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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 and 100 mg of MINO 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 great 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

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TABLE 1. Bactericidal effect of the treatment against *M. leprae* in mice

Group no.	Regimen (dosage [mg/kg])	Proportion of footpads showing multiplication[a] of *M. leprae* with AFB inoculum of:					% of:	
		5×10^3	5×10^2	5×10^1	5×10^0	5×10^{-1}	Viable *M. leprae*[d]	*M. leprae* killed by treatment[e]
1	Untreated (control)	10/10	10/10	10/10	3/10	2/10	13.77	
2	RMP (10)[b]	10/10	9/10	2/10	0/10		0.55	96.0
3	1 mo MDT[c]	2/10	0/10	0/10	0/10		0.007	99.95
4	OFLO (150) + MINO (25)[b]	10/10	10/10	10/10	1/10		5.48, 6.90	49.9, 60.2
5	OFLO (300) + MINO (50)[b]	10/10	10/10	3/10	2/10		1.38, 2.18	84.2, 90.0
6	CLARI (100) + OFLO (150) + MINO (25)[b]	10/10	10/10	9/10	0/10		3.46	74.9
7	RMP (10) + OFLO (150) + MINO (25)[b]	10/10	9/10	0/10	1/10		0.44	96.8
8	RMP (10) + OFLO (300) + MINO (50)[b]	10/10	6/10	2/10	0/10		0.28	98.0
9	RMP (10) + CLARI (100) + OFLO (150) + MINO (25)[b]	10/10	3/10	0/10	0/10		0.09	99.4

[a] Harvested $\geq 10^5$ *M. leprae* cells per footpad.
[b] Single-dose treatment only.
[c] A single dose of RMP (10 mg/kg) plus 0.01% DDS and 0.005% CLO in the mouse diet for 30 days.
[d] Where two values are shown, the first is the minimum estimated value assuming that no multiplication of *M. leprae* would have occurred in mice inoculated with 5×10^{-1} 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.
[e] 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.

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 in nude mice. The results of drug susceptibility testing in the mouse footpad system indicate that the strain is susceptible to both RMP and DDS. An inoculum containing 5×10^3 *M. leprae* cells per 0.03 ml and four serial 10-fold dilutions thereof were prepared, and each hind footpad of 370 normal mice was inoculated. As shown in Table 1, the mice were divided among nine groups, each group consisting of four subgroups that had been inoculated with 5×10^3, 5×10^2, 5×10^1, or 5×10^0 acid-fast bacilli (AFB), except for the untreated control mice, which included a fifth subgroup that had been inoculated with 5×10^{-1} AFB per footpad. Three days after inoculation, a single dose of one of the treatments being tested was administered by gavage to all mice except those of group 3; to mimic the standard MDT regimen, the mice in group 3 were treated with a single 10-mg/kg of body weight dose of RMP plus 0.01% DDS and 0.005% CLO in the mouse diet for 30 days. The control mice were not treated. To evaluate the impact of different dosages of OFLO and MINO, the bactericidal activities of the combinations containing smaller or larger dosages of OFLO (150 or 300 mg/kg) and MINO (25 or 50 mg/kg) were compared. In addition, to assess the impact of excluding CLARI from the combinations, the activities of drug combinations with and without CLARI were also compared.

After 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. Harvesting of *M. leprae* from individual footpads was then performed by the method of Shepard and McRae (23). *M. leprae* was considered to have multiplied (or there were viable bacilli that had survived the treatment) in those footpads found to contain $\geq 10^5$ AFB.

Clinical trial of a single dose of OFLO-MINO, with or without RMP, in patients with lepromatous leprosy. Twenty newly diagnosed lepromatous patients with active skin lesions and high bacterial loads were recruited into the trial at the Institut Marchoux, Bamako, Mali. Of the 20 patients, 16 were male and 4 were female, with a mean age ± standard deviation of 34 ± 14 years (range, 16 to 60 years); 14 were classified as having polar lepromatous leprosy, and 6 were classified as borderline lepromatous. The patients were randomly allocated to one of two groups, with 10 patients in each group; the groups were comparable with regard to patient pretreatment characteristics, including sex, age, classification of the disease, severity of skin lesions, mean bacterial index (BI) in skin smears, and proportion of viable *M. leprae* in the bacterial population, except that, as shown in Table 2, the mean morphological index (MI) of the patients in group II was significantly smaller than that for group I ($P = 0.001$).

