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Activity of Rifampin in Infections of Normal Mice with *Mycobacterium leprae*

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Assessment of the bactericidal activity of rifampin (RMP) against *Mycobacterium leprae* is difficult. As demonstrated by Levy and his colleagues ([5]), and confirmed by day-to-day observation, *M. leprae* recovered from the biopsy specimens of patients who have received nothing more than a single dose of RMP are incapable of multiplying in immunologically normal mice inoculated with 5000–10,000 organisms per foot pad. Thus, employing the normal mouse, it is not possible to determine whether the therapeutic activity of RMP administered daily is different—either larger or smaller—from that of the drug administered intermittently. Similarly, it is not possible to measure additive effects of other drugs—dapsone (DDS), clofazimine (CLO), or a thioamide—administered simultaneously. Moreover, employing the continuous and kinetic methods of Shepard ([7, 8]), administration of RMP to mice inoculated with a small number of *M. leprae* usually prevents subsequent multiplication of the organisms. Even the more discriminating "proportional bactericide" method does not permit one to compare the activity of RMP administered under a variety of conditions. Therefore, it has been difficult to evaluate the role of the rhythm and dosage of RMP administration, and the effects of combining RMP with other drugs.

Because of these difficulties, we undertook to measure the activity of RMP in "established" *M. leprae*-infection of normal mice ([9]), beginning drug administration when the bacterial population reached about 10^6 per foot pad. Bactericidal activity of the drugs was assessed by serially diluting organisms recovered from the infected foot pads of treated mice and sub-inoculating these dilutions into groups of new mice, in order to determine the most probable number (MPN) of surviving *M. leprae*.

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Materials and methods

A single strain of *M. leprae,* originally provided by S. R. Pattyn (strain no. 17547) ([6]) and subsequently maintained in mouse passage, was employed for all experiments. Female Swiss mice were obtained at six weeks of age from the CERJ Breeding Centre, Laval, France.

RMP was administered by gavage with an esophageal cannula in individual doses of 10 mg per kg body weight, contained in a volume of 0.2–0.3 ml. DDS was administered incorporated in the diet in a concentration of 0.01 g per 100 g diet. Prothionamide (PTH) was administered by gavage in individual doses of 25 mg per kg; CLO was administered, also by gavage, in doses of 40 mg per kg; and pefloxacin (PEFLO) was administered by gavage in doses of 150 mg per kg.

The experiments were similar: a large group of mice were inoculated, each in a hind foot pad, with 5000 *M. leprae,* and held without treatment for six months, at which time the number of organisms per foot pad had reached 10^6. At that time, the mice were allocated randomly to small groups, one serving as untreated controls, and the other groups treated by different regimens. At intervals during treatment, five mice of each group were sacrificed, *M. leprae* were harvested from the inoculated foot pads, and the organisms from each group of five mice were pooled, counted, and serially diluted to provide inocula of 5000, 500, 50, 5 and 0.5 organisms per foot pad, which were inoculated into the hind foot pads of groups of passage mice, 10 mice per dilution. One year after sub-inoculation, *M. leprae* were harvested from the foot pads of the passage mice; multiplication was assumed to have occurred in a foot pad when at least 1×10^5 organisms were harvested. The MPN for each inoculum was calculated from the proportion of mice demonstrating multiplication, employing a table published by de Man ([2]).

TABLE 1. *Infective* M. leprae *in mice treated with RMP alone as a function of rhythm and duration of treatment.*

Duration of treatment	Rhythm of administration	No. doses	No. mice showing multiplication[a] / No. mice harvested / No. *M. leprae* inoculated					MPN per 5000 AFB
			5000	500	50	5	0.5	
			Experiment 1					
0	—	0	—	8/8	7/8	3/8	1/8	297
1 day	—	1	—	8/8	6/8	1/8	0/8	137
6 days	Daily	6	8/8	6/8	1/8	0/8	0/8	13.7
4 weeks	—	0	—	—	8/8	6/8	1/8	1375
	Daily[b]	24	3/8	0/8	—	—	—	0.4
	Weekly	5	8/8	5/8	2/8	0/8	—	12.2
	Fortnightly	3	6/8	3/8	—	0/8	—	1.8
	Monthly	2	—	8/8	4/8	0/8	—	62.1
8 weeks	—	0	7/8	2/8	1/8	0/8	—	3.9
	Daily[b]	48	0/8	0/8	0/8	—	—	<0.1
	Weekly	9	8/8	3/8	2/8	0/8	—	7.2
	Fortnightly	5	7/8	3/8	0/8	—	—	2.6
	Monthly	3	8/8	2/8	1/8	0/8	—	4.4
			Experiment 2					
0	—	0	—	—	8/8	7/8	1/8	1940
4 weeks	—	0	—	8/8	1/8	0/8	—	22.8
8 weeks	—	0	8/8	5/8	0/8	—	—	8.4
	Daily[c]	40	0/8	—	—	—	—	<0.1
	Monthly	3	5/8	1/8	1/8	0/8	—	1.2
12 weeks	—	0	8/8	4/8	0/8	—	—	6.2
	Daily[c]	60	0/8	—	—	—	—	<0.1
	Monthly	4	8/8	3/8	0/8	—	—	4.7

