Combination of rifapentine-moxifloxacin-minocycline (PMM) for the treatment of leprosy

B. JI & J. GROSSET
Bactériologie et Hygiène, Faculté de Médicine Pitié-Salpêtrière, Paris, France

Summary To further the development of a multidrug regimen for treatment of leprosy that is suitable for monthly administration and fully supervisable, the bactericidal activities against *Mycobacterium leprae* of HMR 3647 (HMR), moxifloxacin (MXFX) and rifapentine (RPT) were measured by the proportional bactericide technique in the mouse footpad system, and compared with those of the established antileprosy drugs clarithromycin (CLARI), ofloxacin (OFLO) and rifampicin (RMP). Administered in five daily doses of 100 mg per kg body weight, HMR appeared slightly more bactericidal than CLARI, but the difference did not attain statistical significance. Administered as single doses, MXFX in a dosage of 150 mg per kg was more active than OFLO in the same dosage, and displayed the same level of activity as RMP in a dosage of 10 mg per kg; the combination MXFX-minocycline (MINO) (MM) was more bactericidal than the combination OFLO-MINO (OM); RPT in a dosage of 10 mg per kg was more bactericidal than RMP administered in the same dosage, and even more active than the combination RMP-OFLO-MINO (ROM); the combination RPT-MXFX-MINO (PMM) killed 99·9% of viable *M. leprae*, and was slightly more bactericidal than was RPT alone, indicating that the combination PMM showed an additive effect against *M. leprae*. These promising results justify a clinical trial among lepromatous patients, in which MM is being compared with OM, and PMM with ROM, in terms of efficacy and tolerance.

To cope with the serious threat of widespread dapsone-resistant leprosy that had resulted from monotherapy, a World Health Organization (WHO) Study Group recommended in 1981 that leprosy be treated with multidrug therapy (MDT).[1] At that time, only three drugs, rifampicin (RMP), dapsone (DDS) and clofazimine (CLO), all bactericidal to some degree against *Mycobacterium leprae*, were available as potential components of the MDT,[1] permitting little choice in the design of the multidrug regimens. Unlike RMP, which displayed rapid and powerful bactericidal activity against *M. leprae* in mice and in humans, DDS and CLO administered alone showed only weak bactericidal effects.[1] Therefore, the Study Group recommended that patients with paucibacillary (PB) leprosy be treated for 6 months with two drugs, DDS daily plus RMP monthly, and that patients with multibacillary (MB) leprosy be treated for 24 months with a combination of three drugs, DDS and CLO daily plus RMP and a larger, supplemental monthly dose of CLO.[1] Monthly drug-administration is done under supervision, whereas the daily drugs are self-administered. Since 1982, more than 10 million leprosy patients in the world had been cured by this treatment.[2]

Despite the great success of these first MDT regimens, newer regimens are required that are more efficient or operationally less demanding.[3] One of the concerns with regard to the current regimens is that it is difficult to persuade patients to comply with the

self-administered daily component,[4] which is required to ensure elimination before stopping chemotherapy of the spontaneously occurring RMP-resistant mutants; resistance to RMP may develop among MB patients if the daily DDS plus CLO component is not taken regularly. The risk of resistance might be significantly reduced if a fully supervisable MDT regimen were developed, so that all of the components could be administered once monthly under supervision. The demonstration of the promising bactericidal activities against *M. leprae* of ofloxacin (OFLO)[5] and minocycline (MINO)[6,7] led to the development of the monthly administered combined regimen RMP-OFLO-MINO (ROM).[8,9] A single dose of ROM exhibited impressive bactericidal activity against *M. leprae*, both in the mouse footpad system and in clinical trial,[8] and was only marginally less effective, in terms of clinical improvement, for treatment of single-lesion PB leprosy than was the standard 6-month MDT regimen;[9,10] ROM was well tolerated by the patients.[8,9] The enormous operational advantages of single-dose treatment, especially in a country such as India, in which two-thirds of the global leprosy burden is concentrated, and more than 30% of the newly detected patients demonstrate single-lesion PB leprosy, led the WHO Expert Committee on Leprosy to conclude that a single dose of ROM is an acceptable and cost-effective alternative regimen for the treatment of single-lesion PB leprosy.[10] The efficacy of multiple doses of monthly-administered ROM is currently being compared to that of the standard MDT regimens for both PB and MB leprosy in large scale field trials.

However, compared with that of RMP, the bactericidal activities of both OFLO and MINO are rather weak.[8,11] The combination OFLO-MINO (OM) was significantly less active than was RMP alone in both mice and humans, and the combination ROM was no more bactericidal than was RMP alone.[8] To increase further the efficacy of a monthly-administered, fully supervisable MDT regimen, it would be desirable to substitute more powerful bactericidal agents for the components of ROM.

