Lepr Rev (1991) **62,** 280–287

The activity of rifabutin against *Mycobacterium leprae*

L J YODER, R R JACOBSON & R C HASTINGS
Clinical Branch and Laboratory Research Branch, Gillis W. Long Hansen's Disease Center, Carville, Louisiana 70721, USA

Accepted for publication 1 March 1991

Summary Minimal effective doses of rifabutin and rifampicin were determined in *Mycobacterium leprae* isolated from skin biopsies of newly diagnosed, previously untreated lepromatous leprosy patients. Rifabutin was more potent than rifampicin. Our previous report that rifabutin was fully active against rifampicin-resistant *M. leprae* could not be confirmed. Examination of two strains of rifampicin-resistant *M. leprae* from elsewhere, and a repeat experiment on our original strain of rifampicin-resistant bacilli, showed full cross-resistance between rifampicin and rifabutin. A clinical trial in three newly diagnosed, previously untreated lepromatous patients showed that rifabutin has rapid bactericidal activity.

Introduction

Several members of the ansamycin group of antimicrobials have been found to be very active against *M. leprae*.[1–3] Rifampicin is the best known of this group and considerable experience in the use of this drug for the treatment of leprosy has accumulated in the past 15 years.[4] It is the only one of the antileprosy drugs currently available that is rapidly bactericidal for *M. leprae* and it is now considered a standard drug worldwide for the treatment of all types of leprosy in combination with other drugs.[5]

Rifabutin (LM427) is a newer member of the ansamycin group whose activity against *M. leprae* has been demonstrated in the mouse footpad and whose minimal effective dose is several times lower than that of rifampicin.[6] Rifabutin has been used extensively to treat *M. avium-intracellulare* infections in patients with acquired immune deficiency syndrome and it appears to be safe and well tolerated.[7]

We have extended our earlier studies of the minimal effective dose of rifabutin in mouse footpad infections with *M. leprae*,[1] further studied the activity of rifabutin against rifampicin-resistant *M. leprae* in mouse footpads,[6] and we undertook a small clinical trial of rifabutin to measure the rapidity of onset of its bactericidal activity against *M. leprae*.

0305-7518/91/062280+08 $01.00 © Lepra

Materials and methods

DETERMINATIONS OF MINIMAL EFFECTIVE DOSES

Seven consecutive skin biopsies from patients with untreated lepromatous leprosy with a bacterial index (BI) of 4+ or greater and a morphological index (MI) of 1% or greater which were received for routine drug sensitivity studies were used for mouse footpad inoculations. The methods for inoculation, harvest, and bacterial enumerations were those of Shepard[8] and Shepard & McRae.[9] Dapsone (Sigma Chemical Co., St Louis, MO), clofazimine (Geigy, Buffalo Grove, IL), rifampicin (Sigma), and rifabutin (Farmitalia Carbo Erba, Milan, Italy) were mixed in powdered mouse food (Purina Mills, St Louis, MO) at the concentrations indicated and fed continuously. Diets were prepared fresh at weekly intervals and stored at 0–4°C. BALB/c mice were locally bred from breeding stock (Harlan Sprague Dawley, Indianapolis, IN, or Charles Rivers Breeding Labs, Wilmington, MA, or Bantin and Kingman, Fremont, CA), The animals had access to water *ad libitum*.

RIFABUTIN ACTIVITY AGAINST RIFAMPICIN-RESISTANT STRAINS OF *M. LEPRAE*

The activity of rifabutin was tested in continuous feeding experiments on three different occasions against rifampicin-resistant bacilli. In 1983 rifabutin was tested against a mouse footpad passaged strain of *M. leprae* (#3026) isolated originally in 1977 from a patient with rifampicin-resistant disease.[6] Additional mouse footpad passaged, rifampicin-resistant strains were shipped at 0–4°C as amputated mouse footpads from Paris to Carville from the laboratories of Drs Grosset and Guelpa-Lauras.[10] These strains grew in mice given rifampicin 10 mg/kg by gavage at weekly intervals. Finally the Carville passaged strain (#3026) was retested in 1986. The Carville strain had been passaged in mice continuously fed 0·01% w/w dietary rifampicin. We have tested well over 250 isolates of *M. leprae* from leprosy patients' skin biopsies for drug sensitivities in mouse footpad infections. With the exception of the cases of rifampicin resistance which we have reported,[6,11] all these isolates were completely inhibited by 0·01% dietary rifampicin prepared and administered as outlined above.