[a] $\geq 10^5$ *M. leprae* per foot pad.
[b] Six days per week.
[c] Five days per week.

Results

Treatment with RMP alone. In experiment 1, RMP was administered six days per week, weekly, or every two or four weeks for a period of two months. In experiment 2, RMP was administered either five days per week or monthly for three months. As shown in Table 1, the MPN before beginning treatment was 297 and 1940 viable organisms per 5000 *M. leprae* for experiments 1 and 2, respectively. Further inspection of the data of Table 1 reveals that, in experiment 1, the proportion of viable organisms increased in control mice during the first four weeks after beginning treatment, and then rapidly decreased to a value of 3.9 per 5000 during the second four-week period. Spontaneous killing of the *M. leprae* by untreated mice was more dramatic in experiment 2, the MPN decreasing to 22.8, 8.4, and 6.2 viables per 5000 organisms four, eight and 12 weeks, respectively, after beginning the treatment of treated mice. Among treated mice, the values of the MPN were smallest in the mice treated with daily RMP, and largest in those treated with RMP administerd monthly, suggesting a relationship between the number of doses administered and the decrease of the MPN. Two additional points require emphasis: a) the results of experiment 1 do not indicate very rapid killing of *M. leprae* by RMP; and b) in both experiments, only daily administration of RMP appeared capable of reducing the MPN at all intervals to values smaller than those among control mice.

Treatment with monthly RMP in combination with other drugs. In experiment 1, in addition to the mice administered RMP alone, mice of other groups were administered RMP monthly, DDS continuously, and either PTH plus CLO monthly, or PTH administered daily. In experiment 2, the monthly pulses of RMP were combined with

TABLE 2. *Infective* M. leprae *in mice treated with RMP in combination with other drugs.*

Duration of treatment	Drug regimen	No. RMP doses	No. mice showing multiplication[a] No. mice harvested No. *M. leprae* inoculated					MPN per 5000 AFB
			5000	500	50	5	0.5	
		Experiment 1						
0	—	0	—	8/8	7/8	3/8	1/8	297[b]
8 weeks	—	0	7/8	2/8	1/8	0/8	—	3.9[b]
	RMP + PTH + CLO + DDS[c]	3	2/8	3/8	0/8	—	—	0.6
	RMP + PTH + DDS[d]	3	4/8	0/8	—	—	—	0.6
		Experiment 2						
0	—	0	—	—	8/8	7/8	1/8	1940[b]
8 weeks	—	0	8/8	5/8	0/8	—	—	8.4[b]
	RMP + DDS[e]	3	6/8	4/8	0/8	—	—	2.1
	RMP + CLO[f]	3	—	7/8	1/8	0/8	—	18.5
12 weeks	—	0	8/8	4/8	0/8	—	—	6.2[b]
	RMP + DDS[e]	4	2/8	0/8	—	—	—	0.25
	RMP + CLO[f]	4	7/8	1/8	1/6	0/6	—	2.1

[a] $\geq 10^5$ *M. leprae* per foot pad.
[b] The same harvest as that yielding the identical value in Table 1.
[c] RMP, PTH and CLO were administered monthly, and DDS continuously.
[d] RMP was administered monthly, PTH 6 days weekly, and DDS continuously.
[e] RMP was administered monthly and DDS continuously.
[f] RMP was administered monthly and CLO weekly.

DDS administered continuously or CLO administered weekly. As shown in Table 2, the values of the MPN resulting from treatment with regimens that combined RMP and DDS were smaller than those of the *M. leprae* harvested from control mice, and also smaller than those resulting from treatment with monthly RMP and weekly CLO. A monthly supplement of PTH and CLO did not increase the activity of the combination of monthly RMP and continuous DDS. Finally, the values of the MPN observed after treatment with RMP administered monthly and DDS administered continuously were very similar to those observed after treatment with RMP administered alone daily (see Table 1).

