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Antimycobacterial Activities of Two Newer Ansamycins, R-76-1 and DL 473[1]

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The ansamycins are a group of compounds characterized chemically by the presence of a naphthoquinone chromophore spanned by an aliphatic "ansa" chain joining two points of the chromophore. One of these compounds, rifampin (RMP), has proved to be a potent drug in the treatment of leprosy and other infectious diseases. After a single dose of 1200 mg or 1500 mg RMP to adult patients with lepromatous leprosy, *Mycobacterium leprae* in mice could not be isolated from biopsy-specimens taken three or more days later ([16]). This suggested that the drug could be administered intermittently for treatment of leprosy, and intermittent RMP is an important component of the World Health Organization (WHO) multidrug regimens ([28]).

In addition to RMP, large numbers of semisynthetic ansamycins have been prepared. During the last ten years, we have tested a series of these compounds in search of a compound more active than RMP against *M. leprae* ([14]). Initial screening was carried out *in vitro* with a variety of cultivable mycobacteria, and *in vivo* in an experimental infection of mice with *M. lepraemurium* (MLM). When compounds showed activity comparable or superior to that of RMP, they were further evaluated for activity against *M. leprae* in the mouse foot pad.

In 1980, we reported ([15]) the antimycobacterial activity of AF-MO, an oxime of 3-formylrifamycin SV (Fig. 1) synthesized by Lepetit ([9]). This compound demonstrated activity quite similar to that of RMP in the initial screening systems. In addition, ten lepromatous patients were treated with AF-MO in the dosage of 450 mg daily for 12 months; in terms of clinical improvement and change in morphological (MI) and bacterial (BI) indexes, the therapeutic effect was encouraging.

Because of their promising activities, the present report describes the antimycobacterial activity of two other ansamycins: isobutylpiperazinylrifamycin SV, which was synthesized by CIBA-GEIGY (Deutsches Patentamt, Offenlengungsschrift 2444782 10.4.75) (our code number R-76-1), and cyclopentylrifamycin SV, which was synthesized by Lepetit ([8]) and is known as DL 473 or rifapentine.

MATERIALS AND METHODS

Ansamycins. All the ansamycins tested in the present report were provided as gifts by the Sichuan Industrial Institute of Antibiotics, China.

Mycobacteria. For the studies *in vitro,* all of the cultivable mycobacteria were raised on 1% Ogawa egg medium, except for MLM (Hawaii strain) which was raised on 1% Ogawa egg-yolk medium. For studies *in vivo,* MLM were harvested from the spleens of experimentally infected mice, and *M. leprae* susceptible to dapsone were obtained from skin-biopsy specimens of patients with lepromatous leprosy.

Measurement of minimal inhibitory concentration (MIC). For the determination of MIC against MLM, each tube of Ogawa 1% egg-yolk medium was inoculated either with colony transfer (and spread) or with 0.2 ml of an 8 mg/ml suspension of the organism. For determination of the MICs against the other cultivable organisms, each tube of Ogawa 1% egg medium was inoculated with 10^{-3} mg or 10^{-4} mg. The MIC was taken as the smallest concentration of compound that prevented visible growth after incubation at 37°C for 20 weeks for MLM, and

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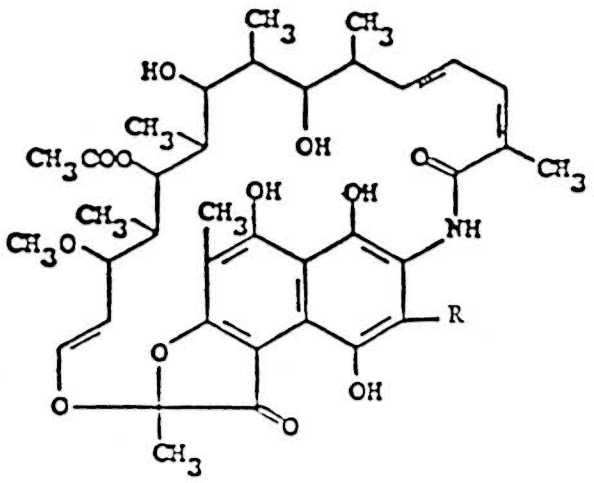

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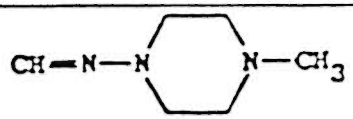
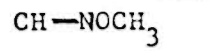
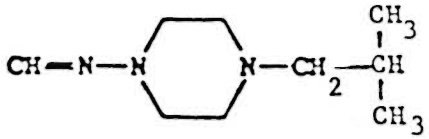
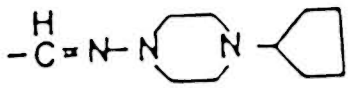
RMP	
AF-MO	
R-76-1	
DL 473	

FIG. 1. Chemical structure of rifampin (RMP), AF-MO, R-76-1, and DL 473.

for 4 weeks for all other cultivable mycobacteria.

Experimental MLM infection. Female Swiss albino mice weighing 18 to 20 g were inoculated intraperitoneally (i.p.) with 0.5 ml of suspension of MLM containing 1×10^9 organisms per ml. The test compounds were either incorporated into the mouse diet or administered by gavage. Treatment either began immediately (on the day of inoculation), was delayed until sufficient time had elapsed to permit established infection, or began when the infection had reached a far-advanced stage and a few animals had already died. Treatment was continued with some of the mice until they were sacrificed; with others, it was discontinued after some time and the mice followed up to learn if the treatment had been bactericidal. Twenty control mice and 10 to 20 treated mice were sacrificed at each interval. Chemotherapeutic activity was evaluated by enumerating the organisms in the spleen by the "pin-head" method ([11]), and a comparison of the number of MLM harvested from the spleens of each group was carried out by the two-tailed Student *t* test; or it was evaluated by the "therapeutic index," which was derived

TABLE 1. *Minimal inhibitory concentrations in μg per ml of DL 473, rifampin (RMP), and R-76-1 against 11 cultivable species of mycobacteria.*

Species	Inoculum size					
	10^{-4} mg			10^{-3} mg		
	DL 473	RMP	R-76-1	DL 473	RMP	R-76-1
M. tuberculosis H37Rv	1.56	6.25	0.39	6.25	6.25	3.13
M. tuberculosis H37Ra	1.56	6.25	0.20	1.56	6.25	0.78
M. bovis	1.56	6.25	0.39	1.56	6.25	0.39
M. avium	3.13	12.5	0.78	3.13	12.5	0.78
M. marinum	0.78	3.13	0.10	1.56	3.13	0.05
M. terrae	25	50	6.25	25	50	6.25
M. scrofulaceum	1.56	0.39	0.39	1.56	1.56	0.78
M. kansasii	3.13	25	0.20	3.13	25	0.20
M. intracellulare	1.56	0.78	0.39	3.13	3.13	0.39
BCG	0.78	0.78	0.39	1.56	3.13	0.20
M. vaccae	50	100	25	50	100	25

from three components: a) the leprosy index, an evaluation of the gross lesions in various organs ([5]); b) the score of infected cells in the mesentery ([6]); and c) the average number of organisms in the spleen. For each component, the ratio of the value for the treated animals to that for the control mice was calculated. The therapeutic index was the reciprocal of the mean of these three ratios; thus, the larger the index, the higher the therapeutic activity of the compound. Therapeutic activity was scored as −, +, ++, +++, or ++++, when the therapeutic index was, respectively, <1.50, 1.51 to 2.50, 2.51 to 3.50, 3.51 to 4.50, or >4.50.

