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Analysis of tear film in cystinosis patients treated with topical viscous cysteamine hydrochloride (Cystadrops®)

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Abstract

Purpose: The aim of this study was to evaluate *in vivo* the tear film in infantile nephropathic cystinosis patients with corneal crystals treated with topical viscous cysteamine hydrochloride (Cystadrops®).

Methods: Ten eyes of five patients with nephropathic cystinosis aged from 10 to 35 years were included in this study. The patients were under treatment with viscous cysteamine hydrochloride formulation containing 3.8 mg/mL cysteamine (vCH 0.55%, equivalent to 0.55% CH; Cystadrops®; Recordati rare Diseases, Puteaux, France) to reduce corneal crystal density. Five age and sex matched individuals were randomly selected as control group. Tear osmolarity testing (TearLab™) was performed to assess the *in vivo* osmolarity of patients under treatment and compared to control group values. Tear film break-up time (TBUT) and basic tear secretion (Schirmer test) were also assessed.

Results: Mean tear osmolarity was 294.8 mOsms/L (±10.4), with a mean absolute difference of 1.85 mOsms/L (±2.13) between the eyes. There was no statistically significant difference between the osmolarity readings of cystinosis and the control group (294.8 ± 10.4 vs 299.4 ± 6.2mOsm/L, respectively; $p = 0.39$). The mean TBUT was 10.2 ± 0.83 s in the study group versus 10 ± 0.7 s in controls ($p = 0.62$). The mean Schirmer test score was 9.2 ± 0.83 mm in the patients versus 10.2 ± 0.83 mm in the controls ($p = 0.14$).

Conclusions: The TearLab™ osmolarity system test showed good reliability and precision in repeated measurements. This is the first report using the TearLab osmolarity system to assess tear film in patients with cystinosis treated with vCH 0.55%. TearLab™ examination showed that the use of vCH 0.55% drops does not determine alterations of the tear film quality.

Keywords

ocular cystinosis, corneal crystal, tear osmolarity

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Introduction

Cystinosis is a rare lysosomal disease featured by the formation and the storage of cystine crystals in several tissues. Brain, kidney, bones, and eyes are often affected. This disorder is due to the mutation of the cystinosin gene (CTNS, on 17p13) that codes for cystinosin, a transmembrane protein that transport the cystine out of the lysosome.[1] In these patients we can find cystine crystals in all structures of the eyes, but the pathognomonic and most frequently described ocular manifestation of cystinosis is crystal deposition in the cornea.[2–4] Consequences of crystal deposition include photophobia, punctate keratopathy, filamentary keratitis, recurrent epithelial erosions that can lead to pain, visual impairment, and

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scarring.[5,6] Filamentous kerato-conjunctivitis, band keratopathy, and corneal neovascularization[1–4] can also arise, specially in older patients. It is known that oral treatment with cysteamine is effective in reducing cystine crystals accumulation in all districts, improving prognosis. But in the cornea, where there is a lack of vascularization, it does not prevent crystal storage. Reducing corneal crystal density and photophobia is possible using a topical treatment with cysteamine hydrochloride (CH) drops.[7,8]

To keep the drug longer on the corneal surface, a viscous CH formulation containing 3.8 mg/mL cysteamine (vCH 0.55%, equivalent to 0.55% CH; Cystadrops®; Recordati rare Diseases, Puteaux, France) was produced, to be instilled less frequently than standard CH drops.[8] The vCH 0.55% drops solution includes a viscous agent, carmellose sodium, that allows to prolong the precorneal permanence time, and can be used up to 7 days after opening, without having to be stored in the refrigerator.[8]

Local adverse drug reactions (LADRs) and their duration and severity were reported in a Phase III Pivotal Study.[8] In this study LADRs were present in all patients and the most frequent ones were stinging, burning, redness, blurred vision and itching.

To better understand the pathophysiology of LADRs we decided to study *in vivo* the tear film osmolarity in cystinosis patients using vCH 0.55%.

Osmolarities and pH of the viscous solutions of cysteamine hydrochloride have been previously reported: osmolality values ranged between 351.2 ± 6.2 and 355.1 ± 7.9 mOsm kg^{-1}, and pH values were between 3.97 ± 0.1 and 3.98 ± 0.2.[9]

Despite the *in vitro* evaluation of the vCH 0.55% drops did not show hyperosmolarity, the tear osmolarity has never been assessed *in vivo* in patients with cystinosis using vCH 0.55%. The variation of tear osmolarity due to local therapy could be one explanation of the previously reported LADRs.

