In Vitro Activity of RP 74501-RP 74502, a Novel Streptogramin Antimicrobial Mixture, against Clinical Isolates of Legionella Species

PAUL H. EDELSTEIN1,2* AND MARTHA A. C. EDELSTEIN1

Department of Pathology and Laboratory Medicine1 and Department of Medicine, 2 University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104-4283

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Agar and broth microdilution MICs of RP 74501-RP 74502, a mixture of streptogramin antimicrobial agents that inhibited 90% of 22 Legionella strains tested, were 0.64 and 0.08 µg/ml, respectively; respective erythromycin values were 1.0 and 0.12 µg/ml. RP 74501-RP 74502 at 1 µg/ml was more active than the same erythromycin concentration in a macrophage system for both L. pneumophila strains studied but at a lower concentration (0.25 µg/ml) was much less active than erythromycin.

RP 74501-RP 74502 is a purified form of pristinamycin, a streptogramin antibiotic (1, 2, 10, 11, 14). RP 74501-RP 74502 is a fixed-combination drug composed of approximately 30% RP 74501 (P1 component) and 70% RP 74502 (P2 component), and is given orally. The combination acts synergistically against most gram-positive cocci and some gram-negative bacilli (13). We tested the effect on Legionella species of RP 74501-RP 74502 by using a variety of susceptibility testing methods designed to determine whether the drug is active against intracellular and extracellular legionellae.

All legionellae studied were clinical isolates. These strains were identical to those used in prior studies and included 2 strains each of L. dumoffii, L. longbeachae, and L. micdadei; 1 strain of L. bozemanii; and 15 strains of L. pneumophila (3, 4). Staphylococcus aureus ATCC 29213 was used as a control organism for susceptibility testing. Legionellae were grown on locally made buffered charcoal yeast extract medium supplemented with 0.1% α-ketoglutarate (BCYEα) (5). Incubation of all media was done at 35°C in humidified air. Standard powders of RP 74501-RP 74502 and erythromycin were obtained from Rhône-Poulenc Rorer, Vitry-sur-Seine, France, and Abbott Laboratories, North Chicago, Ill., respectively.

Agar dilution susceptibility testing was performed as described previously (7). Briefly, antimicrobial-agent-containing BCYEα agar plates were inoculated with ~105 CFU of bacteria. The control S. aureus strain was inoculated on antimicrobial-agent-containing Mueller-Hinton agar plates, as well as BCYEα plates, to determine whether BCYEα medium inhibited antimicrobial agent activity. The plates were incubated for either 24 (S. aureus) or 48 h ( legionellae), at which time MICs were determined; the presence of more than one colony was considered evidence of lack of antibiotic inhibition. Broth microdilution susceptibility testing was performed by using buffered yeast extract broth supplemented with 0.1% α-ketoglutarate (BYEα) (legionellae) or Mueller-Hinton broth (S. aureus), with a final volume of 200 µl and a final bacterial concentration of 5 × 105 CFU/ml (5). Otherwise, the broth microdilution method was performed exactly as described previously for a macrobroth method (7).

Any broth turbidity was considered evidence of growth. All testing was done in duplicate; the geometric mean value was used as the MIC. A MIC found to be less than or equal to the lowest concentration of the antimicrobial agent tested was arbitrarily defined to be the lowest concentration tested. Erythromycin was included as a control; data for the activity of this drug for the Legionella strains we tested have been presented previously (7, 8).

Guinea pig pulmonary alveolar macrophages were harvested and purified as described previously (7). The final concentration of macrophages was ~106 cells per well. Antimicrobial susceptibility testing of intracellular L. pneumophila was performed as described previously (7). Briefly, ~104 CFU of washed BCYEα plate-grown L. pneumophila was added to the purified alveolar macrophages. The bacteria and macrophages were incubated for 1 day after 1 h of incubation with shaking. Antimicrobial agents were added to their respective wells after the wells had been washed three times to remove nonadherent bacteria. Sonic extracts of two replicate, non-antimicrobial-agent-containing wells were quantitatively cultured for use as the day 1 bacterial count. Non-antimicrobial-agent-containing wells were used as growth controls. After two more days of incubation, the supernatants were sampled and quantitatively cultured; all wells were then washed to remove antimicrobial agents. Bacterial counts in the supernatant of each well were determined for another 4 days. To check for antimicrobial agent toxicity, uninfected macrophages were incubated with the highest concentration of antimicrobial agent tested and observed microscopically daily. All experiments were carried out in triplicate, and quantitative plating was carried out in duplicate.

RP 74501-RP 74502 and erythromycin agar MICs for the 22 Legionella strains tested are shown in Table 1. One of the two L. micdadei strains tested did not grow well enough in BYEα broth for broth microdilution testing to be performed, allowing only 21 Legionella strains to be tested by this method (Table 1). The erythromycin broth microdilution MIC for another L. micdadei strain (0.5 µg/ml) was much higher than for any other strain tested.

Both antimicrobial agents were inhibited by BCYEα agar, as determined by their MICs for the control S. aureus strain with BCYEα and Mueller-Hinton agar media. The RP 74501-RP 74502 MIC for S. aureus was 3 log2 greater with
BCYEα agar than it was with Mueller-Hinton agar. The erythromycin MIC for *S. aureus* was 1 log₂ dilution greater with BCYEα agar. BYEα broth did not inhibit RP 74501-RP 74502 activity for *S. aureus*; the erythromycin MIC was 1 log₂ dilution greater with BYEα broth than with Mueller-Hinton broth.