Experimental *M. leprae* infection. Shepard's mouse foot-pad technique ([21]) was employed. The activity of R-76-1, AF-MO, and RMP was compared by the kinetic method ([22, 23]) with three different doses, i.e., 0.0001, 0.001 and 0.01 g of compound per 100 g of mouse diet. Each mouse was inoculated in each hind foot pad with 10^4 *M. leprae*. The activity of the compounds was assessed by comparing the growth curves of *M. leprae* in treated and control mice. Growth delay was defined as the duration of the period between multiplication of *M. leprae* to 10^6 per foot pad in control mice and multiplication to the same level in treated mice.

For the proportional bactericidal test ([7]), the activities of R-76-1, AF-MO, RMP, and DDS were compared in one experiment, and DL-473 and RMP were compared in another. Groups of 10 to 16 mice were inoculated with 10^4, 10^3, 10^2, 10^1 *M. leprae* per foot pad. Twelve months later, all of the mice were sacrificed and organisms were harvested from the inoculated foot pads. *M. leprae* were considered to have multiplied when more than 10^5 organisms per foot pad were harvested. The results were analyzed by calculating the "most probable number" (MPN) ([10, 25]) and median infectious dose (ID_{50}) ([24]). The significance of the differences between the values of ID_{50} in the proportional bactericidal test were calculated by the Spearman-Kärber method ([24]). In one experiment, in order to minimize the number of animals required, only two inocula—10^4 and 10^3—were employed on treated mice, although all four inocula were employed on control animals. Because in some of the groups of treated mice neither of the inocula produced multiplication in all of the inoculated feet, or failed to produce multiplication in all of the feet, some difficulty was experienced in calculating the MPN and the ID_{50}. To solve this problem, two assumptions were made: a) inoculation with 10^2 *M. leprae* per foot pad failed to produce multiplication in any of the inoculated feet, and b) inoculation with 10^2 *M. leprae* per foot pad of treated mice produced the same results as in control mice. Based on these two assumptions, minimal and maximal values for the MPN and ID_{50} were calculated.

Clinical trial. Because the toxicological and pharmacokinetic data ([26]) appeared to justify further study in man, a clinical trial of R-76-1 was carried out. Twenty patients

TABLE 2. *Minimal inhibitory concentrations in μg per ml of DL 473, rifampin (RMP), and R-76-1 against* M. lepraemurium.

Drug	Subculture by	
	Colony transfer	Bacillary suspension (8 mg/ml, 0.2 ml/tube)
DL 473	2	0.5
RMP	10	2
R-76-1	<0.1	<0.5

with lepromatous leprosy (15 LL and 5 BL) were selected. Four had not previously been treated; the remaining patients, none of whom had been treated with an ansamycin, had recently relapsed. All exhibited active skin lesions with high BI and MI. The drug was administered in a daily dosage of 150 mg for from 6 to 18 months, during which time serial skin-biopsy specimens were obtained and mice were inoculated for testing the drug-susceptibility of the patients' *M. leprae* and for measuring the initial rate at which the organisms were killed. In addition, clinical and bacteriological assessments ([27]) were performed at regular intervals, and the side effects and reactions were recorded.

RESULTS

Studies *in vitro*. As shown in Tables 1 and 2, R-76-1 was more active than the other ansamycins against most cultivable mycobacteria, including MLM. The MICs of this compound were, on average, more than three dilutions lower than those of RMP, and more than two dilutions lower than those of DL 473. DL 473 was slightly more active than RMP, with MICs, on average, one dilution lower than those of RMP.

Studies *in vivo* with MLM. Therapeutic activities of R-76-1 and RMP were compared in the experimental infection of mice with MLM by two treatment schedules (immediate and delayed) and seven dosage regimens (Table 3). For each regimen, the therapeutic index was higher for R-76-1 than for RMP. Administered twice weekly in the mouse diet at a concentration of 0.01 g per 100 g diet (equivalent to 20 mg/kg body weight per day), or at 0.003 g per 100 g daily, R-76-1 was at least as effective as RMP administered in a concentration of 0.01 g per 100 g daily. Thus, R-76-1 appears to have been about threefold more effective than RMP.

The activities of DL 473 and RMP were compared in another experiment. Forty-five days after inoculation, in ten mice selected at random, the mean number of organisms per spleen was found to be $8.38 (\pm 6.64) \times 10^8$, indicating that the infection with MLM had become established. Treatment by various regimens was then administered for 84 days, beginning on day 46 after inoculation. On day 137, 20 control mice and 12 mice from each treatment group were randomly selected for sacrifice. As shown in Table 4 and Figure 2, the control mice already showed advanced infection. The average number of organisms per spleen of control mice was significantly greater than that of treated mice, except for the mice treated with smaller dosages and at longer intervals, indicating that the majority of the regimens were effective against MLM infection in mice. No significant difference was found between the number of organisms per spleen

TABLE 3. *Therapeutic activity (therapeutic index) of R-76-1 and rifampin (RMP) in experimental* M. lepraemurium *(MLM).*

	Regimen	R-76-1	RMP
Immediate[a]	0.01% daily	4+ (11.40)	4+ (4.68)
	0.01% daily for 10 consecutive days	2+ (2.75)	1+ (1.54)
	0.01% 2 days per week	4+ (5.88)	1+ (1.97)
	0.003% daily	4+ (6.81)	1+ (1.54)
Delayed[b]	0.01% daily	3+ (3.94)	1+ (2.15)
	0.01% 2 days per week	2+ (2.85)	1+ (1.63)
	0.01% daily for 10 consecutive days, then 2 days per week	2+ (3.15)	1+ (1.86)

[a] Treatment begun immediately after inoculation of MLM.
[b] Treatment delayed for 30 or 60 days, until MLM infection had become firmly established.

