing, concomitant ocular therapies and pregnancy.

Patients on treatment with other medications for dry eye relief were allowed to enter the study following a 5–10 day wash-out period using unpreserved saline as needed. Patients were recruited from three different centres. In all patients, ocular symptoms such as burning, photophobia, foreign body sensation and pain, scored from 0 (absent) to 4 (severe), were recorded before treatment and after 15, 30 and 60 or 90 days of therapy with unpreserved hypotonic 0.4% hyaluronic acid (HHA) or 0.3% hydroxypropylmethylcellulose plus 0.1% Dextran 70 (HPMC). Also a Schirmer I test (mm/5 min), BUT (s), staining of the ocular surface with 1% Bengal Rose (Eagle Vision, Memphis, TN) or 2% fluorescein (Alfa Intes, Casoria, Italy) (scored from 0 to 9) were recorded before treatment and after 15, 30 and 60 or 90 days of therapy with 0.4% HHA or 0.3% HPMC.

While the standard parameters for tear film condition and dry eye symptoms were used by all centres, each centre also analysed the effect of the treatment using different techniques: the tear ferning test, tear osmolarity measurement and conjunctival impression cytology.

Subgroup exclusion criteria were type I and II tear ferning pattern when patients were studied with the tear ferning test, a tear osmolarity lower than 310 mosmol/l for those patients whose tear osmolarity was analysed and a score higher than 1.7 when patients were studied by conjunctival impression cytology.

After recruitment, patients were treated on a random basis with 0.4% HHA or 0.3% HPMC six times a day for 60 or 90 days: tear ferning test and tear osmolarity values were tested at baseline, and after 15, 30 and 60 days of treatment, while conjunctival impression cytology was performed before treatment and after 90 days of therapy to provide the opportunity for full recovery of surface cells.

In the group in which the tear ferning test was performed, 45 of 135 patients were enrolled and tear ferning patterns were evaluated on commencing the study and after 15, 30 and 60 days of therapy, at both 30 min and 90 min after the last instillation of the eye drops. Tear samples were collected by means of a micropipette from the lower fornix of each eye and put on a slide to dry at room temperature. After 5–10 min they were observed by phase-contrast microscopy at ×100 magnification: ferning patterns were classified into four types.[2,6]

In the second group of 58 patients tear osmolarity values were monitored on commencing the study and after 15, 30 and 60 days of treatment with 0.4% HHA or 0.3% HPMC (at both 30 min and 90 min after the last instillation of the drops). Tear samples (2 μl) were collected from the inferior marginal tear strip of each eye using a calibrated microcapillary pipette sampling technique that minimised reflex tearing. Tear osmolarity (m0sm/l) was determined using the cryoscopic osmometer Osmomat 030 (Gonotech, Berlin, Germany).[4,7]

In the third group of 32 patients a conjunctival impression cytology test was performed before treatment and after 90 days of treatment with 0.4% HHA or 0.3% HPMC.[3,8] The imprints were examined by light microscopy at ×400 magnification, in order to classify epithelial cell morphology (0 = flat, 1 = non-keratinised, 2 = cylindrical, 3 = cubic) and goblet cell density (0 = absent, 1 = low, 2 = medium, 3 = high). The final result was obtained by calculating the average of the two scores.

The statistical analysis was conducted by considering the change from baseline of patient's worse eye and between the 0.4% HHA group or 0.3% HPMC one. The data have been analysed using a descriptive analysis, Wilcoxon scores (rank sums) test, Student's *t*-test, covariance analysis and chi-square test.

Results

One hundred and thirteen patients completed the study. Twenty-two were excluded for different reasons: 6 patients had tear ferning patterns I and II at time 0, five patients did not come to the centres for follow-up, four had Bengal Rose allergy, seven for other reasons. One hundred and three were female and 10 male with a mean age of 54 ± 11 years (mean ± SD). Fifty-eight patients (54 female, 4 male) were treated with 0.4% HHA and 55 (49 female and 6 male) with 0.3% HPMC. No significant difference was found in age (52.2 ± 10.6 years and 56.4 ± 12.8 years respectively), weight (59.5 ± 10.4 kg and 64 ± 11.3 kg respectively) and height (161.3 ± 6.9 cm and 162.8 ± 7.3 cm respectively) between patients treated with 0.4% HHA and those treated with 0.3% HPMC.