HMR 3647 (RU 66647, telithromycin) (HMR) is a ketolide, a new class of macrolides possessing a 14-membered ring. In numerous experiments *in vitro*, HMR exhibited strong activity against a wide spectrum of microorganisms, and was the most active macrolide against gram-positive bacteria.[12,13] Moxifloxacin (BAY 12-8039) (MXFX), a new broad-spectrum fluoroquinolone that has recently been marketed in Europe, was found to be by far the most active fluoroquinolone against *M. tuberculosis* in mice.[14] Rifapentine (DL 473) (RPT), a rifamycin derivative, is not a new drug, but its marketing has only recently begun. Our studies have shown that the pharmacokinetic properties of RPT are far more favourable than those of RMP, with a significantly higher serum peak level (C_{max}) and much longer serum half-life ($t_{1/2}$);[15] consequently, RPT was significantly more active in the treatment of murine tuberculosis than was RMP, when both drugs were administered intermittently.[15]

The objectives of this experiment are to measure the bactericidal activities against *M. leprae* of HMR, MXFX, RPT, and the combinations MXFX-MINO (MM) and RPT-MXFX-MINO (PMM) in mice, and to compare these with the activities of established drugs and combinations. Bactericidal activity was determined by the proportional bactericide technique[16] in the mouse footpad system.

Materials and methods

As shown in Table 1, 450 female, immunocompetent Swiss mice (Janvier Breeding Center, le Genest Saint-Isle, France) were divided among 11 groups, each consisting of four subgroups

containing 10 mice each. The mice of each subgroup were inoculated in each hind footpad with an average of 5×10^3, 5×10^2, 5×10^1 or 5×10^0 *M. leprae* of strain no. 17547, a fully drug-susceptible strain. A fifth subgroup of the untreated control group was inoculated with an average of 5×10^{-1} organisms per footpad.

For the treated groups, treatment by the regimens described in Table 1 was begun on day 3 after infection, all drugs being administered by gavage. After completion of treatment, the mice were held for 12 months, a period of time sufficient to permit a single surviving organism to multiply to a readily countable level. Harvests of *M. leprae* from individual inoculated footpads were then performed by the method of Shepard and McRae.[17] *M. leprae* were considered to have multiplied (i.e. viable organisms survived the treatment) in those footpads found to contain $\geq 10^5$ organisms.

The proportion of viable *M. leprae* remaining after the treatment was determined as the median infectious dose (ID_{50}), and the significance of the differences between the groups was calculated by the method of Spearman and Kärber.[18] For multiple comparisons between the groups, Bonferroni's correction[19] was applied. A difference is significant at the 0·05 level if $P < 0{\cdot}05/n$, in which n is defined as the number of primary comparisons; n varied from 1 to 3 for each group, except for untreated control group, in which case $n = 10$.

Table 1. Bactericidal effects against *M. leprae* of various drugs and drug-combinations. *HMR* = HMR 3647; *CLARI* = clarithromycin; *MXFX* = moxifloxacin; *OFLO* = ofloxacin; *MINO* = minocycline; *RPT* = rifapentine; *RMP* = rifampicin

	Regimen (mg/kg/dose)[a]	% viable *M. leprae*[b]	% *M. leprae* killed by treatment
1	Untreated control	21·8[c]	—
2	HMR (100), 5 doses	2·18[d]	90·0
3	CLARI (100), 5 doses	5·48	74·9
4	MXFX (150)	1·73[e]	92·1
5	OFLO (150)	8·69	60·2
6	MXFX (150)-MINO (25) [MM]	1·38[f]	93·7
7	OFLO (150)-MINO (25) [OM]	5·48	74·9
8	RPT (10)	0·09[g]	99·6
9	RMP (10)	1·73[h]	92·1
10	RPT (10)-MXFX (150)-MINO (25) [PMM]	0·02[i]	99·9
11	RMP (10)-OFLO (150)-MINO (25) [ROM]	1·09[j]	95·0

[a] All treatments were administered as a single dose unless otherwise stated.

[b] The proportions of viable *M. leprae* were determined by means of the proportional bactericide technique.[16]

[c] Significantly greater than the results from all other groups, with the exception of those from groups 3, 5 and 7.

[d] Not significantly greater than the result from group 3 ($P > 0{\cdot}05$).

[e] Significantly smaller than the result from group 5 ($P < 0{\cdot}01$), but not different from the results from groups 2 and 3 ($P > 0{\cdot}05$).