CLINICAL BACTERICIDAL ACTIVITY

After giving informed consent three male patients aged from 21 to 49 years with borderline lepromatous or lepromatous leprosy entered the study. All three patients had a BI of at least 4+ and a MI of 1% or more. All were new, previously untreated cases. The patients were hospitalized and all medications were given under supervision. Routine haematological studies, blood chemistries, and urinalysis were done prior to, and on days 2 and 14 of the study. Each patient had baseline skin smears from 12 sites and a skin biopsy for histopathology. Punch skin biopsies for baseline mouse footpad viability and drug sensitivities were performed and four repeat biopsies for mouse footpad viabilities (growth of bacilli detected within 12 months after a footpad inoculum of 5000 bacilli) were done on days 1, 3–6, 7 and 14 after beginning rifabutin. Baseline drug sensitivities were determined in mice fed 0·01%, 0·001% and 0·0001% dapsone, 0·001% clofazimine, 0·01% rifampicin and 0·001%, 0·003%, 0·0001% and 0·00003% rifabutin.

Two patients were given rifabutin 300 mg daily for 14 days and the third patient was given a single dose of 300 mg on day 1 of the study. The rifabutin doses were given without regard for meals since meals do not appear to affect the drug's absorption.[7]

Results

DETERMINATIONS OF MINIMAL EFFECTIVE DOSE

The results from 7 consecutive isolates of *M. leprae* are summarized in Table 1. The minimal effective dose of rifampicin varied from 0·001% to less than 0·00003% w/w in the diet and that of rifabutin varied from 0·0001% to less than 0·00003%. In the isolates which permit comparison, rifabutin seems to be at least 3-fold more potent than rifampicin in mice.

RIFABUTIN ACTIVITY AGAINST RIFAMPICIN-RESISTANT STRAINS OF *M. LEPRAE*

In 1984 we reported that rifampicin-resistant *M. leprae* strain #3026 grew in mice fed 0·01% dietary rifampicin but no growth was seen in mice receiving rifabutin 0·001% to 0·00003% in the diets.[6] This strain was inoculated into mice on 26 July 1983 for this experiment. The bacilli had been passaged in mice continuously fed 0·01% w/w dietary rifampicin since November 1978. They had been passaged in drug-free mice from January 1977 to November 1978. The details of the results of this experiment in 1983 are given in

Table 1. Minimal effective doses of rifampicin and rifabutin against *M. leprae* in mice. Values are the number of acid-fast bacilli per footpad counted as a pool of four footpads

Treatment (continuous)	Isolate						
	B3651	B3657	SI24	SI26	H59	B3661	B2448
Controls	$6{\cdot}12 \times 10^5$	$7{\cdot}56 \times 10^5$	$7{\cdot}97 \times 10^5$	$6{\cdot}20 \times 10^5$	$6{\cdot}04 \times 10^5$	$1{\cdot}09 \times 10^6$	$6{\cdot}43 \times 10^5$
Dapsone (%)							
0·01	N.G.*	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
0·001	$7{\cdot}77 \times 10^4$	N.G.	N.G.	N.G.	N.G.	N.G.	$5{\cdot}52 \times 10^5$
0·0001	$2{\cdot}87 \times 10^4$	N.G.	N.G.	N.G.	$3{\cdot}60 \times 10^4$	N.G.	$9{\cdot}16 \times 10^5$
Clofazimine (%)							
0·001	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
Rifampicin (%)							
0·01	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
0·001	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
0·0001	$1{\cdot}44 \times 10^5$	$2{\cdot}00 \times 10^4$	N.G.	N.G.	$5{\cdot}44 \times 10^5$	N.G.	N.G.
0·000,03	$1{\cdot}76 \times 10^5$	$1{\cdot}60 \times 10^4$	N.G.	N.G.	$8{\cdot}58 \times 10^5$	N.G.	N.G.
Rifabutin (%)							
0·001	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
0·0003	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
0·0001	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.	N.G.
0·000,03	$7{\cdot}75 \times 10^5$	N.G.	N.G.	N.G.	$6{\cdot}72 \times 10^5$	N.G.	N.G.

