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RESEARCH REPORT

Effect of Storage Conditions on Stability of Ophthalmological Compounded Cysteamine Eye Drops

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Abstract Cystinosis is a hereditary genetic disease that results in the accumulation of cystine crystals in the lysosomes, leading to many clinical manifestations. One of these manifestations is the formation of corneal cystine crystals, which can cause serious ocular complications. The only available drug to treat cystinosis is cysteamine, which breaks cystine and depletes its accumulation in the lysosomes. However, the oral form of cysteamine is not effective in treating corneal manifestations. Thus, ophthalmic solutions of cysteamine are applied. Because the commercial cysteamine eye drops are not available in most countries, hospital pharmacies are responsible for preparing "homemade" drops usually without a control of stability of cysteamine in different storage conditions. Hence, we aimed in this study to investigate the effect of different storage conditions on the stability of a cysteamine ophthalmic compounded solution. Cysteamine ophthalmic solution was prepared in the hospital pharmacy and sterilized using a candle filter. The preparations are then stored either in the freezer at −20°C or in the refrigerator at +4°C for up to 52 weeks. The amount of cysteamine hydrochloride in the preparation at different time points was determined using capillary electrophoresis (CE). Storage of the cysteamine ophthalmic preparations at +4° resulted in significant loss of free cysteamine at all time points, from 1 to 52 weeks of storage, when compared with storage in the freezer (−20°C). We demonstrate that cysteamine 0.5% compounded eye drops are easily oxidized within the first week after storage at +4°C, rendering the preparation less effective. Storage at −20°C is recommended to prevent this process.

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Introduction

Cystinosis is a hereditary systemic disease, characterized by accumulation of the amino acid cystine, in a crystalline form, inside the lysosomes of the cells (Elmonem et al. 2016). The disease is caused by bi-allelic mutations in the *CTNS* gene, which encodes for cystinosin protein. Cystinosin is a cystine-proton co-transporter located in the lysosomal membrane (Nesterova and Gahl 2013). Cystinosis is a rare disorder, with a prevalence of 1:100,000 to 1:200,000 live births (Elmonem et al. 2016). However, a higher prevalence was found in some regions, such as French Brittany, with 1:26,000 live births (Bois et al. 1976),

and Canadian Quebec, with 1:62,000 live births (De Braekeleer 1991). The most frequent mutation is the 57-kb deletion, which constitutes 50% of the causing mutations in North Europe and North America (Shotelersuk et al. 1998; Levtchenko et al. 2014). However, this specific mutation is not present in other regions of the world (Soliman et al. 2014).

There are three different forms of cystinosis, depending on the clinical presentation: infantile, juvenile, and corneal non-nephropathic. The infantile nephropathic form, which represents 95% of the cystinosis cases, is the most severe form. This form shows a renal Fanconi syndrome at a very early age (6–12 months of age) with a progressive loss of kidney function, leading to end-stage renal disease (ESRD) before puberty, if left untreated (Schnaper et al. 1992; Brodin-Sartorius et al. 2012). Meanwhile, the juvenile form, which represents less than 5% of the cases, is a milder form that is diagnosed around adolescence with milder symptoms (Servais et al. 2008). Finally, the ocular non-nephropathic form is a rare form in adults, which is only characterized by ocular disease without systemic manifestations. Notably, the mutation type determines the severity of the disease. Severe mutations are causing infantile nephropathic cystinosis, while mild mutations are found in patients with juvenile and ocular non-nephropathic cystinosis (Gahl et al. 2002).

All three allelic forms of cystinosis demonstrate corneal cystine accumulation leading to photophobia (Anikster et al. 2000; Gahl et al. 2002) and blepharospasm (Kaiser-Kupfer et al. 1986). Using slit lamp examination, crystals are first detected at 16 months of age in the peripheral cornea. In vivo confocal microscopy and anterior segment optical coherence tomography have higher resolution in detecting cystine crystals in different corneal layers (Liang et al. 2015). When left untreated, corneal disease progresses causing filamentary keratitis, corneal ulcerations, band keratopathy, corneal neuropathy, corneal vascularization, posterior synechiae, and pupillary block with secondary glaucoma (Liang et al. 2015). Complete loss of transparency can result in corneal blindness. However, corneal transplantation is generally not considered as a valid treatment option because cystine crystals from inflammatory cells can invade the corneal graft resulting in poor vision (Katz et al. 1987). Moreover, cystinosis can lead to retinal blindness in 10–15% of the patients (Gahl et al. 2002).

