OFLOXACIN CONTAINING COMBINED DRUG REGIMENS IN THE TREATMENT OF MULTIBACILLARY LEPROSY

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Abstract. The results of ofloxacin containing combined drug regimens in the treatment of 60 multibacillary leprosy cases from January 1989 to June 1995 are reported. The objective of the trial is to compare the antileprotic property of ofloxacin and rifampicin in multibacillary leprosy patients and to study the killing rate of *M. leprae* by ofloxacin and rifampicin before mass treatment can be recommended.

The complications and side-effects of ofloxacin and rifampicin were of a mild nature and both drugs were well tolerated. Moderate to marked clinical improvement was noticed in a short period with ofloxacin containing regimens in multibacillary leprosy patients. No persisters were detected in any of the 33 specimens (of mouse footpads) that had been obtained after treatment for 6 months. Ofloxacin if added to the currently used WHO recommended MB-MDT regimen may shorten the duration of treatment. Ofloxacin, therefore, may be considered as a suitable alternative in suspected/proven rifampicin resistant cases and where rifampicin is contraindicated.

The results were evaluated on the basis of the clinical conditions, mycobactericidal effectiveness, signs of drug toxicity and side effects.

INTRODUCTION

Once the problem of anti-leprotic drug resistance had been recognized worldwide in 1981 (Ji, 1985; Jacobson and Hastings, 1976), the WHO Study Group on Chemotherapy of leprosy for Control Programs recommended new standard multidrug therapy (MDT) regimens containing rifampicin as a major compoment in order to minimize the drug resistance and duration of treatment (WHO, 1982). This MDT has been accepted worldwide and introduced into leprosy control programs. Studies from various nations have reported satisfactory results with these regimens. However, sporadic reports over the past 10 years have shown that secondary rifampicin resistance develops easily and rapidly in multibacillary leprosy patients, particularly when it is used alone (WHO, 1983). There was also a report of double resistance to both rifampicin and dapsone (Constant-Deports Relonzat, 1983) proved by mouse footpad inoculation test. It is therefore essential to search for or develop new effective antileprotic drugs (Grosset and Ji, 1989; Anonymous, 1987a).

A variety of drugs active against *M. leprae* and other mycobacteria have been tested in mice. The most promising of these seem to be the fluorinated quinolone derivatives, pefloxacin and ofloxacin (Guelpa-Lauras *et al*, 1987, 1988; Pattyn, 1987; Grosset *et al*, 1987; 1988 a,b; Kohsaka *et al*, 1983; Franzblau, and white, 1990). Clinical trials of pefloxacin or ofloxacin monotherapy have confirmed their antileprotic action (Grosset *et al*, 1988 a,b, 1990; N'Deli *et al* 1988, 1990). A randomized clinical trial in multibacillary leprosy patients has been conducted to study the additional therapeutic effects of ofloxacin to the existing MDT regimens containing rifampicin, and the results are reported below.

The objectives of the study were:

1. To compare the antileprotic property of ofloxacin and rifampicin in-multibacillary leprosy patients.

2. To evaluate the additional therapeutic effect of adding ofloxacin to rifampicin containing regimens for multibacillary leprosy patients.

3. To study the killing rate of *M. leprae* by ofloxacin in lepromatous leprosy patients employing mouse footpad inoculation tests.

4. To study the persisting *M. leprae* after treatment using nude mice.

The research hypothesis was:

1. Both rifampicin and ofloxacin have bactericidal action against *M. leprae*, so there should be no difference in cure rate by regimens containing rifampicin or floxacin. Killing rate of *M. leprae* by either drug should be similar.

2. Ofloxacin should potentiate the bactericidal effect of rifampicin. Therefore the duration of treatment should be substantially shortened or early clinical improvement should be observed.

PATIENTS AND METHODS

Patients

Only untreated LL/BL cases with BI of 4+ or above were recruited into the trials. The patients were selected, screened and examined before admission to the research ward in Raj Pracha Samasai Institute.

