Bactericidal Activity of a Single-Dose Combination of Ofloxacin plus Minocycline, with or without Rifampin, against Mycobacterium leprae in Mice and in Lepromatous Patients

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To develop a fully supervisable, monthly administered regimen for treatment of leprosy, the bactericidal effect of a single-dose combination of ofloxacin (OFLO) and minocycline (MINO), with or without rifampin (RMP), against Mycobacterium leprae was studied in the mouse footpad system and in previously untreated lepromatous leprosy patients. Bactericidal activity was measured by the proportional bactericidal method. In mouse experiments, the activity of a single dose of the combination OFLO-MINO was dosage related; the higher dosage of the combination displayed bactericidal activity which was significantly inferior to that of a single dose of RMP, whereas the lower dosage did not exhibit a bactericidal effect. In the clinical trial, 20 patients with previously untreated lepromatous leprosy were treated with a single dose consisting of either 600 mg of RMP plus 400 mg of OFLO or 400 mg of OFLO plus 100 mg of MINO. The OFLO-MINO combination exhibited definite bactericidal activity in 7 of 10 patients but was less bactericidal than the RMP-OFLO-MINO combination. Both combinations were well tolerated. Because of these promising results, a test of the efficacy of multiple doses of ROM in a larger clinical trial appears justified.

The standard regimen of multidrug therapy (MDT) recommended by the World Health Organization for the treatment of multibacillary (MB) leprosy includes three drugs: dapsone (DDS), 100 mg, administered daily; clofazimine (CLO), 50 mg, administered daily (as well as a monthly supplemental dose of 300 mg), and rifampin (RMP), 600 mg, administered monthly (29). Because of its high potency (19, 22, 24, 25), RMP is the key component of the regimen. Its monthly administration permits supervision of each dose, significantly minimizing the problem of patient compliance. The major objective of combining RMP with DDS and CLO is to ensure the elimination of spontaneously occurring RMP-resistant mutants before stopping chemotherapy (14). However, even in the best leprosy control program, it is difficult to persuade patients to adhere to the self-administered daily therapy (3), suggesting that RMP resistance may still develop if the DDS-CLO component is not taken regularly. RMP resistance might be reduced if a fully supervised, monthly administered MDT regimen can be developed, i.e., if all of the components are given under supervision once monthly. Such a regimen would facilitate integration of antileprosy chemotherapy within the general health services.

Because RMP is the drug with by far the most bactericidal activity against Mycobacterium leprae (19), the fully supervised, monthly administered regimens should always contain RMP, except in those instances in which the strain of M. leprae is resistant to RMP. Since the regimens should be effective for all MB patients, including those who have relapsed from previous treatment, and because the patients who have suffered relapses should not be treated with combinations consisting of only RMP plus a single new antimicrobial drug (21), the regimens should include two antimicrobial agents in addition to RMP. The components of the regimens to be added to RMP should meet the following requirements: (i) a single dose must display bactericidal activity against M. leprae, (ii) the additional drugs should not antagonize the activity of RMP, and (iii) the drugs must be well tolerated when administered in an effective dosage (16).

In recent studies, three newer antimicrobial agents—ofloxacin (OFLO; a fluoroquinolone) (6, 12, 13, 18), clarithromycin (CLARI; a macrolide) (5, 15, 17), and minocycline (MINO; a tetracycline derivative) (4, 8–10, 15, 17)—demonstrated very promising bactericidal activities against M. leprae in both mice and patients. Employing the proportional bactericidal method (2), a titrating technique that has proved to be more sensitive for measuring bactericidal effects (20), we have demonstrated in a clinical trial that administration of a single dose of 2,000 mg of CLARI plus 200 mg of MINO, with or without 800 mg of OFLO, to lepromatous patients resulted in bactericidal activity equivalent to that of treatment for 30 days with the DDS-CLO component in the standard MDT regimen for MB leprosy (20). However, gastrointestinal adverse events were frequent, whether or not OFLO was added, suggesting that the adverse events were caused by the large dosage of CLARI (20). Therefore, to improve the tolerance to the treatment, CLARI should not be included in the fully supervised, monthly administered regimens. A previous study showed that a single 800-mg dose of OFLO was bactericidal against M. leprae in three of eight lepromatous patients (13). A clinical trial conducted by other investigators also suggested that a single 200-mg dose of MINO was bactericidal, since the proportion of mouse footpads with viable M. leprae decreased for six of eight lepromatous patients after treatment (10). Thus, OFLO and MINO should be considered as companion drugs with RMP in the fully supervised, monthly administered regimen.

