Telithromycin Is Active against *Mycobacterium avium* in Mice despite Lacking Significant Activity in Standard In Vitro and Macrophage Assays and Is Associated with Low Frequency of Resistance during Treatment

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The activity of telithromycin, a new ketolide, was evaluated in vitro and in vivo against *Mycobacterium avium* complex (MAC) strains. The MIC of telithromycin for several *M. avium* isolates obtained from the blood of AIDS patients ranged from 16 to >128 μg/ml (MIC at which 90% of isolates are inhibited, >128 μg/ml), and the compound did show activity in the macrophage system at concentrations greater than 8 or 16 μg/ml, but this was dependent on the MAC strain used. Telithromycin was then administered to mice infected with MAC strain 101 for 4 weeks at doses of 100, 200, or 400 mg/kg of body weight/day. Treatment with 100 and 200 mg/kg/day was bacteriostatic, but at 400 mg/kg/day telithromycin was bactericidal for MAC strains. The frequency of the emergence of resistance to telithromycin was low despite prolonged usage (12 weeks). This study demonstrates that telithromycin is active in vivo against MAC and warrants further evaluation.

Isolates of the *Mycobacterium avium* complex (MAC) are intracellular pathogens associated with both lung disease and disseminated infection in patients with AIDS (12, 14). Effective treatment for MAC infection is based on the use of new macrolides such as clarithromycin, azithromycin, and roxithromycin (3, 10, 22). However, additional drugs with anti-MAC activity are needed.

Telithromycin (HMR3647) is a ketolide, i.e., a semisynthetic derivative of erythromycin A, which differs from erythromycin A by substitution of a 3-keto group for L-cladinose. Previously, we reported that HMR3004 was active against MAC in vivo, although its effect was bacteriostatic (5). HMR3004 has a C11,C12 carbamate on which a quinoline group is attached through a propyl chain, but it is no longer in clinical development. Telithromycin (formerly, HMR3647) was provided by Roussel Uclaf (Romanville, France).

The potency of the drug was confirmed by using standard American Type Culture Collection (ATCC) quality control strains *Escherichia coli* ATCC 29252 and *Staphylococcus aureus* ATCC 29213. Telithromycin was prepared for administration by gavage by suspending it in 0.2% Tween 80 plus 2.5% gum arabic (Sigma Chemical Co.), as described previously (5).

**Mycobacteria.** The strains of MAC used in the present study (strains 100, 101, 102, 103, 104, 105, 107, 108, 109, 110, 111, 113, 116, 117, 128, 501, 502, 504, 505, 506, 507, and 508) were isolated from the blood of human immunodeficiency virus-infected patients (each strain was isolated from a different AIDS patient) with disseminated MAC disease, and each isolate was identified as *M. avium* by using a commercially available DNA probe (Gen-Probe Inc., San Diego, Calif.). MAC strain 101 (serovar 1), MAC strain 109 (serovar 4), and MAC strain 100 (serovar 8) were used for all macrophage assays. MAC strain 101 was used in the animal studies. MAC 101 is a virulent strain in the beige mouse test system, and this strain causes reproducible levels of infection and mortality in beige mice (8).

MAC organisms were cultured in Middlebrook 7H10 agar medium (Difco Laboratories, Detroit, Mich.) supplemented with oleic acid, albumin, dextrose, and catalase (OADC; Difco) for 10 days at 37°C. Only transparent colony types were used in the studies. For the macrophage and mouse studies, the colonies were harvested and suspended in Hank’s balanced salt solution to concentrations of 1 × 10^7 and 3 × 10^8 CFU/ml, respectively, by comparison with a McFarland no. 1 turbidity standard; samples were plated onto 7H10 agar to confirm the concentration of the inoculum.

Beige mice were infected intravenously with 100 μl of the original suspension (3 × 10^7 MAC 101 bacteria per ml). Before infection of the macrophages, the suspension was vortex agitated for 2 min and passed through a 23-gauge needle five times to disperse clumps. Microscopic observation confirmed the dispersion of the inoculum.

