Bactericidal Activities of HMR 3647, Moxifloxacin, and Rifapentine against Mycobacterium leprae in Mice

SOPHIE CONSIGNY, ABDELHALIM BENTOUCHA, PASCALE BONNAFOUS, JACQUES GROSSET, AND BAOHONG JI*

Faculté de Médecine Pitié-Salpêtrière, Paris, France

Received 20 December 1999/Returned for modification 13 April 2000/Accepted 18 July 2000

Bactericidal activities of HMR 3647 (HMR), moxifloxacin (MXFX), and rifapentine (RPT) against Mycobacterium leprae, measured by the proportional bactericidal technique in the mouse footpad system, were compared with those of the established antileprosy drugs clarithromycin (CLARI), ofloxacin (OFLO), and rifampin (RMP). Administered in five daily doses of 100 mg/kg of body weight, HMR appeared slightly more bactericidal than CLARI. In a single dose, MXFX at 150 mg/kg was more active than the same dose of OFLO and displayed exactly the same level of activity as RMP at 10 mg/kg; the combination MXFX-minocycline (MINO) (MM) was more bactericidal than the combination OFLO-MINO (OM); RPT at 10 mg/kg was more bactericidal than the same dose of RMP and even more active than the combination RMP-OFLO-MINO (ROM); the combination RPT-MXFX-MINO (PMM) killed 99.9% of viable M. leprae and was slightly more bactericidal than RPT alone, indicating that the combination PMM showed an additive effect against M. leprae.

Currently, leprosy is treated with multidrug therapy (MDT). Patients with paucibacillary (PB) leprosy are treated for 6 months with two drugs—dapsone (DDS) daily plus rifampin (RMP) monthly, and those with multibacillary (MB) leprosy are treated for 12 or 24 months with a combination of three drugs—DDS and clofazimine (CLO) daily plus RMP and a larger supplemental dose of CLO monthly (18, 19). The monthly drug administration is under supervision, whereas the daily drugs are self-administered. Since 1982, more than 10 million leprosy patients in the world have been cured by the treatment (20).

Despite the great success of the first MDT regimens, newer regimens are required that are more efficient or operationally less demanding (11). One of the concerns with regard to the current regimens is that it is difficult to persuade patients to comply with the self-administered daily component (3), which is intended to ensure elimination of the spontaneously occurring RMP-resistant mutants before stopping chemotherapy, suggesting that resistance to RMP may still develop among MB patients if the daily DDS-CLO component is not taken regularly. The risk of resistance might be significantly reduced if a fully supervisable, monthly administered MDT regimen were developed, so that all of the components may be administered once monthly under supervision. The recent demonstration of the promising bactericidal activities against Mycobacterium leprae of ofloxacin (OFLO) (6) and minocycline (MINO) (4, 9) led to the development of the monthly administered combined regimen of RMP-OFLO-MINO (ROM) (13). A single dose of ROM exhibited impressive bactericidal activity against M. leprae both in the mouse footpad system and in clinical trial (13); it was only marginally less effective, in terms of clinical improvement, for treatment of single-lesion PB leprosy than the standard 6-month MDT (17, 19), and it was well tolerated by the patients (13, 17). The enormous operational advantages of single-dose treatment, especially in a country such as India, in which 70% of the global leprosy burden is concentrated and more than 50% of newly detected cases are single-lesion PB leprosy, led the World Health Organization (WHO) Expert Committee on Leprosy to conclude that a single dose of ROM is an acceptable and cost-effective alternative regimen for the treatment of single-lesion PB leprosy (19). The efficacy of multiple doses of monthly administered ROM is currently being compared to that of standard MDT regimens for both PB and MB leprosy in large-scale field trials.

However, the bactericidal activities of both OFLO and MINO are rather weak compared with that of RMP (13, 21), the combination OFLO-MINO (OM) was significantly less active than was RMP alone in both mice and humans, and the combination ROM was no more bactericidal than was RMP alone (13). To increase further the efficacy of a fully supervisable, monthly administered MDT regimen, it would be desirable to replace the components of ROM with more powerful bactericidal agents.

The objectives of the experiment were to measure the bactericidal activities against M. leprae of HMR 3647 (RU 66647 or telithromycin) (HMR) (5, 7), moxifloxacin (BAY 12-8039) (MXFX) (14), rifapentine (DL 473) (RPT) (10), and the combinations MXFX-MINO (MM) and RPT-MXFX-MINO (PMM) in mice and to compare these with the activities of established drugs and combinations. Bactericidal activity was determined by the proportional bactericidal technique (2) in the mouse footpad system.

