RIFAMPICIN RESISTANT LEPROSY:
A REVIEW AND A RESEARCH PROPOSAL
OF A PILOT STUDY

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Twenty years ago, dapsone resistance had become so widespread a phenomenon among both treated and untreated leprosy patients, that the achievements of leprosy control obtained during the preceding 30 years by large scale dapsone monotherapy were in serious jeopardy. To avert the further spread of dapsone resistance, immediate implementation of multidrug therapy (MDT) was recommended by the WHO Study Group for the treatment of both paucibacillary (PB) and multibacillary (MB) leprosy patients. The MDT regimens for both PB and MB leprosy contain rifampicin (RMP). Compared with the other components of the MDT regimens, i.e. dapsone (DDS) and clofazimine (CLO), RMP is far more bactericidal against Mycobacterium leprae in mice and in humans. In fact, the bactericidal effect of RMP is greater than that of any combination of the new drugs, ofloxacin, clarithromycin and minocycline; thus, RMP is the backbone of the MDT regimens. Emergence of RMP resistance would create very great difficulties for the treatment of individual patients; its widespread dissemination would pose a serious threat to reaching the leprosy elimination target.

More information about RMP resistant leprosy is needed

Proven RMP resistant leprosy was well documented as early as the 1970s. It was rare at the beginning, probably because, in those days, RMP was seldom employed for the treatment of leprosy. Later, it was reported that among a total of 404 MB patients who had been treated with various RMP-containing regimens, 39 of them relapsed and 22 were resistant to RMP, as proven in the mouse foot pad system. Virtually all of the resistant strains were isolated from patients who had been treated by RMP only after they had relapsed after long-term monotherapy with dapsone or another sulphone derivative, and almost all of the strains were also resistant to DDS, indicating that these patients had essentially been receiving RMP monotherapy, which appeared to be the cause of the emergence of RMP resistance. Because at least 5-5% of the 404 cases developed RMP resistance in the decade after beginning treatment with RMP, it appeared that RMP
resistance developed rather rapidly in a significant proportion of patients whose treatment regimens were inappropriate.

To date, more than 10 million leprosy patients in the world have completed their treatment with MDT. It is extraordinary that the overall relapse-rate after completion of MDT has been about 0.1% per annum, and that RMP resistant leprosy has not been reported among patients who were treated with MDT. However, for the following reasons, one must be cautious in interpreting these very promising results.

1. In the MDT regimen for MB leprosy, because daily DDS and CLO, which are intended to ensure elimination of the spontaneously occurring RMP resistant mutants before stopping chemotherapy, are self-administered, the regimen is not resistance-proof; RMP resistance may still develop among MB patients if the self-administered component is not taken regularly. Some studies had revealed that as many as 30% of patients showed poor compliance with the self-administration of drugs. Despite patients being treated nowadays with blister packs of MDT, it remains to be proved that this formulation substantially improves the compliance of patients with the treatment.

2. In most leprosy control programs, surveillance of relapse after completion of MDT is no longer taking place.

3. Until now, the standard technique for diagnosing drug resistant leprosy has been the mouse foot pad system. Unfortunately, for various reasons, the great majority of the mouse foot pad laboratories established around 1980 for the survey of dapsone resistance have disappeared altogether. As a consequence, during the last decade, which coincided with the intensive implementation of MDT, the RMP-susceptibility test has been carried out but rarely, and the results are not always dependable.

Based on these considerations, one should not exclude the possibility that a number of RMP resistant leprosy cases are currently undetected. Before the problem of RMP resistance could become out of control, more solid information about its magnitude in different parts of the world should be collected.

No longer feasible to monitor RMP resistant leprosy by the mouse foot pad technique

The major constraints in testing drug-susceptibility by the mouse foot pad technique are time (at least 12 months are required to obtain results) and expense (a single test in Europe costs at least several hundreds of US dollars). Another difficulty rarely mentioned is that, to preserve the viability of the \( M. leprae \) contained in biopsy specimens and to prevent growth of contaminants, the interval between performance of the biopsy and mouse inoculation with the extracted bacilli should be no longer than 5 days, during which time the fresh tissue must be maintained at 0–4°C; in other words, the specimens must be shipped on wet ice from the field to the laboratory within 72 h, a requirement that often cannot be met in most leprosy endemic countries. In addition, due to various reasons a proportion of the \( M. leprae \) isolates are unable to multiply in the mouse foot pads, leading to inconclusive results about the susceptibility. More important is the fact that, to date, there are simply not enough qualified laboratories in which the drug susceptibility of a meaningful number of \( M. leprae \) strains might be tested with dependable results, and it seems unrealistic to revive the mouse foot pad laboratories that were established in the past to support dapsone resistance surveys.
In short, it is no longer feasible to undertake a relatively large-scale survey of RMP resistant leprosy by the mouse foot pad technique.

