ATP Synthase Inhibition of Mycobacterium avium Is Not Bactericidal

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The efficacy of ATP synthase inhibitor TMC207 was assessed in early and late Mycobacterium avium infections in mice. In contrast to what was earlier observed for M. tuberculosis, a bacteriostatic effect was obtained. In vitro, the minimal bactericidal concentration (MBC)/MIC ratio was very high. The MBC was more relevant for assessment of pharmacokinetic/pharmacodynamic relationships than the MIC.

Mycobacterium avium is a pathogen that causes disseminated disease in immunocompromised individuals and pulmonary disease in immunocompetent adults (15) and is far less susceptible than Mycobacterium tuberculosis to most antimicrobial agents; treatment options are very limited (7, 10). The most efficacious drugs are clarithromycin (CLA), the azalide antibiotic azithromycin, and amikacin (AMK). They are generally part of a multidrug regimen including rifamycins and ethambutol (10) and need to be administered daily for up to 24 months (10). These regimens are expensive and poorly tolerated (2, 6). TMC207 (also known as R207910) is a diarylquinoxalinone ATP synthase inhibitor with potent activity against M. tuberculosis (1, 3, 13). It has broad antmycobacterial activity, with MICs against several clinical isolates of M. avium ranging from 0.007 to 0.25 μg/ml (1, 8).

Female C57BL/6J mice aged 6 to 7 weeks (Janvier Breeding, France) were infected intraperitoneally with 0.5 ml of a bacterial suspension containing 2.3 × 10^7 CFU of M. avium 101. In a first group of 20 animals (Table 1), treatment started the day after infection (early infection model) and included a negative control, a positive control (CLA), and two test groups (TMC207 or CLA plus TMC207). Mice were sacrificed after 1 month of treatment. A second group of 165 mice was kept untreated for 1 month (late infection model) and then was treated with CLA alone, AMK alone, TMC207 alone, CLA plus AMK, CLA plus TMC207, AMK plus TMC207, or CLA plus AMK plus TMC207 for 4 months (Table 1). Five animals from each group were sacrificed at monthly intervals. All drugs were given five times weekly at the following doses: 25 mg/kg body weight TMC207 orally, 200 mg/kg CLA orally, and 150 mg/kg AMK subcutaneously. Treatment effects were assessed by CFU counts, determined by plating three serial 10-fold dilutions of homogenized spleen suspensions onto Löwenstein-Jensen plates. The Student t test with Bonferroni correction of the P value was used to analyze CFU counts. As four and seven groups were compared, P values were adjusted to 0.0083 and 0.0024, respectively.

In the early infection model, untreated control mice had 6.53 ± 0.56 log_{10} CFU counts at day 0, increasing to 8.0 ± 0.9 log_{10} CFU 1 month later (P = 0.02). Monotherapy with CLA and TMC207 decreased CFU counts by 1.99 and 2.56 log_{10} compared to late controls (P = 0.005 and P = 0.002, respectively). The combination of TMC207 and CLA did not improve the activity of the individual compounds (P > 0.05) (Table 2).

### TABLE 1. Experimental design

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of mice (n = 185)</th>
<th>Date of sacrifice</th>
<th>Day −28 (n = 5)</th>
<th>Day −27 (n = 20)</th>
<th>Day 0 (n = 40)</th>
<th>Mo 1 (n = 40)</th>
<th>Mo 2 (n = 40)</th>
<th>Mo 3 (n = 40)</th>
<th>Mo 4 (n = 40)</th>
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<tbody>
<tr>
<td>Control</td>
<td>30</td>
<td>Infection</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
</tr>
<tr>
<td>CLA</td>
<td>5</td>
<td>Infection</td>
<td>Treatment (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
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<tr>
<td>TMC207</td>
<td>5</td>
<td>Infection</td>
<td>Treatment (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
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<tr>
<td>CLA + TMC207</td>
<td>5</td>
<td>Infection</td>
<td>Treatment (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
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<tr>
<td>CLA</td>
<td>20</td>
<td>Infection</td>
<td>Treatment (5)</td>
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<tr>
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<td>Treatment (5)</td>
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<tr>
<td>AMK</td>
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<td>Treatment (5)</td>
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<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
<td>Sacrifice (5)</td>
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<td>CLA + AMK</td>
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<td>Infection</td>
<td>Treatment (5)</td>
<td>Sacrifice (5)</td>
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<td>Treatment (5)</td>
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<td>Infection</td>
<td>Treatment (5)</td>
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* CLA, 200 mg/kg; TMC207, 25 mg/kg; AMK, 150 mg/kg.

