Original article

POTENCY OF EXTEMPORENEOUS
CEFAZOLIN SODIUM EYE DROPS USED
IN MAHARAJ NAKORN CHIANG MAI HOSPITAL

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Abstract To assess the potency of extemporaneous cefazolin sodium (33 mg/mL) eye drops stored at 2-8 °C and 28 °C and used in Maharaj Nakorn Chiang Mai Hospital. This prospective study of the potency of extemporaneous cefazolin sodium (33 mg/mL) eye drops, used in Maharaj Nakorn Chiang Mai Hospital, was divided into 2 groups. Group I comprised eye drops stored at 2-8 °C and group II thus stored at 28 °C. The minimum inhibitory concentration (MIC) was assessed on the day of preparation and then day 3, 7, 14, 21 and 28. The MIC was examined by the broth dilution techniques. Cefazolin sodium (33 mg/mL) eye drops stored at 2-8 °C were stable for 28 days, but eye drops stored at 28 °C were stable for 14 days. The potency of extemporaneous cefazolin sodium (33 mg/mL) eye drops was less stable at 28 °C and they therefore should be stored in a refrigerator. Chiang Mai Med J 2007;46(3):101-105.

Keywords: eye drop, potency, minimum inhibitory concentration, cefazolin sodium

Extemporaneous preparations of ophthalmic antibiotics are often prescribed for the treatment of severe ocular infectious disease. Infectious keratitis is a potentially blinding condition caused by a variety of microorganisms. Frequent instillation of topical antimicrobial agents is the mainstay of treatment. The drug concentration in commercially available antimicrobial ophthalmic solutions is generally too low for adequate treatment, and solutions with higher drug concentrations are often prepared extemporaneously. These medications are usually prepared by combining standard parenteral or lyophilized antibiotic preparations with compatible vehicles that will not precipitate out of the antibiotic at extemporaneous concentrations.(1) Guidelines on the extemporaneous preparation of ophthalmic solutions have been published.(2) Extemporaneous preparations of ophthalmic antibiotics are

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prepared under aseptic conditions in a laminar-airflow hood. Because the process is labor-intensive, these solutions are costly to prepare. A common recommendation is to refrigerate and discard them no later than seven days after preparation. It is unknown how long extemporaneous preparations of ophthalmic antibiotics remain safe. The purpose of our investigation was to determine whether prolonged storage of cefazolin sodium in Cellufresh MD™ (33 mg/mL) is feasible.

In this study, antimicrobial potency, stability of extemporaneous preparations of cefazolin sodium in an artificial tear drop (33 mg/mL), and Cellufresh MD™, was evaluated for 28 days.

**Materials and methods**

**Microorganism**

The bacterial strain used in this study was *Staphylococcus aureus* American Type Culture Collection (ATCC) 29213. The microorganism was susceptible to cefazolin sodium, as tested by broth dilution, which was described in the National Committee for Clinical Laboratory Standards (NCCLS), 2003.(3)

**Chemicals and reagents**

Cefazolin sodium for injection and Cellufresh MD™ were purchased from Fujisawa Pharmaceutical, Japan and Allergan, USA, respectively. Trypticase soy broth (TSB), BBL™ was purchased from Voigt Global Distribution Inc., USA.

**Methods**

A stock solution of cefazolin sodium was diluting with water for injection and Cellufresh MD™ to a concentration of 33 mg/mL in a Class 100 clean-room environment.

Stock solution was divided in half and placed into Cellufresh™ containers. One set of solution was stored at room temperature (28 °C) and the other refrigerated (2-8 °C) on each test day, day 0, 3, 7, 14, 21 and 28. The containers were wrapped in aluminum foil and stored in the dark.

Standard quality control reference strains of *Staphylococcus aureus* ATCC 29213,(3) with sensitivity to cefazolin sodium, were 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. Cefazolin sodium solutions were diluted further to a concentration of 16 µg/mL by water for injection before serial dilutions with trypticase soy broth were performed, as shown in Table 1. For each dilution tube, 0.5 mL 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) was defined as the lowest concentration of antibiotic, which yielded no growth in the trypticase soy broth.

**Results**

This study was concerned with antimicrobial potency and the stability of extemporaneous preparations of cefazolin sodium in Cellufresh MD™ (33 mg/mL). On examining the MIC on day 0, 3, 7, 14, 21 and 28, it was found that concentration of cefazolin sodium in the eye drops stored at 2-8 °C was 0.5 µg/mL, which indicated no loss of MIC during the 28 day period. However, at 28 °C, there was an increase in MIC from 0.5 to 1.0 µg/mL from day 21 onwards (Table 2). According to the
National Committee for Clinical Laboratory Standards (NCCLS), the standard MIC of cefazolin sodium is 0.25-1.00 µg/mL. All positive controls did not have cefazolin sodium added, which showed positive results. Negative controls of were not inoculated with Staphylococcus aureus ATCC 29213, which showed negative results.

Discussion

Extemporaneously prepared ophthalmic antimicrobial solutions are not only costly, but inconvenient for the patient. The solutions must be used frequently, often hourly throughout the day and night. Requiring patients to keep their solutions refrigerated may hinder them from going to work if a refrigerator is not available there. Even at home, having to retrieve an ophthalmic solution from a refrigerator is much more disruptive to sleep than keeping it on the beside table. Since most community pharmacies in Thailand do not have laminar-airflow hoods, solutions prepared by the aseptic technique in laminar air flow may only be available from hospital pharmacies. If storage-time and temperature guidelines of extemporaneously prepared ophthalmic antimicrobial solutions could be safely relaxed, their cost might decrease and patient compliance increase.