The total number of patients was 60, randomly allocated into 3 different regimens A, B and C.

Regimens

Regimen A: WHO/MDT regimen for multibacillary leprosy, *ie* rifampicin 600 mg/month, clofazimine 300 mg/month plus daily 100 mg dapsone and 50 mg clofazimine

Regimen B: WHO/MDT regimen for multibacillary leprosy plus daily 400 mg. Ofloxacin for 1 month then shift to regimen A.

Regimen C: Ofloxacin 400 mg/day, clofazimine 300 mg/month plus daily 100 mg. Dapsone and 50 mg clofazimine for 1 month then shift to regimen A.

Regimen A was served as standard control. The therapeutic effect of ofloxacin in regimen C was compared with rifampicin in regimen A. Regimen B provided information whether ofloxacin had additional therapeutic effect on top of WHO/MDT regimen. The drugs had been administered under supervision.

Duration of the trials

phase 1: Initial intensive period of 4 weeks during which all the patients were admitted for

1) initial screening tests and assessment
2) careful monitoring and observation for signs of drug toxicity and side effects.

phase 2: After phase 1 till stopping treatment which was 2 years for all regimens. All the patients were treated and examined as out patients at the interval of 1 month. The therapeutic effectiveness, side effect and all complications were evaluated.

phase 3: A period of 5 years for surveillance were assessed for clinical and bacteriological improvement and for possible reactivation/relapse after stopping treatment.

Exclusion from the trials

1. **Before treatment** (at the screening)

a) Intercurrent life-shortening disease such as cancer, severe diabetes, hypertension, cardiac, renal or hepatic diseases,
b) Pregnant female patients
c) History of drug hypersensitivity
d) Psychologically unstable patients

2. During the trials

a) Drug toxicity and side effects which require cessation or alteration of the assigned regimens.
b) Severe reaction (RR or ENL)
c) Serious intercurrent diseases
d) Voluntary withdrawal or non-cooperation by patients.
e) Adverse clinical status or mouse footpad inoculation test.

Frequency of skin biopsies

1. **Histological examinations:** Skin biopsy at day

0, 6 months and at 2 years of treatment were assessed the histological improvement. Skin biopsy every other year after stopping treatment.

2. Mouse footpad inoculation test: Skin biopsy at day 0 for titration of viability of *M. leprae* and tested for dapsone or rifampicin resistance was performed. Skin biopsy at day 14 and day 28 of treatment was done for titration of viability of *M. leprae* after treatment to see the bactericidal effect of drugs and to detect persisters as well. Skin biopsy at 6 months and 2 years of treatment was done to detect persisters.

M. leprae organisms recovered from skin biopsies were diluted to a required number in a volume of 0.05 ml which was inoculated into both hind footpads of each mouse either normal or nude one according to the following scheme (Table 1).

Harvest: In order to allow a single viable organism to multiply to a detectable level, all mice, in general they were harvested at 12 months after inoculation.

Clinical laboratory procedures

The following clinical laboratory procedures were performed on all patients at the time of admission to the trial, and at the later intervals prescribed by the Standard Protocol as mentioned below:

Table 1

Scheme of mice inoculated per patient in each group.

	Normal mice (No. required)					Maximal AFB recovered	Nude mice (No. required)				
	10^4	10^3	10^2	10^1	10^0		10^4	10^3	10^2	10^1	10^0
Day 0	25*	10	10	10	10		15**	10	10	10	10
Day 14	10	10				10	10	10			
Day 28	10					20					
6 months						20					
2 years						20					
Total			95						155		

* 15 mice were used to test for DDS resistance.

** 5 mice were used to test for rifampicin resistance.

Table 2

The status of patients in each group (as of June, 1995).