* N.G. = No growth detected.

Table 2. The activity of rifabutin against rifampicin-resistant *M. leprae* strain No. 3026 inoculated in 1983*[6]

Treatment (continuous)	No.+/ Total	AFB/Footpad ($\times \pm$SEM)‡
Controls	6/6	$8{\cdot}34\ (\pm 0{\cdot}65) \times 10^5$
Rifampicin (%)		
0·01	6/6	$1{\cdot}76\ (\pm 0{\cdot}46) \times 10^5$
0·001	2/5	$1{\cdot}92\ (\pm 1{\cdot}27) \times 10^5$
0·0001	5/6	$3{\cdot}07\ (\pm 0{\cdot}87) \times 10^5$
0·000,03	5/6	$1{\cdot}89\ (\pm 0{\cdot}16) \times 10^5$
Rifabutin (%)		
0·001	0/6	$< 1{\cdot}6 \times 10^4$
0·0003	0/6	$< 1{\cdot}6 \times 10^4$
0·0001	0/6	$< 1{\cdot}6 \times 10^4$
0·000,03	0/6	$< 1{\cdot}6 \times 10^4$

* Inoculated 7/26/83, harvested 2/10/84.
‡ Means of positive footpads only.

Table 3. The activity of rifabutin against rifampicin-resistant *M. leprae* strains No. 83004 and No. 82026 from Drs Grosset and Guelpa-Lauras

Treatment (continuous)	Isolate			
	No. 83004*		No. 82026†	
	No.+/Total	AFB/Footpad ($\times \pm$SEM)‡	No.+/Total	AFB/Footpad ($\times \pm$SEM)
Controls	5/6	$2{\cdot}39\ (\pm 0{\cdot}46) \times 10^5$	5/6	$6{\cdot}18\ (\pm 2{\cdot}16) \times 10^5$
Dapsone				
0·01	0/6	$< 1{\cdot}6 \times 10^4$	0/6	$< 1{\cdot}6 \times 10^4$
0·001	6/6	$2{\cdot}59\ (\pm 0{\cdot}57) \times 10^5$	6/6	$1{\cdot}81\ (\pm 0{\cdot}79) \times 10^5$
0·0001	6/6	$5{\cdot}94\ (\pm 0{\cdot}95) \times 10^5$	6/6	$7{\cdot}34\ (\pm 1{\cdot}29) \times 10^5$
Clofazimine (%)				
0·001	0/6	$< 1{\cdot}6 \times 10^4$	0/6	$< 1{\cdot}6 \times 10^4$
Rifampicin (%)				
0·01	6/6	$9{\cdot}53\ (\pm 2{\cdot}72) \times 10^5$	6/6	$1{\cdot}79\ (\pm 0{\cdot}74) \times 10^5$
0·001	4/6	$5{\cdot}26\ (\pm 1{\cdot}93) \times 10^5$	6/6	$1{\cdot}31\ (\pm 0{\cdot}13) \times 10^6$
0·0001	6/6	$9{\cdot}35\ (\pm 2{\cdot}50) \times 10^5$	6/6	$1{\cdot}49\ (\pm 0{\cdot}18) \times 10^6$
0·000,03	6/6	$1{\cdot}05\ (\pm 0{\cdot}22) \times 10^6$	6/6	$2{\cdot}74\ (\pm 0{\cdot}49) \times 10^6$
Rifabutin (%)				
0·001	6/6	$3{\cdot}86\ (\pm 1{\cdot}37) \times 10^5$	6/6	$3{\cdot}71\ (\pm 0{\cdot}86) \times 10^5$
0·0003	6/6	$4{\cdot}58\ (\pm 1{\cdot}34) \times 10^5$	6/6	$2{\cdot}29\ (\pm 0{\cdot}82) \times 10^5$
0·0001	6/6	$8{\cdot}41\ (\pm 1{\cdot}93) \times 10^5$	6/6	$1{\cdot}04\ (\pm 0{\cdot}13) \times 10^6$
0·000,03	6/6	$4{\cdot}10\ (\pm 1{\cdot}51) \times 10^5$	6/6	$1{\cdot}71\ (\pm 0{\cdot}17) \times 10^6$