The objectives of these studies were as follows: (i) to measure the bactericidal effect of a single dose of OFLO-MINO, with or without added RMP, against M. leprae in immunocompetent (normal) mice; and (ii) to evaluate the bactericidal
activity of single doses of these combinations and their adverse effects in lepromatous patients.

MATERIALS AND METHODS

The procedures for measuring the bactericidal effects of treatments in the mouse experiment (15) and in the clinical trial (13, 17, 18, 20) have been described at length elsewhere.

Bactericidal activity of a single dose of OFLO-MINO, with or without RMP, in the footpads of normal mice. M. leprae C6 was isolated from a previously untreated lepromatous patient in 1990 and has since been maintained by passage described at length elsewhere (15) and in the clinical trial (13, 17, 18, 20). To determine the proportion of viable M. leprae would have occurred in mice inoculated with 5 × 10^2 AFB while the second is the maximum estimated value assuming that multiplication of M. leprae would have occurred in the same proportion of footpads in mice inoculated with 5 × 10^1 AFB and in mice inoculated with 5 × 10^0 AFB.

Where two values are shown, the first is the minimum killing rate, calculated from the maximum estimated proportion of viable organisms, and the second is the maximum-killing rate, calculated from the minimum estimated proportion of viable organisms.

### Table 1. Bactericidal effect of the treatment against M. leprae in mice

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Regimen (dosage [mg/kg])</th>
<th>Proportion of footpads showing multiplication* of M. leprae with AFB inoculum of:</th>
<th>% of:</th>
<th>Viable M. leprae&lt;sup&gt;a&lt;/sup&gt;</th>
<th>M. leprae killed by treatment&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated (control)</td>
<td>10/10 10/10 10/10 3/10 2/10</td>
<td>13.77</td>
<td>96.0</td>
<td>99.0</td>
</tr>
<tr>
<td>2</td>
<td>RMP (10)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10/10 9/10 2/10 0/10 0/10</td>
<td>0.55</td>
<td>99.9</td>
<td>99.9</td>
</tr>
<tr>
<td>3</td>
<td>1 mo MDT&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2/10 0/10 0/10 0/10 0/10</td>
<td>0.007</td>
<td>99.9</td>
<td>99.9</td>
</tr>
<tr>
<td>4</td>
<td>OFLO (150) + MINO (25)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10/10 10/10 10/10 1/10 0/10</td>
<td>5.48</td>
<td>49.9</td>
<td>60.2</td>
</tr>
<tr>
<td>5</td>
<td>OFLO (300) + MINO (50)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10/10 10/10 3/10 2/10 0/10</td>
<td>1.38</td>
<td>84.2</td>
<td>90.0</td>
</tr>
<tr>
<td>6</td>
<td>CLARI (100) + OFLO (150) + MINO (25)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10/10 10/10 9/10 9/10 0/10</td>
<td>3.46</td>
<td>74.9</td>
<td>81.6</td>
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<td>7</td>
<td>RMP (10) + OFLO (150) + MINO (25)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10/10 9/10 0/10 0/10 0/10</td>
<td>0.44</td>
<td>96.8</td>
<td>98.2</td>
</tr>
<tr>
<td>8</td>
<td>RMP (10) + OFLO (300) + MINO (50)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10/10 6/10 2/10 0/10 0/10</td>
<td>0.28</td>
<td>98.0</td>
<td>99.9</td>
</tr>
<tr>
<td>9</td>
<td>RMP (10) + CLARI (100) + OFLO (150) + MINO (25)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10/10 3/10 0/10 0/10 0/10</td>
<td>0.09</td>
<td>99.4</td>
<td>99.9</td>
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</table>

* Harvested ≥10^5 M. leprae cells per footpad.

<sup>a</sup> Single-dose treatment only.

<sup>b</sup> A single dose of RMP (10 mg/kg) plus 0.01% DDS and 0.005% CLO in the mouse diet for 30 days.

<sup>c</sup> A single dose of RMP (10 mg/kg) plus 0.01% DDS and 0.005% CLO in the mouse diet for 30 days.

<sup>d</sup> Where two values are shown, the first is the minimum killing rate, calculated from the maximum estimated proportion of viable organisms, and the second is the maximum-killing rate, calculated from the minimum estimated proportion of viable organisms.

