Stability of insulin eye drops in the treatment of refractory corneal ulcers

Estabilidad del colirio de insulina para el tratamiento de úlceras corneales refractarias

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Keywords: Insulin; Corneal ulcers; Stability; Pharmaceutical compounding; Ophthalmology; Ophthalmic Solutions.

Abstract

Objective: To determine and compare the physicochemical and microbiological stability of two 25 IU/mL insulin eye drop formulations made with normal saline and a balanced salt solution, respectively, stored for 120 days under various conditions.

Method: Eye drops were compounded in triplicate with 100 IU/mL Actrapid® insulin and either normal saline or a balanced salt solution as vehicles, and they were stored alternatively at room temperature (25 °C), in a refrigerator (2-8 °C) or in a freezer (−20 °C) for 120 days. Insulin concentrations were determined by ultra-high resolution liquid chromatography, and osmolality and pH values were measured at days 0, 3, 7, 15, 30, 60, 90 and 120. Likewise, samples were extracted for microbiological studies on days 0, 30, 60, 90 and 120.

Results: The formulation made with normal saline maintained insulin concentrations above 90% of the baseline level after 120 days across all temperature conditions. In the case of the balanced salt solution-based eye drops, insulin concentration when stored at room temperature or in the freezer remained stable after 120 days, although insulin concentration when stored in the refrigerator fell below 90% on day 90 of the study. Osmolality and pH values remained constant in both formulations and across all storage conditions. No microbiological growth was observed in any of the samples.

Keywords: Insulin; Corneal ulcers; Stability; Pharmaceutical compounding; Ophthalmology; Ophthalmic Solutions.

PALABRAS CLAVE

Insulina; Úlceras corneales; Estabilidad; Formulación magistral; Oftalmología; Soluciones oftálmicas.
Conclusions: 25 IU/mL insulin eye drops made with normal saline remain stable for 120 days whether they are stored at room temperature, in a refrigerator or in a freezer, provided that they are protected from light. When made with a balanced salt solution, they remain stable for 120 days at room temperature and in a freezer, their shelf life being reduced to 90 days in the case of storage in a refrigerator.

Conclusions: El colirio de insulina 25 UI/ml elaborado con suero fisiológico es estable 120 días, conservado tanto a temperatura ambiente como en nevera o congelador, protegido de la luz. Con solución salina balanceada permanece estable 120 días a temperatura ambiente y congelador, reduciéndose el periodo de validez a 90 días en el caso de la conservación en nevera.

Introduction

Corneal ulcers, which arise as a result of a rupture or a defect in the corneal epithelium, can cause an underlying inflammation and often give rise to necrosis of the corneal stroma. The condition is a common cause of ocular morbidity at a world level. Associated symptoms include irritation, foreign body sensation, conjunctival edema, hyperemia and blurred vision. Early diagnosis and early initiation of appropriate treatment are essential as lack of treatment may lead to vision loss due to corneal opacity, as well as persistent epithelial defects.

Persistent epithelial defects are defined as corneal alterations not showing signs of improvement following two consecutive weeks of treatment. This absence of epithelialization of the corneal surface may result from multiple causes such as infections, adverse drug reactions, poor epithelial adhesion or trauma. Treatment of this kind of lesion should start with intensive lubrication, the withdrawal of treatments leading to epithelial toxicity, prophylactic antibiotic therapy as well as the use of occlusive bandages and therapeutic contact lenses. In the event of refractiveness, recourse can be made to ophthalmic administration of autologous serum or platelet-rich plasma.

The last few years have seen an increasing interest in the search of growth factors capable of promoting corneal wound healing, given the presence of receptors for these molecules in the epithelial cells of the cornea.

Recent studies have shown the effectiveness of the epidermal growth factor, the nerve growth factor and insulin for treating this kind of lesion due to their epithelial promoting properties.

The use of insulin in corneal ulcers was first proposed by Aynsley in 1945 in a study on the reepithelialization of ulcers refractory to standard treatment. Topical use of insulin in corneal ulcers is currently reserved mainly to diabetic patients with either postoperative corneal epithelial defects or nonsurgical epithelial defects. In the case of non-diabetic patients, the use of insulin has been described for the treatment of neurotrophic corneal ulcers refractory to conventional treatment and in persistent corneal defects following resection of a neurotoma. As regards the safety of insulin, no side effects or alterations to blood sugar levels, intraocular pressure or the corneal epithelium have been reported as a result of its long-term administration. When no other treatment is available, it is not uncommon to resort to the formulation of existing medicines to adapt them to an administration route different from the one approved in the drugs’ summary of product characteristics (SmPC).

Given that no scientific studies have been published to date on the stability of the ophthalmic insulin formulations used to treat persistent epithelial defects, this study can be considered the first of its kind. Its purpose is to determine the stability of two kinds of 25 IU/mL insulin eye drops: one using normal saline (NS) and the other using a balanced salt solution (BSS). 200 mL of each formulation was compounded by adding 50 mL Actrapid® insulin to 150 mL of BSS® or NS in a 250 mL Vacuflas®. Three batches of each vehicle were prepared. The solutions were homogenized by shaking them for 30 seconds and later introduced into 5 mL type 1 amber vials. The whole process was conducted in aseptic conditions under horizontal laminar flow, the day when the formulations were compounded being considered day 0 of the study.

Conservation conditions

Conservation conditions were as follows: room temperature (25°C), refrigeration (2°C to 8°C) or frozen storage (−20°C). To ensure that temperature remained constant throughout the study, vials stored at room temperature were kept in an ICH L climate chamber (Memmert GmbH + Co.®, Schwabach, Germany), which maintains stable temperature (25°C) and humidity (60%) conditions. Moreover, the fridge and the freezer were equipped with a Siemens® temperature sensor. All vials were protected from light.