* Inoculated 3 February 1986, harvested 19 November 1986.
† Inoculated 3 February 1986, harvested 8 January 1987.
‡ Means of positive footpads only.

Table 2. Our interpretation of these results was that rifabutin was fully active against this rifampicin-resistant strain of *M. leprae*.

On 28 August 1985 we received two *M. leprae* strains from Drs Grosset and Guelpa-Lauras which had been found to be rifampicin-resistant in their laboratory.[10] Unfortunately these two strains (#82061 and #82073) did not grow in our control mice, presumably due to loss of viability during shipment. A further shipment was received on 30 January 1986. These strains (#83004 and #82026) were viable and were tested against various doses of dapsone, clofazimine, rifampicin, and rifabutin. The results are presented in Table 3. Clearly there was full cross-resistance between rifampicin and rifabutin in these two strains of *M. leprae*.

We then re-examined our strain #3026 which had remained in passage in mice continuously fed 0·01% w/w dietary rifampicin. The mice were inoculated on 1 December 1986 when the results with strain #83004 became available, and the experiment of 26 July 1983 was repeated. The results are given in Table 4. In contrast to the 1983 results, rifabutin now showed no activity in doses up to 0·001% w/w dietary concentrations against this rifampicin-resistant strain of *M. leprae*.

CLINICAL BACTERICIDAL ACTIVITY

The results of the clinical study are summarized in Table 5. The sensitivity pattern in the pretreatment biopsy in patient No. 1 showed full sensitivity to all drugs in all concentrations studied except for growth in the two lowest rifabutin levels of 0·0001% and 0·00003%. Patients No. 2 and No. 3 showed full sensitivities to all drugs in all concentrations tested. In patient No. 1, inoculation of 5000 bacilli became noninfectious for mice 4–7 days after starting rifabutin. In patients No. 2 and No. 3, 5000 bacilli became noninfectious within 24 hr of a single dose of 300 mg of rifabutin.

Table 4. Repeat drug sensitivity studies with Carville No. 3026 strain of rifampicin-resistant *M. leprae* inoculated 1986*

Treatment (continuous)	No. +/Total	AFB/Footpad (x+SEM)†
Controls	6/6	$7{\cdot}33\ (\pm 1{\cdot}70) \times 10^5$
Rifampicin (%)		
0·01	6/6	$2{\cdot}29\ (\pm 0{\cdot}38) \times 10^5$
0·001	6/6	$9{\cdot}24\ (\pm 1{\cdot}87) \times 10^5$
0·0001	6/6	$6{\cdot}87\ (\pm 0{\cdot}90) \times 10^5$
0·000,03	4/4	$5{\cdot}88\ (\pm 0{\cdot}74) \times 10^5$
Rifabutin (%)		
0·001	6/6	$1{\cdot}12\ (\pm 0{\cdot}12) \times 10^6$
0·0003	6/6	$7{\cdot}19\ (\pm 1{\cdot}31) \times 10^5$
0·0001	2/2	$5{\cdot}19\ (\pm 3{\cdot}59) \times 10^5$
0·000,03	6/6	$1{\cdot}22\ (\pm 0{\cdot}43) \times 10^6$

* Mice inoculated 1 December 1986, harvested 21 October 1987.