In vitro susceptibility testing. MICs were determined by a radiometric broth macrodilution method and the T100 method of data analysis (16). The inoculum for susceptibility testing was prepared by picking 5 to 10 colonies from a 7H11 agar plate and placing them into 7H9 broth; it was then tested directly or frozen at −70°C. The inoculum was adjusted to approximately 5 × 10^4 CFU/ml by

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for the final suspension was determined by quantitative plate counts. Four hours with 100
and dose. Blood was obtained via the tail vein. Drug levels in tissue have been
n and 2m ML-glutamine. The cells were grown to a density of 5
Chicago, Ill.) supplemented with 5% fetal bovine serum (Sigma Chemical Co.)
MAC 101 was tested against amikacin to control for overall test performance.
control and test cultures, but a period of 7 days was sufficient for most isolates.
cluded the inoculum undiluted without added drug (no-drug control), the inoc-
that could not be easily dispersed were shaken with glass beads. Controls in-
- defined by using the beige mouse test system as described above. If more
- resistant colonies per mouse were taken into consideration to calculate the
- the number of CFU were counted. Both the total number of colonies and the number of
- ing the number of CFU per gram of tissue growing on plates with and without
- tative plate counts to establish the initial inoculum (baseline), as reported previ-
- the infected monolayers were then treated with telithromycin (concentration
- Drug and medium were replenished daily for 4
days. After the treatment period (4 days), the medium was removed and the
- were lyzed as described previously (4). Briefly, (0.5 ml) was added to each monolayer well, and the mixture was allowed to stand
- for 10 min at room temperature. Then, 0.5 ml of a second lysing solution (1 ml of
- of Middlebrook 7H9 broth plus 0.4 ml of 0.25% sodium deoxycholate (SDS) in
- phosphate buffer) was added to each well, and the mixture was allowed to stand for
- an additional 10 min. The wells were vigorously scraped with a rubber
- and the macrophage lysates were resuspended in 0.5 ml of 20% bovine serum in sterile water to neutralize the SDS. The suspension was agitated for 2 min to ensure complete lysis of the macrophages. Finally, the
- sonicated for 5 s to disrupt clumps of bacilli. To control for the osmotic
- of the mycobacteria, a suspension of mycobacteria alone was plated for
- quantitation before and after being subjected to the lysis procedure as described
- and in each instance there was no change in the number of CFU per
- before or after the lysis treatment procedure.

The final macrophage lysate suspension was serially diluted, and 0.1 ml was plated onto
H10 agar. The plates were allowed to dry at room temperature for
15 min and incubated at 37°C in 5% CO2 for 2 weeks. Duplicate plates were
prepared for each well, and the results were reported as the mean number of
CFU per milliliter of macrophage lysate. Each assay was performed in triplicate, and
each experiment was repeated six times.

Animal studies. The potential therapeutic efficacy of telithromycin was deter-
- using by the beige mouse test system as described previously (4, 6, 20).
- seven-week-old female C57BL/6 bg bg mice (Jackson Laboratories, Bar Harbor, Maine).
- were infected through the
- infection, the livers
- the treatment period (up to 12 weeks). In mice that received the
- were killed, and the spleens were removed by aseptic dissection. The organs were
- the MIC of telithromycin with the BACTEC system, as described above. If more
- on the similarity of pharmacokinetics between telithromycin and azithro-
- based on the similarity of pharmacokinetics between telithromycin and azithro-