Tear film hyperosmolarity is regarded as a crucial pathophysiological component in dry eye disease (DED)[10,11] and has been proposed as the single best marker of DED severity.[12] It has been reported as more sensitive and specific for diagnosis and management of DED than other tear film tests.[13–15]

A recent technique using electrical impedance to measure tear osmolarity has been developed.[16] The TearLab™ osmometer (OcuSense Inc., San Diego, CA, USA) assesses small volumes (nanoliters) of tears with similar analytical performance as laboratory-based osmometers.[17]

The aim of our study is to evaluate tear osmolarity by means of the TearLab™ osmometer in patients with cystinosis treated with vCH 0.55% eye drop solution and to compare obtained data to age-matched control values. Additionally, quantitative parameters such as tear film break-up time (TBUT) and basic tear secretion (Schirmer test) are also assessed.

Methods

Ten eyes of five patients affected by infantile nephropathic cystinosis were included in this study: 3 females and 2 males with a mean age of 18.4 years (range, 10–35). For all patients, the diagnosis was based on a typical clinical history associated with an intraleukocyte cystine concentration above 3 nmol half-cystine/milligram protein.

Five age and sex matched individuals without nephropatic cystinosis were randomly selected as control group.

All patients underwent a complete ophthalmologic evaluation including best corrected visual acuity (BCVA), slit-lamp examination with corneal imaging, fundoscopy with 90D lens and intraocular pression (IOP) measurement. Based on corneal examination Gahl's corneal cystine crystal score (0–3)[18] was determined. All ophthalmologic evaluations were performed under controlled standardized room illumination conditions. A semi quantitative measure of corneal crystal deposits was performed according to Gahl's scale.

At study initiation, each patient was under treatment with vCH 0.55% eye drop solution (Cystadrops®) for more than one month: 1 drop was instilled four times a day in both eyes.[8,19] Local therapy's compliance was assessed.

Tear osmolarity testing (TearLab™) was assessed during the ophthalmic evaluation in patients under treatment and in age-matched control group. Tear film break-up time (TBUT) and basic tear secretion (Schirmer test, through Shirmer strips) were also assessed. All procedures were performed before the instillation of mydriatic drop in order to prevent ocular surface exposure to preservatives. TBUT was measured introducing a fluorescein strip into the conjunctival sac with minimal conjunctival irritation and asking to the patient to keep the eyes open after a few blinks. The tear film breaking time was evaluated with a slit lamp with cobalt blue filter.

To measure tear osmolarity we used the TearLab™ osmometer (OcuSense Inc., San Diego, CA, USA), using single-use test cards containing microchannels to collect tear fluid held by a pen designed to facilitate tear collection.

Measurements were performed an hour after the instillation of the therapy. Tear samples were collected by passive capillary action from the inferior tear meniscus near the lateral canthus, without manipulating the lower eyelid. Firstly, was tested right eye with three repeated measurements. The same procedure was then performed on the fellow eye. The time between two consecutive measurements on the same eye has never been greater than 1 min and patients were invited to blink normally when not being tested. Osmolarity readings were expressed in mOsm/L. Measurement was repeated in case the value was out of the osmometer's range (275–400 mOsm/L).

To reduce bias and modifications induced by the investigator on osmolarity evaluations, all measurements were

Table 1. Demographic data and ocular surface parameters of the patient.

Patient no.	Age	Gender	Tear Film Break-up time (sec)	Basic tear secrection (Schirmer) (mm)	Tear Osmolarity (msomol/L)
1	14	Male	11	9	301
2	12	Male	10	8	285
3	10	Female	9	10	310
4	21	Female	10	9	290
5	35	Male	10	10	288

performed in the same examination room. The examiner (SP) was trained to use the device by the same TearLab company representative. In accordance with manufacturer indications, quality control procedures were performed upon receipt of the test cards and before each session. The conditions of the room in which testing was performed, including relative humidity (%) and ambient temperature (°C), were constant and recorded before each measurement session.

Patient and control group data were compared by independent T-test and a value <0,05 was considered as statistically significant.

This study was conducted at the Bambino Gesù Children's Hospital in Rome, Italy.

All subjects were asked to sign a written consent form after explanation of the procedures of the study. The study was conducted according to the Declaration of Helsinki ethical principles.