[f] Significantly smaller than the result from group 7 ($P < 0{\cdot}05$).

[g] Significantly greater than the result from group 10, but significantly smaller than the results from all other groups.

[h] Not significantly different from the result from group 4 ($P > 0{\cdot}05$).

[i] Significantly smaller than the results from all other groups.

[j] Not significantly different from the result from group 9 ($P > 0{\cdot}05$).

Results

The results from the untreated control group indicated that the inoculum used for the experiment included 21·8% viable *M. leprae* (Table 1), a rather large proportion.

Among the 10 treated groups, the proportions of viable *M. leprae* were all smaller than that of untreated control group; however, by multiple comparisons between the groups, the difference between control mice and those treated with CLARI alone, OFLO alone and combination OFLO-MINO did not attain statistical significance. All of the remaining regimens displayed some degree of bactericidal activity against *M. leprae* in mice.

As shown in Table 1, the proportion of viable *M. leprae* in mice that had been treated with five daily doses of HMR was significantly smaller than that in the untreated controls; this was not the case in mice treated with CLARI, indicating that HMR displayed significant bactericidal activity against *M. leprae* whereas CLARI did not. However, the difference of the proportion of viable *M. leprae* between the groups treated with two different macrolides did not reach statistical significance.

Administered as a single dose, MXFX was far more bactericidal than OFLO, and was not significantly less active than five daily doses of HMR or CLARI. The bactericidal activity of a single dose of MXFX was identical to that of a single dose of RMP; both killed 92·1% of the viable *M. leprae* originally present. On the other hand, a single dose of MXFX was less bactericidal than was a single dose of RPT. Similarly, administered in a single dose, the combination MM was more bactericidal than the combination OM, but was not significantly more bactericidal than MXFX alone.

A single dose of RPT killed 99·6% of the viable organisms, significantly more than were killed by RMP alone or the combination ROM. A single dose of the combination PMM killed 99·9% of the viable *M. leprae*, and was more bactericidal than a single dose of ROM or of RPT alone.

Discussion

In the current experiment, the bactericidal activity against *M. leprae* of three newer antimicrobials HMR, MXFX and RPT has been unequivocally demonstrated, justifying our approach to the identification of new antileprosy drugs by screening compounds that exhibit powerful activity either against a wide spectrum of microorganisms in general (e.g. HMR), or against cultivable mycobacteria in particular (e.g. MXFX), or possess pharmacokinetic properties much more favourable than those of the member of the class presently employed (e.g. RPT versus RMP).

Because the bactericidal activity against *M. leprae* of a single dose of CLARI alone was rather weak,[11] and because no information was available regarding the activity of HMR against *M. leprae* when the experiment was designed, the mice were treated with the two macrolides for 5 consecutive days instead of a single dose, to facilitate comparison of the activities of the two compounds. Compared to the untreated control group, 5 consecutive days of treatment with HMR displayed significant bactericidal activity against *M. leprae* whereas CLARI did not. Nevertheless, although the proportion of viable *M. leprae* was smaller in the mice treated by HMR than in those treated by CLARI, the difference between the two treatments did not reach statistical significance. This is the first evidence that a ketolide macrolide is bactericidal against *M. leprae*. Whether HMR will eventually replace CLARI for

the treatment of leprosy is unclear, and depends primarily upon the tolerance of patients to the treatment with HMR, because gastrointestinal side-effects were common among patients treated with CLARI.[20]

Because MXFX is by far the most active fluoroquinolone against *M. tuberculosis* in mice,[14] that it is the most active fluoroquinolone against *M. leprae* was not unexpected. The observation that the bactericidal activity of a single dose of MXFX was identical to that of a single dose RMP is most encouraging, because RMP has been by far the most bactericidal of the established drugs against *M. leprae*;[3,20] the current experiment is the first to yield evidence that MXFX, a non-rifamycin compound, displays a level of bactericidal activity against *M. leprae* similar to that of RMP.

Just as MXFX was much more active than OFLO, a single dose of the combination MM was significantly more bactericidal than the combination OM. In fact, that a single dose of OM did not show statistically significant bactericidal effect against *M. leprae* was completely in agreement with the results of our previous experiment, which demonstrated that the activity of a single dose OM was dosage-related; the larger dosage displayed a bactericidal effect, whereas the smaller dosage (that employed in this experiment) did not.[8] The killing effect of MM appeared slightly greater than that of MXFX alone, but the difference did not attain statistical significance, indicating that the addition of MINO did not significantly enhance the bactericidal activity of MXFX.