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When compared with the early controls, all regimens were bacteriostatic.

In the late infection model, untreated control mice reached $8.0 \pm 0.9 \log_{10}$ CFU counts 1 month after infection and remained stable over the next 4 months (Table 2). The regimens CLA alone, AMK alone, TMC207 alone, CLA plus TMC207, CLA plus AMK, AMK plus TMC207, and CLA plus AMK plus TMC207 did not show any activity after 1 month of treatment. After 2 months of treatment, all regimens achieved a bacteriostatic activity. After 3 months of treatment, the triple combination CLA plus AMK plus TMC207 achieved bactericidal activity ($P = 0.001$) and was more active than CLA or TMC207 alone, but not more active than AMK alone. After 4 months of treatment, AMK alone, CLA plus AMK, AMK plus TMC207, and CLA plus AMK plus TMC207 all achieved bactericidal activity. The activity of the TMC207 monotherapy was still bacteriostatic. The activity of the triple combination CLA plus AMK plus TMC207 was better than that of CLA alone or TMC207 alone but not better than that of AMK alone.

The MIC of TMC207 against the M. avium strain used in the present study was 0.015 $\mu$g/ml, consistent with earlier estimates (1, 8). Based on the impressive in vivo results obtained with TMC207, which has a MIC of 0.060 $\mu$g/ml (1), the weak in vivo activity of TMC207 against M. avium was disappointing. We decided to determine the minimal bactericidal concentration (MBC) of TMC207 against M. avium and found it to be much higher than its MIC (128 $\geq$ 0.015 $\mu$g/ml). A bacteriostatic activity of TMC207 against M. avium was also confirmed in a killing study in which M. avium was exposed to 100$\times$ and 1,000 $\times$ the MIC, which resulted in a 1-log$_{10}$CFU kill after 14 days of exposure. An M. tuberculosis sample exposed to the same concentrations of TMC207 was reduced by 5 log$_{10}$ CFU, clearly illustrating that TMC207 has bactericidal activity against M. tuberculosis and bacteriostatic activity against M. avium.

Our data reveal that the mechanism by which mycobacteria are inhibited in their growth (as reflected by the MIC) may be different from the mechanism by which some mycobacterial species are killed. It is currently not clear why some mycobacteria are not killed by TMC207, despite their growth being inhibited at low concentrations, while others do get killed, albeit after a delay of a few days.

Many pharmacokinetic/pharmacodynamic parameters used to estimate efficacy of antimicrobial agents refer to the MIC: e.g., area under the inhibitory curve (AUC), area under the concentration-time curve (AUC/MIC ratio, time above the MIC ($T > MIC$), and maximum concentration of drug in serum ($C_{max}$/MIC ratio) (14). For drugs with a high MBC/MIC ratio, parameters referring to the MBC (such as the area under the bactericidal curve [AUBC]) (14) are probably more relevant. Based on the pharmacokinetic parameters of TMC207 on the one hand and the MICS for M. tuberculosis and M. avium on the other hand, one would not have been able to predict the dramatic difference in efficacy in the mouse model, using exactly the same dosing schedule as that for M. tuberculosis (1).

Very few compounds have shown bactericidal efficacy against M. avium complex infections in the beige or the C57BL/6J mouse models. Many drugs have been tested, but with the exception of macrolides (4, 11, 16) and aminoglycosides (5, 9, 11, 12), none were bacteriostatic. AMK is the only antimicrobial tested which was able to increase the activity of CLA in the mouse model and to prevent the selection of resistant mutants of M. avium to CLA (12). Since AMK cannot be used for longer than 2 or 3 months for safety reasons (ototoxicity and nephrotoxicity), an alternative orally available compound is desirable. Provided that TMC207 has an acceptable safety profile, it could be used as a companion drug of CLA because of its bacteriostatic activity. The ability of TMC207 to prevent the selection of resistant mutants to CLA should be investigated in future studies.

REFERENCES


4. Fernandes, P. B., D. J. Hardy, D. McDaniel, C. W. Hanson, and R. N.


