Lepr Rev (2012) **83,** 261–268

OFLOXACIN multicentre trial in MB leprosy FUAM-Manaus and ILSL-Bauru, Brazil

MARIA DA GRAÇA SOUZA CUNHA*,
MARCOS VIRMOND**,
ANTONIO PEDRO SCHETTINI*,
ROSSILENE CONCEIÇÃO CRUZ*, SOMEI URA**,
CASSIO GHUIDELLA***,
FRANCISCO DOS REIS VIANA****,
JOÃO CARLOS REGAZZI AVELLEIRA***** &
ANTÔNIO A.AUGUSTO CAMPOS
BRASIL FILHO******
**Fundação de Dermatologia Tropical Alfredo da Matta (FUAM), Manaus, Amazonas, Brazil*
***Instituto Lauro de Souza Lima (ILSL), Bauru, São Paulo, Brazil*
****Municipal Health Department of Rondonopolis, Mato Grosso, Brazil*
*****Hospital Curupaiti, Rio de Janeiro, Brazil*
******Instituto de Dermatologia Rubem D. Azulay, Rio de Janeiro, Brazil*
*******Universidade para Desenvolvimento do Estado e Região do Pantanal (UNIDERP), Mato Grosso, Brazil*

Accepted for publication 24 September 2012

Summary Recently antimicrobials of the fluoroquinolone class (pefloxacin and ofloxacin) were found far more effective against *Mycobacterium leprae* in studies with both mice and patients than dapsone and clofazimine. As multicentre trial participants, we evaluated the therapeutic efficacy, in terms of rate of relapse, of two new multidrug regimens containing ofloxacin, comparing them to 1 year and 2 years of standard WHO-MDT regimen in multibacillary (MB) leprosy patients. A total of 198 MB patients were recruited to participate in a randomized, double-blind trial. Among those, 53 patients were treated with 1 year of WHO-MDT (a regimen including dapsone, clofazimine, and rifampin), 55 patients received 1 year of WHO-MDT plus an initial 1 month of daily ofloxacin, 63 patients were treated with 1 month of daily rifampin and daily ofloxacin, whereas 27 were treated with 2 years of

Correspondence to: Maria da Graça Cunha, Fundação de Dermatologia Tropical Alfredo da Matta (FUAM), Rua Codajás, 24 Cachoeirinha. 69.056-130 Manaus, Amazonas, Brazil (Tel: +559299855161; e-mail: mcunha@fuam.am.gov.br)

0305-7518/12/064053+08 $1.00

WHO-MDT. Patients were regularly monitored for signs of relapse, in at least 7 years follow-up after being released from treatment.
Results: Relapse occurred in those treated with 1-month regimen alone at a significant higher rate ($P < 0{,}001$): 38,8%, whereas in the other three regimens that included WHO-MDT it ranged from 0 to 5%. This study found that a short-course treatment for MB patients with rifampicin-ofloxacin combination had a higher failure rate. The addition of one month of daily ofloxacin to 12 months MB WHO-MDT did not increase its efficacy.

Introduction

In 1982 the WHO[1] advocated multidrug therapy (MDT) for all forms of leprosy combining three drugs, dapsone, clofazimine, and rifampin; the last drug being the only one bactericidal for *Mycobacterium leprae* in clinical trials.[2–4] Despite the success of the MDT regimens, the duration of MDT treatment should be relatively long to ensure elimination of drug-resistant mutants, especially those resistant to rifampicin, and to reduce the number of viable organisms to undetectable levels. Therefore, one of the needs is to shorten the treatment period mostly for MB leprosy. Mouse and clinical trials with the new fluoroquinolones, pefloxacin and ofloxacin, have shown the high bactericidal activity of these drugs against *M. leprae*. Ofloxacin displayed promising bactericidal activity against *M. leprae* (in the murine model) and produced remarkable clinical and laboratory improvements in LL patients.[5–10] A regimen based on ofloxacin plus rifampin might increase the effectiveness/shorten the duration of chemotherapy. Against this background in 1991, the WHO organised a multicentre initially double-blinded trial of four regimens to treat MB leprosy based on the TDR/THELEP protocol and WHO/CIOMS guidelines. Its main objective was to compare two new multidrug regimens containing ofloxacin with 1 year and 2 years of standard WHO-MDT regimens. This paper deals with the results of two Brazilian Centres participating in the multicentre trial: the Fundação Alfredo da Matta, in Manaus and Instituto Lauro de Souza Lima, in São Paulo.