At baseline no significant difference was found for signs and symptoms between the group treated with 0.4 HHA and that treated with 0.3% HPMC.

With the introduction of artificial tears, a significant decrease in all symptoms was found between baseline and day 60. In addition a significant difference was found for foreign body sensation, burning, pain and photophobia between the group of patients treated with 0.4% HHA tears and those with 0.3% HPMC tears, on days 15, 30 and 60 (Fig. 1). Also all the signs improved comparing those at day 0 with those at day 60 and

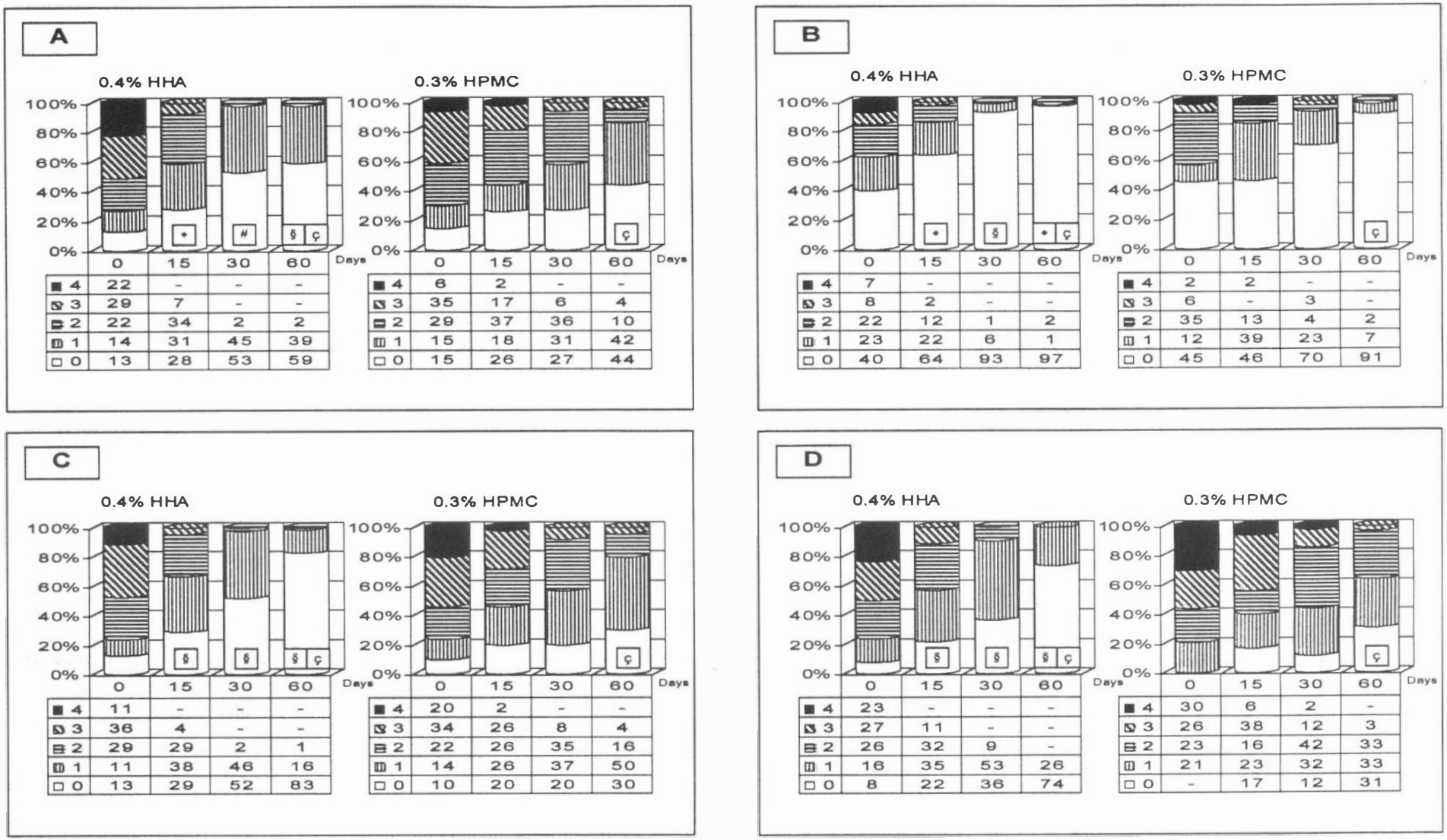

Fig. 1. *Symptoms in KCS patients before and after treatment. Percentage of KCS patient eyes with photophobia (A), pain (B), burning (C), foreign body sensation (D) scored from 0 (absent) to 4 (severe) before treatment and after 15, 30 and 60 days of therapy with 0.4% HHA (left column)* (n = *116 eyes) or 0.3% HPMC (right column)* (n = *110 eyes).* ♦ = $p < 0.05$: *0.4% HHA vs 0.3% HMPC (Wilcoxon rank sum test).* # = $p < 0.01$: *0.4% HHA vs 0.3% HMPC (Wilcoxon rank sum test).* § = $p < 0.0001$: *0.4% HHA vs 0.3% HMPC (Wilcoxon rank sum test).* ç = $p < 0.0001$: *baseline vs 60 days (paired Student's* t*-test).*