Currently, the only available treatment for cystinosis is β-mercaptoethylamine or cysteamine (Thoene et al. 1976). It is an amino thiol found naturally as a constituent of the coenzyme A (Cavallini et al. 1968). However, it is undetectable in normal individuals' plasma samples. Cysteamine is easily oxidized to form a disulfide bond, which enables it to break each cystine molecule accumulated in the lysosome into two cysteine molecules; one is transported outside the lysosome as itself, and the other is transported as a cysteamine-cysteine disulfide, both of which use other transporters than cystinosin (Gahl et al. 1985). Oral cysteamine administration will deplete crystals in vascularized structures as the retina, but unfortunately not the corneal cystine crystals (Cantani et al. 1983). Hence, cystinosis patients should apply ophthalmic cysteamine preparations, in order to deplete the corneal crystals (Kaiser-Kupfer et al. 1987, 1990).

Since cysteamine is easily oxidized to cystamine, it was worthy to investigate whether the oxidized form (cystamine) is effective, as an ophthalmic preparation, in depleting the corneal crystals. Regrettably, it has been shown that cystamine is not as effective as cysteamine in depleting the cystine corneal crystals (Iwata et al. 1998).

The development of commercial cysteamine eye drops has been hindered by the difficulties to obtain a stable cysteamine solution. To the moment, two commercial formulations have reached market authorization: 0.44% aqueous cysteamine hydrochloride solution (Cystaran™, Sigma-Tau Pharmaceuticals) and 0.55% cysteamine gel (Cystadrops®, Orphan Europe). Unfortunately, these commercial drops are not available in most countries, and most cystinosis patients around the globe rely on cysteamine ocular solutions prepared by their hospital pharmacies. These "homemade" drops are cheaper and are frequently considered as a valid alternative by local health-care authorities. In this study, we examined the stability of cysteamine ocular solution prepared in the pharmacy of our university hospital upon different storage conditions.

Methods

Formulation

All chemicals were of analytical grade. Ammonium acetate was sourced from Fisher Scientific (Loughborough, UK). The simulated formulation matrix was prepared with ascorbic acid (Acros Organics, Geel, Belgium), benzalkonium chloride (Sigma-Aldrich, Steinheim, Germany), KH_2PO_4 (Merck, Darmstadt, Germany), methanol (Acros Organics), NaCl (Fisher Scientific), and $Na_2HPO_4{\cdot}12\ H_2O$ (Chem-Lab, Zedelgem, Belgium). All solutions and sample dilution were performed in Milli-Q (Millipore, Massachusetts, USA) water. The cysteamine hydrochloride powder was obtained from Acros Organics (Belgium) for standard preparation, and the sodium benzoate (as internal standard (IS)) was from Sigma-Aldrich. Sodium hydroxide was sourced from Fisher Scientific for the preparation of capillary rinsing solutions. The preparation was sterilized using a candle filter to assure sterility.

Cysteamine Measurement

A stock solution of the simulated formulation matrix was prepared by incorporating all the components at the specified concentration: NaCl (3 mg/mL), $Na_2HPO_4 \cdot 12\ H_2O$ (8.25 mg/mL), KH_2PO_4 (1.16 mg/mL), ascorbic acid (0.2 mg/mL), and benzalkonium chloride (0.1 mg/mL). Afterward, a standard solution of cysteamine hydrochloride at the corresponding concentration in the formulation (0.5% w/v) was prepared by accurately weighing and diluting it with the simulated matrix. Finally, both the sample and the standard (1 mL) were diluted (10×) with Milli-Q water along with the transfer (0.3 mL) of the solution of sodium benzoate (0.6 mg/mL) as IS. The background electrolyte (BGE) of the experiment was a mixture of 15 mM ammonium acetate (with pH adjusted to 8.85) and methanol (90:10 v/v).

Cysteamine concentrations were determined using capillary electrophoresis (CE). A P/ACE MDQ, Beckman Coulter CE (Fullerton, CA, USA), instrument was used for the electrophoresis. Karat 32® software was used for instrument operations and UV data acquisition (195 nm). A bare fused silica capillary was used for the assay (total length 40 cm, effective length 30 cm, id 50 μm). All the weighings and the pH adjustment of the BGE were performed by a Mettler Toledo (Greifensee, Switzerland) electric balance and a Metrohm 691 pH meter (Herisau, Switzerland).