Objective

To compare the efficacy in terms of rate of relapse, feasibility and tolerance, of two new multidrug regimens containing ofloxacin with 1 year and 2 years of standard WHO-MDT for MB leprosy.

Materials and Methods

The different regimens used were conducted in an initially double-blind trial sponsored by WHO/THELEP with several centres carrying it out through a common protocol. Inclusion criteria was previously untreated MB patients with bacteriological index ([BI], a logarithmic measure of the number of AFB in the dermis) $\geq 2+$ at any site and the period of follow up was at least 7 years after the completion of therapy. As found by Gelber *et al.*,[11] MB leprosy relapse after 2 years of MDT only begins 6 years after the completion of therapy and often much later. Herein we present data on relapse frequencies in MB patients followed up for at least 7 years and at times as many as 12 years after they were released from treatment.

Drug Regimens Tested

a. WHO MDT for 1 year consisting of daily dapsone 100 mg and daily clofazimine 50 mg (unsupervised) and monthly supervised rifampin 600 mg and clofazimine 300 mg (supervised).
b. WHO MDT for 1 year as just described plus an initial month of supervised daily ofloxacin 400 mg.
c. 1 month of daily rifampin 600 mg and daily ofloxacin 400 mg (supervised).
d. 2 years of the WHO MDT regimen.

The regimens are shown diagrammatically on Table 1.

Patients were excluded if they could not be expected to be supervised daily for the first month of the trial, received rifampin or fluoroquinolone previously, were less than 15 years old or more than 65 years of age, were pregnant or breast feeding, had a previous leprosy reaction requiring prolonged steroid therapy, had tuberculosis, severe lung disease, or bronchial asthma requiring theophylline, and had cancer, diabetes, severe hypertension, renal, hepatic or cardiac disease, epilepsy, or any other medical condition that adversely affects compliance to treatment or follow-up.

FOLLOW UP AFTER COMPLETION OF TREATMENT

After completing 1 year of the trial, the study was un-blinded, but only for Regimen D, which was continued for another year. After completion of the scheduled therapy (1 or 2 years), all anti-leprosy treatments were stopped. Patients were physically examined once every 6 months for clinical change and any evidence of relapse. In addition, skin smears examination (six sites) was carried out once every year for at least 7 years for bacteriological progress and whenever necessary to verify clinical relapse.

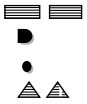
Either the appearance of new skin lesions or an increase of BI at any skin smear site was cause to suspect relapse. Relapse was confirmed if a BI at any site increased 2+ over the previous values, new skin lesions appeared with a BI greater than in pre-existing lesion and/or viable *M. leprae* were demonstrated by mouse footpad inoculation.

M. leprae viability and drug susceptibility obtained from relapse cases were assessed by mouse footpad inoculation. The tests using the mouse model were carried out at the Instituto Lauro de Souza Lima, Bauru, SP, Brazil, where the susceptibility of the bacillus to dapsone, rifampicin and ofloxacin was conducted.

The protocol for this study was approved by the WHO Steering Committee of The Therapy of Leprosy (THELEP), local institutional Ethical Committee and the Brazilian Health Ministry. Patients were enrolled in the trial only after obtaining written informed consent. Patients were assigned to one of four regimens through a system of pre-coded treatment packs, incorporating identical-looking placebo preparations as appropriate.

