POWERFUL BACTERICIDAL ACTIVITY OF MOXIFLOXACIN IN HUMAN
LEPROSY

Short Title: MOXIFLOXACIN IN LEPROSY

Fe Eleanor F. Pardillo, Jasmin Burgos, Tranquilino T. Fajardo, Eduardo Dela Cruz,
Rodolfo M. Abalos, Rose Maria D. Paredes, Cora Evelyn S. Andaya, and Robert H.
Gelber

Leonard Wood Memorial Center for Leprosy Research, Cebu, Philippines

Preliminary results of this study were presented at the 46th Interscience Conference on

* Corresponding Author. Mailing Address: 220 Scenic Avenue, San Anselmo, California 94960 U.S.A.

Phone: (415) 454-8765. Fax: (415) 454-8191. Email: ikgelber@hotmail.com.
ABSTRACT

In a clinical trial of moxifloxacin in 8 multibacillary (MB) leprosy patients, moxifloxacin proved highly effective. In all trial patients a single 400 mg dose of moxifloxacin resulted in significant killing (p ≤ 0.006) of *M. leprae* ranging from 82% to 99%, mean 91%. In all instances, no viable bacilli were detected with an additional three weeks of daily therapy—this observed rapid bactericidal activity being only matched previously by rifampin. On moxifloxacin therapy skin lesions cleared exceedingly rapidly with definite improvement consistently observed after eight doses and progressive resolution continuing for the 56 days of the trial. Side effects, toxicities and laboratory abnormalities were mild, not requiring discontinuation of therapy.
INTRODUCTION

Fluoroquinolones have proved active against *M. leprae* in rodents (10, 13, 18, 20, 31) and in clinical trials (9, 19, 26) in leprosy patients. The first studies of fluoroquinolones in *M. leprae*-infected mice found that ciprofloxacin was inactive, while pefloxacin and ofloxacin were bactericidal (18, 20). We (13) tested several fluoroquinolones against *M. leprae* in mice finding some, namely WIN5727, temafloxacin and, particularly, sparfloxacin, superior to pefloxacin and ofloxacin. Furthermore in the heavily infected neonatally thymectomized Lewis rat the combination of rifampin and ofloxacin was more regularly sterilizing than both the combination of rifampin plus dapsone and rifampin plus clofazimine (10), rifampin, dapsone and clofazimine being the three components of the widely implemented WHO-recommended regimens for multibacillary (MB) leprosy (36, 37). Clinical trials of pefloxacin and ofloxacin in leprosy have demonstrated encouraging clinical responses and clearance of viable *M. leprae* within 2 months (9, 19, 26), this being more rapid than dapsone and clofazimine, several months (32), similar to minocycline (8, 12) and clarithromycin (3), but much slower than rifampin (29, 32, 34).

Moxifloxacin against *M. tuberculosis*, (6, 14, 23, 25) has been found in vitro to be more bactericidal than other quinolones and similar to rifampin. Also, moxifloxacin has been demonstrated in a murine model of tuberculosis to add to the sterilizing activity of isoniazid, rifampin, and pyrizinamide (27) and to provide significant bactericidal activity in the first few days of treatment of human tuberculosis, both as monotherapy (17, 24, 28) and multidrug therapy (2, 15). As a result, trials with moxifloxacin in active pulmonary
tuberculosis are currently in progress, both using it to replace established agents and to
shorten the course of effective therapy of active pulmonary tuberculosis.

In a murine model of leprosy moxifloxacin has been demonstrated to be more
bactericidal than ofloxacin, and, in that regard, as potent as rifampin (5). Because the one
year WHO (37) regimen for MB leprosy is still quite lengthy, some studies have found
double-digit relapse rates even after two years of this regimen (11, 16, 22), and
emergence of rifampin-resistant *M. leprae* is of concern, the discovery of alternative
bactericidal agents to treat leprosy remains a high priority. Thus we embarked on this
present clinical trial of moxifloxacin in leprosy.
MATERIALS AND METHODS

At the Clinical Branch of the Leonard Wood Memorial Leprosy Research Center, Cebu, Philippines, 8 previously untreated MB leprosy patients without a previous history of leprosy treatment or lepra reactions and having a high bacteriologic index (BI) were recruited to this moxifloxacin trial. Fertile females and those under 18 years of age were excluded. The protocol for this study was approved by a local Institutional Review Board (licensed by the National Institutes of Health), and written informed consent, including permission to publish photographs, was obtained from volunteers. Participants were initially carefully and thoroughly examined with emphasis on dermatologic and neurologic status, lesions mapped and clinical photographs obtained. Prior to therapy study patients, also, underwent routine 6-site skin smears, a skin biopsy from an active lesion both for dermatopathologic evaluation by the method of Ridley and Jopling (30) and determination of both M. leprae viability, as well as, dapsone and moxifloxacin sensitivity in mice, and laboratory evaluations, including a complete hematogram, SGPT, BUN, and urinanalysis.