The new capillary was activated by a sequence of rinsing with different solutions (10 min each) in the following order: water, methanol, water, 1 mM sodium hydroxide, water, 0.1 mM sodium hydroxide, water. A simple daily capillary rinsing with 0.1 mM sodium hydroxide followed by water was adopted for the capillary activation. For the proper equilibration of BGE components, a BGE rinsing followed by electrophoresis at the working voltage (28 kV) for 10 min each was performed before the actual analysis. Finally, the optimum electrophoresis involved 3 min of inter-injection BGE rinsing followed by a hydrodynamic sample injection at 0.5 psi for 5 s and 5 min of electrophoresis at 28 kV.

Statistical Analysis

The statistical analysis was done using Sigma Plot 12.0 software (Systat Software Inc., IL, USA). Student's t-test was performed on at least three replicates from each experimental condition, while means and standard deviations were used in the graph, as stated in the figure legend. The p value was considered significant if it was ≤ 0.05.

Results

Cysteamine ophthalmic solutions (0.5%) were sterilized using a candle filter. The preparations were stored either at +4°C or at −20°C. Samples from ophthalmic preparations at two different storage conditions were collected at different time points: 1, 2, 4, 12, 26, and 52 weeks. Cysteamine levels in the different samples were determined using the CE technique.

Statistical analysis showed that the percentage of cysteamine left of label claim was significantly less at +4°C compared to −20°C storage at all time-points (Fig. 1), with p value <0.001. The results also showed that the percentage of cysteamine left of the label claim decreased with time upon refrigerator storage from 61.6% (±2%) after 1 week of storage to 22.9% (±1.9%) after 52 weeks of storage. Meanwhile, the freezing conditions tended to keep the same level of cysteamine at the different time points, from 85.1% (±0.9%) after 1 week of storage to 85.7 (±2.5%) after 52 weeks of storage.

Discussion

Cystinosis patients have to use ophthalmic cysteamine preparation in order to eliminate cystine crystals accumulated in the cornea because systemically used cysteamine does not reach avascular structures (Elmonem et al. 2016). Because cysteamine eye drops (Liang et al. 2017) are not commercially available in Belgium, we tested the stability of pharmacy-made cysteamine preparation (0.5% solution) compounded in our institution.

We followed a validated protocol using a candle filter to assure sterile preparation and added 0.01% benzalkonium chloride to prevent bacterial contamination. Based on previously published data, eye drops were kept frozen before analysis (Iwata et al. 1998).

As the patient has to apply the preparation frequently every day, ophthalmic solution is usually stored in the freezer once purchased. Upon usage, the preparation is thawed in the refrigerator or even at room temperature (and kept there for 1 week or more) for patients' convenience as it is not feasible to unfreeze the whole solution (for multiple doses) every time before administration. At these conditions, cysteamine is easily oxidized to cystamine. It has been shown that cystamine is not effective in eliminating the cystine crystals from the cornea (Iwata et al. 1998).

We mimicked these storage conditions in our laboratory and evaluated the percentage of free cysteamine remaining after storage.

The results showed that, when stored at +4°C, a significant amount of cysteamine in the ophthalmic preparation is oxidized within 1 week. This might explain that the majority of our patients continue to complain of photophobia and other ocular complications of cystinosis despite their compliance with "homemade" eye drops.

In contrast, novel aqueous 0.44% cysteamine hydrochloride solution Cystaran™ (Sigma-Tau Pharmaceuticals) can