† Means of positive footpads only.

Table 5. Clinical mouse footpad studies

	Patient		
	1	2	3
Rifabutin dose	300 mg daily	300 mg daily	300 mg single dose
Average BI*	4·7	4·0	4·5
Footpad results—AFB/FP†			
Pretreatment	9×10^5	2×10^5	6×10^5
Day 1	5×10^4	0	0
Day 4	6×10^4	0	0
Day 7	0	0	0
Day 14	0	0	0

* BI = Bacterial index.
† AFB/FP = Acid-fast bacilli per footpad.

Discussion

As measured by minimal effective dose determinations in mice, rifabutin is highly active against *M. leprae* and is more potent than rifampicin.

We are unable to offer a satisfactory explanation for our inability to repeat our observation of rifabutin sensitivity of the rifampicin-resistant *M. leprae* isolate No. 3026. The 1983 data (Table 2) appeared satisfactory, although in retrospect the lack of uniform takes or growth at the lower dosage levels of rifampicin are unusual. We have rechecked all records and found no evidence of technical errors. The technical and animal caretaker personnel are very experienced and reliable.

The primary effect of the rifamycins is inhibition of DNA-dependent RNA polymerase. Mutations of a single amino acid in the enzyme diminish or completely abolish the ability of rifamycins to bind to RNA polymerase, resulting in drug-resistant organisms.[12] One could speculate that rifabutin acted on the rifampicin-resistant *M. leprae* isolate No. 3026 at a site other than DNA-dependent RNA polymerase in 1983. There is evidence that rifabutin can inhibit the biosynthesis of DNA in a rifampicin-resistant mutant of *M. tuberculosis* H37Rv.[12] One could further speculate that another mutation occurred between 1983 and 1986 in this strain, resulting in rifabutin resistance at this site of action also. We have no evidence for such a biologic explanation, just as we have no evidence for a technical explanation for these findings.

We undertook a trial of rifabutin in a small number of leprosy patients to measure its bactericidal activity and to compare the results with similar trials previously carried out with rifampicin by Levy *et al.*[13] and the rifampicin trial at Carville in the early 1970s. In these studies, single doses of 600–1500 mg of rifampicin and daily doses of 600 mg and 300 mg were given to newly diagnosed lepromatous or borderline lepromatous patients and a series of skin biopsies were obtained for mouse footpad studies. Single doses of 600 mg of rifampicin or more resulted in loss of growth of 5000 *M. leprae* in almost all of the mouse footpads within 3 to 5 days. Daily rifabutin, in doses of 300 mg, similarly prevented the growth of 5000 of the patients' *M. leprae* within 1 to 7 days.

The results obtained in these 3 patients indicate a level of bactericidal activity of

rifabutin against *M. leprae* which is comparable to that observed with rifampicin. The clinical study is too small to adequately define the role of rifabutin in the treatment of leprosy, but it suggests that rifabutin could have a role similar to rifampicin.

Acknowledgments

We wish to thank Adria Laboratories, Columbus, Ohio, for providing the rifabutin for this clinical study. We are grateful to Vernon B Richard for his expert technical work in the mouse footpad studies, and to Willard Brown and his colleagues for expert animal care. We are indebted to Professor J. Grosset who suggested that we test additional strains of rifampicin-resistant *M. leprae* for susceptibility to rifabutin.