Extemporaneous preparations of ophthalmic antibiotic preparation of cefazolin sodium in Cellufresh MD™ (33 mg/mL) are

### Table 1. Preparing dilutions of cefazolin sodium for use in broth dilution susceptibility tests

<table>
<thead>
<tr>
<th>Tube No</th>
<th>Working Solution (mL)</th>
<th>TSB (mL)</th>
<th>TSB from previous tube (mL)</th>
<th>Inoculum (mL)</th>
<th>Final concentration of cefazolin sodium solution (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.0</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>6</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>7</td>
<td>0.0</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>8</td>
<td>0.0</td>
<td>0.5</td>
<td>0.0</td>
<td>0.5</td>
<td>Positive control</td>
</tr>
<tr>
<td>9</td>
<td>0.0</td>
<td>0.5</td>
<td>0.0</td>
<td>0.0</td>
<td>Negative control</td>
</tr>
</tbody>
</table>

### Table 2. Minimum inhibitory concentration of extemporaneous cefazolin sodium eye drops stored at different temperatures over time

<table>
<thead>
<tr>
<th>Day</th>
<th>Minimum inhibitory concentration (2-8°C) (µg/mL)</th>
<th>Minimum inhibitory concentration (28°C) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>14</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>21</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>28</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
commonly used today for the treatment of severely infectious ocular disease. Previous experimental studies have reported that some extemporaneous preparations of antibiotics have maintained stable potency for 7 days.\(^{(4)}\) Charltons \textit{et al}\(^{(5)}\) and Bowe \textit{et al}\(^{(1)}\) demonstrated that the maximum storage time for cefazolin solution was 28 days under refrigeration. Under this condition, the potency and physical properties of cefazolin solution was maintained. In our study, we found that the MIC of cefazolin sodium in Cellufresh MD\(^{TM}\) (33 mg/mL) did not change and remained stable at 2-8°C for at least 4 weeks.

For stored ophthalmic solutions, patients should be taught and encouraged to use appropriate solution-administration technique in order that the likelihood of contamination be minimized.

\textbf{Conclusion}

Our results show that the potency of cefazolin sodium eye drops (33 mg/mL), as measured by minimum inhibitory concentration, remains at a clinically effective level for 28 days at 2-8°C and 14 days at 28°C. Subsequently, it is recommended that cefazolin sodium (33 mg/mL) eye drops should be discarded after 14 days storage at room temperature or after 28 days under refrigeration. This recommendation is according to the reducing potency of cefazolin sodium eye drops.

\textbf{Acknowledgements}

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\textbf{References}

การศึกษาความแรงของยาหยอดตาเซฟาโซลินโซเดียมที่เตรียมสำหรับผู้ป่วยเฉพาะราย

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'ฝ่ายเภสัชกรรม คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่, 'งานปฏิบัติการกับ_MEMBER' โรงพยาบาลสุราษฎร์นครชัยนาท, 'คณะเวชศาสตร์ มหาวิทยาลัยเชียงใหม่, สายวิชาวิทยาศาสตร์เภสัชกรรม คณะเภสัชศาสตร์ มหาวิทยาลัยเชียงใหม่'

บทคัดย่อ งานวิจัยนี้ศึกษาความแรงของยาหยอดตาเซฟาโซลินโซเดียม 33 มิลลิกรัม/มิลลิลิตรที่เตรียมสำหรับผู้ป่วยเฉพาะรายที่ใช้ในโรงพยาบาลสุราษฎร์นครชัยนาท โดยศึกษาความแรงของยาหยอดตาที่อุณหภูมิ 2-8 องศาเซลเซียส และ 28 องศาเซลเซียสเป็นเวลา 28 วัน การศึกษานี้เป็นการศึกษาไปข้างหน้า โดยศึกษาความแรงของยาหยอดตาเซฟาโซลินโซเดียม 33 มิลลิกรัม/มิลลิลิตรที่เตรียมสำหรับผู้ป่วยเฉพาะรายที่ใช้ในโรงพยาบาลสุราษฎร์นครชัยนาท โดยยาหยอดตาที่เตรียมไว้ที่อุณหภูมิ 2-8 องศาเซลเซียสและกลุ่มที่ 2 เก็บที่อุณหภูมิ 28 องศาเซลเซียส ได้แก่การใช้broth dilution technique ในวันที่ 0, 3, 7, 14, 21 และ 28 ยาหยอดตาเซฟาโซลินโซเดียม 33 มิลลิกรัม/มิลลิลิตรที่เตรียมสำหรับผู้ป่วยเฉพาะรายที่เก็บไว้ในกลุ่มที่ 2 คงตัวได้ 28 วัน กลุ่มที่ 1 เก็บที่อุณหภูมิ 28 องศาเซลเซียส คงตัวได้ 14 วัน

ยาหยอดตาเซฟาโซลินโซเดียม 33 มิลลิกรัม/มิลลิลิตรที่เตรียมสำหรับผู้ป่วยเฉพาะรายที่เก็บไว้ในกลุ่มที่ 1 อุณหภูมิ 2-8 องศาเซลเซียส คงตัวได้ 28 วัน ส่วนกลุ่มที่ 2 อุณหภูมิ 28 องศาเซลเซียส คงตัวได้ 14 วัน

ยาที่ใช้ในการวิจัย ยาแคทของยาความเข้มข้นต่ำสุดที่ยับยั้งเชื้อ เซฟาโซลินโซเดียม

คำสำคัญ: ยาหยอดตา ความแรงของยา ความเข้มข้นต่ำสุดที่ยับยั้งเชื้อ เซฟาโซลินโซเดียม