Results

Mean Gahl's corneal cystine crystal score was 2.75.

Mean tear osmolarity in cystinosis patients was within physiological values as shown in Table 1. Consequently, no statistically significant difference was found between patient and control group (294.8 ± 10.4 and 299.4 ± 6.2mOsm/L, respectively; $P = 0.39$). (Table 1 and 2).

The mean TBUT was 10.2 ± 0.83 s in the study group versus 10 ± 0.7 s in controls ($p = 0.62$). The mean Schirmer test score was 9.2 ± 0.83 mm in the patients versus 10.2 ± 0.83 mm in the controls ($p = 0.14$). (Table 1 and 2).

There was no statistically significant difference between the two eyes of the patients in the TBUT and Schirmer test results (t-test, $p > 0.05$).

Discussion

Given that cystinosis is a rare disease, there are only a few studies reporting the effect of topical cysteamine hydrochloride in the treatment of corneal cystine crystals. In the last years, the availability of more advanced technology, such as in vivo confocal microscopy, has permitted the measurement of corneal cystine crystals both quantitatively and qualitatively.

Table 2. Demographic data and ocular surface parameters of the patient and control groups and comparisons using an independent samples t-test.

	Cystinosys Group	Control Group	P values
Age	18.4 ± 10.1	18 ± 8.1	0.83
Tear Film Break-up time (sec)	10.2 ± 0.83	10 ± 0.7	0.62
Basic tear secrection (Schirmer) (mm)	9.2 ± 0.83	10.2 ± 0.83	0.14
Tear Osmolarity (msomol/L)	294.8 ± 10.4	299.4 ± 6.2	0.39

It is demonstrated that topical cysteamine hydrochloride at 0.55% is efficacious as well as safe in treating corneal cystine crystals.[20]

The reduction in corneal cystine crystal density determines a minor risk of complications, such as corneal erosions, scarring, and neovascularization[7] with consequent improvements in photophobia and, thence, quality of life. However, treatment-related discomforts are reported causes frequently following instillation. Most frequent local adverse drug reactions (LADRs) are stinging, burning, redness, itching and blurred vision. Their duration and severity were reported in a Phase III Pivotal Study.[8]

To better understand pathophysiology of LADRs we decided to study *in vivo* the tear film osmolarity in patients using vCH 0.55%.

Two central factors to determine dry eye and hyperosmolarity are tear flow and evaporation. Decreasing tear secretion and increasing evaporation improve tear osmolarity, reducing tear turnover rate and tear film thickness.[21,22]

Tear film hyperosmolarity directly causes cell injury and nerve stimulation, triggering inflammatory cascades. These cascades then contribute to further cell damage, including loss of mucin-producing goblet cells. This increases tear film instability and drives the circle further.[23]

Parra et al. affirmed that tear film hyperosmolarity could increase corneal nerve terminal impulses and act as an effective stimulus.[24]

The main aim of this study was to evaluate *in vivo* the tear osmolarity among infantile nephropathic cystinosis patients with corneal crystals treated with topical viscous cysteamine hydrochloride.

This is the first analysis of tear osmolarity in patients under treatment with vCH 0,55%.

The results of this study showed that tears osmolarity readings fall within the normal range.

Tear osmolarity has been widely investigated to determine the normal range of osmolarity. It has been reported that the normal range varies between 293 and 318 mOsm/l.[25,26]

According to literature there is no uniformity in the estimation of the osmolarity normal range. Lemp and colleagues have suggested as the most sensitive cutoff value between normal and hyperosmolarity 308 mOsm/l, whereas the most specific was 315 mOsm/l.[13] Another cutoff value reported by Tomlinson and colleagues was 316 mOsm/l.[17]

In the present study, the mean tear osmolarity in cystinosis patients was 294 mOsm/l. The other tests (TBUT and Schirmer) were within normal range confirming the absence of quantitative tear film alteration in cystinosis patients. The results of this study suggest that the use of viscous cysteamine hydrochloride (Cystadrops®) to reduce corneal crystal density in patients with ocular cystinosis do not affect tear osmolarity.

The main limitation of this study was the relatively small sample size due to the rarity of this disease. This study showed that the use of viscous cysteamine hydrochloride (Cystadrops®) to reduce corneal crystal density in patients with cystinosis does not affect tear osmolarity.

Declaration of conflicting interests

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