<table>
<thead>
<tr>
<th>Drug dose (µg/ml)</th>
<th>No. of CFU/ml of macrophage lysate at day 4a</th>
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<tbody>
<tr>
<td>MAC 100</td>
<td>MAC 101</td>
</tr>
<tr>
<td>None</td>
<td>(9.8 ± 0.1) × 10^6</td>
</tr>
<tr>
<td>1</td>
<td>(9.6 ± 0.1) × 10^6</td>
</tr>
<tr>
<td>2</td>
<td>(9.0 ± 0.1) × 10^6</td>
</tr>
<tr>
<td>4</td>
<td>(7.9 ± 0.1) × 10^6</td>
</tr>
<tr>
<td>8</td>
<td>(5.9 ± 0.1) × 10^6</td>
</tr>
<tr>
<td>16</td>
<td>(4.8 ± 0.2) × 10^6</td>
</tr>
<tr>
<td>32</td>
<td>(2.6 ± 0.3) × 10^6</td>
</tr>
<tr>
<td>64</td>
<td>(1.6 ± 0.1) × 10^6</td>
</tr>
<tr>
<td>128</td>
<td>(6.5 ± 0.1) × 10^5</td>
</tr>
</tbody>
</table>

a The number of intracellular bacteria at time zero (after infection but before treatment) were as follows: MAC 100, (8.5 ± 0.2) × 10^6; MAC 101, (7.9 ± 0.3) × 10^6; MAC 109, (7.4 ± 0.2) × 10^6.

b P < 0.5 compared with the value for controls at 4 days (bacteriostatic activity).

c P < 0.5 compared with the baseline level at time zero (bactericidal activity).

comparison with a McFarland no. 1 turbidity standard. Isolates that clumped and
that could not be easily dispersed were shaken with glass beads. Controls in-
cluded the inoculum undiluted without added drug (no-drug control), the inoc-
ulum diluted 1:100 (99% control), and the inoculum diluted 1:1,000 (99.9%
control). In addition, one vial was inoculated with a suspension of mycobacteria
which were boiled for 5 min prior to inoculation in order to monitor the non-
growth-related release of carbon dioxide in the BACTEC system. The period of
observation and the end points were determined by daily monitoring of the
control and test cultures, but a period of 7 days was sufficient for most isolates.
MAC 101 was tested against amikacin to control for overall test performance.

Human macrophage studies. The source of macrophages was the human
monocyte cell line U937 cultured in RPMI 1640 medium (pH 7.2) (Gibco,
Chicago, Ill.) supplemented with 5% fetal bovine serum (Sigma Chemical Co.)
and 2 mM l-glutamine. The cells were grown to a density of 5 × 10^5 cells per ml
and were then centrifuged, washed, and resuspended in supplemented RPMI
1640 medium. The concentration of cells was adjusted to 10^6 cells per ml, and 1
ml of the cell suspension was added to each well of a 24-well tissue culture plate
(Costar, Cambridge, Mass.). Monolayers were treated with 0.5 mg of phosphol
myristate acetate per ml for 24 h to stimulate maturation of the monocytes. The
monolayers were monitored for the number of cells, and no difference in the
extent of cell detachment was observed among the several treatment and control
groups.

M. avium strains were cultured for 10 days in Middlebrook 7H10 agar (Difco
Laboratories). On the day of the experiment, the bacteria were harvested,
washed twice in Hank’s balanced salt solution, and suspended in Hank’s bal-
anced salt solution; and a dispersed inoculum was prepared as described above.
The turbidity of the suspension was adjusted so that it was equivalent to that of
a McFarland no. 1 turbidity standard, and the suspension was diluted to a final
concentration of approximately 5 × 10^7 CFU/ml. Each monolayer was infected with
100 µl of the final suspension, and the actual number of CFU per milliliter for
the final suspension was determined by quantitative plate counts. Four hours
after infection, the number of CFU of mycobacteria per well of the macrophage
monolayer was determined by lysing the macrophages and performing quantita-

TABLE 2. Telithromycin concentration in the blood of micea

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Conc in blood (µg/ml) for the following dose:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>100 mg/kg</td>
</tr>
<tr>
<td>0.5</td>
<td>3.90</td>
</tr>
<tr>
<td>1</td>
<td>3.95</td>
</tr>
<tr>
<td>2</td>
<td>4.91</td>
</tr>
<tr>
<td>4</td>
<td>3.97</td>
</tr>
<tr>
<td>8</td>
<td>2.16</td>
</tr>
</tbody>
</table>

a Three uninfected mice were given telithromycin orally for each time point and
dose. Blood was obtained via the tail vein. Drug levels in tissue have been
published previously (9).
points were determined by the Mann-Whitney nonparametric test. The statistical significance of the differences between the number of organisms recovered from the spleens, livers, and blood was evaluated by one- or two-variable analysis of variance. Differences between the results for experimental groups and between the results for the experimental groups and the control groups were considered statistically significant if P values were <0.05.