### Table 2. Clinical responses and changes of BI and MI in skin smears of patients treated with a single dose of RMP-OFLO-MINO

<table>
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<tr>
<th>Group no.</th>
<th>No change</th>
<th>Improvement</th>
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<tr>
<td>I</td>
<td>0</td>
<td>7</td>
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<td>II</td>
<td>0</td>
<td>10</td>
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<table>
<thead>
<tr>
<th>Mean BI ± SD on day:</th>
<th>Mean MI (%) ± SD on day:</th>
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<tbody>
<tr>
<td>4.82 ± 0.37</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td>4.76 ± 0.37</td>
<td>2.7 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.66 ± 0.72</td>
<td>2.4 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.65 ± 0.55</td>
<td>2.2 ± 1.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Significantly smaller than the pretreatment (day 0) value for the same group (P = 0.012).

<sup>b</sup> Significantly smaller than the corresponding value for group I (P = 0.001).
performed. A bactericidal effect of the treatment was defined as a significant decrease in the proportion of viable *M. leprae* from the pretreatment value.

Statistical analysis. Except for the determination of the proportion of viable *M. leprae*, results were analyzed and compared by the use of Student’s t test and Fisher’s exact probability calculation. In the mouse experiment and the clinical trial, the proportion of viable organisms remaining after the treatment and the significance of their differences between the groups were calculated by the Spearman and Kärber method (26), employing the results of the harvesting of *M. leprae* from mouse footpads that had been inoculated with the serial 10-fold-diluted suspensions prepared from the same sample. When the maximum inoculum was 5 × 10³ bacilli per footpad, a proportion of viable *M. leprae* as small as 0.006% could be measured. Differences were considered significant at the 95% level of confidence.

Among the treated groups of *M. leprae*-infected mice, in which multiplication of the organism was detected even in those footpads that had been inoculated with the smallest number of bacteria, i.e., 5 × 10⁰ organisms per footpad, the minimal and maximum values for the proportion of viable *M. leprae* were estimated assuming either that no multiplication would have occurred in footpads inoculated with 5 × 10⁻¹ organisms per footpad or that multiplication would have occurred in the same proportion of footpads inoculated with 5 × 10⁻¹ bacteria as observed for the footpads than had been inoculated with 5 × 10⁰ AFB.

RESULTS

Bactericidal activities of a single dose of OFLO-MINO, with or without RMP, in the footpads of normal mice. As shown in Table 1 by the results of harvests from untreated control mice, the proportion of viable *M. leprae* in the inoculum was 13.77%. Although the proportions of viable organisms harvested after treatment in the groups treated with regimens that did not include RMP (groups 4, 5, and 6) appear to be smaller than in the untreated controls, only the differences between the minimum or maximum estimated value for group 5 (whose members were each administered a single dose of a higher dosage of OFLO-MINO) and group 6 (whose members were each given a single dose of CLARI plus the lower dosage of OFLO-MINO) and that of the untreated control mice were statistically significant; neither of the differences between either estimated value of group 4 (whose members were administered a single dose of the lower dosage of OFLO-MINO) and that of the untreated control mice attained significance. The proportions of viable *M. leprae* in these three groups were significantly higher than those in the groups treated with RMP-containing regimens (groups 2, 3, 7, 8, and 9) (P < 0.01). In fact, none of the regimens that did not include RMP killed more than 90% of the viable organisms, whereas all of the RMP-containing regimens killed at least 96.0% of the *M. leprae*. The minimum estimated value for the proportion of viable organisms in group 4 was significantly higher than that for group 5 (P < 0.01); however, the difference between the maximum estimated values for the two groups was not statistically significant. Neither the minimum nor the maximum estimated proportion of viable organisms in group 4 differed significantly from the corresponding value for group 6.

As expected, the proportions of viable organisms in all five groups treated with RMP-containing regimens were significantly smaller than that of the control mice (P < 0.01). The differences among groups 2, 7, and 8 (treated, respectively, with RMP alone, RMP plus the lower dosage of OFLO-MINO, and RMP plus the higher dosage of OFLO-MINO) were not significant, indicating that the addition of either dosage of OFLO-MINO neither enhanced nor antagonized the bactericidal activity of RMP against *M. leprae*. The proportion of viable organisms in group 9 (whose members were each administered a single dose of RMP-CLARI-OFOLO-MINO) was significantly smaller than those of groups 2, 7, and 8 (P < 0.05 or P < 0.01). Finally, similar to the results observed in our previous experiment (19), the proportion of viable organisms among the mice administered MDT for 1 month (group 3) was smaller than that of among those administered any of the remaining four RMP-containing regimens.