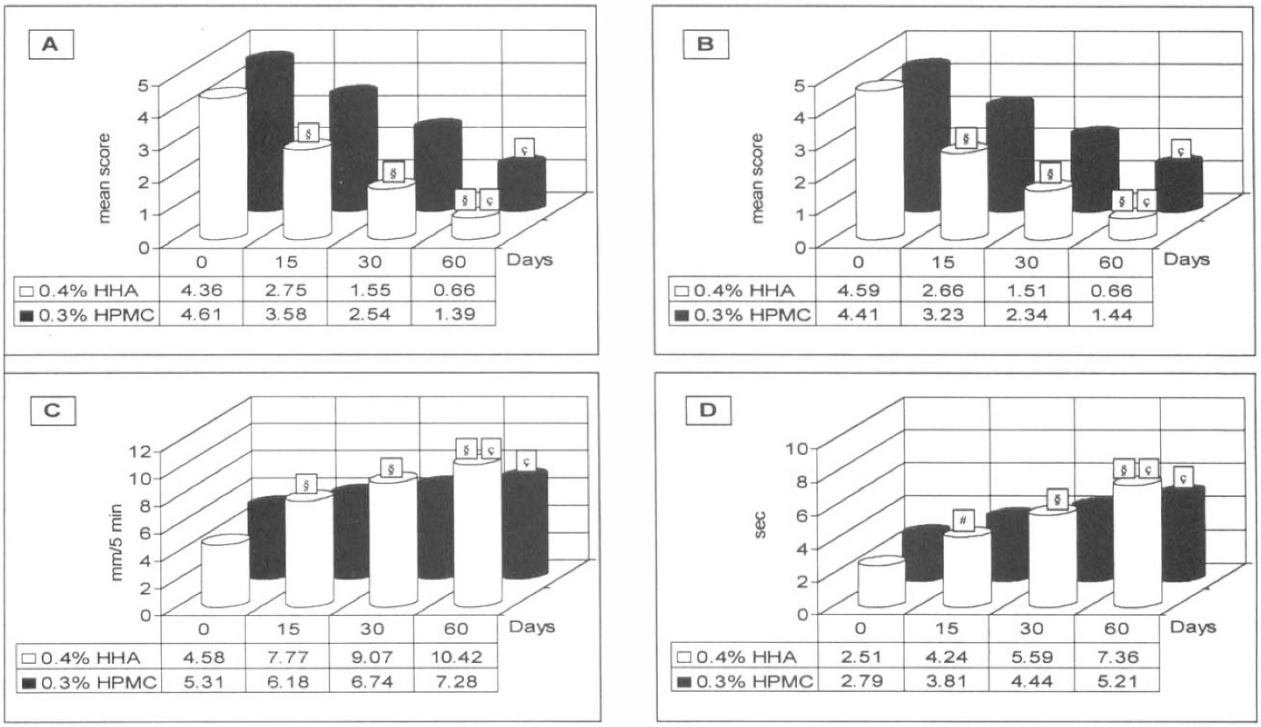

Fig. 2. *Signs in KCS patients before and after treatment. (A) Fluorescein staining of ocular surface (mean score) in KCS patients before treatment and after 15, 30 and 60 days of therapy with 0.4% HHA (outer columns) or 0.3% HPMC (inner columns). (B) Bengal Rose staining of ocular surface (mean score) in KCS patients before treatment and after 15, 30 and 60 days of therapy with 0.4% HHA (outer columns) or 0.3% HPMC (inner columns). (C) Mean values of Schirmer I test (mm/5 min) in KCS patients before treatment and after 15, 30 and 60 days of therapy with 0.4% HHA (outer columns) or 0.3% HPMC (inner columns). (D) Mean values of tear film break-up time (s) in KCS patients before treatment and after 15, 30 and 60 days of therapy with 0.4% HHA (outer columns) or 