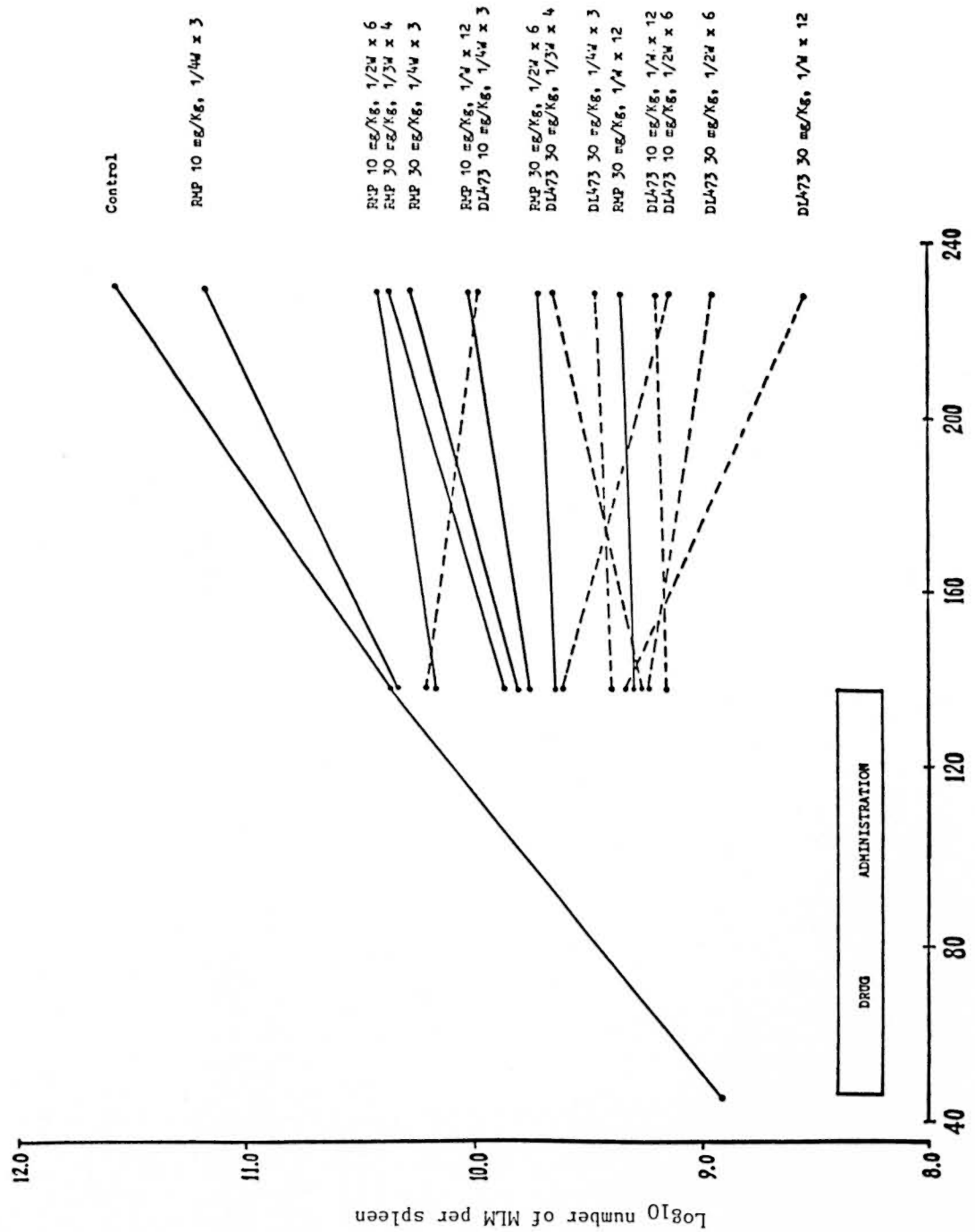


FIG. 2. Effects of rifampin (RMP) and DL 473 in established *M. lepraemurium* (MLM) infection.

TABLE 4. *Number of* M. lepraemurium *($\times 10^8$) per spleen of mice treated with rifampin (RMP) or DL 473.*

Regimen		Autopsied at 137th day	Autopsied at 228th day
Controls		230.8 ± 183.7	3737.5 ± 2453.8
30 mg/kg, 1/week × 12	RMP	19.8 ± 14.7[b]	22.1 ± 46.0
	DL 473	21.3 ± 23.5[b]	3.6 ± 1.8
30 mg/kg, 1/2 weeks × 6	RMP	[c] 44.0 ± 25.3	51.4 ± 41.7 [c]
	DL 473	17.3 ± 13.6[b]	9.1 ± 3.7
30 mg/kg, 1/3 weeks × 4	RMP	74.4 ± 132.6	227.3 ± 449.8
	DL 473	18.3 ± 11.4	45.0 ± 65.1
30 mg/kg, 1/4 weeks × 3	RMP	63.8 ± 63.0	186.0 ± 81.7 [c]
	DL 473	24.8 ± 19.4	28.9 ± 29.2
10 mg/kg, 1/week × 12	RMP	[c] 57.3 ± 27.0	102.9 ± 255.0
	DL 473	14.4 ± 5.0	15.3 ± 24.6
10 mg/kg, 1/2 weeks × 6	RMP	[c] 149.3 ± 101.2[d]	258.8 ± 349.9 [c]
	DL 473	42.9 ± 48.8	14.1 ± 13.4
10 mg/kg, 1/4 weeks × 3	RMP	220.5 ± 168.2[d]	1511.3 ± 2634.3[d]
	DL 473	159.7 ± 193.5[d]	100.6 ± 73.9

[a] Drugs administered from day 46 to day 130 after inoculation.
[b] Not significantly different from the pretreatment (day 45) value.
[c] Significantly different from each other ($p < 0.05$).
[d] Not significantly different from the corresponding control value.

determined on day 45 (before treatment) and that on day 137 (after treatment) for the groups of animals treated with the following regimens: a) RMP 30 mg/kg, once weekly for 12 doses (1/week × 12); b) DL 473 30 mg/kg, 1/week × 12; and c) DL 473 30 mg/kg, once in 2 weeks for six doses (1/2 weeks × 6). In the case of three regimens—30 mg/kg, 1/2 weeks × 6; 10 mg/kg, 1/week × 12; and 10 mg/kg, 1/2 weeks × 6—fewer organisms were recovered on day 137 from DL 473-treated mice than from RMP-treated mice. Moreover, there were no significant differences among the four regimens employing DL 473 in a dosage of 30 mg/kg; whereas the number of organisms per spleen increased (although not all the differences were significant) with prolongation of the interval between 30 mg/kg doses of RMP from once weekly to once in 4 weeks, suggesting that DL 473 exerted a more prolonged activity against MLM infection than did RMP. Finally, the number of organisms in the spleens of mice administered the regimen RMP 30 mg/kg, 1/2 weeks × 6 was significantly greater than those of the following DL 473 regimens: 30 mg/kg, once in 3 weeks for four doses (1/3 weeks × 4); 30 mg/kg, once in 4 weeks for three doses (1/4 weeks × 3); and 10 mg/kg, 1/week × 12, suggesting, again, that DL 473 was more effective than RMP, and that an equivalent therapeutic effect could be obtained by fewer doses of DL 473 than of RMP.