References

[1] Hastings RC, Jacobson RR. Activity of ansamycin against *Mycobacterium leprae* in mice (Letter). *Lancet*, 1983; **2:** 1079–80.
[2] Pattyn SR. A comparison of the bactericidal activity of a series of rifampicins against *Mycobacterium leprae*. *Arzneimittelforschung/Drug Res,* 1982; **32:** 15–17.
[3] Waters MFR, Rees RJW, Pearson JMH, Laing ABG, Helmy HS, Gelber RH. Rifampicin for lepromatous leprosy: Nine years experience. *Br Med J,* 1978; **1:** 133–6.
[4] Bullock WE. Rifampicin in the treatment of leprosy. *Rev Infect Dis,* 1983; **5:** S 606–S 613.
[5] WHO Expert Committee on Leprosy. *Sixth Report*. Technical Report Series No. 768. WHO: Geneva, 1988.
[6] Hastings RC, Richard VR, Jacobson RR. Ansamycin activity against rifampicin-resistant *Mycobacterium leprae* (Letter). *Lancet*, 1984; **1:** 1130.
[7] O'Brien RJ, Lyle MA, Snider DE. Rifabutin (ansamycin LM 427): A new rifamycin-S derivative for the treatment of mycobacterial diseases. *Rev Infect Dis* 1987; **9:** 519–30.
[8] Shepard CC. The experimental disease that follows the injection of human leprosy bacilli into the footpads of mice. *J Exp Med* 1960; **112:** 445–54.
[9] Shepard CC, McRae DH. A method for counting acid-fast bacteria. *Int J Lepr*, 1968; **36:** 78–82.
[10] Guelpa-Lauras CC, Grosset JH, Constant-Desportes M, Brucker G. Nine cases of rifampicin-resistant leprosy. *Int J Lepr,* 1984; **52:** 101–2.
[11] Hastings RC, Jacobson RR. Rifampicin resistant leprosy. *Health Cooperation Papers, Quaderni di cooperazione sanitaria. Associazione Italiana Amici di Raoul Follereau*, 1981; **nl:** 47.
[12] Ungheri D, Della Bruna C, Sanfilippo A. Studies on the mechanism of action of the spiropiperidylrifamycin LM 427 on rifampicin-resistant *M. tuberculosis. Drugs Exptl Clin* Res, 1984; **X**(10): 681–9.
[13] Levy L, Shepard CC, Fasal P. The bactericidal effect of rifampicin on *Mycobacterium leprae* in man: (a) Single doses of 60, 900 and 1200 mg; and (b) Daily doses of 300 mg. *Int J Lepr,* 1976; **44:** 183–7.

L'activite de la rifabutine vis-à-vis de *Mycobacterium leprae*

L J Yoder, R R Jacobson et R C Hastings

Résume Des doses minimales efficaces de rifabutine et rifampicine furent détectées dans de *Mycobacterium leprae* isolé par biopsies cutanées chez des patients non traités, récemment diagnostiqués comme souffrant de lèpre lépromateuse. La rifabutine était plus efficace que la rifampicine. Il n'a pas été possible de confirmer notre rapport précédent sur l'efficacité totale de la rifabutine vis-à-vis de *M. leprae* résistant à rifampicine. L'examen des deux souches de *M. leprae* résistantes à la rifampicine provenant d'une autre source et une seconde expérimentation sur la souche originale de bacilles résistants à la rifampicine a révélé une résistance croisée totale entre la rifampicine et la rifabutine. Un essai clinique effectué sur trois patients non traités et récemment diagnostiqués comme souffrant de lèpre lépromateuse indiquait que l'activité bactéricide de la rifabutine était plus rapide.

La actividad de la rifabutina en contra del *Mycobacterium leprae*

L J Yoder, R R Jacobson y R C Hastings

Resumen Las dosis efectivas mínimas de rifabutinay rifampicina fueron determinadas en *Mycobacterium leprae* aislados de biopsias de piel de pacientes leprosos lepromatosos, no tratados previamente y con diagnóstico reciente. La rifabutina fue más potente que la rifampicina. Nuestro informe previo de que la rifabutina era completamente activa en contra del *M. leprae* resistente a la rifampicina no pudo ser confirmado. El estudio de dos cepas de *M. leprae* resistentes a la rifampicina de otro lugar, y una repetición del experimento en nuestra cepa original de bacilo resistente a la rifampicina, mostró una resistencia cruzada completa entre rifampicina y rifabutina. Un ensayo clínico en tres pacientes lepromatosos no tratodos previamente y con diagnóstico reciente, mostró que la rifabutina tiene una actividad bactericida rápida.