The finding that a single dose of RPT was significantly more bactericidal than RMP was also very encouraging. RPT appears to be the most active bactericidal drug against *M. leprae* that has ever been tested. The greater activity of RPT in mice appears mainly to result from its very favourable pharmacokinetic properties.[15] It is also possible that the inherent anti-*M. leprae* activity of RPT may be greater than that of RMP, because RPT exhibited lower MICs against various cultivable mycobacteria.[21]

Possibly the most important result of this experiment is that a single dose of the combination PMM killed 99·9% of the viable *M. leprae*, significantly more than the combination ROM, which killed 95·0%. Heretofore, no RMP-containing multidrug regimen has been found to be more bactericidal than RMP alone,[3,20] presumably because the activities of all of the accompanying drugs were relatively weak compared to that of RMP. The combination PMM was slightly more bactericidal than RPT alone, indicating that the addition of MM enhanced the activity of RPT, probably because of the rather powerful bactericidal activity of MXFX.

To confirm the promising bactericidal activities against *M. leprae* of MXFX and RPT in humans, and, more important, to promote development of the combination PMM as a monthly-administered, fully supervisable multidrug regimen, a clinical trial is being conducted among patients with lepromatous leprosy. Four different regimens, all administered as a single dose, are compared in the trial: 400 mg OFLO plus 100 mg MINO (OM); 400 mg MXFX plus 100 mg MINO (MM); 600 mg RMP plus 400 mg OFLO and 100 mg MINO (ROM); and 600 mg RPT plus 400 mg MXFX and 100 mg MINO (PMM). The efficacy and side-effects of the treatments are monitored by standard methods,[5,7,8,20] and the bactericidal effects of the treatments are measured by the proportional bactericide technique.[16]

Today, leprosy patients are increasingly being classified on the basis of their clinical appearance; PB leprosy refers to those newly diagnosed patients who have no more than five skin lesions, and is further divided into PB single-lesion (one skin lesion) and PB multiple-lesion (two to five skin lesions) leprosy.[10] It is possible that the response to chemotherapy of

PB patients with two to five skin lesions may not be very different from the PB single-lesion cases. Because a single dose of ROM has been officially recommended for the treatment of PB single-lesion cases,[10] because preliminary results indicated that its efficacy for the treatment of multiple-lesion PB leprosy was similar to that of standard MDT regimen (see p. 77), and also because a single dose of PMM is more bactericidal than a single dose of ROM, it may be that a single dose of PMM is more efficient than ROM for the treatment of PB single-lesion leprosy, and is even sufficient for the treatment of PB multiple-lesion cases. Therefore, we propose to compare the therapeutic effects of a single dose of PMM, a single dose of ROM and the standard 6-month MDT regimen among newly diagnosed PB patients with two to five skin lesions in a multicentre field trial.

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DISCUSSION

Dr Gupte: If treatment of single-lesion leprosy by a single dose of ROM is shown to be successful in the ongoing clinical trials, couldn't this regimen be adopted immediately for use in the field? Will it then be necessary to undertake a new trial of PMM?

Professor Ji: In the past, only two regimens have been recommended without prior careful study in the field—dapsone monotherapy and WHO/MDT. These regimens were recommended at a time of crisis—dapsone monotherapy, at a time when no other effective antimicrobial therapy was available, and WHO/MDT, at a time when dapsone monotherapy was failing because of spreading dapsone-resistance. I believe that, once a regimen has been shown to be active, any alternative regimen must be studied in a field trial before it may be recommended for routine use.

Dr Lockwood: I support Professor Ji's insistence that any new regimen be subjected to field trial. I believe that, should complications of treatment or unexpectedly high rates of relapse appear, one could be severely criticised for having proceeded without a trial. There remains considerable uncertainty about the response to ROM of patients with three or four lesions. Dr Gupte's data suggest that ROM represents adequate treatment for patients with only one or two lesions, but similar data are lacking for patients with more widespread disease.

Professor Ji: We know something about the mechanism of relapse among patients with MB leprosy, but very little about the mechanism of relapse among patients with PB leprosy. These two situations are probably quite different.

Professor Grosset: I agree with Dr Lockwood that, for patients with more than one or two lesions, single-dose treatment may not be sufficient. This suggests that the bacterial load may be larger when multiple lesions are present.

Dr Van Brakel: You have shown rather impressive results of treatment by PMM, and you've told us of the trial currently in progress at the Institut Marchoux. Do you have any initial data on the response to these new drugs in man?

Professor Ji: Both rifapentine and moxyfloxacin have been marketed, indicating that they have been carried through phase III studies. No data are available as yet on leprosy patients who have been treated with these drugs.