Erythromycin Bioactivity Is Stable in Ophthalmic Ointment Used for Prophylaxis of Neonatal Gonococcal Conjunctivitis

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Erythromycin ophthalmic ointment (E. Fougera & Co., Melville, N.Y.) and erythromycin gluceptate standards prepared in ointment base were stored at room temperature and heated at temperatures up to 45°C for as long as 6 h before being assayed for bioactivity. We were unable to detect any significant loss of antibiotic bioactivity.

Although the incidence of neonatal gonococcal conjunctivitis has been dramatically reduced by the use of neonatal ocular prophylaxis (6), cases of neonatal gonococcal conjunctivitis (4, 5, 10) and Chlamydia conjunctivitis (7) still occur. Erythromycin ointment appears to be as effective as silver nitrate in preventing neonatal gonococcal conjunctivitis (2, 16) and does not cause chemical conjunctivitis (14); several reports have therefore recommended its adoption for routine prophylactic use (3, 7, 11). The effects of warming the ointment under the radiant warmer in the delivery room, as is often done before application, or the long-term stability of bioactive erythromycin in such ointment has not been established, although several studies have examined certain other relevant aspects of antibiotic ointments (12, 15, 17, 20). We examined the biological activity of erythromycin in ophthalmic ointment under conditions similar to those used for routine prophylactic administration to neonates for prevention of conjunctivitis and found erythromycin activity to remain stable under all conditions tested.

The bioactivity of erythromycin in ointment was determined by using a modified agar diffusion assay, as previously described (1). In brief, four plates of 1% modified Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) containing 1.5 × 10⁷ CFU of "Micrococcus lysodeikticus" per ml were prepared in polystyrene petri dishes (15 by 150 mm). Wells were produced in the agar with a single cork borer (no. 1 size) with a 3-mm external diameter, the agar plugs were removed by suction, and the erythromycin solutions being tested were placed in the wells (5 μl of solution per well). Each solution was tested in duplicate on two separate bioassay plates (four determinations). After 1 h of refrigeration at 4°C to allow diffusion of the antibiotic, the plates were incubated at 35°C in air for 24 h. Zones of inhibition of bacterial growth surrounding the erythromycin ointment-containing wells were measured using calipers and transmitted light. Zone diameters were fitted to a standard curve of erythromycin concentrations versus diameter derived by using the Cooper equation and methods described by Kavanagh (8). A standard curve (Fig. 1) was determined for each test day using erythromycin solutions made up in ointment base, as described below. Statistical methods used to assess results included analysis of variance and multiple regression analysis.

Ointment base (without antibiotic) was prepared by manually mixing white petrolatum and mineral oil at a ratio of 79.5:19.9 by weight (to simulate Ilotycin ointment base; Eli Lilly & Co., Indianapolis, Ind.). Erythromycin gluceptate standard powder (Eli Lilly & Co.) was dissolved in ethanol and diluted in phosphate-buffered saline (pH 7.5) to a concentration of 50 mg of active erythromycin per ml; aliquots were stored at −70°C. After thawing, dilutions were prepared in phosphate buffer (pH 7.1) for addition to the ointment base. Standard curves of erythromycin bioactivity in phosphate buffer were determined initially, but the mea-

FIG. 1. Standard curves (generated on 2 different test days) of concentration of erythromycin in ophthalmic ointment base and zones of inhibition of "M. lysodeikticus." The values for $x^2$ were derived from the zone diameter (d) by the formula: $x = 0.5 (d - 3)$, in which 3 mm was the diameter of the wells. Each point represents the mean and standard error of the mean of four separate determinations. Lines were derived by linear regression.

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TABLE 1. Percent bioactivity of erythromycin in ophthalmic ointment after repackaging into tuberculin syringes

<table>
<thead>
<tr>
<th>Additional incubation before test</th>
<th>% Bioactivity after the following no. of days at room temp:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>37°C, 24 h</td>
<td>103</td>
</tr>
<tr>
<td>45°C, 1 h</td>
<td>103</td>
</tr>
<tr>
<td>45°C, 3 h</td>
<td>100</td>
</tr>
<tr>
<td>45°C, 6 h</td>
<td>97</td>
</tr>
</tbody>
</table>

*Results are expressed as percent bioactivity in relation to that of erythromycin ointment control, as determined by measuring zones of inhibition and extrapolating bioactivity from standard curves generated on the same day, as described in the text.

The surable activity of the antibiotic in ophthalmic ointment was so decreased compared with that of the same concentration of erythromycin in buffer that only ointment standards were used for preparation of the standard curves.

Erythromycin ophthalmic ointment (0.5%; E. Fougera & Co., Melville, N.Y.) in a 1/8-oz (ca. 3.5-g) tube was aseptically repackaged into 1-ml tuberculin syringes in aliquots of 0.2 ml under a laminar flow hood as described by Bryant (3) and Wyatt (19). In experiment 1, sets of two syringes were held at room temperature for 1, 4, 6, 13, or 15 days after repackaging and before testing. All samples were assayed for bioactivity on the same day. One of each pair of syringes was heated at a temperature-controlled 37°C water bath for 24 h, and the bioactivity of the erythromycin was compared with that of the erythromycin in the syringe held at room temperature. In experiment 2, erythromycin ointment repackaged into syringes was heated at 37, 40, and 45°C for 1, 3, and 6 h before the bioactivity of the erythromycin was measured. These temperatures were chosen for testing because the maximum attainable temperatures measured at bed level for four radiant warmers in routine use at the hospital (as might be used for warming the ointment before application to the neonate) were found to be between 30 and 39°C.

Storage of erythromycin ophthalmic ointment in syringes for up to 15 days at room temperature did not affect bioactivity, nor did heating the repackaged ointment to 37°C for 24 h (Table 1). There was no significant difference between the bioactivities of erythromycin in heated and unheated ophthalmic ointment samples or between the bioactivities in samples with various storage times as determined by analysis of variance. In fact, the bioactivity of erythromycin ophthalmic ointment as determined in our assay was stable even at 45°C for 6 h (Table 1). There was no significant difference between the bioactivities of erythromycin in ointment heated at different temperatures or for different lengths of time as determined by multiple regression analysis.

The bioactivity of an antibiotic ointment depends not only on the concentration of antibiotic but also on the particle size, solubility, and diffusibility of the antibiotic in the ointment base and the biological substrate at the surface (tears or agar) (9, 15). Because bioactivity detected in our assay was greater in phosphate buffer than in ointment (results not shown) and because erythromycin is more diffusible in tears than in agar, better activity might be predicted in tears in vivo than in agar in our in vitro assay.

Increased nongonococcal neonatal conjunctivitis after erythromycin ointment prophylaxis has been reported and was believed to be related to difficulty in applying the ointment (10, 13). After initially suggesting warming the ointment (3), Bryant later abandoned this technique (18). Softening the ointment by briefly placing it in a radiant warmer may allow easier application of the ointment, and we have shown here that this treatment does not interfere with its antibiotic activity. The availability of unit-dose ophthalmic ointment obviates the need for repackaging (18), and storage for as long as 2 weeks at room temperature does not affect the bioactivity of erythromycin. Proper delivery of a sufficient quantity of erythromycin in the ointment vehicle therefore becomes the most important factor for effective prophylaxis against neonatal infectious conjunctivitis.

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LITERATURE CITED