Clinical trial of a single dose of OFLO-MINO, with or without RMP, in lepromatous patients. (i) Clinical response. As shown in Table 2, clinical improvement (e.g., partially regression of infiltration and/or flattening of nodules, lepromas, or plaques) was observed in the great majority of patients of both groups. Because posttreatment assessment was carried out only 7 days after treatment with a single dose, improvement was slight, even among the patients of group I, who had been treated with RMP-OFOLO-MINO.

(ii) Changes of BSs and MIs in skin smears. As is also shown in Table 2, after the single dose of treatment with either regimen, the mean BI values were virtually unchanged from the pretreatment values. On the other hand, the mean MI value was significantly smaller than the pretreatment value for the patients of group I (P < 0.05) but remained basically the same as the pretreatment value for the patients of group II.

(iii) Bactericidal activities of the treatments against *M. leprae*. As shown in Table 3, the pretreatment skin biopsy samples from all 20 patients harbored proportions of viable *M. leprae* large enough to be detected by inoculation into the footpads of normal mice. However, the mean proportion of viable organisms ± the standard deviation among the 20 patients was only 1.35 ± 1.36%, again demonstrating that in newly detected, previously untreated lepromatous patients, the great majority of the organisms are dead (13). Although the proportion of viable organisms in the pretreatment samples varied widely among the patients, ranging from a barely detectable level (0.006%) to 4.35%, the mean values for the two groups did not differ significantly (1.19 ± 1.36% for group I and 1.51 ± 1.40% for group II).

As expected (20), the proportion of viable *M. leprae* in the posttreatment biopsy samples had significantly decreased to an undetectable level (<0.006%), and the treatment resulted in ≥95.7% killing of the AFB in 9 of 10 patients in treatment group I; in case no. 12, however, the proportion of viable organisms remained unchanged after treatment. On the other hand, a decrease in the proportion of viable organisms in the posttreatment samples from 7 of 10 patients in group II was observed; the proportion decreased to an undetectable level in only a single patient (case no. 20), a phenomenon significantly less frequent than that observed in group I (P < 0.01). Among six other patients, viable organisms were still detected in the posttreatment samples and, in fact, the proportion of footpads showing multiplication of *M. leprae* after being inoculated with 5 × 10³ organisms was virtually unchanged from that of footpads that had been inoculated with bacilli recovered from the pretreatment samples, but the proportions of footpads demonstrating viable organisms among those inoculated with smaller inocula—i.e., 5 × 10², 5 × 10¹, or 5 × 10⁰ AFB, were significantly smaller than those from the pretreatment samples, therefore revealing a killing effect ranging from 68.2 to 98.7%.

(iv) Leprosy reaction. During the 7-day trial, erythema nodosum leprosum developed in two patients of group II; a reversal reaction was not observed in any patient of either group.

(v) Adverse events associated with the treatment. Six patients, four from group I and two from group II, had gastrointestinal complaints; these included nausea (four cases, all from Group I), diarrhea (three cases, one from group I and two from group II), and abdominal pain (one case, from group I). All of the events were mild and transitory, and they were not accompanied by significant findings on physical examination.
In the mice, a single dose of 300 mg of OFLO and 50 mg of MINO per kg of body weight displayed bactericidal activity against *M. leprae*. However, the proportion of viable organisms in the mice administered half that dosage, i.e., 150 mg of OFLO and 25 mg of MINO per kg, did not differ significantly from that of untreated controls. Therefore, the activity of a single dose of the OFLO-MINO combination was dose related, being bactericidal for *M. leprae* at only the higher dosage. While OFLO and MINO have been commonly used clinically, their pharmacokinetics in mice have not been well studied (28), and it is difficult to define the effective dose in humans by extrapolating the results from mouse experiments. The murine infection in mice are quite different from those in patients, the potential lead of a new drug or a new treatment identified in the mouse footpad system must be evaluated in clinical trials before moving toward field application. In the present clinical trial, the most encouraging observation was that a single dose consisting of the combination of 400 mg of OFLO plus 100 mg of MINO displayed a definite bactericidal effect against *M. leprae* in 7 of 10 patients. In one patient, the organisms lost their infectivity for normal mice inoculated with $5 \times 10^3$ organisms per footpad, the maximum inoculum, indicating that $>99.6\%$ of the viable *M. leprae* cells had been killed; the bactericidal activity of this treatment in six other patients ranged from 68.2 to 98.7%. As expected, a single dose of the OFLO-MINO combination was less bactericidal than a single dose of the RMP-OFLO-MINO combination; in the latter group, the bacilli from 9 of 10 patients lost their infectivity for normal mice, as had been observed in a clinical trial of other RMP-containing regimens (20).