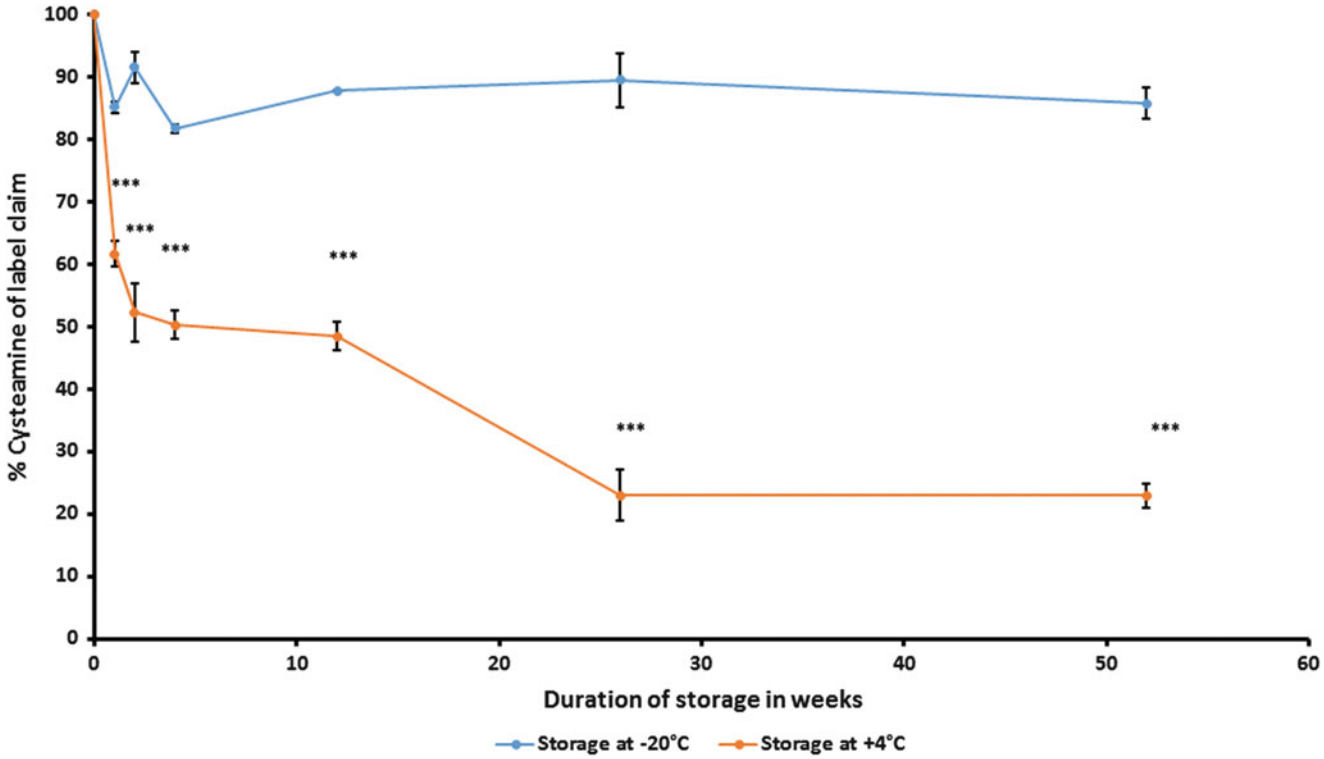

Fig. 1 Percentage of cysteamine left of label claim after 1, 2, 4, 12, 26, and 52 weeks of storage using different storage conditions; freezer (−20°C, in blue) or refrigerator (+4°C, in orange). The points represent the average values of three samples with the error bars representing the standard deviations (SD). For statistical analysis, student's t-test was applied between the two different conditions at the different time points ($p < 0.001$)

be kept at 4°C for at least 1 week and showed efficiency in reducing the corneal cystine score (CCCS) overtime. However, it has to be applied every waking hour complicating therapy compliance (Gahl et al. 2000). Another novel viscous preparation Cystadrops® (Orphan Europe) containing carmellose sodium as a viscous agent prolongs the precorneal residence time and can be stored at room temperature, protected from light, up to 7 days after opening. This gel-like solution showed excellent efficiency when applied four times daily when assessed using slit lamp examination, optical coherence tomography, and highly sensitive in vivo confocal microscopy (Liang et al. 2017). Moreover, clinical evaluation showed a significant reduction of photophobia in patients using Cystadrops® compared to standard cysteamine hydrochloride (0.1%) preparation.

Conclusion

In conclusion, we have demonstrated that cysteamine hydrochloride (0.5%) formulated ophthalmic preparation is easily oxidized within the first week after storage at +4°C, rendering the preparation less effective. Hence, storage at −20°C is recommended to preserve the efficiency of this preparation. Thus, in ideal setting, the preparation should be aliquoted, and each aliquot (for a single dose) should be thawed before each application.

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Synopsis

Cystinosis patients may get less effective ophthalmological treatment than what is expected.

Details of the Contributions of Individual Authors

Ahmed Reda, Lambertus van den Heuvel, and Elena Levtchenko contributed in planning. Ahmed Reda, Ann Van Schepdael, Erwin Adams, Prasanta Paul, David Devolder, and Ingele Casteels contributed in conducting. Ahmed Reda, Mohamed A. Elmonem, Koenraad Veys, Lambertus van den Heuvel, and Elena Levtchenko contributed in reporting.

Name of One Author Who Serves as Guarantor

Prof. Elena Levtchenko.

A Competing Interest Statement

Ahmed Reda, Ann Van Schepdael, Erwin Adams, Prasanta Paul, David Devolder, Mohamed A. Elmonem, Koenraad Veys, Ingele Casteels, and Lambertus van den Heuvel declare that they have no conflict of interest.

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Details of Ethics Approval and Patient Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

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