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ORIGINAL ARTICLE

Formulation and stability of an extemporaneous 0.02% chlorhexidine digluconate ophthalmic solution

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Background/Purpose: *Acanthamoeba* keratitis is difficult to treat because *Acanthamoeba* cysts are resistant to the majority of antimicrobial agents. Despite the efficacy of 0.02% chlorhexidine in treating *Acanthamoeba* keratitis, a lack of data in the literature regarding the formulation's stability limits its clinical use. The objective of this study was to develop an optimal extemporaneous 0.02% chlorhexidine digluconate ophthalmic formulation for patients in need.
Methods: With available active pharmaceutical ingredients, 0.02% chlorhexidine digluconate sample solutions were prepared by diluting with BSS Plus Solution or acetate buffer. Influences of the buffer, type of container, and temperature under daily-open condition were assessed based on the changes of pH values and chlorhexidine concentrations of the test samples weekly. To determine the beyond-use date, the optimal samples were stored at 2–8°C or room temperature, and analyzed at time 0 and at Week 1, Week 2, Week 3, Week 4, Week 5, Week 8, Week 12, and Week 24.
Results: Despite chlorhexidine exhibiting better stability in acetate buffer than in BSS solution, its shelf-life was $<$ 14 days when stored in a light-resistant low-density polyethylene container. The acetate-buffered 0.02% chlorhexidine digluconate solution stored in light-resistant high-density polyethylene eyedroppers did not exhibit significant changes in pH or strength at any time interval.
Conclusion: The acetate-buffered 0.02% chlorhexidine digluconate ophthalmic solution stored in light-resistant high-density polyethylene eyedroppers demonstrated excellent stability at 2

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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−25°C for 6 months after being sealed and for 1 month after opening. This finding will enable us to prepare 0.02% chlorhexidine digluconate ophthalmic solutions based on a doctor's prescription.

Introduction

Acanthamoeba keratitis, a corneal infectious disease, is difficult to treat because *Acanthamoeba* cysts are resistant to the majority of existing antimicrobial agents. Compared with other pharmaceutical agents, biguanides have the lowest minimal amebicidal and cysticidal concentration, and 0.02% biguanide ophthalmic preparations are the drug of choice for *Acanthamoeba* keratitis.[1,2] However, these products are not commercially available in Taiwan.

Chlorhexidine is a biguanide (Fig. 1) and the drug of choice for *Acanthamoeba* keratitis.[3] It is a strong base, practically insoluble in water at 20°C, and available in different salt forms, including dihydrochloride, diacetate, and digluconate. Different chlorhexidine salts have varying water solubilities.[4,5] Chlorhexidine digluconate is freely soluble in water, is manufactured as a 20% w/v stock aqueous solution, and should be protected from light during storage.[6] Some licensed 20% chlorhexidine digluconate products obtained from domestic manufacturers and chlorhexidine digluconate concentrate imported from the reagent supplier Sigma-Aldrich (Steinheim, Germany) can be used as raw materials.

Chlorhexidine digluconate is extensively used as an antiseptic because of its broad-spectrum coverage of Gram (+) and Gram (−) bacteria.[7] Chlorhexidine 0.005% has been used as a pharmaceutical preservative, particularly in ophthalmic solutions.[5,6] However, high concentrations of chlorhexidine are irritating to mucous membranes, and a concentration not to exceed 0.05% is recommended when applied to wounds and burns to decrease the risk of anaphylactic reaction.[8] Recently, several studies have demonstrated that 0.02% chlorhexidine possesses excellent amebicidal activity and minimal corneal epithelial toxicity.[1−3] Despite reports of the efficacy of 0.02% chlorhexidine in the treatment of *Acanthamoeba* keratitis, a lack of data in the literature regarding the formulation's stability limits its clinical use. To the best of our knowledge, only Moorfields Pharmaceuticals (London) in the United Kingdom has commercialized 0.02% chlorhexidine ophthalmic products. Ordering the product from abroad is not only expensive, but also labor and time intensive, which may result in a delay in treatment. Therefore, extemporaneous compounding of chlorhexidine digluconate 0.02% ophthalmic solutions is needed in Taiwan.

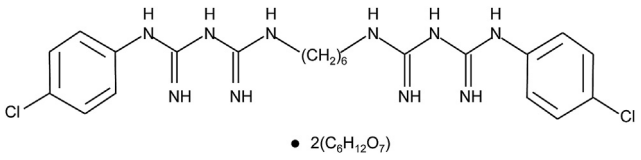

Figure 1 Chemical structure of chlorhexidine digluconate.

The aim of this study was to develop an optimal extemporaneous 0.02% chlorhexidine digluconate ophthalmic formulation with an appropriate pH value and osmolality for patients with *Acanthamoeba* keratitis, to ensure that the sterility, particulate matter, and assay of the final product meet the general characteristics of ophthalmic solutions in pharmacopoeia, and to determine the type of container, storage condition, and beyond-use date of the final product. This is the very first report on the formulation and stability of an optimal extemporaneous chlorhexidine digluconate 0.02% ophthalmic solution. Our hospital, with 60-year experiences in extemporaneous compounding and in-house quality control for medications, would like to share this formula with the world.[9−12]

Methods

Materials

Chlorhexidine acetate standard was obtained from US Pharmacopeia (USP; Rockville, MD, USA). Chlorhexidine digluconate 20% was provided by Schutz Dishman Biotech Limited (Ahmedabad, India). Anhydrous sodium acetate (CH_3COONa), glacial acetic acid (CH_3COOH), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$), triethylamine, and acetonitrile were purchased from Merck (Darmstadt, Germany). Light-resistant low-density polyethylene (LDPE) containers for ophthalmic solutions were provided by Sinphar (I-Lan, Taiwan), and light-resistant high-density polyethylene (HDPE) containers were provided by Chin-Tai Plastic Ind. Co., Ltd (Changhua, Taiwan). Balanced salt solution (BSS Plus Solution), which contains sodium chloride, potassium chloride, calcium chloride, magnesium chloride hexahydrate, sodium acetate, and sodium citrate, was obtained from Alcon Laboratories, Inc. (Fort Worth, TX, USA).

Experimental design and sample preparation

With available active pharmaceutical ingredients, we formulated a 0.02% chlorhexidine digluconate ophthalmic solution and investigated the effects of temperature and buffer on its stability in two types of containers, HDPE eyedroppers and LDPE eyedroppers. Other parameters, such as pH value, osmolality, particulate matter concentration, and sterility, were also assessed. The beyond-use date was determined through these tests.

Experimental implementation of various physical conditions encountered clinically is listed in Table 1 and can be divided into three stages. In the first stage, eight sets of