Physicochemical characterization

Insulin determination and quantification

Determination and quantification of insulin were carried out by reverse phase ultra-high performance liquid chromatography (UHPLC) using an ACQUITY UPLC H Class Plus® (Waters) with a photodiode array (PDA) detector at days 0, 3, 7, 15, 30, 60, 90 and 120. The analytical method used was validated for linearity, accuracy, precision and detection and quantification limits. A linear calibration curve was obtained for both diluents over a concentration range of 0.3-10.0 IU/mL (R² = 0.999). The detection and quantification limits were 0.15 IU/mL and 0.30 IU/mL, respectively, for both diluents. An analysis was also made to ensure that the method met standard accuracy and precision criteria. Compliance with the analytical validation standards of the European Medicines Agency was also ascertained.

An ACQUITY UPLC BEH C18 column (2.1 x 50.0 mm, 1.7 µm) was used at a temperature of 35°C. The temperature of the sample was 8°C, the flow rate 0.5 mL/min and the injected volume of the sample 10 µL. The mobile phase comprised the use of 0.1% formic acid (FA) in water (Milli-Q® UHPLC Systems, Merck Millipore®, Madrid, Spain) and 0.1% FA in acetonitrile (ACN) (VWR Chemicals®, Pennsylvania, USA). The chromatographic method used was a gradient elution, starting with 80% 0.1% FA in water and 20% 0.1% FA in ACN and reaching a 30-70% proportion of the respective components at minute 6. Quantification of the insulin required a 1:10 dilution of the samples and their subsequent filtration by 13 mm low protein adsorption Acrodisc® filters (0.2 µm). The data obtained was processed using Empower® 3 software. Under such conditions, insulin retention time stood at 2.1 minutes at a wavelength of 220 nm (Figure 1).

Osmolality and pH determination

Osmolality was determined using a cryoscopic osmometer (Osmo- Special 1, Aston Tecnica®, Poncarale, Italy) where a 150 µL aliquot of each sample was introduced. pH measurements were carried out with a Basic 20 pHmeter (Crison®, Barcelona, Spain). Both variables were determined at days 0, 3, 7, 15, 30, 60, 90 and 120.

Microbiological stability

Three mL were removed from each formulation at days 0, 15, 30, 60, 90 and 120. The culture media used to evaluate sterility of the formulations...
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were thioglycolate broth (Merck®, Darmstadt, Germany), Columbia blood agar (Merck®, Darmstadt, Germany) and Sabouraud agar (Merck®, Darmstadt, Germany). Samples were incubated in a stove at 37 °C in anaerobic conditions. The thioglycolate was incubated for 10 days, whereas the blood and Sabouraud agars were incubated for 48 hours. Once stove incubation was completed, the plates with Sabouraud agar were incubated again for 13 days in aerobic conditions.

Variation range and statistical analysis

The shelf-life of formulations was established in accordance with the provisions of the Pharmaceutical Codex20. The active pharmaceutical ingredient was considered stable if the compounded formulation retained 90-110% of the initial concentration21-23. As far as osmolality and pH values were concerned, any variation outside the limits accepted for ophthalmic formulations was considered unacceptable, as was the presence of microbial contamination in the analyzed samples21-24.

Results

Insulin quantification

Figure 2 shows the insulin concentration of the NS and BSS® formulations measured at each time point, expressed as percentages of the initial concentration (25 IU/mL).

Although the insulin concentration of the BSS® formulation fell below 90% at 120 days in the refrigerated samples, concentration at 120 days remained within the accepted range in the samples kept at room temperature or in frozen storage. Conversely, the NS formulation showed insulin concentration within the accepted range until the 120th day in all the conservation temperatures analyzed (room temperature, refrigeration and frozen storage), with physicochemical stability being guaranteed for the entire study period.

pH and osmolality quantification

pH remained constant, without significant variations, throughout the study (Figure 3). In eye drops elaborated with the BSS® formulation, the mean pH value in the different temperature conditions was 7.21 ± 0.21, whereas the mean pH value obtained for eye drops elaborated with the NS formulation was 7.05 ± 0.20.

As regards osmolality, no significant variations were detected during the study period (Figure 4). The osmolality of the BSS® formulation was 296.01 ± 9.04 mOsm/kg considering the three storage conditions.
**Discussion**

The conventional therapeutical arsenal available to address persistent corneal epithelial defects sometimes falls short of the patients’ needs. For that reason, practitioners must resort to therapeutic alternatives such as insulin, endowed with properties capable of promoting epithelial growth. The absence of commercially available insulin-based eye drops makes it necessary to compound them as extemporaneous preparations. To date, several studies have shown the efficacy of this treatment\(^2\), although data on physicochemical or microbiological stability remain scarce. As regards the insulin concentration in the preparation, the literature reports highly variable concentrations, ranging from 1 IU/mL to 50 IU/mL\(^2\,3\). A decision was made to select the 25 IU/mL concentration for this analysis, based on Fai et al.\(^4\). Given that insulin does not feature as an active ingredient in the Spanish register of manufacturers, importers, and distributors of injectable insulin preparations, it can be ascertained that no contaminations occurred during the formulation and conservation process.

**Microbiological stability**

No microbiological contamination was observed in any of the samples at the different temperature conditions and time points analyzed. As a result, it can be ascertained that no contaminations occurred during the formulation and conservation process.

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