0.3% HPMC (inner columns). * = p < 0.05: 0.4% HHA vs 0.3% HMPC (Wilcoxon rank sum test). # = p < 0.01: 0.4% HHA vs 0.3% HMPC (Wilcoxon rank sum test). § = p < 0.0001: 0.4% HHA vs 0.3% HMPC (Wilcoxon rank sum test). ç = p < 0.0001: baseline vs 60 days (Paired Student's t-test).*

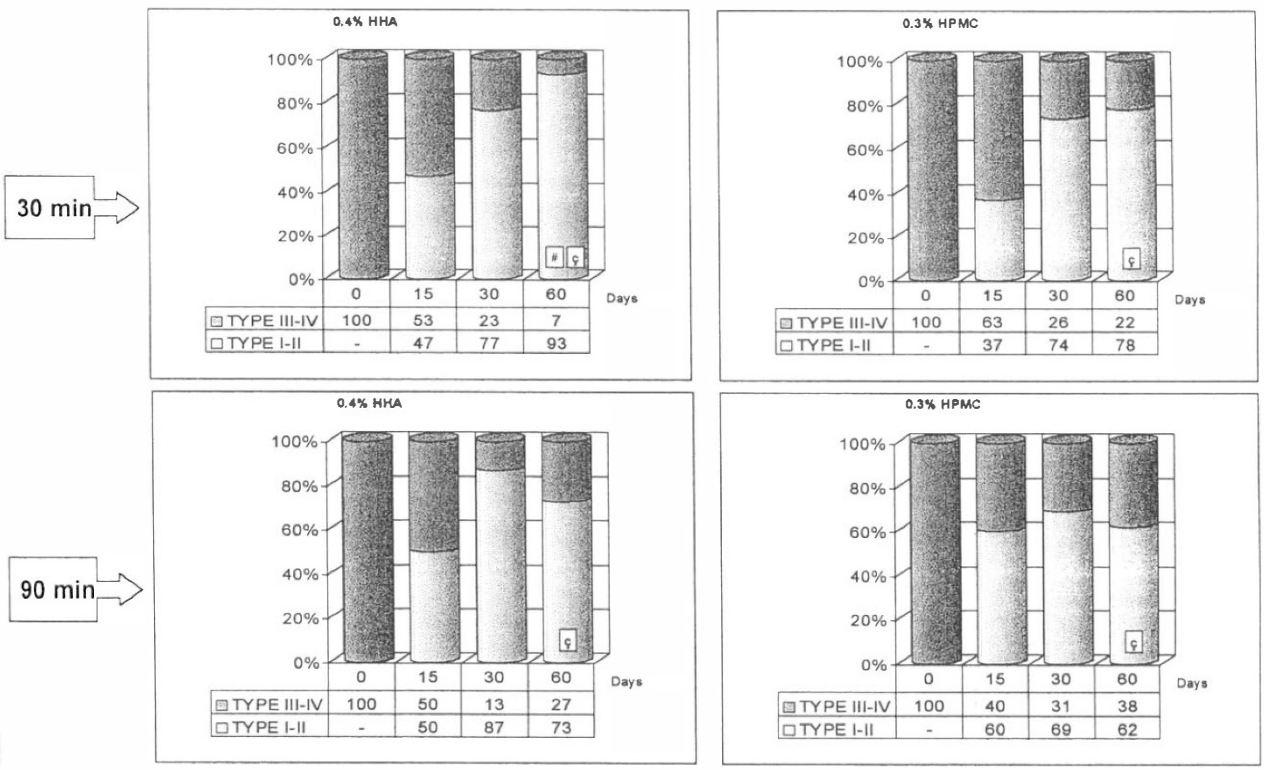

Fig. 3. *Tear mucus ferning before and after treatment. Incidence of normal (type I and II) and pathological (type III and IV) tear mucus ferning in KCS patients before treatment and after 15, 30 and 60 days of therapy with 0.4% HHA (left column) (n = 30 eyes) or 0.3% HPMC (right column) (n = 32 eyes), 30 and 90 minutes after the last eye drop application. # = p < 0.01: 0.4% HHA vs 0.3% HMPC (chi-square test). ç = p < 0.0001: baseline vs 60 days (Wilcoxon signed rank sum test).*