The patients were hospitalized at least 25 days for the trial. Patients of group I were treated with a single dose of 600 mg of RMP plus 400 mg of OFLO and 100 mg of MINO on day 1, whereas those of group II were administered a single dose of 400 mg of OFLO plus 100 mg of MINO. All of the drugs were administered under the supervision of medical personnel. No treatment was given from days 2 to 6. On day 7, immediately after the posttreatment clinical, bacteriological (skin smears and skin biopsy), and other laboratory examinations had been completed, the patients were all started on a 2-year course of standard MDT.

The methods employed for the pre- and posttreatment clinical, bacteriological, and other laboratory examinations have been described at length elsewhere (13, 17, 18, 20). To determine the proportion of viable *M. leprae*, the organisms recovered from a skin biopsy specimen obtained from a lesion were serially diluted, and groups of 10 mice were inoculated with 0.03 ml of a suspension containing 5×10^3, 5×10^2, 5×10^1, or 5×10^0 AFB. Twelve months after inoculation, harvesting of *M. leprae* (23) from the inoculated footpads was performed. For those mice that had been inoculated with organisms recovered from biopsy specimens taken before treatment (day zero), harvesting of organisms from mice that had been administered all four inocula was performed. For those that had been inoculated with organisms recovered from biopsy specimens taken after the single-dose treatment, however, harvesting of *M. leprae* from mice inoculated with 5×10^3 organisms per footpad was performed first, followed by that from the footpads inoculated with higher dilutions. When no multiplication of *M. leprae* (defined as an increase to $\geq 10^5$ organisms per footpad) was found in any of the footpads inoculated with either of two consecutive dilutions, harvesting from the footpads that had been inoculated with higher dilutions was not

TABLE 2. Clinical responses and changes of BI and MI in skin smears of patients treated with a single dose of RMP-OFLO-MINO (group I) or OFLO-MINO (group II)

Group	No. of patients showing clinical response on day 7		Mean BI ± SD on day:		Mean MI (%) ± SD on day:	
	No change	Improvement	0	7	0	7
I	1	9	4.82 ± 0.37	4.75 ± 0.37	4.0 ± 1.2	2.7 ± 1.4[a]
II	0	10	4.66 ± 0.72	4.65 ± 0.55	2.4 ± 0.6[b]	2.2 ± 1.2

[a] Significantly smaller than the pretreatment (day 0) value for the same group ($P = 0.012$).
[b] 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 serially 10-fold-diluted suspensions prepared from the same sample. When the maximum inoculum was 5×10^3 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., 5×10^0 organisms per footpad, the minimum 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 5×10^0 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-OFLO-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-OFLO-MINO.

(ii) Changes of BIs 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 5×10^3 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., 5×10^2, 5×10^1, or 5×10^0 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.

TABLE 3. Proportion of viable *M. leprae* in patients before (day 0) and after (day 7) treatment

