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## In Vitro Activities of 2,2'-Bipyridyl Analogs against *Mycobacterium leprae*

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**In vitro susceptibility of *Mycobacterium leprae* to two bipyridyl analogs was studied by using two biochemical parameters to measure the metabolic activity of the organism. VUF-8514 at 0.16 µg/ml, but not VUF-8842, completely inhibited the metabolic activity of *M. leprae*, and the action was bactericidal. When compared to rifampin (MIC 0.3 µg/ml), VUF-8514 was equally bactericidal against *M. leprae*.**

The World Health Organization estimates that there are 5.5 million cases of leprosy worldwide (18). Even though the World Health Organization first recommended the use of multi-drug therapy with dapsone, clofazimine, and rifampin in 1981 (21), *Mycobacterium leprae* resistance to dapsone and rifampin is still widely encountered and is of considerable concern (15). Thus, there is an urgent need for new antimycobacterial agents to treat leprosy (1, 14). De Zwart and associates (3–5) have reported a new class of compounds, 2,2'-bipyridyl analogs, exhibiting potent antimycoplasmal properties. Subsequently, in preliminary studies, Timmerman and coworkers (20) have demonstrated antimycobacterial activities of these analogs, and Seydel and associates (19) have studied relationship between inhibitory activities of these analogs and membrane composition of various bacteria and fungi. Recently, we have demonstrated the bactericidal activities of these analogs against several clinical isolates of *M. avium* and *M. tuberculosis* (16).

The aim of the present study was to evaluate the activities of these analogs, in comparison to that of rifampin, against *M. leprae* in an in vitro culture system.

**Antimicrobial agents.** The 2,2'-bipyridyl analogs, VUF-8514 (isoquinoline derivative) and VUF-8842 (1,10-phenanthroline derivative) were obtained from H. Timmerman of Free University, Amsterdam, The Netherlands. The structural formulae and chemistry of these compounds have been described earlier (3–5). Stock solutions were prepared in dimethyl sulfoxide and then appropriately diluted in DH medium. Rifampin (Sigma Chemical Co., St. Louis, Mo.) was used as a standard bactericidal anti-leprosy agent.

**Isolation of *M. leprae*.** *M. leprae* cells were harvested from livers of nine-banded armadillos (*Dasyurus novemcinctus*), inoculated earlier with human- or armadillo-derived *M. leprae*. *M. leprae* suspension was purified with DNase and Percoll gradient (8). Cell counts were determined microscopically by the pinhead method (8) and inoculated into DH medium to obtain 10<sup>7</sup> cells per ml of medium.

**Growth medium.** The culture medium (DH medium) and conditions used for the maintenance and growth of *M. leprae* were the same as described earlier (12).

**Measurement of growth.** Two biochemical parameters were used to measure the metabolism (and growth) of *M. leprae*—

intracellular ATP content of *M. leprae* and uptake of [<sup>3</sup>H]thymidine by *M. leprae* cells (7). ATP determinations were carried out by the firefly bioluminescent technique described earlier (8), while the procedure of Khanolkar and coworkers (17) was followed for the measurement of thymidine uptake.

The effect of drug on *M. leprae* in primary culture was evaluated after incubating cultures for 4 weeks at 34°C. To determine if the effects were bacteriostatic or bactericidal, the cells from 4-week-old primary cultures were washed twice with fresh DH medium and then suspended in fresh DH medium without the drug and incubated at 34°C for four more weeks. At this time, ATP and [<sup>3</sup>H]thymidine uptake assays were again performed.

Sampling for the assays from primary and subcultures was done as described previously. The results presented here were derived from three separate experiments, using different *M. leprae* strain in each experiment. In each experiment, triplicate assays were done for each concentration of every drug including rifampin.

**Susceptibility of *M. leprae* to bipyridyls.** In the control cultures (without drugs), metabolic activity of *M. leprae* in primary cultures at 4 weeks was 143% of that at zero hour (Table 1). When the cells from these primary cultures were transferred to fresh DH medium, the activity in subcultures was 134% of the original at zero hour of these subcultures (143% increase in primary cultures was adjusted to 100% at zero hour for subcultures). With VUF-8514 at concentrations of 0.08 µg/ml and below, the metabolic activity of *M. leprae* in primary cultures as well as in subcultures was the same in control cultures. However, when the concentration of VUF-8514 in primary cultures was 0.16 µg/ml and higher, no metabolic activity could be detected, suggesting the MIC of VUF-8514 against *M. leprae* was 0.16 µg/ml. When these cells from primary cultures were transferred to fresh drug-free DH medium, cells failed to exhibit any metabolic activity, even after 4 weeks. This suggests the bactericidal activity of VUF-8514 on *M. leprae*. On the other hand, VUF-8842 had no inhibitory effect on the metabolic activity of *M. leprae* even at 0.32 µg/ml—the highest concentration tested to compare against rifampin, whose MIC against *M. leprae* has been shown to be 0.3 µg/ml (10). In subsequent studies, no growth inhibition of *M. leprae* was observed even when the concentration of VUF-8842 in the DH medium was raised up to 2.56 µg/ml.