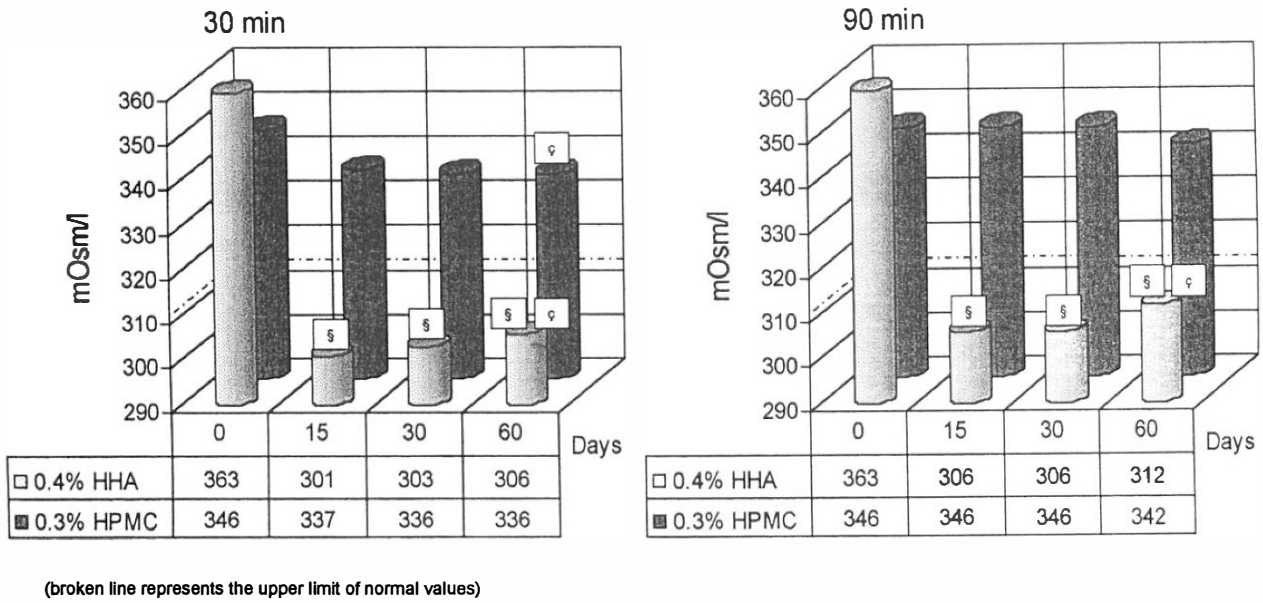

Fig. 4. *Tear film osmolarity before and after treatment. Mean values of tear film osmolarity in KCS patients before treatment and after 15, 30 and 60 days of therapy with 0.4% HHA (outer columns)* (n = *58 eyes) or 0.3% HPMC (inner columns)* (n = *56 eyes), 30 and 60 minutes after the last eye drop application.* § = p < *0.0001: 0.4% HHA vs 0.3% HMPC (covariance analysis).* ç = p < *0.001: baseline vs 60 days (paired Student's t-test).*

significant differences were found between baseline and day 60; in particular 1% fluorescein staining and Bengal rose decreased significantly, while the Schirmer test and BUT increased significantly. When patients treated with 0.4% HHA tears and those treated with 0.3% HPMC tears were compared, a significant difference was found on days 15, 30 and 60 between the groups. The patients treated with 0.4% HHA had a subjectively greater improvement of symptoms than those treated with 0.3% HPMC (Fig. 2).

Thirty-one patients had the tear ferning test. On day 60, 30 min after instillation of the drops, tear ferning patterns changed from 100% pathological (types III–IV) to 93% physiological (types I–II) in the 0.4% HHA group