On day 228, 98 days after stopping treatment, the surviving mice were all sacrificed. Since all of the untreated control mice had died before day 204, with the last ten autopsied and the organisms in the spleens enumerated, the mice which were therefore treated until day 130, and which died during the same period as the last ten control mice, were also examined and the results included with the results of the mice sacrificed on day 228. As is also shown in Table 4 and Figure 2, the pattern of differences between the numbers of MLM harvested from the spleens of control and treated mice was much the same as that described for day 137. Significantly smaller numbers of MLM were produced in the spleens of treated mice than in those of control mice by all of the regimens except RMP 10 mg/kg, 1/4 weeks × 3. No significant differences were noted in the results of mice sacrificed on day 137 and those on day 228, with the exception of the control mice and those treated with RMP 30 mg/kg, 1/4 weeks × 3, both of which showed a progressive in-

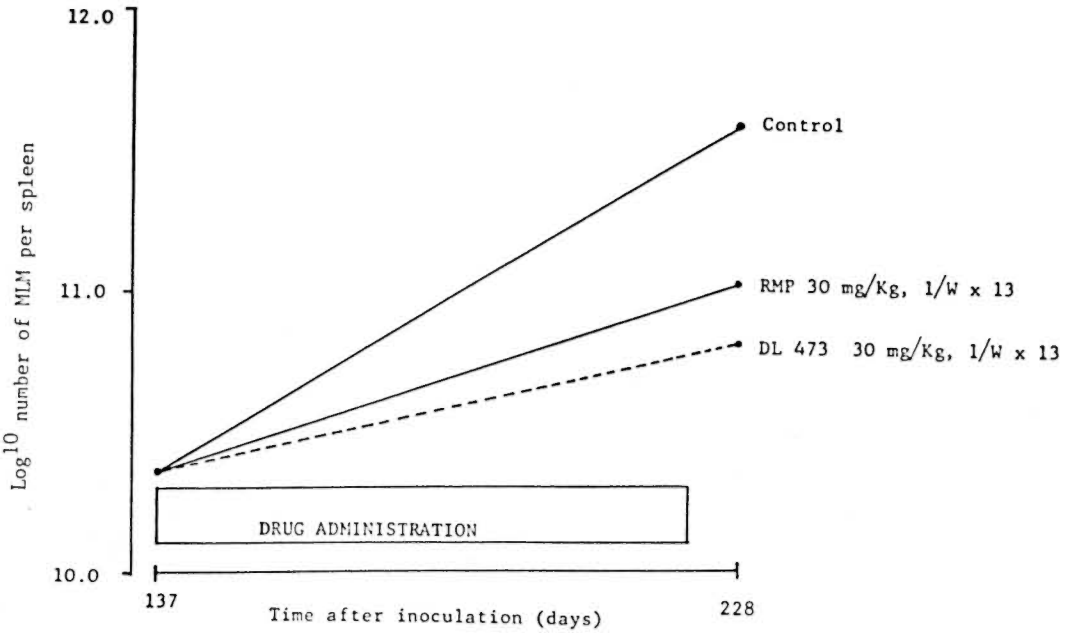


FIG. 3. Effects of rifampin (RMP) and DL 473 in far-advanced *M. lepraemurium* (MLM) infection.

crease in the numbers of MLM per spleen, and for the mice treated with DL 473 30 mg/kg, 1/week × 12, for which the number of organisms per spleen decreased significantly despite the fact that these animals were not treated during the interval. Thus, for most of the regimens, MLM had been killed during the period of treatment and did not resume multiplication during the 13 weeks following cessation of treatment on day 130.

Also in the same experiment, to evaluate the therapeutic effects of DL 473 and RMP in far-advanced infection, two groups of ten mice each were randomly selected from among the control mice on day 137 and treated with one of these two compounds at the dosage of 30 mg/kg once a week until day 221. All of the surviving mice were sacrificed and examined under the same conditions as above. As shown in Figure 3, both RMP and DL 473 were highly effective against far-advanced MLM infection; in each case, the number of organisms per spleen was significantly smaller than that of untreated control mice. The apparent difference between the two treatments was not significant.

Studies against *M. leprae in vivo*. Studied by the kinetic technique, neither R-76-1 nor RMP exhibited bactericidal activity against *M. leprae* when administered in a concentration of 0.0001 g per 100 g diet (equivalent to 0.2 mg/kg body weight per day) (Table 5). Administered in a concentration ten times higher, however, R-76-1 demonstrated a bactericidal-type effect but RMP did not. Both drugs exhibited bactericidal activity at a concentration of 0.01 g per 100 g.

The results of a proportional bactericidal test, in which four drugs were administered by two treatment schedules, are summarized in Table 6. The significance of differences between the groups was tested by calculating the value of p (probability) for each pair of $\log_{10}$ ID_{50} values ([24]). From Table 7, it may be seen that all of the regimens show bactericidal activity but, in reality, the ansamycins were more active than the dapsone. Of the ansamycins, no significant difference was detected between R-76-1 and AF-MO and both were more active than RMP. Finally, although no significant differences were found between the two treatment schedules for each drug, the regimens employing R-76-1 or AF-MO in a concentration of 0.003 g per 100 g were more active than those employing RMP in a concentration of 0.01 g per 100 g, suggesting that both

TABLE 5. *Effects of rifampin (RMP), AF-MO, and R-76-1 on multiplication of* M. leprae *in mice by kinetic technique.*

Drug concentration in mouse diet[a]	Drug	Harvest (AFB/foot pad) ($\times 10^5$)						Days of growth delay[c]
		Day 90	Day 150	Day 190	Day 234	Day 250	Day 280	
0	—	1.69 (4)[b]	20.79 (28)			12.10 (6)		—
0.0001%	RMP		18.98 (8)	27.42 (4)	21.87 (4)		20.21 (4)	0
	AF-MO		32.92 (8)	18.86 (4)	27.26 (8)		32.76 (2)	0
	R-76-1		9.07 (4)	28.45 (4)	6.12 (4)		20.60 (4)	16
0.001%	RMP		10.20 (4)	30.46 (4)	19.66 (4)		39.99 (4)	10
	AF-MO		3.08 (4)	15.37 (4)	4.44 (4)		31.01 (4)	44
	R-76-1		1.42 (4)	2.51 (4)	2.52 (4)		5.27 (4)	>160
0.01%	RMP		2.79 (4)	7.45 (6)	6.01 (4)		8.58 (4)	>146
	AF-MO		0.59 (4)	0.58 (6)	1.72 (4)		2.09 (4)	>186
	R-76-1		0.81 (4)	1.86 (6)	0.55 (4)		2.09 (4)	>186

[a] The drug-containing diet was administered from day 90 to day 140 after inoculation of *M. leprae.*
[b] Number of foot pads harvested are shown in parentheses.
[c] Delay, relative to control curve, in the time the growth curve reached the level 10^6.

of the former drugs possessed about three times the bactericidal activity of RMP against *M. leprae.*

Two comparisons of the bactericidal activity of RMP and DL 473 were performed. In the first experiment, we determined the dose of each drug, administered once in every 4 weeks, which was required to prevent multiplication of *M. leprae* in the foot pads of half of the mice. As shown in Table 8, the required concentration of RMP appears to fall in the range of 1.25 to 2.5 mg/kg and that of DL 473 is less than 0.625 mg/kg; thus, DL 473 appears to be at least twice as potent as RMP.