As shown in Table 1, 450 female, immunocompetent Swiss mice (Janvier Breeding Center, le Genest Saint-Isle, France) were divided among 11 groups, each consisting of four subgroups with 10 mice each. The mice of each subgroup were inoculated in each hind footpad with $5 \times 10^2$, $5 \times 10^3$, $5 \times 10^4$, or $5 \times 10^5$ M. leprae organisms of strain 17547, a strain fully susceptible to DDS, CLO, and RMP. A fifth subgroup of the untreated control group was inoculated with $5 \times 10^{-1}$ bacilli per footpad. Treatment by the regimens specified in Table 1 was begun on day 3 after infection; all drugs were administered by gavage. After completion of the treatment, the mice were held for 12 months, a period of time sufficient to permit a single surviving organism to multiply to a readily countable level. Harvests of M. leprae from individual inoculated footpads were then performed by the method of Shepard and...
HMR, because gastrointestinal side effects were common among patients treated with CLARI (12).

As administered as a single dose, MXFX was more bactericidal than OFLO and was not significantly less active than five daily doses of HMR or CLARI. Because MXFX is by far the most active fluoroquinolone against M. tuberculosis in mice (14), that it is the most active fluoroquinolone against M. leprae was not unexpected. The observation that the bactericidal activity of a single dose of MXFX was identical to that of a single dose of RMP, both killing 92.1% of the viable M. leprae bacteria originally present, is most encouraging, because it was concluded that RMP was by far the most bactericidal of the established drugs against M. leprae (11, 12). The present experiment is the first to yield evidence that MXFX, a non-rifamycin compound, displays a level of bactericidal activity similar to that of RMP. On the other hand, a single dose of MXFX was significantly less bactericidal than was a single dose of RPT.

Just as MXFX was much more active than OFLO, a single dose of the combination MM was significantly more bactericidal than the combination OM. In fact, a single dose of OM did not show clear bactericidal effect compared with that of the untreated control group, which was in agreement with the results of a previous experiment which demonstrated that the activity of a single dose of OM was dosage related: the higher dosage of the combination displayed bactericidal activity whereas the lower dosage (same as in this experiment) did not (13). The killing effect of MM appeared slightly greater than that of MXFX alone, but the difference did not attain statistical significance, suggesting that the addition of MINO did not enhance the bactericidal activity of MXFX.

The finding that a single dose of RPT at 10 mg/kg of body weight killed 99.6% of viable organisms, significantly more than were killed by RMP or the combination ROM, was also very encouraging. RPT appears to be the most powerful bactericidal drug against M. leprae that has ever been tested. The greater activity of RPT in mice appears to result mainly from its very favorable pharmacokinetic properties (10). It is also possible that the inherent anti-M. leprae activity of RPT is

---

**TABLE 1.** Comparing the bactericidal effects against M. leprae of various drugs or drug combinations by the proportional bactericidal method

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Regimen (dose [mg/kg])</th>
<th>No. of footpads showing multiplication of M. leprae/ no. of footpads harvested, by inoculum&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Viability of M. leprae&lt;sup&gt;b&lt;/sup&gt;</th>
<th>% M. leprae killed by treatment&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated control</td>
<td>10/10 10/10 10/10 10/10 0/10</td>
<td>21.82</td>
<td>Greater than all groups, except no. 3, 5, and 7</td>
</tr>
<tr>
<td>2</td>
<td>HMR (100), 5 doses</td>
<td>10/10 10/10 7/10 10/10 0/10</td>
<td>2.18</td>
<td>vs no. 3, P &gt; 0.05 90.0</td>
</tr>
<tr>
<td>3</td>
<td>CLARI (100), 5 doses</td>
<td>10/10 10/10 8/10 10/10 3/10</td>
<td>5.48</td>
<td>74.9</td>
</tr>
<tr>
<td>4</td>
<td>MXFX (150)</td>
<td>10/10 10/10 6/10 10/10 0/10</td>
<td>1.73</td>
<td>vs no. 5, P &gt; 0.002 92.1</td>
</tr>
<tr>
<td>5</td>
<td>OFLO (150)</td>
<td>10/10 10/10 10/10 10/10 3/10</td>
<td>8.69</td>
<td>vs no. 9, P &gt; 0.001 99.6</td>
</tr>
<tr>
<td>6</td>
<td>MXFX (150)–MINO (25)</td>
<td>10/10 9/10 6/10 10/10 0/10</td>
<td>1.38</td>
<td>vs no. 8, P &lt; 0.001 99.9</td>
</tr>
<tr>
<td>7</td>
<td>OFLO (150)–MINO (25)</td>
<td>10/10 8/10 10/10 10/10 3/10</td>
<td>5.48</td>
<td>74.9</td>
</tr>
<tr>
<td>8</td>
<td>RPT (10)</td>
<td>9/10 3/10 1/10 10/10 0/10</td>
<td>0.09</td>
<td>vs no. 7, P &lt; 0.005 93.7</td>
</tr>
<tr>
<td>9</td>
<td>RMP (10)</td>
<td>9/10 10/10 7/10 10/10 0/10</td>
<td>1.73</td>
<td>vs no. 9, P &lt; 0.001 92.1</td>
</tr>
<tr>
<td>10</td>
<td>RPT (10)–MXFX (150)–MINO (25)</td>
<td>5/10 2/10 0/10 10/10 0/10</td>
<td>0.02</td>
<td>vs no. 11, P &lt; 0.001 99.9</td>
</tr>
<tr>
<td>11</td>
<td>RMP (10)–OFLO (150)–MINO (25)</td>
<td>10/10 9/10 5/10 10/10 0/10</td>
<td>1.09</td>
<td>95.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> HMR, HMR 3647; CLARI, clarithromycin; MXFX, moxifloxacin; OFLO, ofloxacin; MINO, minocycline; RPT, rifapentine; RMP, rifampin. Unless specified, all the treatments were given as a single dose.