Thus, the results in the present study clearly indicate that VUF-8514, at 0.16 µg/ml, completely inhibits the metabolic activity of *M. leprae*. It has been demonstrated previously with *M. lepraemurium* (9) and *M. tuberculosis* (2) that increases in

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TABLE 1. Effect of 2,2'-bipyridyl analogs on metabolic activity (in vitro growth) of *M. leprae* in DH medium

Agent and concn ( $\mu\text{g/ml}$ )	Metabolic activity of <i>M. leprae</i>			
	In primary cultures at 4 wk <sup>a</sup>		In subcultures at 4 wk	
	ATP <sup>b</sup>	[ <sup>3</sup> H]thymidine <sup>c</sup>	ATP	[ <sup>3</sup> H]thymidine
None	307 $\pm$ 46	0.90 $\pm$ 0.12	399 $\pm$ 44	1.24 $\pm$ 0.14
VUF-8514				
0.01	314 $\pm$ 44	0.93 $\pm$ 0.11	430 $\pm$ 56	1.35 $\pm$ 0.15
0.02	331 $\pm$ 39	0.95 $\pm$ 0.10	470 $\pm$ 61	1.46 $\pm$ 0.19
0.04	340 $\pm$ 44	0.98 $\pm$ 0.14	496 $\pm$ 54	1.49 $\pm$ 0.18
0.08	309 $\pm$ 43	0.90 $\pm$ 0.13	408 $\pm$ 57	1.26 $\pm$ 0.16
0.16 <sup>d</sup>	0 <sup>e</sup>	0	0	0
0.32	0	0	0	0
VUF-8842				
0.01	309 $\pm$ 40	0.90 $\pm$ 0.11	402 $\pm$ 48	1.21 $\pm$ 0.13
0.02	336 $\pm$ 44	0.98 $\pm$ 0.12	470 $\pm$ 65	1.45 $\pm$ 0.20
0.04	323 $\pm$ 35	0.93 $\pm$ 0.14	446 $\pm$ 49	1.34 $\pm$ 0.20
0.08	303 $\pm$ 45	0.88 $\pm$ 0.12	373 $\pm$ 44	1.17 $\pm$ 0.13
0.16	327 $\pm$ 46	0.95 $\pm$ 0.12	445 $\pm$ 58	1.35 $\pm$ 0.17
0.32	316 $\pm$ 47	0.91 $\pm$ 0.14	441 $\pm$ 62	1.26 $\pm$ 0.15
Rifampin				
0.1	285 $\pm$ 40	0.86 $\pm$ 0.09	353 $\pm$ 46	1.31 $\pm$ 0.16
0.2	199 $\pm$ 22	0.62 $\pm$ 0.09	221 $\pm$ 29	0.79 $\pm$ 0.09
0.3 <sup>d</sup>	0	0	0	0

<sup>a</sup> Zero hour values: ATP, 221  $\pm$  26 pg/10<sup>7</sup> cells; [<sup>3</sup>H]thymidine, 0.61  $\pm$  0.08 pmol/5  $\times$  10<sup>7</sup> cells.

<sup>b</sup> ATP: pg/10<sup>7</sup> cells.

<sup>c</sup> [<sup>3</sup>H]thymidine: pmol/5  $\times$  10<sup>7</sup> cells.

<sup>d</sup> MIC of each compound.

<sup>e</sup> 0 indicates no metabolic activity.

intracellular ATP values parallel microscopic counts and viable colony-forming unit counts, respectively, during the in vitro growth of these organisms. Recently, we have also observed parallelism between ATP levels, [<sup>3</sup>H]thymidine uptake values, microscopic counts, and phenolic glycolipid (PGL-1) levels of *M. leprae* cells incubated in DH medium. Using this system, we have evaluated anti-leprosy activities of several compounds, and the in vitro susceptibility results were confirmed by the established mouse footpad system (10, 11, 13). On the basis of these results, it is safe to suggest that the increase in metabolic activity of *M. leprae* observed in present studies is an indication of increase in cell numbers in primary cultures, as seen in control cultures with no added drugs. Similar increases in metabolic activity of *M. leprae* were also observed in cultures containing VUF-8842 as well as in cultures containing lower concentrations of VUF-8514 and rifampin, implying normal growth of *M. leprae* and thus, no growth-inhibitory effects. On the other hand, when exposed to 0.16  $\mu\text{g}$  of VUF-8514 per ml or 0.3  $\mu\text{g}$  of rifampin per ml, *M. leprae* lost all metabolic activity and thus its ability to multiply in such an environment.

One of the basic concepts of multidrug therapy for leprosy is that the treatment be administered for a short period of time to accomplish excellent compliance and avoid developing resistance. For this purpose, it is recommended to include only bactericidal drugs in the multidrug treatment regimen (16) so that each drug will kill *M. leprae* resistant to another drug. VUF-8514 has been shown here to be bactericidal and inhibits the metabolic activity of *M. leprae* at a concentration which is equal to or even lower than that of rifampin when used to achieve similar results. Thus, it seems VUF-8514 will be a good candidate for inclusion in the multidrug regimen in the

treatment of leprosy and should be evaluated further for its pharmacokinetics and toxicity, etc.

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