All 8 MB patients recruited to participate in the trial were males and ranged in age from 22 to 49 years. Trial subjects had an average bacteriologic index of 3.8 to 5.1; seven were histologically found to be polar lepromatous (LL), and one was borderline lepromatous (BL). Patients were treated with a single initial dose of 400 mg moxifloxacin, no therapy for 7 days and a daily observed 400 mg dose from Day 8 to Day 56. For the two month duration of the trial patients were hospitalized, supervised in their
intake of medication, seen on a daily basis, underwent the previously described laboratory evaluations every two weeks, had serial clinical photographs taken and were evaluated as to clinical progress, as well as any adverse side effects / toxicities.

Serial skin biopsies for *M. leprae* viability were obtained prior to therapy and on Day 7, 14, 28 and 56 after the initiation of therapy, and *M. leprae* therein inoculated in groups of hind footpads of mice in amounts of 50 (not on Day 28 and 56), 500 (not on Day 56) and 5,000 *M. leprae* / footpad. Also, from the biopsy obtained prior to therapy *M. leprae* sensitivity to dapsone and moxifloxacin was, also, assessed. For these determinations of drug sensitivity groups of mice infected with 5,000 *M. leprae* were either untreated, treated continuously with three levels of dietary dapsone (0.0001%, 0.001% and 0.01%) or moxifloxacin 50mg/kg five times weekly by gavage. From these mice six months after footpad infection four hind feet from two mice were harvested, pooled and the numbers of *M. leprae* therein enumerated microscopically. In these and all other instances growth of *M. leprae* to the $\geq 10^5$ was considered evidence that the initial inoculum was viable, or in the case of drug sensitivity assessment, drug resistant. For purposes of assessing *M. leprae* viability from each biopsy, the number of *M. leprae* obtained from the hind feet of two mice (4 feet) infected with 5,000 *M. leprae* was enumerated both eight and twelve months after footpad infection. Furthermore, in order to quantitate *M. leprae* killing and whether significant killing had occurred, 10 or more individual footpads from each inoculum size were harvested, preferably at one year after infection or, at times, at mouse death six or more months after footpad infection. From these results the percentage of viable *M. leprae* was obtained, the percentage bactericide
calculated, the probability that killing occurred, and the probability that differences in *M. leprae* viability were recognized was assessed by the method of Spearman and Karber (33). On Day 28 and 56 where certain lower *M. leprae* inocula were not evaluated, these calculations assumed if higher ones showed no growth in all footpads, lower ones would as well.

At the completion of this trial, all subjects were treated with multidrug therapy as currently recommended by the WHO (37).
RESULTS

In all eight patients prior to therapy (Day 0) viable *M. leprae* was consistently observed in four foot harvests of mice infected with 5,000 *M. leprae* both eight and twelve months after footpad infection and the large majority of single foot harvests. In all 8 patients, their *M. leprae* obtained prior to the moxifloxacin trial was fully susceptible to both dapsone and moxifloxacin, because multiplication of *M. leprae* was inhibited in mice treated with each of the three levels of dietary dapsone and 50 mg / kg moxifloxacin five times weekly gavage (data not shown).

The results of *M. leprae* viability and killing obtained from sequential biopsies in the 8 trial patients obtained from both four foot harvests and single foot harvests are presented in Table 1. Single dose therapy (Day 7) resulted in significant *M. leprae* killing (p ≤ 0.006) in all, ranging from 82%-99%, (mean 91%). After an additional one week of daily moxifloxacin (Day 14) significant killing (p< 0.001) was even more impressive, ranging from 90% in one patient to 99% in six patients (mean 97%). Between Day 7 and Day 14 significant *M. leprae* killing (p< 0.0001) was observed in Patients 4-8, ranging from 84% to 97% (mean 93%), but not in Patients 1-3. Utilizing the results of both four foot harvests and single foot harvests, in these eight patients, after a single dose of moxifloxacin (Day 7) some viable bacilli were detected in seven of eight patients (not Patient 2), and on an additional one week of daily moxifloxacin (Day 14) in four patients (Patient 2, 4, 7, and 8) but not four others (Patient 1, 3, 5 and 6). In all eight patients no viable *M. leprae* were detected at Day 28 and 56.
The detection of viable *M. leprae* from four pooled footpads and from any individual footpad were quite similar, individual footpads being marginally more sensitive—that difference found only at the 14 day harvest in three of eight patients. As harvesting mouse footpads at twelve months as opposed to eight months allows a greater period of time for *M. leprae* multiplication to be observed, it is not surprising that harvesting at twelve months proved more sensitive in detecting viable *M. leprae* than harvesting at eight months; from the seven day biopsy, three patients (Patient 3, 6, and 8) demonstrated viable *M. leprae* at twelve months but not at eight months. Also, no biopsy was found to harbor viable *M. leprae* in the eight month harvest and not at twelve months.