This report presents data on relapse frequencies in patients treated with those four regimens in Brazil (Manaus and Bauru), for a minimum of 7 years active follow up after they had completed treatment regimens and at times as many as 12 years after they were release from treatment.

Table 1. Trial Regimens Schedule

REGIMENS	First Month		Month 2–12		Month 13–24
WHO-MDT	Day 1	Day 2 − 28	Day 1	Day 2–28	NIL
					NIL
WHO MDT PLUS OFLOXACIN					NIL
					NIL
OFLOXACIN PLUS RIFAMPICIN					NIL
					
WHO MDT 2 years	Same as A		Same as A		Same as month 2–12
	Active	Placebo			
Rifampicin 300 mg					
Clofazimine 100 mg					
Clofazimine 50 mg					
Dapsone 100 mg					
Ofloxacin 200 mg					

Results

CLINICAL PROFILE OF PATIENTS

A total of 198 MB patients were recruited and sequentially enrolled in the trial between 1992 and 1994, in Manaus and Bauru, Brazilian trial centres. On enrollment, patients were randomly allocated with pre-coded treatment packs.

The intake, treatment and follow up details of the MB patients according to the Brazilian centres are showed in Table 2. Among 183 patients who completed treatment, the minimum 7 years scheduled follow-up after being released from treatment was available for 114 patients. The other 69 patients, recruited and assigned to one of four regimens, were lost to follow up, after a period ranging from 1 to 4 years after release from treatment, mainly because of migration.

Clinical classification of patients on admission according to centre, participant and allocated regimen in the two centres are presented in Table 3.

The majority of patients recruited in Brazil were found to be BL. The mean BI taken from six sites at time of enrollment was 3,1 ± 1,4.

DRUG TOLERANCE

The incidence of side effects was low. Among 183 patients who completed the course of treatment, three patients were decoded, because of severe side effects. Only one of these patients was receiving an ofloxacin regimen.

RELAPSES

During follow up after treatment, 23 MB patients were diagnosed as relapse cases. All relapses occurred in patients who were initially BL or LL and the mean bacteriological index (BI) of these patients, on the trial intake, was 4,2 ± 0,84. Table 4 presents findings of these individuals who relapsed. Higher relapse rate (38,8%) was observed in MB cases treated with regimen C consisting of 1 month of daily rifampin 600 mg and daily ofloxacin 400 mg (supervised).

After performing skin biopsy for histology and mouse footpad inoculation, all 23 patients were put on standard WHO-MDT and improved.

The relapse prevalence according to treatment regimen is presented on Table 5.

No relapses were detected in the 2 years standard WHO MDT group during the follow up period. Nevertheless, it could be due to the size of the group (24 cases). All relapses cases were detected after 6 years of follow up except for one patient who showed new active lesions

Table 2. Ofloxacin trial: Brazilian centres participants, patients intake and active follow up after completion of treatment regimen

CENTRES	INTAKE	TREATMENT COMPLETED	ACTIVE 7 YEAR FOLLOW-UP
BAURU (Campo Grande, Rondonópolis, Rio de Janeiro)	98	89	47
MANAUS	100	94	67
TOTAL	198	183	114

Table 3. Clinical classification of the patients on admission and regimens allocated

CENTRE	BAURU(Bauru, Campo Grande, Rio de Janeiro, Rondonópolis)	MANAUS	TOTAL
MB patients	98	100	198
Clinical classification	BT 07		BT 07
	BB 26	BB 19	BB 45
	BL 25	BL 53	BL 78
	LL 42	LL 28	LL 42
Regimens tested	A 25	A 28	A 53
	B 29	B 26	B 55
	C 32	C 31	C 63
	D 12	D 15	D 27

and increased BI before the third year of follow up. The shortest period for relapse occurred at 2·5 years and the longest at 10 years, after completion of trial therapy. One of the patients treated with 1-month regimen of daily rifampin and ofloxacin relapsed again, after been treated with 2 years of WHO-MDT. This patient is receiving a second course of 2 years WHO-MDT regimen is improving.