Regimen	Total no. of patients (M : F)*	No. of patients completing treatment						
		1M	1Yr	2Yr	3Yr	4Yr	5Yr	6Yr
A	19 (15:4)	19	19	19	18	5	4	2
B	20 (14:6)	20	20	19	15	7	2	0
C	21 (17:4)	21	21	20	17	7	3	1
Total	60 (46:4)	60	60	58	50	19	9	3

* six cases excluded:

One patient was dead from heart failure, another from other disease; one patient developed viral hepatitis after treatment for 3 months with regimen A, another in regimen A developed jaundice with urinary tract infection with *Trichomonas vaginalis*, two were lost of follow up.

1. Complete blood count
2. Complete urinalysis
3. Blood chemistry (BUN, SGOT, SGPT, Serum bilirubin and alkaline phosphatase)
4. Stool examination
5. G-6-PD level and fasting blood sugar
6. Chest X-ray
7. Lepromin test
8. Skin smear at 6 selected sites
9. Nasal swab

RESULTS

Thirty-six cases from Raj Prachcha Samasai Institute and 30 cases from Leprosy Zonal Center 6 Khon Kaen have been enroled in this study since January 1989, a total of 66 cases. Of these, six patients were excluded due to various reasons, including lost to follow up, death, complications, etc. The remaining 60 cases consisted of 46 males and 14 females, aged 12-75 years. There were 46 lepromatous, 12 borderline lepromatous, and 2 mid borderline cases. Fifty patients were followed up for 3 years whereas only 19 cases were followed up for 4 years (Table 2).

Clinical results

Among 60 patients selected for the trial at the first month of treatment, 14 improved (definite improvement DI, slight improvement SI) 73.68% in regimen A, 15 improved (DI + SI) 75% in regimen B, and 18 improved (DI + SI) 85.71% in regimen C.

But at the 2 years of treatment, 9 improved (DI + SI) 47.37% in regimen A, 15 improved (DI + SI) 78.94% in regimen B, and 17 improved (DI + SI) 85% in regimen C. At the 3 years of trial 12 improved (DI + SI) 66.66% in regimen A, 11 improved (DI + SI) 73.33% in regimen B, and 13 improved (DI + SI) 76.47% in regimen C as shown in Table 3.

Bacteriological results

Bacterial Index (BI) after 1st month of treatment in regimen A total 19 cases, the BI was reduced 3.72% from an average of 4.30 before treatment to 4.1, in regimen B, total 20 cases; the BI was reduced 4.78% from an average of 4.61 before treatment to 4.39; and in regimen C total 21 cases, the BI was reduced 2.05% from an average of 4.38 before treatment to 4.29.

After 1 year of treatment, in regimen A total 19 cases the BI was reduced 33% from 4.3 (before treatment) to 2.88; in regimen B also total 19 cases the BI was reduced 26% from 4.61 (before treatment) to 3.43; and in regimen C total 20 cases the BI was reduced 30% from 4.38 (before treatment) to 3.07.

After 2 years, the BI was reduced 60% in regimen A, 58% in regimen B and 55% in regimen C. At 3 years the BI was reduced 90% in regimen A, 82.43% in regimen B and only 56.39% in regimen C. At 5 years the skin smears in all patients in

Table 3

Comparing change of cutaneous manifestations in each group at 1 month, 2 years, and 3 years after trial.

Regimen	1 month				2 years				3 years			
	No. of cases	Impr*	Not Impr	% Impr	No. of cases	Impr	Not Impr	% Impr	No. of cases	Impr*	Not Impr	% Impr
A	19	14	5	73.68	19	9	10	47.37	18	12	6	66.66
B	20	15	5	75.00	19	15	4	78.94	15	11	4	73.33
C	21	18	3	85.71	20	17	3	85.00	17	13	4	76.47
Total	60	47	13	78.33	58	41	17	70.69	50	36	14	72.15

* Impr = Improved (DI + SI), DI = definite improvement, SI = slight improvement,
Not Impr = NC + DE , NC = no change, DE = deterioration

regimen A and B were negative, only in regimen C the average BI was still positive 1.05 as shown in Table 4.

