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Bactericidal Activities of R207910 and Other Newer Antimicrobial Agents against *Mycobacterium leprae* in Mice

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As measured by a proportional bactericidal technique in the mouse footpad system, the bactericidal activity against *Mycobacterium leprae* of R207910 was equal to that of rifapentine, rifampin, or moxifloxacin and significantly greater than those of minocycline, PA-824, and linezolid. These data suggest that R207910 may play an important role in treatment of leprosy.

To simplify and to facilitate the direct observation of treatment, a fully supervisable, monthly-administered multidrug regimen for leprosy is highly desirable (6). One of the requirements of such a regimen is that a single dose of each of the components displays bactericidal activity against *Mycobacterium leprae*. Therefore, new drugs with more powerful bactericidal activity against *M. leprae* than that of those comprising the current multidrug regimens (17, 18) are needed.

Because R207910 (a diarylquinoline) (2), PA-824 (a nitroimidazopyran) (10, 14, 16), and linezolid (an oxazolidinone) (1, 5) had displayed promising activity against *Mycobacterium tuberculosis* both in vitro and in vivo, we measured the bactericidal activities of these three compounds against *M. leprae* in mice by the proportional bactericidal technique (3). In three experiments, we compared these compounds to rifampin, the most bactericidal component of the current multidrug regimens (7, 8), and to rifapentine, moxifloxacin, and minocycline, the three components of the combination rifapentine-moxifloxacin-minocycline (PMM), the most active regimen against *M. leprae* in mice when administered once monthly (4).

In the first two experiments, mice were infected with *M. leprae* isolate 17543 (4); in the third experiment, mice were infected with *M. leprae* isolate Thai-53 (15). In each of the three experiments, female 4-week-old Swiss mice were inoculated with *M. leprae* (11) and randomly allocated to an untreated control group and a number of treated groups. Each group of mice consisted of three subgroups that were inoculated, respectively, with 5×10^3, 5×10^2, and 5×10^1 *M. leprae* bacilli per hind footpad. Treatments, all by oral gavage, were begun 24 h after inoculation. Drugs were administered either as a single dose or once daily for five consecutive days in the first experiment and only as single doses in the second and third experiments. Dosages were as follows: R207910, 25 or 100 mg/kg of body weight (2); PA-824 (10, 16) and linezolid (5), 100 mg/kg; moxifloxacin, 150 mg/kg (4); rifampin and rifapentine, 10 mg/kg (4); and minocycline, 25 mg/kg (4). After treatment, the mice were held for 12 months. Harvests of *M. leprae* from individual inoculated footpads were then performed by the method of Shepard and McRae (12). *M. leprae* bacilli were considered to have multiplied (i.e., survived the treatment) in those footpads found to contain $\geq 10^5$ acid-fast bacilli, regardless of the size of the inoculum. The proportion of viable *M. leprae* remaining after treatment was determined in terms of the median infectious dose (13). The significance of differences between the groups was calculated by the method of Shepard (13); differences were considered significant at the 95% level of confidence.

In the first experiment (Table 1), single doses of R207910 and moxifloxacin were highly bactericidal against *M. leprae* isolate 17543; 94.9% to >95.7% of viable *M. leprae* bacilli were killed. The activity of a single 25-mg/kg dose of R207910 did not differ significantly from that of a single 150-mg/kg dose of moxifloxacin. Neither the difference between the proportion of viable organisms among the mice treated with a single 25- or 100-mg/kg dose of R207910 nor the difference between the mice treated with a single dose or five daily doses of moxifloxacin attained statistical significance, because the proportions of viable *M. leprae* among these groups were either very close to or below the lower limit of detection (0.005% or 0.006% in our model, depending upon the number of harvested footpads); thus, no conclusion can be drawn regarding the comparative bactericidal activities of these treatments. Although single 100-mg/kg doses of PA-824 and linezolid failed to show significant bactericidal activity against *M. leprae*, when both drugs were given for five consecutive days, the proportions of viable *M. leprae* were significantly lower than that of untreated control; however, the activities of PA-824 and linezolid administered for five consecutive days were significantly weaker than that of single doses of R207910 and moxifloxacin.

The second experiment (Table 2) revealed that single doses of R207910, moxifloxacin, or rifapentine displayed potent and similar bactericidal activity against *M. leprae* isolate 17543, whereas a single dose of minocycline failed to exert significant bactericidal activity.

Although the mice in the third experiment (Table 2) were infected with a different isolate of *M. leprae*, the results among groups treated with single doses of monotherapy were very similar to those of the corresponding groups in the first two experiments. Single doses of R207910, moxifloxacin, rifampin, or rifapentine displayed significant bactericidal activity against

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TABLE 1. Bactericidal activities against *M. leprae* 17543 of several antimicrobial agents measured with mice by the proportional bactericidal method (first experiment)

Group no.	Treatment	No. of footpads showing multiplication[a] of *M. leprae*/no. of footpads harvested, by inoculum			% Viable *M. leprae*[b]	% Viable *M. leprae* killed by treatment[c]
		5×10^3	5×10^2	5×10^1		
1	None (control)	10/10	5/10	0/10	0.138	
2	Single 25-mg/kg dose of R207910	1/10	0/10	0/10	0.006[d,f]	95.7
3	Single 100-mg/kg dose of R207910	0/10	0/10	0/10	<0.006[d,f]	>95.7
4	Single 150-mg/kg dose of moxifloxacin	1/10	1/10	0/10	0.007[d,f]	94.9
5	5 daily 150-mg/kg doses of moxifloxacin	0/10	0/10	0/10	<0.006[d,f]	>95.7
6	Single 100-mg/kg dose of PA-824	9/10	4/10	1/10	0.109[e]	21.0
7	5 daily 100-mg/kg doses of PA-824	6/10	2/10	0/10	0.028[d]	79.7
8	Single 100-mg/kg dose of linezolid	9/10	2/10	1/10	0.069[e]	50.0
9	5 daily 100-mg/kg doses of linezolid	9/10	1/10	0/10	0.044[d]	68.1