The results of the comparison by means of the proportional bactericidal test, shown in Table 9, showed significant bactericidal activity for all regimens except for the single 5 mg/kg dose of RMP. Therefore, even a single dose of 1.25 mg/kg of DL 473 was active; whereas the smallest single active dose of RMP was 10 mg/kg. Among the intermittent regimens, no significant difference was observed between RMP at 20 mg/kg and DL 473 at 0.625 mg/kg. As shown by the values for the MPN, except for RMP at 5 mg/kg, all of the intermittent regimens killed at least 99% of the *M. leprae,* and single 20 mg/kg doses of RMP and 10 mg/kg doses of DL 473 were equally effective. DL 473 in single doses of 5 and 10 mg/kg was significantly more effective than RMP in equal doses and, for both drugs, the 10

TABLE 6. *Bactericidal activity of R-76-1, AF-MO, rifampin (RMP), and dapsone (DDS) against* M. leprae *in the proportional bactericidal test.*

Drug regimen	*M. leprae* per foot pad Inoculum size				MPN[a] of viable *M. leprae*	% Survival	Log ID_{50}
	10^4	10^3	10^2	10^1			
Controls	11/11[b]	13/13	10/12	8/15	310	—	1.14 ± 0.18
0.01% RMP, 0–30 days	8/8	1/7	1/7	1/9	4	1.3	3.11 ± 0.23
0.01% R-76-1, 0–30 days	1/12	0/12	0/9	0/8	0.08	0.03	4.42 ± 0.08
0.01% AF-MO, 0–30 days	1/13	1/9	0/12	0/6	0.15	0.05	4.31 ± 0.14
0.01% DDS, 0–30 days	10/10	9/9	2/7	0/6	40.5	13	2.21 ± 0.19
0.003% RMP, 0–45 days	10/13	7/7	1/10	0/10	13.7	4.4	2.63 ± 0.16
0.003% R-76-1, 0–45 days	0/13	0/5	0/8	0/7	<0.08	<0.03	>4.42 ± 0.08
0.003% AF-MO, 0–45 days	2/12	1/13	0/7	0/5	0.25	0.08	4.25 ± 0.14
0.003% DDS, 0–45 days	8/9	8/10	1/10	1/9	11.1	3.6	2.60 ± 0.23

[a] Most probable number per 10^4 bacilli inoculated.
[b] Number of mice showing growth of *M. leprae*/number of mice harvested.

TABLE 7. *P values for between-group differences of* log_{10} ID_{50}.[a]

Group	0.01%, 0–30 days				0.003%, 0–45 days		
	RMP[b]	R-76-1	AF-MO	DDS[c]	RMP	R-76-1	AF-MO
0.01% RMP, 0–30 days	—						
0.01% R-76-1, 0–30 days	<0.001	—					
0.01% AF-MO, 0–30 days	<0.001	>0.05	—				
0.01% DDS, 0–30 days	<0.01	<0.001	<0.001	—			
0.003% RMP, 0–45 days	>0.05	<0.001	<0.001	>0.05	—		
0.003% R-76-1, 0–45 days	<0.001	>0.05	>0.05	<0.001	<0.001	—	
0.003% AF-MO, 0–45 days	<0.001	>0.05	>0.05	<0.001	<0.001	>0.05	—
0.003% DDS, 0–45 days	>0.05	<0.001	<0.001	>0.05	>0.05	<0.001	<0.001

[a] All regimens were statistically different from controls, $p < 0.001$.
[b] RMP = rifampin.
[c] DDS = dapsone.

mg/kg dose was significantly more effective than the 5 mg/kg dose. On the other hand, no significant difference in bactericidal activity was noted among the three smallest single doses of DL 473.

Clinical trial of R-76-1. Before beginning treatment with R-76-1, *M. leprae* strains isolated from 10 of the 20 patients had been tested for drug susceptibility. Two of the patient-strains were found to be susceptible to dapsone, seven were fully resistant to dapsone, and the tenth strain was resistant to both thiacetazone and thiambutosine. The estimated duration of treatment required with R-76-1 before the patient's organisms had been rendered incapable of multiplying in mice—i.e., the midpoint between the day of the last biopsy-specimen yielding multiplication of *M. leprae* ("last positive specimen") in mice and the first subsequent specimen which failed to yield multiplication ("first negative specimen")—was 3.5 to 21 days, with a median of 9 days after beginning treatment (Fig. 4). The clinical response was excellent during the first 6 months of treatment, and 15 of the 20 patients were determined to have shown marked improvement at the end of this period (Table 10). Many of the skin lesions disappeared in the course of the first 6 months of treatment, particularly nodules, plaques, and histoid lesions which regressed and flattened during the first 2 weeks of treatment. It was noted that improvement was not so rapid during the second and third 6-month periods of treatment and because the lesions were no longer distinctive, further improvement was difficult to discern. The MI fell rapidly, usually reaching baseline within the first 4 weeks of treatment (Table 11); however, the BI fell more slowly, decreasing by about one unit during the first year of treatment. As shown in Table 12, erythema nodosum leprosum (ENL) was as severe during the first 6 months of treatment as it had been prior to treatment with R-76-1, but it appeared to become more severe during the second and third 6-month periods of treatment. The drug appeared to be well tolerated throughout the 18 months of treatment. Jaundice and an elevation of serum glutamic pyruvate transaminase (SGPT) were observed in two cases at the third and 12th months of treatment, respectively. Because there was an outbreak of infectious hepatitis in that area, and because the two patients did not show any sign or symptom of liver injury when they resumed the R-76-1 treatment after the jaundice disappeared and liver function returned to normal, the hepatitis could not be definitely attributed to R-76-1.