RESULTS

MIC studies. The MICs at which 50 and 90% of isolates were inhibited for 24 MAC strains (blood isolates) were 128 and 128 \( \mu \text{g/ml} \), respectively. Seventeen of the individual strains tested were resistant to telithromycin at concentrations of 128 \( \mu \text{g/ml} \) or higher, and the range of MICs for the 23 strains tested varied from 32 to \( >128 \mu \text{g/ml} \).

Human macrophage studies. As shown in Table 1, telithromycin had no inhibitory activity against intracellular MAC strains 100 and 101 at concentrations less than 16 \( \mu \text{g/ml} \), while inhibitory activity against strain 109 was observed in the presence of 8 \( \mu \text{g/ml} \) after 4 days of treatment. Only the concentration of 128 \( \mu \text{g/ml} \) was bactericidal for MAC 100. No other bactericidal activity was observed in this system with the concentrations used.

Therapeutic studies with mice. Telithromycin was administered orally at doses of 100, 200, and 400 mg/ml daily; these doses were based on the levels obtained in serum (Table 2). Telithromycin was not toxic, and its administration was not associated with increased rates of mortality. While 35% of the untreated control mice survived the experiment, 60% of the mice receiving 100 mg of telithromycin per ml, 75% of the mice receiving 200 mg of telithromycin per ml, and 85% of the mice receiving 400 mg of telithromycin per ml survived the period of the study. As shown in Fig. 1A to C, there was a dose-related effect according to the dose of telithromycin used. While concentrations of 100 and 200 mg/kg/day were bacteriostatic compared to the level of infection at day 7 (before treatment), the dose of 400 mg/kg showed bactericidal activity in both the livers and the spleens, and treatment at these doses resulted in a significant reduction (approximately 1 log) in the number of bacteria in the blood.

Emergence of resistance. Treatment with telithromycin resulted in a low frequency of resistance. Table 3 shows the number of mice from which telithromycin-resistant MAC strains were isolated over time and the frequency of resistance to the drug among these isolates. While the frequency of the emergence of strains resistant to telithromycin was statistically significantly greater compared with the frequency of emergence of resistant MAC strains isolated from untreated mice at both weeks 8 and 12, the frequency was nonetheless low. The telithromycin MICs for the resistant strains were 512 \( \mu \text{g/ml} \) or greater.

DISCUSSION

Telithromycin showed weak activity against MAC in vitro, with MICs of 16 or greater, but it was significantly active against MAC infection in vivo. The activity in vivo was dose dependent. Telithromycin was bacteriostatic at doses of 100 and 200 mg/kg/day, but it was bactericidal at a dose of 400 mg/kg/day. Telithromycin was significantly more active on a weight basis than HMR3004 for the treatment of MAC infection in a similar mouse system (5).
with consistent results. Although limited, our observations thus far suggest that compounds that achieve very high concentrations within cells may be associated with a lower frequency of emergence of resistance among MAC strains than compounds that achieve high concentrations in serum.

Clinical resistance to macrolides is primarily due to a single mutation in the 23s rRNA gene (19). Thus far, all macrolide-resistant strains that we have tested are also resistant to ketolides (data not shown). Therefore, ketolides cannot be used as alternative therapies when macrolides fail.

In summary, we described the anti-MAC activity of telithromycin, an antibiotic that, despite its poor activity in vitro, showed significant efficacy in the treatment of MAC infection in mice, likely due to its pharmacokinetic properties.

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REFERENCES