Differences in MICs with agar and broth dilution such as those seen in this study have been uniformly observed with *Legionella* species (see reference 8 for more detail). This has been attributed to antimicrobial agent inhibition by charcoal-containing media, as was observed in this study. Whether agar or broth dilution susceptibility testing of *Legionella* species is more accurate is unknown. The RP 74501-RP 74502 broth MICs obtained for the *Legionella* species tested in this study are somewhat lower than those observed for a related compound, RP 59500, with a different testing method (4).

The intracellular activity of RP 74501-RP 74502 and erythromycin against two *L. pneumophila* serogroup 1 strains grown in guinea pig alveolar macrophages is shown in Fig. 1 and 2. *Legionella pneumophila* F889 was inhibited by both antimicrobial agents tested at concentrations ≥0.25 μg/ml after 48 h of incubation. Strain F2111 was inhibited by RP 74501-RP 74502 at a concentration of 1.0 μg/ml, but not by 0.25 μg/ml. Erythromycin (0.25 μg/ml) was more active than was the same concentration of RP 74501-RP 74502, in contrast to the relative activities of both drugs at a higher concentration (1.0 μg/ml). For both bacterial strains tested, RP 74501-RP 74502 (1.0 μg/ml) had a longer-lasting inhibitory effect than did the same concentration of erythromycin after drug removal by washing. The decrease in viable counts of strain F2111, after drug removal (day 3), in the presence of no antibiotic or RP 74501-RP 74502 (0.25 μg/ml), is artifactual because of macrophage killing by the bacteria by day 3; many of the bacteria in these tissue culture wells were washed out with the damaged macrophages. Macrophage toxicity caused by antimicrobial agents alone was not observed.

These studies show that RP 74501-RP 74502 is about as active as erythromycin for both extracellular and intracellular legionellae. Both drugs were solely inhibitory, as has been observed for macrolides and other drugs previously (7-9). For intracellular *L. pneumophila* infection, RP 74501-RP 74502 was more active than erythromycin when either drug was tested at a concentration of 1.0 μg/ml. However, the converse was true at drug concentrations of 0.25 μg/ml. The reason for this paradox is unknown. One possibility is that there is a threshold below which RP 74501-RP 74502 is not taken up into cells or below which it is inactive in the phagosome. It is known that RP 74501-RP 74502 is relatively unstable at 35°C at the concentrations tested, which may have led to an underestimation of its intracellular activity under the conditions of prolonged incubation used in the macrophage studies (12).

The cellular uptake kinetics of RP 74501-RP 74502 are unknown, although a related combination streptogramin drug, RP 59500, is concentrated in J 774 cells, with a range of intracellular/extracellular concentration ratios of 10 to 50 (3).

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### TABLE 1. Agar and broth microdilution susceptibilities of *Legionella* strains

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC &lt;sub&gt;50&lt;/sub&gt; (μg/ml) with:</th>
<th>MIC &lt;sub&gt;90&lt;/sub&gt; (μg/ml) with:</th>
<th>Range</th>
<th>MIC &lt;sub&gt;50&lt;/sub&gt; (μg/ml) with:</th>
<th>MIC &lt;sub&gt;90&lt;/sub&gt; (μg/ml) with:</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BCYEα agar</td>
<td></td>
<td></td>
<td>BYEα broth</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RP 74501-RP 74502</td>
<td>0.3</td>
<td>0.6</td>
<td>0.16–0.64</td>
<td>0.04</td>
<td>0.08</td>
<td>0.04–0.08</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.1</td>
<td>1.0</td>
<td>≤0.06–1.0</td>
<td>0.06</td>
<td>0.10</td>
<td>≤0.06–0.5</td>
</tr>
</tbody>
</table>

* Twenty-two strains were tested by using agar dilution, and 21 were tested by using broth microdilution.

*<sup>a</sup> MIC<sub>50</sub> and MIC<sub>90</sub>, MICs for 50 and 90% of strains tested, respectively.

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**FIG. 1.** Growth of *L. pneumophila* serogroup 1 strain F889 in guinea pig alveolar macrophages at postinfection days 1 to 7. □, growth control; △ and ○, 0.25 and 1.0 μg of erythromycin per ml, respectively; □ and x, 0.25 and 1.0 μg of RP 74501-RP 74502 per ml, respectively.

**FIG. 2.** Growth of *L. pneumophila* serogroup 1 strain F2111 in guinea pig alveolar macrophages at postinfection days 1 to 7. □, growth control; △ and ○, 0.25 and 1.0 μg of erythromycin per ml, respectively; □ and x, 0.25 and 1.0 μg of RP 74501-RP 74502 per ml, respectively.
Because cellular uptake of antimicrobial agents is required for the inhibition of *L. pneumophila* growth in this model, RP 74501-RP 74502 must be present intracellularly in appreciable concentrations (7). Unknown are the relative intracellular amounts of the two components of this drug combination, which could be different in vivo than in tissue culture, because of the different pharmacokinetics of the components. Because the results of antimicrobial agent treatment of this cell model of *L. pneumophila* infection are generally predictive of clinical effectiveness, RP 74501-RP 74502 is of potential use for the treatment of Legionnaires’ disease and possibly other intracellular infections (6).

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REFERENCES