DISCUSSION

Except for RMP, only a few studies of the activity of ansamycins against *M. leprae* in the mouse foot pad have been reported

TABLE 8. *Growth of* M. leprae *in mice administered various doses of rifampin (RMP) or DL 473 every 4 weeks.*

Dosage (mg/kg)	RMP	DL 473
10	1/10	0/8
5	1/10	
2.5	3/10	0/10
1.25	7/7	0/10
0.625		1/9

TABLE 9. *Proportional bactericidal test of rifampin (RMP) and DL 473 against* M. leprae.

Drug regimen		*M. leprae* per food pad Inoculum size				MPN[a] of viable *M. leprae*	% Survival	Log ID_{50}
		10^4	10^3	10^2	10^1			
Controls		14/14	12/12	3/12	0/16[b]	35.5	–	2.25 ± 0.13
1/4 weeks × 4 (mg/kg)								
20	RMP	0/7	0/9			<0.13	<0.4	>4.36 ± 0.14
10	RMP	1/9	0/8			0.11	0.3	4.39 ± 0.11
	DL 473	0/11	0/7			<0.09	<0.2	>4.41 ± 0.09
5	RMP	4/10	1/9			0.57	1.6	3.99 ± 0.20
	DL 473	0/10	0/10			<0.10	<0.3	>4.40 ± 0.10
2.5	DL 473	0/10	0/10			<0.10	<0.3	>4.40 ± 0.10
1.25	DL 473	1/9	0/8			0.11	0.3	4.39 ± 0.11
0.625	DL 473	2/8	0/9			0.25	0.7	4.25 ± 0.16
Single dose (mg/kg) 30 days after infection								
40	RMP	0/10	0/9			<0.10	<0.3	>4.40 ± 0.10
20	RMP	2/7	0/10			0.28	0.8	4.21 ± 0.18
	DL 473	1/9	0/8			0.11	0.3	4.39 ± 0.11
10	RMP	4/8	1/8			0.76	2.1	3.87 ± 0.23 [c]
	DL 473	1/10	0/9			0.10	0.3	4.40 ± 0.10 [c]
5	RMP	9/10	6/8			4.15–5.81	11.7–16.4	2.60–2.85[d] [c]
	DL 473	4/12	3/8			0.66–0.96	1.9–2.7	3.54–3.79 [c]
2.5	DL 473	9/12	2/9			1.5–2.0	4.2–5.6	3.28–3.53
1.25	DL 473	9/10	3/7			2.81–4.04	7.9–11.4	2.92–3.17

[a] Most probable number of viable *M. leprae* per 10^4 inoculated.
[b] Number of mice showing growth of *M. leprae*/number of mice harvested.
[c] Significantly different ($p < 0.05$).
[d] Not significantly different from the results in control mice.

TABLE 10. *Clinical assessment after treatment with R-76-1, 150 mg daily.*

Case no.	Disease type	Duration of treatment (mo.)		
		0–6	7–12	13–18
1	LL	I[a]	I	I
2	LL	M	I	I
3	BL[b]	I	I	N
4	LL	M	I	I
5	LL[b]	M	I	I
6	LL	I	I	N
7	LL[b]	M		
8	LL[b]	M		
9	LL[b]	M		
10	LL[b]	M		
11	BL	M	I	
12	LL	M	M	
13	LL	M	M	
14	LL	M	M	
15	BL	M	I	
16	BL[b]	I	I	
17	LL[b]	M	I	
18	LL[b]	M	M	
19	BL	M		

[a] I = improved; M = markedly improved; N = no change.
[b] Accompanied by histoid lesions.

([12, 13, 19, 20]). For the ansamycins, the initial screening system described in this report appeared useful. For the three compounds for which we now present data, activity *in vitro* against a number of cultivable mycobacteria and *in vivo* against MLM correlated well with the activity of these compounds against *M. leprae* in the mouse foot pad.

In vitro, R-76-1 was found to have consistently lower MICs than RMP and, *in vivo,* R-76-1 demonstrated more potent bactericidal activity than RMP against both MLM and *M. leprae.* Because the half-time of elimination (17.1 hr) of R-76-1 from the serum ($T_{1/2}$) of mice is twice that of RMP (8.1 hr) ([18]), it appears likely that the greater effectiveness of R-76-1 *in vivo* is the result of both its greater antimicrobial potency and its longer-maintained effective serum concentration.

The activity of DL 473 against *M. leprae* has been studied by Pattyn, *et al.* ([19, 20]), who, despite having used a different dosage