Histopathological results

All skin biopsies at the end of 2nd year treatment showed histological improvement with reduction of the volume of the granuloma infiltration, presence of fibrosis and decrease in the number and density of bacilli. Among 60 patients, signs of histopathologic involution were seen in all cases, as shown in Table 5.

The results of leprosy reaction in the trial

At the end of 1st year treatment we had detected leprosy reactions (ENL - Erythema Nodosum Leprosum; RR = Reversal Reaction and Neuritis) 31.58% in regimen A, 25.00% in regimen B and 28.57% in regimen C, as shown in Table 6.

But after the second year of treatment the evidence of lepra reaction was decreased, only a few erythema nodosum leprosum and neuritis cases were seen 9.52% in regimen A.

Side-effects: Ofloxacin and rifampicin were well-tolerated. There were mild nausea which disappeared without withdrawal of treatment.

Table 4

Bacteriological clearance in the patients in each group during the course of treatment.

Regimen	Mean initial BI	Mean BI after treatment						
		1M	1Yr	2Yr	3Yr	4Yr	5Yr	6Yr
A	4.30 (n=19)	4.14 (n=19)	2.88 (n=19)	1.72 (n=19)	0.43 (n=18)	0.25 (n=5)	0 (n=4)	0 (n=2)
B	4.61 (n=20)	4.39 (n=20)	3.43 (n=19)	1.95 (n=19)	0.81 (n=15)	0.33 (n=7)	0 (n=2)	- (n=0)
C	4.38 (n=21)	4.29 (n=21)	3.07 (n=20)	1.99 (n=19)	1.19 (n=17)	0.52 (n=7)	1.05 (n=3)	0 (n=1)
Total	4.43 (n=60)	4.27 (n=60)	3.13 (n=58)	1.89 (n=58)	0.81 (n=50)	0.36 (n=19)	0.35 (n=9)	0 (n=3)

n = No. of patients

Table 5

Histopathological results.

Regimen	Before treatment				2 years after treatment			
	No. of cases	Active	Not active	% Active	No. of cases	Active	Not active	% Active
A	19	18	1*	94.73	19	-	19	0
B	20	20	-	100	20	-	20	0
C	21	21	-	100	21	-	21	0
Total	60	59	1	98.33	60	-	60	0

Active = AU+AI
Not active = RI+RD
AU = Active unimproved, AI = Active improved, RI = Resolving, RD = Resolved
*LLs (RI : case TC from Khon Kaen)

Analysis of mouse data

This analysis of mouse data from the ofloxacin trial is limited to those 38 patients-13 treated by Regimen A, 14 treated by Regimen B, and 11 by Regimen C-whose *Mycobacterium leprae*, derived from pretreatment biopsy specimens, multiplied in the footpads of mice.

As shown in Table 7, "persisting" *M. leprae* were detected in biopsy specimens obtained from 5 of the 38 patients and in 6 of 37 of the biopsy specimens that had been obtained after treatment by one of the regimens for 2 or 4 weeks.

These calculations are based on numerous assumptions (Anonymous, 1987a, b). The values shown are similar to those already published. The apparent differences among the regimens are probably not significant. What is important are the following: 1) the similarity of these results with those published earlier; and 2) that such small populations of viable *M. leprae* cases are unlikely to include even single drug-resistant individuals. Thus, should such a patient relapse after release from treatment, his organisms will be susceptible to the drugs originally employed and retreatment employing the same regimen as that originally employed will be successful.

DISCUSSION

A multicenter study was conducted in Raj Pracha Samasai Institute, Phra Pradaeng Hospital, Samut-Prakan Province and Leprosy Zonal Center 6, Khon Kaen Province to determine the clinical efficacy, the antileprotic activity and safety of ofloxacin and rifampicin in leprosy patients. The total of 60 multibacillary leprosy patients (46 males, 14 females) age between 12-75 years were in the trial from January 1989 to June 1995 for 6 1/2 years.