Group[a]	Case no.	Proportion of footpads showing multiplication of *M. leprae* at day 0 after administration of AFB inoculum of:				% of viable *M. leprae* at day 0	Proportion of footpads showing multiplication of *M. leprae* at day 7 after administration of AFB inoculum of:				% of viable *M. leprae* at day 7	% of viable *M. leprae* killed by treatment
		5×10^3	5×10^2	5×10^1	5×10^0		5×10^3	5×10^2	5×10^1	5×10^0		
I	4	9/10	8/10	1/10	0/10	0.28	0/10	0/10	ND[b]	ND	<0.006	>97.9
	5	7/10	6/10	2/10	0/10	0.14	0/10	0/10	ND	ND	<0.006	>95.7
	7	10/10	8/10	5/10	0/10	0.87	0/10	0/10	ND	ND	<0.006	>99.3
	9	10/10	4/10	3/10	0/10	0.22	0/10	0/10	ND	ND	<0.006	>97.3
	12	10/10	9/10	5/10	0/10	1.09	10/10	8/10	6/10	0/10	1.09	0
	13	10/10	6/10	3/10	1/10	0.44	0/10	0/10	0/10	ND	<0.006	>98.6
	14	10/10	7/10	7/10	1/10	1.38	0/10	0/10	ND	ND	<0.006	>99.6
	15	9/10	7/10	3/10	0/10	0.35	0/10	0/10	ND	ND	<0.006	>98.3
	17	10/10	10/10	4/10	4/10	2.75	0/10	0/10	ND	ND	<0.006	>99.8
	18	10/10	10/10	7/10	3/10	4.35	0/8	0/10	ND	ND	<0.006	>99.9
II	1	10/10	8/10	3/10	2/10	0.87	8/10	2/10	0/10	ND	0.044	94.9
	2	10/10	6/10	1/10	0/10	0.22	8/10	3/10	1/10	0/10	0.069	68.6
	3	10/10	10/10	6/10	0/10	1.73	8/10	6/10	7/10	0/10	0.55	68.2
	6	8/10	9/10	1/10	1/10	0.35	10/10	10/10	9/10	2/10	5.48	0
	8	10/10	10/10	3/10	2/10	1.38	8/10	5/10	1/10	2/10	0.17	87.7
	10	7/10	7/10	5/10	6/10	1.38	10/10	9/10	6/10	2/10	2.18	0
	11	7/10	10/10	7/10	5/10	3.46	7/10	4/10	2/10	1/10	0.087	97.5
	16	1/10	0/10	0/10	0/10	0.006	2/10	0/10	0/10	ND	0.007	0
	19	10/10	8/8	10/10	0/10	4.35	6/6	5/6	1/8	0/4	0.39	91.0
	20	10/10	9/10	5/10	1/10	1.38	0/10	0/10	ND	ND	<0.006	>99.6

[a] Group I members were treated with RMP (600 mg) plus OFLO (400 mg) plus MINO (100 mg), and group II members were treated with OFLO (400 mg) plus MINO (100 mg).
[b] ND, not done; see Materials and Methods.

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 combinations 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 sparfloxacin (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 5×10^3 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 organisms 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., 5×10^3 organisms per footpad, while we always apply the more complicated, but also more sensitive, titrating technique, i.e., administering at least four differently sized inocula (5×10^3, 5×10^2, 5×10^1, and 5×10^0 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 combination, would be revealed 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., 5×10^2, 5×10^1, or 5×10^0 AFB, but not in the footpads that had been administered an inoculum of 5×10^3 AFB. The moderate bactericidal effect of a single-dose treatment was masked by the inoculum containing 5×10^3 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 monotherapy 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 single 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 in a larger-scale clinical trial appears justified. With respect to the treatment of MB leprosy, the optimal duration of treatment with RMP-OFLO-MINO remains to be determined. As with the DDS-CLO component in the standard MDT regimen, the major role of OFLO-MINO as a component of RMP-OFLO-MINO is to ensure the elimination of RMP-resistant mutants before stopping chemotherapy. Because a patient with untreated lepromatous leprosy is believed to harbor no more than 10^4 of these mutants (14), a useful approach to determine an acceptable minimum duration of treatment with RMP-OFLO-MINO is to measure in previously untreated lepromatous patients the duration of OFLO-MINO treatment that is required to kill 99.99% of the viable *M. leprae* initially present, i.e., to reduce the proportion of viable organisms by 4 orders of magnitude. Until such information becomes available, the duration of treatment for patients with MB leprosy with an RMP-OFLO-MINO regimen should not be shorter than that with the MDT regimen. Furthermore, the long-term efficacy of the RMP-OFLO-MINO regimen relies on the measurement of the relapse rate after treatment is ended (21).

Finally, in the mouse experiment, the finding that 1 month of the standard MDT regimen (group 3 in Table 1) was more effective than a single dose of a combined regimen consisting of RMP and two or three of the new drugs was very similar to that from our previous experiment (19). However, it is unclear whether the differences still exist after a longer duration (e.g., 3 to 6 months) of treatment. Because multiple doses of RMP alone are so potently bactericidal, comparing the longer duration of an MDT regimen with the same duration of multiple monthly doses of an RMP-containing combined regimen will rapidly exceed the limit of sensitivity of the mouse footpad system, even employing the nude mouse (19). Thus, it seems reasonable to compare the bactericidal effects of 3 to 6 months of daily treatment with the DDS-CLO component of the MDT regimen with those of the same duration of monthly doses of the OFLO-MINO combination in *M. leprae*-infected nude mice (19) or in previously untreated lepromatous patients.

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