Treatment with a single drug other than RMP. In experiment 2, as shown in Table 3, treatment of mice for eight or 12 weeks with PTH administered five days weekly or CLO administered once weekly did not produce values of the MPN smaller than those for control mice. On the other hand, treatment with DDS administered continuously or daily PEFLO yielded the smallest values of the MPN observed. Because PEFLO yielded values of the MPN that did not differ from those after treatment with DDS, the former drug may be of considerable interest for the treatment of leprosy.

Discussion

The results of these experiments show clearly that established infection of normal mice with *M. leprae* is not a convenient system by which to compare the activities of different drug regimens; rapid spontaneous killing of the organisms in untreated mice, once the *M. leprae* have multiplied to the plateau level, renders the assessment of drug activity very difficult. For this reason, the results reported here must be interpreted with caution. It may be that the rapid spontaneous killing demonstrated in this experiment is peculiar to the strain of mice or the strain of *M. leprae* employed. Welch and her colleagues reported ([12]) that the half-time of spontaneous killing was 25 days, a rate much slower than that observed in the present experiments.

Three main findings have emerged from the experiments reported here. The first is the limited activity of a single dose of 10 mg RMP per kg, compared to the great activity of the same dosage in man ([5]), despite

TABLE 3. *Infective* M. leprae *in mice treated with a drug other than RMP.*

Duration of treatment	Drug	Rhythm of administration	No. mice showing multiplication[a] No. mice harvested / No. *M. leprae* inoculated					MPN per 5000 AFB
			5000	500	50	5	0.5	
			Experiment 2					
0	—	—	—	—	8/8	7/8	1/8	1940[b]
8 weeks	—	—	8/8	5/8	0/8	—	—	8.4[b]
	DDS	Continuously	5/8	2/8	0/8	—	—	1.2
	PTH	Daily[c]	8/8	8/8	2/8	2/8	0/8	52
	CLO	Weekly	8/8	7/8	2/8	0/7	—	22
	PEFLO	Daily[c]	0/8	0/8	—	—	—	<0.1
12 weeks	—	—	8/8	4/8	0/8	—	—	6.2[a]
	DDS	Continuously	0/8	0/8	—	—	—	<0.1
	PTH	Daily[c]	7/8	3/8	0/8	—	—	2.6
	CLO	Weekly	8/8	7/8	2/8	1/8	0/8	38.5
	PEFLO	Daily[c]	1/7	0/8	—	—	—	0.1

[a] $\geq 10^5$ *M. leprae* per foot pad.
[b] The same harvest as that yielding the identical value in Table 1.
[c] Five days per week.

the fact that the pharmacokinetics of the drug are more favorable in the mouse than in man. In patients, a single 600-mg dose of RMP has been found to kill about 99.9% of the viable *M. leprae* present before treatment ([5]), whereas we observed killing only to the extent of about 90%. Moreover, even six consecutive daily doses of 10 mg RMP per kg were incapable of killing 99% of the viable organisms originally present. Similar results were observed by Fieldsteel and Levy, in studies of the effects of RMP-treatment of the *M. leprae*-infected neonatally thymectomized rat ([3]).

The second important finding is that, in the mouse, RMP administered daily is more active than when the drug is administered intermittently. Although much data obtained from the chemotherapy of tuberculosis in man attest to greater activity of daily RMP ([4]), similar data have not been reported from studies of the chemotherapy of leprosy in man. In fact, no differences among regimens could be demonstrated in a comparison of regimens as different as daily RMP + CLO + DDS and daily DDS supplemented by a single initial 25 mg/kg dose of RMP ([10]). It is important to learn whether the similarity of two so different regimens resulted because their activities were indeed similar, or because the mouse foot pad represents too insensitive a measuring device.

The final point of importance is the excellent activity in the mouse of the combination daily DDS + monthly RMP, a finding consistent with the considerable activity in man of DDS administered alone. It may be that, when the two drugs are administered in combination, the activity of DDS masks any real difference that may exist between RMP administered daily and the drug administered monthly. Even more important is the experimental support provided by the results reported here to the regimen, which includes monthly RMP together with daily DDS + CLO, recommended by the WHO Study Group for treatment of patients with multibacillary leprosy ([13]).

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