Improvement in skin lesions occurred remarkably rapidly. In Figure 1 we present sequential clinical photographs of three representative trial patients. Though subtle improvements in skin lesions were noted in some patients on Day 7 after but a single dose of moxifloxacin, in all eight patients definite improvement was observed with an additional week of daily moxifloxacin (Day 14). All eight patients by that time had loss of erythema in skin lesions and in four patients fading of macules, nodules and / or plaques. In all eight patients continued improvements in skin lesions was observed throughout the 56 day trial period.

During the trial period evanescent dizziness was noted in three patients, and diarrhea and epigastric pain each in one patient. These symptoms did not lead to moxifloxacin discontinuation. Patient 1 developed mild erythema nodosum leprosum (ENL) on Day
which lasted 3 days and did not require corticosteroids. Reversal reactions were not observed during moxifloxacin therapy in any of the trial patients. Of the seven patients who did not develop lepra reactions during moxifloxacin administration, four had ENL and two RR after the completion of moxifloxacin therapy and while on standard WHO MDT.

Laboratory abnormalities found during this trial were confined to elevations of SGPT of 2-3 times normal in three patients on Day 28 and/or 56 without symptoms of hepatitis, one of these patients experiencing a fall hematocrit from 39% to 27% on Day 56 of unknown etiology. All laboratory abnormalities found during the course of the trial normalized after discontinuation of moxifloxacin.
DISCUSSION

In this clinical trial of moxifloxacin in MB leprosy we found it to be rapidly bactericidal for *M. leprae* (matched only previously by rifampin), clear skin lesions regularly and uniformly quickly (perhaps a function of moxifloxacin’s anti-inflammatory and immunomodulating effects [4, 35]), and to be devoid of serious side effects or toxicities. Thus, if our findings are confirmed by others in clinical trial, moxifloxacin presents an opportunity to be included as a component of a new generation of multidrug therapy.

The key to the short course chemotherapy of tuberculosis has been regimens including two or more bactericidal agents (1, 7, 21). In the one year WHO (37) regimen for MB leprosy only rifampin (29, 32, 34), and not dapsone (32) and clofazimine (32), are bactericidal for *M. leprae* in patients. Though pefloxacin / ofloxacin (9, 19, 26), minocycline (8, 12) and clarithromycin (3) clear viable *M. leprae* in MB patients more rapidly more than dapsone and clofazimine (32), in the Philippines they do not result in significant killing following a single dose, and each requires a few months to reliably clear all viable *M. leprae* (3, 8, 9). On the other hand, in this present study moxifloxacin was demonstrated to consistently result in killing of *M. leprae* in singe dose and clear viable bacilli in days or weeks, similar to the rate previously found only by rifampin (29, 32, 34). Thus as demonstrated herein moxifloxacin may provide the key and only other drug but rifampin which is consistently bactericidal for *M. leprae* in clinical trial—providing for leprosy the important second bactericidal agent with the analogous
prospects provided previously by rifampin for tuberculosis, a more reliably efficacious
and shorter course of treatment.

If the results of this current study can be confirmed by others, a regimen including daily
observed rifampin and moxifloxacin, perhaps with an additional agent such as
minocycline, of one or a few months, presents sufficient promise for MB leprosy to
suggest that such a clinical trial would be warranted. Ideally this regimen would be
compared in a randomized double-blind trial with one year WHO MDT, wherein relapse
rates after the completion of therapy would be the only valid measure of treatment
efficacy. Because in our (11) experience relapse in MB leprosy only begins to occur six
years after the completion of therapy and generally ten years thereafter, and we (11) and
others (16, 22) have found relapse to be much higher in patients with a high bacterial
burden, in order to maximize the potential of the proposed study at least 100 patients who
are LL or BL would need to be recruited in each arm and undergo annual follow-up for at
least fifteen years. If such a rifampin / moxifloxacin regimen proves effective in MB
leprosy, a short course regimen, including rifampin and moxifloxacin, might provide the
long-sought single effective regimen for all forms of leprosy.
ACKNOWLEDGEMENTS

This study was supported by the Leonard Wood Memorial Leprosy Research Foundation and the American Leprosy Missions. Moxifloxacin, utilized in this study, was graciously supplied by the Bayer Corporation.
REFERENCES


Table 1. M. leprae Viability

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Figure 1: Clinical Photographs