In mouse experiments or in clinical trials, there is a consensus that multiple, daily doses of OFLO (12, 13, 18), CLARI (1, 5, 11, 15, 17), and MINO (4, 8–10, 15, 17) display bactericidal effects against *M. leprae*. However, opinions among investigators differ with regard to the bactericidal effects of single doses of the new agents and thus their potential for intermittent therapy of leprosy. In clinical trials in which bactericidal activity was monitored by mouse footpad inoculation of organs recovered from biopsy specimens taken before and after treatment, several groups concluded that a single dose of MINO (4, 10) or CLARI (1, 11) did not exert a bactericidal effect. Previously, we observed that a single 800-mg dose of OFLO exerted a significant bactericidal effect against *M. leprae* in three human trials (7); nevertheless, its inactivity in mice has not been documented by the investigator and has yet to be confirmed by others. On the other hand, because the pharmacokinetics of the drugs and the pathogenesis of *M. leprae* infection in mice are quite different from those in patients, the

<table>
<thead>
<tr>
<th>Group</th>
<th>Case no.</th>
<th>Proportion of footpads showing multiplication of <em>M. leprae</em> at day 0 after administration of AFB inoculum of:</th>
<th>% of viable <em>M. leprae</em> at day 0</th>
<th>Proportion of footpads showing multiplication of <em>M. leprae</em> at day 7 after administration of AFB inoculum of:</th>
<th>% of viable <em>M. leprae</em> at day 7</th>
<th>% of viable <em>M. leprae</em> killed by treatment</th>
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**DISCUSSION**

In the mice, a single dose of 300 mg of OFLO and 50 mg of MINO per kg of body weight displayed bactericidal activity against *M. leprae*. However, the proportion of viable organisms in the mice administered half that dosage, i.e., 150 mg of OFLO and 25 mg of MINO per kg, did not differ significantly from that of untreated controls. Therefore, the activity of a single dose of the OFLO-MINO combination was dose related, being bactericidal for *M. leprae* at only the higher dosage. While OFLO and MINO have been commonly used clinically, their pharmacokinetics in mice have not been well studied (28), and it is difficult to define the effective dose in humans by extrapolating the results from mouse experiments. The murine experiment also indicated that the activity of a single dose of the OFLO-MINO combination was inferior to that of a single dose of RMP, and the addition of either dosage of OFLO-MINO did not compromise the activity of RMP against *M. leprae*. Although the proportion of viable *M. leprae* in mice administered the combination RMP-CLARI-OFLO-MINO was smaller than that in mice administered RMP-OFLO-MINO, the value for mice administered CLARI-OFLO-MINO did not differ significantly from that for mice administered OFLO-MINO. Therefore, in terms of bactericidal effect, the consequence of excluding CLARI from the drug combination is marginal.

In leprosy chemotherapy research, the results of murine experiments almost invariably run parallel to those of human trials. The bactericidal activities of all the major antileprosy drugs—RMP, DDS, CLO, CLARI, MINO, OFLO, and sparflaxacin (SPFX)—against *M. leprae* have been demonstrated in the mouse footpad system. The rationale for testing CLARI, MINO, OFLO, and SPFX in clinical trials derived from their promising bactericidal effects in mice. Fusidic acid probably is the only drug which was said to be inactive against *M. leprae* in mice but exhibited a weak bactericidal activity against *M. leprae* in a human trial (7); nevertheless, its inactivity in mice has not been documented by the investigator and has yet to be confirmed by others. On the other hand, because the pharmacokinetics of the drugs and the pathogenesis of *M. leprae* infection in mice are quite different from those in patients, the potential lead of a new drug or a new treatment identified in the mouse footpad system must be evaluated in clinical trials before moving toward field application. In the present clinical trial, the most encouraging observation was that a single dose consisting of the combination of 400 mg of OFLO plus 100 mg of MINO displayed a definite bactericidal effect against *M. leprae* in 7 of 10 patients. In one patient, the organisms lost their infectivity for normal mice inoculated with $5 \times 10^3$ organisms per footpad, the maximum inoculum, indicating that $>99.6\%$ of the viable *M. leprae* cells had been killed; the bactericidal activity of this treatment in six other patients ranged from 68.2 to 98.7%. As expected, a single dose of the OFLO-MINO combination was less bactericidal than a single dose of the RMP-OFLO-MINO combination; in the latter group, the bacilli from 9 of 10 patients lost their infectivity for normal mice, as had been observed in a clinical trial of other RMP-containing regimens (20).