[a] *M. leprae* bacilli were considered to have multiplied if the harvest from a footpad yielded $\geq 10^5$ acid-fast bacilli.
[b] Derived from the following equation: % viable *M. leprae* = 0.69/50% infectious dose (13).
[c] Calculated from the comparison of the proportions of viable organisms between untreated controls and the treated group.
[d] Significantly smaller than that of the untreated control group.
[e] Not significantly different from that of the untreated control group.
[f] Significantly smaller than those of groups 6 to 9.

M. leprae isolate Thai 53, whereas a single dose of minocycline did not. A single 25-mg/kg dose of R207910 killed 95.1% of viable *M. leprae* isolate Thai 53 bacilli originally presented in the mouse footpads, a degree of bactericidal effect virtually identical to that against *M. leprae* isolate 17543 from the first two experiments. The proportions of viable organisms among groups administered rifapentine monotherapy or any of the two-drug or three-drug combinations were <0.006%, below the lower limit of detection for the proportion of viable organisms in our system.

The results of these experiments demonstrated unequivocally that R207910 exhibited powerful bactericidal effect against two different isolates of *M. leprae*; a single 25-mg/kg dose killed more than 95% of viable *M. leprae* bacilli originally inoculated into the mouse footpads, an activity which was indistinguishable from those of rifapentine, rifampin, and moxifloxacin and significantly greater than those of a single dose of minocycline or five daily doses of PA-824 or linezolid, suggesting that R207910 may play an important role in the treatment of leprosy. Additional experiments to define further its activity against *M. leprae* are warranted.

TABLE 2. Bactericidal activities against *M. leprae* 17543 (second experiment) or Thai 53 (third experiment) of several antimicrobial agents, measured with mice by the proportional bactericidal method

Expt	Treatment[a]	% Viable *M. leprae*	% Viable *M. leprae* killed by treatment
Second	None (control)	0.047	
	R207910, 25 mg/kg	<0.005[b]	>89.4
	MIN, 25 mg/kg	0.029[c]	38.4
	MXF, 150 mg/kg	0.005[b]	89.4
	RFP, 10 mg/kg	0.006[b]	87.2
Third	None (control)	0.123	
	R207910, 25 mg/kg	0.006[b]	95.1
	MIN, 25 mg/kg	0.028[c]	77.2
	MXF, 150 mg/kg	0.011[b]	91.1
	RIF, 10 mg/kg	0.014[b]	88.6
	RFP, 10 mg/kg	<0.006[b]	>95.1
	MXF-MIN	<0.006[b]	>95.1
	MXF-R207910	<0.006[b]	>95.1
	RFP-MXF-MIN (PMM)	<0.006[b]	>95.1
	RFP-MXF-R207910	<0.006[b]	>95.1

[a] All drugs were administered by gavage as a single dose. Abbreviations: MIN, minocycline; MXF, moxifloxacin; RFP, rifapentine; RIF, rifampin.
[b] Significantly lower than that for untreated control group in the same experiment.
[c] Not significantly different from that for untreated control group in the same experiment.

The combination rifampin-ofloxacin-minocycline is the first once-monthly multidrug regimen for treatment of leprosy (9). However, the activities of both ofloxacin and minocycline are rather weak compared to that of rifampin (9). To increase the efficacy of a fully supervisable, monthly-administered multidrug regimen, rifampin and ofloxacin in the rifampin-ofloxacin-minocycline combination were replaced, respectively, by their more bactericidal analogs, rifapentine and moxifloxacin, yielding the combination PMM (4). However, PMM still included minocycline, as no suitable alternative to minocycline was available. Now, however, because our experiments have demonstrated that a single 25-mg/kg dose of R207910 was far more bactericidal than a single 25-mg/kg dose of minocycline, substitution of R207910 for minocycline in the PMM may yield a more effective treatment for leprosy.

Nevertheless, in the third experiment, our attempts to compare the bactericidal activity of the combination moxifloxacin-minocycline to that of moxifloxacin-R207910 and that of the combination PMM to that of rifapentine-moxifloxacin-R207910 failed, because the proportions of viable *M. leprae* in mice treated with all four combinations were below the lower limit of detection in immunologically competent mice, in which the maximal inoculum does not exceed 5×10^3 bacilli per footpad. It appears necessary to compare the bactericidal activities of these combined regimens with a more sensitive system, such as the *M. leprae*-infected nude mouse (7).

The activity of PA-824 or linezolid against *M. leprae* was rather modest: a single 100-mg/kg dose did not show significant bactericidal activity, and the bactericidal effect after five consecutive days of treatment was significantly weaker than that after a single dose of treatment with R207910 or moxifloxacin, thus confirming

the observation that PA-824 possesses a narrow spectrum of activity, limited primarily to the *M. tuberculosis* complex (14), and indicating that neither PA-824 nor linezolid is a suitable component of a once-monthly-administered combined regimen for the treatment of leprosy.

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