The patients were allocated into 3 different regimens. The regimen A (WHO/MDT regimen) served as a standard. The regimen B was WHO/MDT regimen plus 400 mg ofloxacin for 1 month in order to provide the additional effect of ofloxacin on top of WHO/MDT regimen, and the effect of ofloxacin in regimen C was compared with rifampicin in regimen A.

The results of microbiologic and clinical responses indicated a high level of efficacy against *M. leprae* in all regimens. At the initial intensive

Table 6

Leprosy reaction report in the trial.

Regimen	At the end of 1st yr treatment							
	No. of cases	ENL	(%)	RR	(%)	Neuritis	(%)	% Reaction & Neuritis
A	19	2	(10.52)	2	(10.52)	2	(10.52)	31.58
B	20	3	(15.00)	1	(5.00)	1	(5.00)	25.00
C	21	2	(9.52)	2	(9.52)	2	(9.52)	28.57
Total	60	7	(11.66)	5	(8.33)	5	(8.33)	28.33

Table 7

Persisting *M. leprae* were detected in biopsy-specimens before and after treatment 2 or 4 week.

	Regimen			Total
	A	B	C	
Number of patients	13	14	11	38
Number of "positive" patients	1	2	2	5
Number of 2 or 4 week specimens	11	13	12	37
Number of "positive" specimens	1	2	3	6

Persisters were not detected in any of the 33 specimens that had been obtained after treatment for 6 months. The frequency of persisters is not significant among the regimens ($p > 0.3$, Table 8).

Table 8

The absolute numbers of organisms, both viable and dead, and of viable organisms (persisters).

Number *M. leprae*	Regimen A	Regimen B	Regimen C	Total
Total	1.12×10^{13}	1.28×10^{13}	2.12×10^{13}	4.52×10^{13}
Average number per patient	8.64×10^{11}	9.14×10^{11}	1.93×10^{12}	1.19×10^{12}
Total viable	1.80×10^{5}	3.01×10^{7}	6.43×10^{6}	3.66×10^{7}
Average number per patient	1.38×10^{4}	2.15×10^{6}	5.84×10^{5}	9.64×10^{5}

period of 1 month there was clinical improvement (DI + SI) 73% in regimen A, 75% in regimen B and 85.75% in regimen C. Bacterial index (BI) was reduced 3.72% in regimen A, 4.78% in regimen B and 2.05% in regimen C. This indicated that there was no difference in cure rate of regimens containing rifampicin or ofloxacin, as shown in Tables 3, 4. The changes of cutaneous manifestations in the 2nd year of treatment showed clinical improvement (DI + SI) 47.37% in regimen A, 78.94% in regimen B and 85% in regimen C with 60% decreased in the average BI in regimen A, 58% decreased in regimen B and 55% decreased in regimen C. So the clinical improvement in regimens B and C was much better than in regimen A.

At 3 years of trial, 66% improved (DI + SI) in regimen A, 73.33% improved in regimen B and 76.47% also improved in regimen C. But the BI was reduced 90% in regimen A, 82.43% regimen B and 56.39% in regimen C. The bacterial clearance was fell as shown in Table 4. For the period of surveillance the skin smear were negative in regimen A and B at the 5th year of trial, except in regimen C the BI was still positive (1.05); the results of histopathological examination showed signs of histopathologic involution in all cases at the 2nd year of treatment. And no persisters were detected in any of 33 specimens of mouse footpads obtained after treatment for 6 months.

The results of lepra reaction in the trial showed 31.58% in regimen A, 25% in regimen B and 28.58% in regimen C during the 1st year of treatment, as shown in Table 6. In the 2nd year the lepra reaction was decreased only 9.52% in regimen A.

The authors conclude that both ofloxacin and rifampicin are highly effective anti-leprosy drugs with the most rapid, active and some what well tolerated. This trial thus documents a role for ofloxacin as an effective alternative to the anti-leprosy drugs currently utilized in leprosy control program in Thailand.

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Appendix

Participating institutes and co-researchers during 1989-1995.

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