In mouse experiments or in clinical trials, there is a consensus that multiple, daily doses of OFLO (12, 13, 18), CLARI (1, 5, 11, 15, 17), and MINO (4, 8–10, 15, 17) display bactericidal effects against *M. leprae*. However, opinions among investigators differ with regard to the bactericidal effects of single doses of the new agents and thus their potential for intermittent therapy of leprosy. In clinical trials in which bactericidal activity was monitored by mouse footpad inoculation of organs recovered from biopsy specimens taken before and after treatment, several groups concluded that a single dose of MINO (4, 10) or CLARI (1, 11) did not exert a bactericidal effect. Previously, we observed that a single 800-mg dose of OFLO exerted a significant bactericidal effect against *M. leprae* in three
of eight lepromatous patients (13); administration of a single
dose of 2,000 mg of CLARI plus 200 mg of MINO to 10
lepromatous patients resulted in bactericidal activity similar to
that resulting from treatment for 30 days with the DDS-CLO
component in the standard MDT regimen (20). In the present
study, a single dose of 400 mg of OFLO plus 100 mg of MINO
achieved a bactericidal effect in 7 of 10 patients. We attribute
the discrepancy mainly to the different techniques employed by
us and others. When monitoring the bactericidal effect by
mouse footpad inoculations, others have almost invariably
used a single inoculum size, i.e., $5 \times 10^3$ organisms per foot-
pad, while we always apply the more complicated, but also
more sensitive, titrating technique, i.e., administering at least
four different sized inocula ($5 \times 10^3, 5 \times 10^2, 5 \times 10^1,$
and $5 \times 10^0$ AFB) to different groups of mice. Because the new
drugs are significantly less bactericidal than RMP, our studies
(19, 20) (Table 3) clearly demonstrated that the effect of a
single dose of one of the new drugs, either alone or in combi-
nation, would be limited only by a significant reduction in the
proportion of mouse footpads showing multiplication of $M. leprae$
in those of mice that had been inoculated with one of the
smaller-sized inocula, i.e., $5 \times 10^2, 5 \times 10^1,$ or $5 \times 10^0$ AFB,
but not in the footpads that had been administered an inocu-
ulum of $5 \times 10^3$ AFB. The moderate bactericidal effect of a
single-dose treatment was masked by the inoculum containing $5 \times 10^3$ AFB; this appears to be the main reason why other
investigators have missed it. Another possible explanation for
the discrepancy is that others employed a single dose of mono-
therapy while we administered combined therapy.

Gastrointestinal adverse events were mild and transitory
among patients treated with the RMP-OFLO-MINO or
OFLO-MINO combination. These findings are in agreement
with that of excellent tolerance among patients with single-
lesion paucibacillary leprosy who had been treated with a sin-
gle dose of RMP-OFLO-MINO (27). In that double-blind
trial, 739 patients were treated with a single dose of RMP-
OFLO-MINO (at the same dosages as in the present clinical
trial) and 744 patients were given 6 months of standard MDT for
paucibacillary leprosy; the side effects were very rare (0.5%-
in the former, 1.1% in the latter group, and 0.8% overall) and
mild, and their frequencies were very similar for the two
groups (27).

Because a single dose of the OFLO-MINO combination,
with or without RMP, displayed promising bactericidal activity
against $M. leprae$ in patients with lepromatous leprosy and was
well tolerated, a test of the efficacy of multiple doses of RMP-
OFLO-MINO combination. These findings are in agreement
with that of excellent tolerance among patients with single-
lesion paucibacillary leprosy who had been treated with a sin-
gle dose of RMP-OFLO-MINO (27). In that double-blind
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