<sup>b</sup> M. leprae was considered to have multiplied if the harvest from a footpad yielded ≥10<sup>5</sup> acid-fast bacilli.

<sup>c</sup> Percentage of viable cells was derived from the following equation: % viable M. leprae = 0.69/50% infectious dose (15).

Comparisons were done by the Spearman and Kärber method (15). For multiple comparisons between the groups, Bonferroni’s correction was applied, i.e., difference was significant at the 0.05 level only if the P value was <0.05/n (1), in which n was defined as the number of primary comparisons, which varied from one to three for each group, except 10 for the untreated control group.

Calculation based on the proportions of viable organisms between untreated controls and treated group.

---

Among the 10 treated groups, their proportions of viable organisms were all smaller than that of the untreated control group; however, by multiple comparisons between the groups, Bonferroni’s correction was applied, i.e., the difference would be significant at the 0.05 level only if the P value was <0.05/n (1), in which n was defined as the number of primary comparisons, which varied from one to three for each group, except 10 for the untreated control group.

The results from the untreated control group indicated that the inoculum used for the experiment included 21.8% viable M. leprae organisms (Table 1), a rather large proportion. Among the 10 treated groups, their proportions of viable M. leprae organisms were all smaller than that of the untreated control group; however, by multiple comparisons between the groups, the differences between control mice and those treated with CLARI alone, OFLO alone, and combination OM did not attain statistical significance. In other words, all of the remaining tested regimens displayed some degree of bactericidal activity against M. leprae in mice.

Because the bactericidal activity against M. leprae of a single dose of CLARI alone was rather weak (21) and because no information was available regarding the activity of HMR against M. leprae when the experiment was designed, the mice were treated with the two macrolides for five consecutive days instead of a single dose, to facilitate comparison of the activities between the two compounds. Compared with untreated control mice, treatment with HMR did show significant bactericidal activity against M. leprae whereas CLARI did not. Nevertheless, despite HMR appearing to be slightly more bactericidal than CLARI, the difference between the two macrolides did not attain statistical significance. This is the first evidence that a ketolide macrolide is bactericidal against M. leprae. However, the likelihood that HMR may eventually replace CLARI for the treatment of leprosy remains unclear, depending primarily upon the tolerance of patients to treatment with
greater than that of RMP, because RPT exhibited lower MICs against various cultivable mycobacteria (8).

Possibly the most important result of this experiment is that a single dose of the combination PMM killed 99.9% of viable M. leprae organisms, significantly more than the combination ROM, which killed 95.0%. Therefore, no RMP-containing multidrug regimen had been found to be more bactericidal than RMP alone (11, 12), presumably because the activities of all of the accompanying drugs were relatively weak compared to that of RMP. The combination PMM was more bactericidal than RPT alone (P < 0.05), indicating that the addition of MM enhanced the activity of RPT, probably because of the rather powerful bactericidal activity of MXFX.

To confirm the promising bactericidal activities against M. leprae of MXFX and RPT in humans and, more importantly, to promote development of the combination PMM as a fully supervisable, monthly administered multidrug regimen, a clinical trial is being conducted among patients with lepromatous leprosy. Four different regimens, all administered as a single dose, are compared in the trial: 400 mg of OFLO plus 100 mg of MINO (OM); 400 mg of MXFX plus 100 mg of MINO (MM); 600 mg of MXFX plus 400 mg of OFLO and 100 mg of MINO (ROM); and 600 mg of RPT plus 400 mg of MXFX and 100 mg of MINO (PMM). The efficacy and side effects of the treatments are monitored by the standardized method (6, 9, 12, 100 mg of MINO (PMM). The efficacy and side effects of the three additional antimicrobials, i.e., HMR, MXFX, and RPT, was demonstrated in the present experiment justifies our approach to the identification of new antileprosy drugs by screening compounds which exhibit powerful activity either against a wide spectrum of microorganisms in general (e.g., HMR) or against cultivable mycobacteria in particular (e.g., MXFX) or possess pharmacokinetic properties much more favorable than those of the member of the class presently employed (e.g., RPT versus RMP).

This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases and Hoechst Marion Roussel, France.

We thank J. Y. Mary, Universite Paris 7, for his advice on statistical analysis.

REFERENCES