

Potency of Extemporaneous Gentamicin Eye Drops Used in Maharaj Nakorn Chiang Mai Hospital

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ABSTRACT

This study evaluated the potency of extemporaneous gentamicin (13.6 mg/ml) eye drops, stored for 4 weeks. The effects of different storage temperatures on the antimicrobial potency were examined. Gentamicin (13.6 mg/ml) was prepared with aseptic technique and the solutions stored at 2-8°C and 28°C for 28 days.

The fortified stock solutions of gentamicin (13.6 mg/ml) were prepared by reconstituting gentamicin (0.3%) eye drops with gentamicin (40 mg/ml) injection. The potency was evaluated by measuring the minimum inhibitory concentration.

Throughout the 28-day period, no change was observed in the minimum inhibitory concentration of gentamicin (13.6 mg/ml) stored at 2-8°C and 28°C.

Extemporaneously - prepared gentamicin (13.6 mg/ml) eye drops remained stable at 2-8°C and 28°C for 28 days.

Key words: Gentamicin eye drops, Potency

INTRODUCTION

In Maharaj Nakorn Chiang Mai Hospital, the traditional treatment of bacterial keratitis includes the use of antibiotic eye drops, often a combination of cefazolin sodium (33 mg/ml) and gentamicin (13.6 mg/ml) (Tananutvat et al., 2004). These formulations are prepared by Division of Pharmacy in Maharaj Nakorn Chiang Mai Hospital. Commercial preparations of low-dose topical antibiotics are generally for many superficial infections but extemporaneous preparations of ophthalmic antibiotics are required for the treatment of severe sight-threatening ocular infection. These medications are usually prepared by combining standard parenteral or lyophilized antibiotic preparations with 0.9% sodium chloride injection or

an artificial tear solution (Reynolds and Closson, 1993). However, to maintain antimicrobial potency of extemporaneous eye drops during the treatment period, it is not clear to what extent the formulation potency is influenced by storage temperatures.

In this study, we tested gentamicin (13.6 mg/ml) eye drops that is commonly used in Maharaj Nakorn Chiang Mai Hospital. We examined the potency of gentamicin eye drops by measuring the minimum inhibitory concentration against common ophthalmic pathogens over a four-week period. We also tested the effect of storage temperature at room temperature (28°C) and at 2-8°C.

Table 1. Preparing dilutions of gentamicin to be used in broth dilution susceptibility tests.

Tube No	Working Solution(ml)	TSB(ml)	TSB from previous tube(ml)	Inoculum(ml)	Final concentration of gentamicin solution (ug/ml)
1	0.5	0.5	0.0	0.5	8
2	0.0	0.5	0.5	0.5	4
3	0.0	0.5	0.5	0.5	2
4	0.0	0.5	0.5	0.5	1
5	0.0	0.5	0.5	0.5	0.5
6	0.0	0.5	0.5	0.5	0.25
7	0.0	0.5	0.5	0.5	0.12
8	0.0	0.5	0.0	0.5	Positive control
9	0.0	0.5	0.0	0.0	Negative control

Appendix A

Procedure for compounding gentamicin 13.6 mg/ml ophthalmic solution

1. Clean all work and container surface with 70% isopropyl alcohol.
2. Assemble all pharmaceuticals and materials in the laminar-air flow hood.
3. Add 2 ml of gentamicin injection (40 mg/ml) to commercial gentamicin 0.3% ophthalmic solution. Cap the dropper bottle, shake to mix, and label. The vial contains 13.6 of gentamicin per milliliter.

MATERIALS AND METHODS

Materials

Microorganism

The bacterial strain used in this study was *Staphylococcus aureus* American Type Culture Collection (ATCC) 29213. Microorganism is susceptible to gentamicin as tested by broth dilution which was described in the National Committee for Clinical Laboratory Standards (NCCLS, 2003).

Chemicals and Reagents

Gentamicin eye drops (0.3%) and gentamicin injection (40 mg/ml) were purchased from Seng Thai Company and Atlantic Labs, respectively. Trypticase soy broth (TSB), BBL™ was purchased from Voigt Global Distribution Inc., United States of America.

Methods

A stock solution of gentamicin was prepared by adding gentamicin injection (40 mg/ml) 2 ml into gentamicin eye drops (0.3%) 5 ml in Class 100 clean-room environment to a concentration of 13.6 mg/ml in gentamicin eye drops containers (Reynolds and Closson, 1993). Stock solution was divided into half. One set of solutions was stored at room temperature (28°C) and the other set was refrigerated (2-8°C) throughout studied period. Solutions were test at day of preparation and day 3, 7, 14, 21 and 28.

Standard quality control reference strains of *Staphylococcus aureus* ATCC 29213 (NCCLS, 2003) with sensitivity to gentamicin was chosen for this study. The bacteria were transferred daily to ensure purity and good growth.

On each test day, a bacterial suspension equal to the 0.5 McFarland turbidity standard was prepared in trypticase soy broth. Gentamicin solutions were further diluted to a concentration of 16 ug/ml by water for injection before serial dilutions with trypticase soy broth were performed (table 1). For each dilution tube, 0.5 ml each of bacterial suspension and the antimicrobial agent were incubated together at 35°C in an aerobic environment for 24 hours.

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of antibiotic that yields no growth in the trypticase soy broth.

RESULTS AND DISCUSSION

Extemporaneously-prepared ophthalmic antimicrobial solutions of gentamicin (13.6 mg/ml) are commonly used today for the treatment of severe ocular infection disease. Previous experimental studies reported that some antibiotic extemporaneous preparations could maintain stable potency for 7 days (Arici et al., 1999). However, it is unknown to what extent the potency of these formulations can be influenced by the storage temperatures. In this work, we therefore studied the effects of different storage temperatures that could influence antibacterial potency of gentamicin (13.6 mg/ml) ophthalmic eye drops.

In the potency studies, minimum inhibitory concentration of gentamicin (13.6 mg/ml) ophthalmic solutions was found to be 2.0 ug/ml which exhibited no loss of potency during the entire four-week period when stored at room temperature (28°C) and refrigerated (2-8°C). The authors (McBride et al., 1991) demonstrated that the storage time for gentamicin (13.6 mg/ml) ophthalmic solutions was three months at 4-8°C. According to the National Committee for Clinical Laboratory Standards (NCCLS), standard minimum inhibitory concentration of gentamicin

is 0.12-1.00 ug/ml. The minimum inhibitory concentration of gentamicin (13.6 mg/ml) ophthalmic solutions in our study is slightly different from that of the National Committee for Clinical Laboratory Standards (NCCLS) which could be due to the stock solutions of gentamicin.

CONCLUSION

Studies of the potency of gentamicin eye drops (13.6 mg/ml), as measured by minimum inhibitory concentration, indicated that it could remain at a clinically-effective level for 28 days at 2-8°C and 28°C. Consequently, it is recommended that gentamicin (13.6 mg/ml) eye drops should be discarded after 28-day storage at room temperature and refrigeration. This recommendation is made to maintain the potency of gentamicin (13.6 mg/ml) eye drops.

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