DRUG SUSCEPTIBILITY BY MOUSE FOOTPAD INOCULATION

Drug susceptibility was performed with specimen from skin biopsies of 10/23 patients who relapsed. Multiplication of *M. leprae* was observed in 8/10 samples tested and, among those, drug resistance was detected in two samples: one case of ofloxacin resistance and one case of dapsone resistance. The patient with ofloxacin-resistant isolates had been treated with the 1 month regimen, comprising of daily rifampin and ofloxacin, and was found to relapse earliest – less than 3 years after completion of treatment.

M. leprae susceptibility from the skin biopsy relapse case treated with 1 month regimen of daily rifampin and ofloxacin, who relapsed again after 2 years of WHO-MDT, was assessed in the mouse model. *M leprae* from this patient grew in untreated mice and was susceptible to rifampicin, dapsone and ofloxacin.

Discussion

This study shows that a short duration regimen for MB patients with the rifampicin-ofloxacin combination had a high failure rate (38,8%). The relapse rate obtained by the 1-month

Table 4. Overall relapse and allocated regimens

CENTRE	BAURU (Bauru, Rondonópolis, Rio de Janeiro)	MANAUS	TOTAL
N° of Relapses	11	12	23
Allocated Regimens		A 02	A 02
	B 01	B 01	B 02
	C 10	C 09	C19

Table 5. Relapse prevalence by MB regimens

Regimens	f_1/n	%	IC95%
WHO MDT 12 months (A)	2/46	4·3(a)*	0,8–16,0
WHO MDT 12 months + Ofloxacin (B)	2/40	5·0(a)*	0,3–18·2
OflE»t + Rifampinn (C)	19/49	38·3(b)*	25,5–53,3
WHO MDT 24 months (D)	−/24	-	-
Total	23/169	13,6	3,9–19,9

* $P <$ 0,001 ($\chi 2 = 25,7$); Distinct letters indicate statistic difference at a 5% level

regimen of daily rifampin and ofloxacin was found significantly higher than observed in the three other regimens that included at least 1 year of the MB regimen recommended by the WHO. Fajardo *et al.*[12] published their findings from the same WHO multicentre trial. In that publication, they found out that the 1 month regimen resulted in 11% relapse rate at 9 years and 25% at 12 years from the initiation of therapy. This Philippine experience mirrors our results, showing that 1 month of rifampin and ofloxacin is insufficient for treatment of MB leprosy cases. Also, Pattyn and Grillone[13] found out that a 6 week regimen of daily rifampin, clofazimine and ofloxacin plus weekly minocycline had previously been found to result in 13% of the patients experiencing relapse. Therefore, this study also supports our thesis. However, although small in number, relapses still occurred after 1 year of WHO-MDT and 1 year of WHO-MDT plus 1 month of daily ofloxacin with no statistically significant difference between these two regimens. So, the addition of 1 month of daily ofloxacin to 12 months MB WHO MDT did not increase its efficacy.

No relapses were detected in the 2 years WHO-MDT group during the follow-up period. This could be due to the small size of the group (24 cases). All relapses occurred in patients who were initially BL and LL and had an initial average BI of 4,2 ± 0,84. These results indicate close correlation between relapse and high initial BI. As suggested by Pattyn[14] and Rocha *et al.*,[15] a short incubation period for relapse could have correlation with drug resistance. The short incubation period (2·5 years follow-up) for relapse in the case of ofloxacin resistance seems to suggest the possibility of relapse due to drug resistance. Late relapses observed irrespective of which one regimen had been used could most likely result from the re-growth of persisting *M. leprae*. Persistent organisms lie dormant and or are located in inaccessible tissues of the body such as the nerves. They are not affected by treatment because of their non-metabolic state or dormancy, and/or because of their inaccessible location in the body. Once treatment is stopped however, the organisms may start to replicate and eventually produce a disease with organisms still sensitive to the drugs used earlier in the treatment of the case.

