Stability of Rifampin in Dimethylsulfoxide

ALFRED G. KARLSON AND JOHN A. ULRICH
Mayo Clinic and Mayo Foundation, Section of Microbiology, Rochester, Minnesota 55901

Received for publication 18 July 1969

Stock solutions of rifampin remain stable in dimethylsulfoxide for at least 8 months.

Rifampin is only slightly soluble in water but is reported to be soluble in "most of the organic solvents" (2). For in vitro tests of the antibacterial effect of rifampin, dimethylformamide was recommended as the primary solvent (5). However, our experience with this toxic solvent for amphotericin and for other agents revealed that dimethylformamide has a relatively high antimicrobial activity. Ethyl alcohol may be used to dissolve rifampin, but this solvent also has antibacterial activity; furthermore, ethyl alcohol is highly volatile and stock solutions of rifampin could be altered by evaporation.

To avoid the use of dimethylformamide and of ethyl alcohol in the preparation of stock solutions of rifampin, we tried dimethylsulfoxide (DMSO) which has low antibacterial activity (6). Stock solutions containing 10 mg of rifampin per ml of DMSO appeared to remain stable for at least 8 months when tested in vitro sensitivity of mycobacteria.

We recently tested a stock solution containing 10 mg of rifampin per ml of DMSO, which had been stored at 15 C for 8 months, against gram-positive cocci. The minimal inhibitory concentrations (MIC) for 24 different gram-positive cocci were the same as those obtained with a freshly prepared solution. The stock solution, which crystallizes at 15 C, was melted at room temperature and diluted in sterile 0.2 M KH2PO4 buffer to make working solutions of 1,000 and of 10 μg of rifampin per ml. Appropriate amounts of the working solutions were added to 100-ml amounts of melted and cooled (approximately 50 C) Trypticase Soy Agar (BBL) to make concentrations of 10, 5, 1, 0.2, 0.05, and 0.01 μg/ml of agar. The agar was poured into conventional plastic petri dishes and, after hardening, was stored at 15 C. In addition to a control plate of plain Trypticase Soy Agar, a control plate was used containing 1.0 ml of 10% DMSO in the buffer per 100 ml of agar.

For each strain, the inoculum was a 4-hr culture in Mueller-Hinton broth (4) diluted with the broth to a turbidity of tube no. 1 of McFarland's nephelometer. The inoculated plates were incubated at 37 C and were read in about 16 hr.

Table 1 shows the comparison of the MIC from eight cultures representative of the 24 cultures tested simultaneously in medium made with a fresh stock solution and stored at 15 C for 8 months.

<table>
<thead>
<tr>
<th>Culture</th>
<th>MIC of rifampin*</th>
<th>Fresh solution</th>
<th>Stored solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Enterococcus</td>
<td>10.0</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

* Expressed as micrograms per milliliter of agar.

b The minimal inhibitory concentration of this strain was <0.01 μg when tested 8 months previously by using a portion of the same stock solution of rifampin in DMSO.

c Resistant to penicillin and to vancomycin.

d Resistant to ampicillin.

e Resistant to penicillin and to dicloxacillin.

...
of rifampin remain stable in DMSO for at least 8 months.

This investigation was supported by grant from the Minnesota Respiratory Health Association.

Rifampin used in our experiments was provided by R. S. Janecki, Pittman-Moore Division of Dow Chemical Co., Zionsville, Ind.

LITERATURE CITED