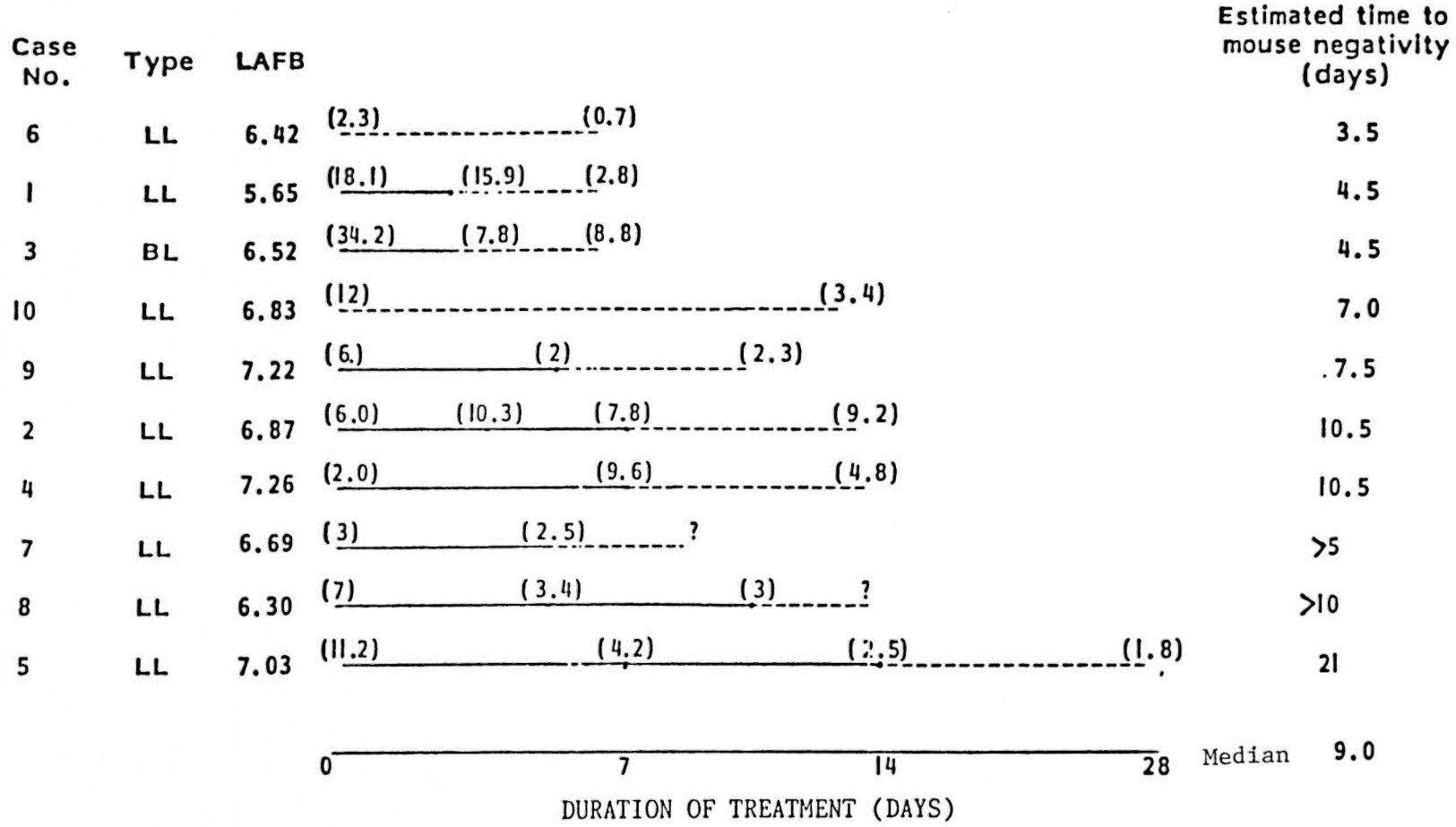


FIG. 4. Rate of loss of infectivity of *M. leprae* for the mouse foot pad during treatment with R-76-1, 150 mg daily. LAFB = $\log_{10}$ of the number of *M. leprae* per mg of tissue in the pretreatment biopsy-specimen. Figures enclosed in parentheses are the MIs measured in the specimens; (——) = time between beginning of treatment and the last positive specimen; (– – –) = time between the last positive and the first negative specimen.

TABLE 11. *Effect of treatment with R-76-1, 150 mg daily, on the bacterial index (BI) and morphological index (MI) of patients with lepromatous leprosy.*

Treatment period	BI (mean ± S.D.)	MI (mean ± S.D.)
0	4.66 ± 0.85	12.9 ± 6.7
7 days	4.51 ± 0.80	7.8 ± 3.8
14 days	4.16 ± 1.35	5.8 ± 3.4
30 days	4.26 ± 0.82	3.5 ± 2.1
2 mos.	4.16 ± 0.86	1.5 ± 1.0
3 mos.	4.13 ± 0.77	0.9 ± 0.8
6 mos.	3.82 ± 1.05	0.2 ± 0.3
12 mos.	3.73 ± 1.10	0
18 mos.	3.30 ± 0.87	0

TABLE 12. *Severity of erythema nodosum leprosum (ENL) before and after treatment with R-76-1.*

Case no.	Before treatment	During treatment (mo.)		
		0–6	7–12	13–16
1	0	0	+	0
2	0	0	+	0
3	+	++	+	++
4	+	+	++	++
5	0	0	0	0
6	+	+	++	++
7	0	0		
8	0	0		
9	++	+		
10	0	0		
11	0	0	+	
12	0	0	0	
13	0	0	0	
14	0	0	+	
15	+	+	+	
16	+	++	++	
17	0	0	++	
18	0	0	0	
19	0	0		
20	0	++		

schedule, reported approximately the same values for the concentration of RMP and DL 473 required to prevent multiplication in half of the mice. On the other hand, these authors reported that RMP in a single dose of 10 mg/kg body weight was inactive, whereas we found that this dose killed 97.9% of the *M. leprae.* Moreover, they found DL 473, in single doses of 2.5 mg/kg and 5 mg/kg, more active than reported here. The reasons for these differences are not obvious; however, the results from studies in both laboratories indicate that DL 473 is more active than RMP *in vivo.*

DL 473 is eliminated by experimental animals ([2, 3, 18]) and man ([4]) more slowly than is RMP; its half-life in mice is more than 30 hr, which is almost five times longer than that of RMP ([2, 18]). In man, its half-life is 19.9 hr after a dose of 8 mg/kg ([4]). Moreover, 24 hr after the dose was administered, tissue concentrations of DL 473 found in animals were 40 times those of RMP ([3]). Thus, the slightly greater activity of DL 473 *in vitro,* together with its much more favorable pharmacokinetic properties, suggests that the latter accounts for its greater effectiveness against *M. leprae in vivo.*

The clinical trial of R-76-1 demonstrated that the drug, administered in a daily dose of 150 mg, was very effective in lepromatous leprosy. We had chosen this relatively low dosage, one third to one quarter of the dose routinely employed for RMP, for two reasons. Animal studies had indicated that R-76-1 was as active as RMP administered in a dosage threefold greater. Moreover, RMP administered in a dosage of 600 mg daily is so rapidly effective in man ([1]) that an alternative drug would be interesting only if it were active in a considerably smaller dosage.

In retrospect, there were several defects in the design of the clinical trial of R-76-1. First, the duration of the monotherapy was unnecessarily long, risking the selection of drug-resistant mutants. Second, the relationship between the dosage of RMP and the peak serum level is nonlinear because of the need to saturate the biliary excretory mechanism ([1]); thus, even if the bactericidal activity of RMP in a daily dose of 150 mg had been shown to be inferior to that of R-76-1 in the same dosage, this would not have confirmed the greater potency of the latter compound. Finally, the comparison between RMP and R-76-1 should have been carried out in a trial in which immunosuppressed mice (thymectomized-irradiated or congenitally athymic) were inoculated with larger numbers of *M. leprae* in order to detect smaller proportions of viable organisms.

Recently, two new ansamycins have been shown to have potential for application to the chemotherapy of leprosy. LM 427 (spiropiperidylrifamycin SV, rifabutin) may be

effective against *M. leprae* resistant to RMP ([12, 13]), and FCE 22250 (piperidinomethylazinomethylrifamycin SV), which has been shown *in vitro* to possess an antibacterial spectrum and MIC against *M. tuberculosis* similar to that of RMP, is, like DL 473, eliminated by mice much more slowly ($T_{1/2}$ = 19 hours) than RMP ([17]). These two compounds, together with those described in this report, should be further studied in animals—e.g., their activity against strains of *M. leprae* resistant to RMP, and the ability of single doses to eradicate infection with *M. leprae* in immunocompetent and immunosuppressed mice.