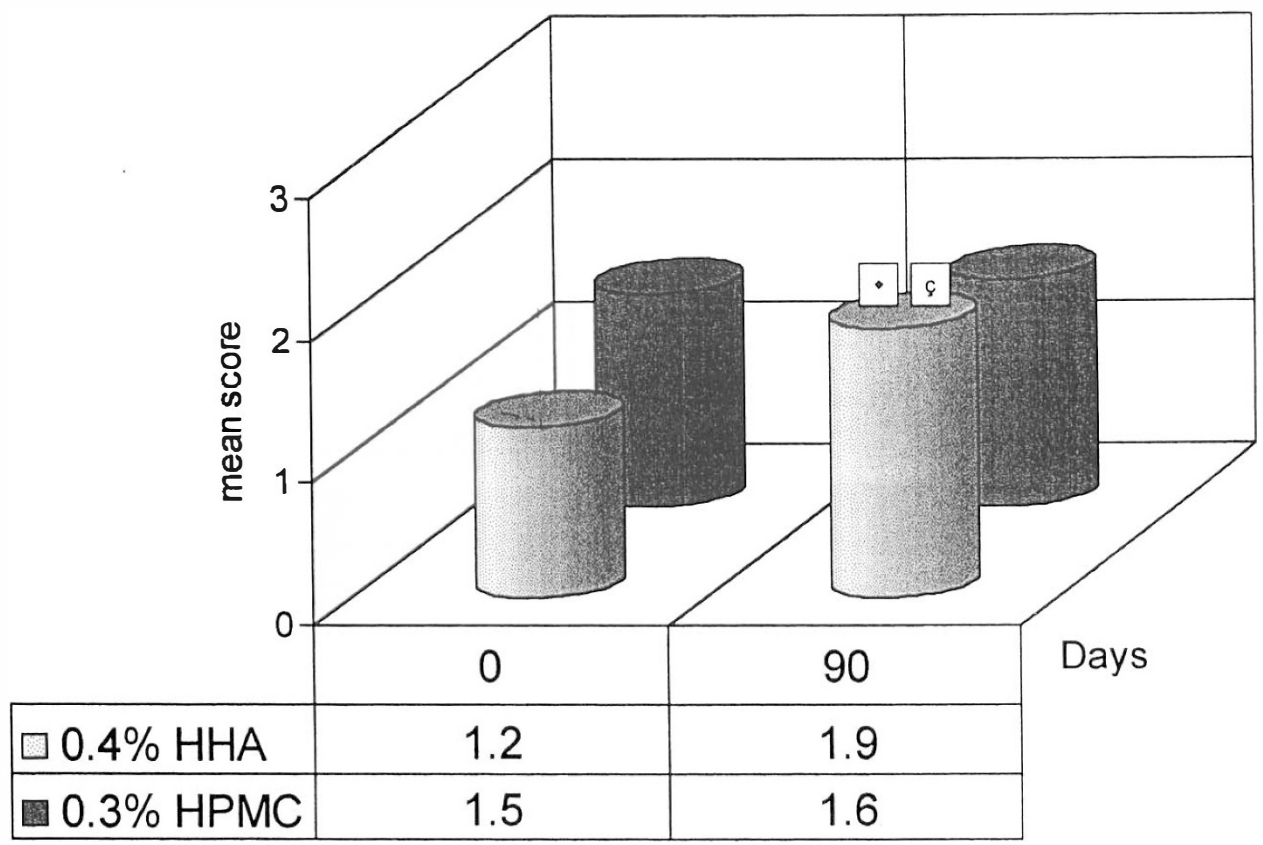

Fig. 5. *Impression cytology mean score before and after treatment. Average of goblet cell density and epithelial cell morphology scores in KCS patients before treatment and after 15, 30 and 60 days of therapy with 0.4% HHA (outer columns)* (n = *28 eyes) or 0.3% HPMC (inner columns)* (n = *22 eyes).* ♦ = p < *0.05: 0.4% HHA vs 0.3% HMPC (covariance analysis).* ç =p < *0.0001: baseline vs 60 days (paired Student's t-test).*

and from 100% pathological to 78% physiological in the 0.3% HPMC group. The difference between groups was significant ($p < 0.001$). No other significant difference was found (Fig. 3).

Fifty-seven patients had the tear osmolarity test. In 0.4% HHA-treated eyes, a significant improvement in tear osmolarity towards normality was achieved and maintained over time, while in the 0.3% HPMC-treated eyes tear osmolarity values were abnormal at each follow-up examination. On day 60, 30 min after instillation, tear osmolarity shifted from 353 ± 23 to 305 ± 6 mOsm/l in the 0.4% HHA group and from 346 ± 15 to 336 ± 8 mOsm/l in the 0.3% HPMC group ($p < 0.001$ between the groups) (Fig. 4).