**PCR-based, DNA sequence analysis of the rpoB gene might be a cost-effective alternative technique for diagnosing RMP resistant leprosy**

The target of RMP is the beta subunit of DNA-dependent RNA polymerase, which is encoded by the *rpoB* gene. Within the last decade, knowledge of the molecular genetic basis of drug resistance in mycobacteria has advanced rapidly. The region of the *M. leprae* genome containing the *rpoB* gene was one of the first to be sequenced and analysed. Based on DNA sequence analysis of the polymerase chain reaction (PCR) amplified *rpoB* genes, an in vitro technique for detecting RMP resistant *M. leprae* has been developed. The molecular genetic analysis of *M. leprae* from RMP resistant patients, proven by the mouse foot pad technique, has revealed that RMP resistance resulted from a limited number of missense mutations, all located in a short region of *rpoB*, codons 500–540 (numbering system used for *E. coli*), which is known to be the site of mutations that confer RMP resistance in *E. coli* and *Mycobacterium tuberculosis*. Compilation of data available from various studies demonstrated that the *rpoB* mutations were identified from all 25 RMP resistant strains of *M. leprae*, whereas no *rpoB* mutation was detected in any of the 44 RMP-susceptible strains, also proven by the mouse foot pad technique, thus suggesting that the results of DNA sequence analysis of the *rpoB* genes were in full concordance with those of the susceptibility tests carried out in the mouse foot pad system. The most common mutation affected the serine codon at position 531, very often substituted by leucine, as has been identified in 23 (92%) of the 25 RMP resistant strains of *M. leprae*. Other mutations affecting the codons at other positions have also been observed but less frequently, the mutation affecting the histidine codon at position 526, which is rather common in RMP resistant *M. tuberculosis*, has been rarely identified in RMP resistant *M. leprae*.

As mentioned already, due to various reasons a proportion of *M. leprae* strains are unable to multiply in the foot pads of untreated control mice, and hence their drug susceptibility cannot be determined by the mouse foot pad technique. However, the DNA sequence analysis of *rpoB* gene does not depend upon the viability of *M. leprae*; it can be performed on strains that are unable to multiply in mice, and is probably the only method available to determine the RMP susceptibility of such strains. Among 37 strains that failed to multiply in the foot pads of untreated mice, Cambau and colleagues have successfully amplified the *rpoB* gene for DNA sequence analysis in 33, almost 90%; no mutation was identified in 32 strains, but an *rpoB* mutation leading to amino acid substitution Ser531Leu was identified in one strain, and therefore established the diagnosis of RMP resistance.

Other advantages of PCR-based DNA sequence analysis over the mouse foot pad technique are also obvious. Because the biopsy specimens can be kept in 70% alcohol, and be maintained at room temperature for several weeks, the conditions required for storage and shipment of the biopsy specimens for DNA sequence analysis are less demanding than those for mouse inoculation. The cost of such an analysis is only 5–10% of the cost for a test employing mouse foot pad inoculation. More importantly, the results become available after as few as 2–4 days.

The available data regarding RMP resistance associated mutations in *M. leprae* is so striking, it is reasonable to propose that, based on identification of *rpoB* mutation with amino
acid substitution of Ser531, the DNA sequence analysis might be applied as an alternative
technique for diagnosing RMP resistant leprosy. This approach may lead to the diagnosis
of a good 80% of RMP resistant strains of *M. leprae*, and the risk of over-diagnosis of
RMP resistance seems small. In the meantime, more studies on the association between RMP
resistance and *rpoB* mutation, particularly at positions other than Ser531, should be pursued.
It may be possible that on testing an increased number of *M. leprae* strains, no *rpoB* mutations
may be observed in a small proportion of RMP resistant strains, as has been demonstrated in
4% of RMP resistant strains of *M. tuberculosis*.15,16

Monitoring RMP resistant leprosy by DNA sequence analysis of the *rpoB* gene
of *M. leprae*

**OBJECTIVE**

To measure the magnitude of the threat to leprosy elimination presented by RMP resistant
leprosy, we propose to monitor the presence of RMP resistant leprosy by the PCR-based,
DNA sequence analysis of the *rpoB* gene of *M. leprae*. The key information to be collected
is the minimal point-prevalence of RMP resistance; it is not feasible to collect information
on incidence or risk factors of RMP resistance. Because the feasibility of the project remains
unclear, as there are so many uncertainties about the operational difficulties and possible
workloads, a pilot study of the project is required. Aiming to be the starting point for
discussion, leading to the creation of a coordinated monitoring program, an outlined protocol
of the pilot study is presented.