Acknowledgements

A competing interest statement – This investigation received financial support from the UNDP/World Bank/WHO special Programme for Research and Training in Tropical Diseases (TDR).

References

[1] World Health Organization, *Chemotherapy of Leprosy for Control Programmes*. World Health Organization, Geneva, 1982.

[2] Shepard CC. A brief review of experiences with short-term clinical trials monitored by mouse-footpad inoculation. *Lepr Rev*, 1981; **52**: 299–308.

[3] Rees RJW, Pearson JMH, Waters MFR. Experimental and clinical studies on rifampicin in treatment of leprosy. *BMJ*, 1970; **1**: 89–92.

[4] Shepard CC, Levy L, Fasal P. Rapid bactericidal effect of rifampicin on *Mycobacterium leprae*. Am J Trop Med Hyg, 1977; **21**: 446–449.

[5] Guelpa-Lauras CC, Perani EG, Giroir AM, Grosset TH. Activity of pefloxacin and ciprofloxacin against *Mycobacterium leprae* infection induced in mice. *Int J Lepr Other Mycobact Dis*, 1986; **54**: 560–562.

[6] Grosset JH, Guelpas-Lauras CC, Perani EG, Beoletto C. Activity of ofloxacin against *M. leprae* in the mouse. *Int. J. of Lepr*, 1988; **56**: 259–264.

[7] Gelber RH, Iranmanesh A, Murray L *et al.* Activities of various quinolone antibiotics against *Mycobacterium leprae* in infected mice. *Antimicrob Agents Chemother*, 1992; **36**: 2544–2547.

[8] Grosset JH, Ji B, Guelpas-Lauras CC *et al.* Clinical Trial of Perfloxacin and Ofloxacin in the treatment of lepromatous leprosy. *Int J Lepr Other Mycobact Dis*, 1990; **58**: 281–295.

[9] Ji B, Perani EG, Petinom C, Grosset JH. Bactericidal activities of combinations of new drugs against *Mycobacterium leprae* in nude mouse. *Antimicrob. Agents Chemother*, 1996; **40**: 393–399.

[10] Fajardo TT, Jr, Vllahermosa LG, Dela Cruz EC *et al.* A clinical Trial of pefloxacin and ofloxacin in lepromatous leprosy. *Lepr Rev*, 2005; **73**: 389–391.

[11] Gelber RH, Balagon VF, Cellona RV. The relapse rate in MB leprosy patients treated with 2 years of WHO-MDT is not low. *Int J Lepr Other Mycobact Dis*, 2004; **72**: 493–500.

[12] Fajardo TT, Jr, Vllahermosa LG, Partdillo FEF *et al.* A comparative Clinical Trial in Multibacillary Leprosy with Long-Term Relapse Rates of Four Different Multidrug Regimens. *Am J Trop Med Hyg*, 2009; **81**: 330–334.

[13] Pattyn S, Grillone S. Relapse rates and a 10-year follow-up of a 6-week quadruple drug regimen for multibacillary leprosy. *Lepr Rev*, 2002; **73**: 245–247.

[14] Pattyn SR. Persisting *Mycobacterium leprae* among THELEP trial patients in Bamako and Chingleput. Subcommittee on Clinical Trials of the Chemotherapy of Leprosy (THELEP) Scientific Working Group of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. *Lepr Rev*, 1987; **58**: 325–337.

[15] Rocha SA, Cunha MG, Diniz LM *et al.* Drug and Multidrug Resistance among *Mycobacterium leprae* Isolates from Brazilian Relapsed Leprosy Patients. *J. Clin Microbiol*, 2012; **50**: 1912–1917. DOI:10.1128/JCM.06561-11.