Twenty-five patients underwent conjunctival impression cytology. On day 90 the impression cytology score improved from 1.2 to 1.9 in the 0.4% HHA group while it did not change in the 0.3% HPMC group. This improvement was significant in comparison with the improvement produced by 0.3% HPMC therapy, although the conjunctival cell status of HHA was worse than that of HPMC patients before starting the therapy (Fig. 5).

Discussion

KCS is a disease of the ocular surface consequent on tear film alterations and giving rise to typical symptoms and changes in the conjunctiva and cornea.[9,10] Epithelial changes which disturb the adhesion of natural or artificial tears to the ocular surface are maintained as a result of interactions of altered tears with the epithelium.[11]

In particular, it has been suggested that the presence of a reduced tear volume or of an unstable tear film will increase water evaporation from the tear film, leading to an increased osmolarity and consequent damage.[12] In time, this results in damage to the ocular surface epithelia with loss of the glycocalyx and a squamous metaplastic change in the conjunctiva. As a consequence, the adhesion of normal or artificial tears to the abnormal ocular surface is prevented, allowing both a faster evaporation rate of the water from the ocular surface and a direct traumatic or inflammation-mediated damage with build up of a series of vicious circles.

On the basis of this it is proposed that tear substitutes for KCS should provide an adequate amount of fluid for the ocular surface, to guarantee regular flushing and waste renewal for the ocular surface, and allow adequate lubrication to the lid/globe system by reducing the shearing forces at the interfaces, and protect and facilitate the natural healing process of the ocular surface cells so that the glycocalyx can be rebuilt and the ability of the ocular surface to grasp water can be restored.[13]

These alterations can also create a decrease in psychophysical performance. Indeed it has been shown that in patients with KCS, spatial contrast sensitivity is lower than in normal age-matched subjects. But the use of artificial tears can significantly improve spatial contrast sensitivity, suggesting the importance of having an intact tear film surface for correct vision.[14]

Current management of KCS includes the use of artificial tear substitutes, and punctal occlusion and other surgical interventions in more severe cases.[15,16]

We found significant improvements in symptoms (burning, foreign body sensation, photophobia and pain) and clinical signs (Bengal Rose or fluorescein staining, BUT, tear ferning test, conjunctival impression cytology and tear osmolarity) in patients treated with either 0.4% HHA or 0.3% HPMC; however, 0.4% HHA also provided a clinically significant improvement over 0.3% HPMC for both symptoms and signs.

One hundred and nine patients improved using one of the two artificial tear preparations: in particular, when 0.4% HHA was used, 56 patients improved, while 53 patients improved when they were treated with 0.3% HPMC. These data were not significantly different, but a significant difference was found when the assessment of the improvement was considered. Indeed patients treated with 0.4% HHA had a greater improvement than the control group.

In order to obtain reproducible results, this multicentre trial was split over three centres according to the abilities of the different groups to deal with the specific test used. This reduced the overall number of the patients examined by each test, but gave the opportunity for greater consistency.

Since one of the pathogenetic pathways of damage in KCS is increased tear water evaporation from the tear film[9] inducing a higher than normal (298 mosmol) tear osmolarity[7] and in turn leading to goblet cell loss and epithelial damage, the hypotonicity of the tear substitute may have been of help in restoring a more normal environment for the ocular surface.[17]

Tear ferning has been reported to be the result of the equilibrium between electrolyte concentration and high-molecular-weight glycoproteins of the tear film. The improvement of ferning patterns suggests a corresponding improvement in the electrolyte/glycoprotein ratio, possibly related to the electrolyte-driven, osmolarity correction.[18] The ocular improvement in the surface environment is confirmed by the striking changes in the conjunctival samples obtained by impression cytology.

The 0.4% HHA-based tear substituted seemed to create a more correct, albeit artificial environment for the ocular surface, able to stop the deteriorating vicious circles and to allow healing and regeneration of a healthier ocular surface epithelium.

The 0.4% HHA exhibits non-Newtonian behaviour, that is, its viscosity decreases as the velocity of lid movement increases, and reduces shearing forces and consequent trauma to the epithelium of the ocular surface. It has also a very long tear adherence (adsorption and absorption) to the ocular surface with considerable blurring effects on vision because of its adsorption capabilities. The 0.4 HHA drop was more efficacious than 0.3% HPMC in improving symptoms and clinical signs in the patients studied, and was as safe as 0.3% HPMC over the period of the trial.

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