**CRITERIA IN SELECTING THE STUDY AREA FOR THE PILOT STUDY**

1. The study area should cover a whole country, or one or two states in major endemic
countries like India or Brazil.
2. Reasonable health infrastructure for providing MDT services.
3. High MDT coverage, i.e. >80%, has been reached for more than 8 years.
4. Existence of at least 2000 eligible patients (see below).
5. Approval from the national or state authorities.

**ELIGIBLE PATIENTS**

As a first step, the study should focus on acquired or secondary RMP resistance among
MB leprosy patients, because it is unclear whether primary RMP resistance has already
emerged. Although secondary RMP resistance may occur during treatment with MDT, and
may also exist among patients with a low bacterial index (BI) or even with a negative BI,
it probably emerges mostly among MB patients who have relapsed after completion of
MDT, with a relatively high BI. Because relapse occurs late, at least 5 ± 2 years after
completion of MDT,24 the major criteria for selecting eligible patients in the study are: (i) a
history of MB leprosy (here, MB refers to a patient who was skin smear positive before
treatment, regardless of the degree of positivity) and (ii) completion of a full course of
24-month MDT for MB leprosy at least 4 years earlier.

It may only be possible to scrutinize a proportion of the eligible patients in the
project. Because the number of such patients varies widely, selection of the sample and determining its size may differ from country to country. In most countries, approximately 10–20% or at least 1000 eligible patients should be examined both clinically and bacteriologically. It must be emphasized that the skin smears must be taken from at least four sites; and to facilitate the later selection of a biopsy site, at least half of the skin smears should be taken from sites other than on the face. Whenever possible, these eligible patients should be randomly but proportionally selected from the major geographical areas.

DETECTION OF THE SUSPECTED RELAPSED CASES AMONG THE ELIGIBLE PATIENTS

Cases of suspected relapse are those whose skin smears reveal a BI of ≥3+ in at least one site, with or without obvious skin lesions. All these suspects will be biopsied.

MAJOR OPERATIONAL DIFFICULTIES IN DETECTING SUSPECTED RELAPSE CASES AMONG ELIGIBLE PATIENTS

Currently, the eligible patient is no longer considered as a ‘case of leprosy’ by leprosy control programmes;8 after completion of MDT, patients are invariably removed from the register and are often not being followed, and may be difficult to trace. Therefore, one of the initial activities of the project is recreating the register of ex-MB patients who have been released from treatment.

Another major difficulty is that within the last few years, skin smears have been virtually abandoned, and the required skills and facilities for bacteriological examination of skin smears are no longer available in a significant proportion of the national leprosy programmes. Before starting the project, it is therefore necessary to revive the skin smear services and upgrade the skills.

DIAGNOSIS OF RMP RESISTANT LEPROSY

A skin biopsy will be taken from each suspect of MB relapse at the site with a BI of ≥3+, and the biopsy is dispatched to the laboratory for DNA sequence analysis of the rpoB gene of M. leprae.18,19 RMP resistance will be diagnosed if the rpoB mutation with amino acid substitution of Ser531 is identified. If the mutation is identified at a position other than Ser531, no diagnosis should be made immediately; a second biopsy is taken for validating the RMP resistance by mouse foot pad technique.

QUALITY CONTROL OF THE PROJECT

Creation of a specialized team, with two to four experienced health workers, may be required. Its main tasks would be training; organization, coordination and supervision of the field activities; and controlling the quality of clinical and bacteriological examinations.

To standardize the techniques for clinical examination, skin smear taking and reading, biopsy taking, and data collection, all health workers involved in the project should attend the standardization workshops. If more than one laboratory is involved for the DNA sequence analysis of the rpoB gene, a standardization workshop for the techniques and materials is also required.
Conclusions

Relapse and emergence of drug resistance are common phenomena in antimicrobial therapy, and there is no reason to believe that the MDT for treatment of leprosy would be an exception. Because RMP is the irreplaceable key component of the MDT regimens for both PB and MB leprosy, and as >10 million leprosy patients have completed their treatment with MDT, it is time to monitor carefully the magnitude of the emergence of RMP resistance. Although much remains to be learned about the molecular genetic basis of RMP resistance in *M. leprae*, the PCR-based DNA sequence analysis of the *rpoB* gene represents the first *in vitro* method for rapid indirect identification of RMP resistant *M. leprae*, and could be employed for documenting the epidemiological situation of RMP resistant leprosy, despite operational difficulties.

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