SUMMARY

The antimycobacterial activities of two newer ansamycins, isobutylpiperazinylrifamycin SV (R-76-1) and cyclopentylrifamycin SV (DL 473), were compared with those of rifampin (RMP) both *in vitro* and *in vivo*. In terms of minimal inhibitory concentrations against a number of cultivable mycobacteria, R-76-1 was about eight times more active *in vitro* than RMP; whereas DL 473 was only slightly more active than RMP. Therapeutic activities of R-76-1 versus RMP and DL 473 versus RMP were compared, respectively, in the experimental infection of mice with *Mycobacterium lepraemurium* by different treatment schedules (immediate and delayed) and dosage regimens. R-76-1 appeared to have been three times more effective than RMP; DL 473 was also more effective than RMP in that an equivalent therapeutic effect could be obtained by fewer doses of DL 473 than of RMP, and in that DL 473 exerted a more prolonged activity than RMP. With the kinetic method and a dosage of 0.001% in the diet, R-76-1 demonstrated a bactericidal-type effect against *M. leprae* whereas RMP did not; with the proportional bactericidal method, R-76-1 possessed about three times the bactericidal activity of RMP against *M. leprae*. When drugs were administered once in 4 weeks, the RMP dose required to prevent multiplication of *M. leprae* in the foot pads of half of the mice was in the range of 1.25 to 2.5 mg/kg; whereas that of DL 473 was less than 0.625 mg/kg. With the proportional bactericidal method, even a single dose of 1.25 mg DL 473 per kg was active against *M. leprae*; whereas the smallest single active dose of RMP was 10 mg/kg. DL 473 in single doses of 5 mg/kg and 10 mg/kg was significantly more effective than RMP in equal doses and, among the intermittent regimens administered four times, once every 4 weeks, no significant differences of bactericidal activity were observed between RMP at 20 mg/kg and DL 473 at 0.625 mg/kg. A preliminary clinical trial of R-76-1 in 20 patients with lepromatous leprosy showed that the compound, administered in a dosage of 150 mg daily, was very effective.

RESUMEN

Se compararon las actividades antimicobacterianas de dos nuevas ansamicinas, la isobutilpiperazinilrifamicina SV (R-76-1) y la ciclopentilrifamicina SV (DL 473), con aquellas de la rifampina (RMP), tanto *in vitro* como *in vivo*. En términos de las mínimas concentraciones inhibitorias contra diversas micobacterias cultivables, la R-76-1 fue casi 8 veces más activa *in vitro* que la RMP en tanto que la DL 473 fue sólo ligeramente más activa que la RMP. También se compararon las actividades terapéuticas de R-76-1 contra la RMP y de DL 473 contra la RMP, en la infección experimental del ratón con *Mycobacterium lepraemurium*, bajo diferentes esquemas de tratamiento. La R-76-1 pareció ser 3 veces más efectiva que la RMP; la DL 473 también fue más efectiva que la RMP ya que se obtuvo un efecto terapéutico equivalente usando dosis menores de DL 473 que de RMP, además de que el efecto de la DL 473 fue más prolongado. Con el método cinético y a una dosis de 0.001% en la dieta, la R-76-1 mostró un efecto bactericida contra el *M. leprae* mientras que ésto no ocurrió con la RMP; con el método bactericida proporcional, la R-76-1 presentó casi 3 veces más actividad bactericida que la RMP contra el *M. leprae*. Cuando las drogas se administraron una vez en 4 semanas, la dosis de RMP requerida para prevenir la multiplicación del *M. leprae* en el cojinete plantar de la mitad de los ratones, estuvo en el rango de 1.25 a 2.5 mg/kg, mientras que con la DL 473 tal dosis fue menor de 0.625 mg/kg. Con el método bactericida proporcional, la DL 473 fue activa contra el *M. leprae* aún a dosis de 1.25 mg/kg, mientras que la mínima dosis efectiva de RMP fue de 10 mg/kg. La DL 473 en dosis únicas de 5 mg/kg y 10 mg/kg fue significativamente más efectiva que la RMP en dosis iguales. Con los programas de administración intermitentes, una vez cada 4 semanas por 4 ocaciones, no se observaron diferencias significativas de la actividad bactericida entre la RMP a 20 mg/kg y la DL 473 a 0.625 mg/kg. Un ensayo clínico preliminar de la R-76-1 en 20 pacientes con lepra lepromatosa mostró que el compuesto administrado en una dosis de 150 mg diarios fue muy efectivo.

RÉSUMÉ

On a comparé, *in vitro* et *in vivo,* les activités antimycobatériennes de deux nouvelles ansamycines, l'isobutylpiperazinylrifamycine SV (R-76-1) et la cyclopentylrifamycine SV (DL 473), avec celle de la rifampine (RMP). Quand on considère les concentrations minimales requises pour l'inhibition d'un certain nombre de mycobactéries cultivables, R-76-1 était environ huit fois plus actif *in vitro* que la rifampine (RMP). Par contre, DL 473 n'était que légèrement plus actif que la rifampine. On a comparé également les activités thérapeutiques de R-76-1 par rapport à RMP, et de DL 473 par rapport à RMP, dans l'infection expérimentale de la souris par *Mycobacterium lepraemurium.* A cet effet, différentes posologies et schémas de traitement (immédiat et retardé), ont été utilisés. Il est apparu que R-76-1 était trois fois plus actif que la rifampine (RMP); DL 473 était également plus efficace que RMP, car un effet thérapeutique équivalent pouvait être obtenu à la suite de l'administration de doses plus faibles de DL 473 que de RMP; de plus, DL 473 témoignait d'une activité plus prolongée que RMP. Par la méthode cinétique, à un dosage de 0,001% dans la ration, on a démontré que R-76-1 possédait une action de type bactéricide contre *M. leprae,* alors que cette action faisait défaut avec RMP. La méthode bactéricide proportionnelle a montré que R-76-1 était dotée d'une activité bactéricide contre *M. leprae* trois fois plus élevée que RMP. Lorsque les médicaments étaient administrés une fois toute les quatre semaines, la dose requise de RMP pour empêcher la multiplication de *M. leprae* dans les coussinets plantaires de la moitié des souris, se situait entre 1,25 et 2,5 mg/kg; par contre, les doses de DL 473 nécessaires pour obtenir le même effet étaient inférieures à 0,625 mg/kg. Avec cette même méthode bactéricide proportionnelle, on a pu démontrer que même une dose unique de 1,25 mg de DL 473 par kg était active contre *M. leprae*; alors que la dose active unique la plus faible de rifampine était de 10 mg/kg. DL 473 à doses uniques de 5 mg/kg et de 10 mg/kg était significativement plus actif que la rifampine à des doses identiques. En comparant des posologies administrées de façon intermittente toutes les quatre semaines, à quatre reprises successives, il n'a pas été possible de mettre en évidence des différences significatives dans l'activité bactéricide entre RMP à la dose de 20 mg/kg et DL 473 à la dose de 0,625 mg/kg. Des essais cliniques préliminaires de R-76-1 chez 20 malades atteints de lèpre lépromateuse ont montré que ce produit, administré à une dose quotidienne de 150 mg, était très actif.

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