

Loss of viability of *Mycobacterium leprae* isolated from nasal secretions of lepromatous patients following daily rifampicin and DDS therapy

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Summary Excreta from blowing their noses was collected from 4 previously untreated multibacillary (LL) patients in the ALERT hospital, immediately before and during daily treatment with 600 mg rifampicin and 100 mg dapsone (DDS). The *Mycobacterium leprae* recovered from the nasal secretions were enumerated and inoculated into the footpads of normal mice. Bacilli recovered from 2 of the patients failed to infect mice after 1 day's treatment, and all infectivity of the bacilli from the other 2 patients was lost after 2 days' treatment. These findings demonstrate the rapidity with which rifampicin-containing multidrug treatment is likely to reduce a patient's level of infection to their contacts.

Introduction

It is generally agreed that the nose is the main exit route of leprosy bacilli from the body.¹⁻⁴ Therefore, the quicker *M. leprae* shed from the nose lose their viability because of chemotherapy, the quicker multibacillary patients become non-infectious to their contacts. Previous studies have demonstrated that daily treatment with 600 mg rifampicin results in extremely rapid killing of leprosy bacilli recovered from skin biopsies of multibacillary patients.⁵⁻⁶ Loss of infectivity for normal mice occurred within 3–7 days, the time the first post-treatment biopsies were taken, indicating that at least 99% of the initial population of viable *M. leprae* had been killed. Similar results were achieved with single doses of 1200 mg rifampicin, while single doses of 900 and 600 mg rifampicin were only slightly less bactericidal.⁷ Because there have been no parallel studies to determine how quickly *M. leprae* that have been excreted from the nose are killed by rifampicin-containing treatment, we studied the length of time taken for leprosy bacilli, recovered after the subjects had blown their noses, to lose their infectivity to normal mice.

Table 1. Loss of infectivity of *M. leprae* isolated from nasal secretions of lepromatous leprosy patients

Duration of treatment (days)	Patient number			
	1	2	3	4
0	8/8	8/8	8/8	8/8
1	0/8	8/8	8/8	0/8
2, 3, 8, 15	0/8	0/8	0/8	0/8

Results are expressed as number of mice showing multiplication/number of mice inoculated with 10^4 AFB (8/8 or 0/8).

Materials and methods

We selected 4 newly-diagnosed and previously untreated multibacillary patients for the study and in 1991 they were admitted to the All Africa Leprosy and Rehabilitation Training Centre (ALERT) hospital for an initial period of daily treatment with 600 mg rifampicin and 100 mg dapson. Nasal secretions were collected immediately before dose 1 of rifampicin (day 0) and 24 hours after doses 1, 2, 3, 5, 8 and 15 (days 1, 2, 3, 5, 8 and 15) by patients blowing their noses into Petri dishes. The nasal collections were decontaminated by treating with 0.5 N sodium hydroxide for 20 minutes and washed twice with phosphate-buffered saline (PBS) pH 7.2 at a room temperature of 21°C. The leprosy bacilli were then pelleted by centrifugation and resuspended in 2 ml PBS containing 0.1% bovine serum albumin (BSA). The numbers of *M. leprae* present in each nasal excretion were determined by acid-fast staining and 8 BALB/c mice inoculated with 10^4 *M. leprae* in both hind foot-pads.⁸ The mice were killed 6 months later, the infected foot-pads washed with alcohol, minced and then homogenized in PBS containing 0.1% BSA until a homogeneous suspension was obtained. The number of bacteria in the homogenates were then determined after acid-fast staining. Multiplication of *M. leprae* was considered to have occurred when more than 10^5 acid-fast bacilli were recovered per foot-pad.

Results and Discussion

The number of *M. leprae* recovered from each nasal excretion of the 4 patients fell from an average of 2.7×10^6 per ml from days 0–3 to about 0.6×10^6 from days 5–15. While the numbers of bacilli shed from each nose fell relatively slowly, the daily treatment with rifampicin and DDS caused a large fall in bacilli viability (Table 1)—i.e. as expected, bacilli from the 4 patients were infectious for mice pretreatment, but 24 hours after dose 1 leprosy bacilli recovered from 2 patients were no longer infectious and 24 hours after dose 2 and on all subsequent occasions all mouse foot-pad infectivity was lost. Because of the incubation with NaOH, and because *M. leprae* were collected only 6 months after the mice had been inoculated, the proportions of viable organisms may have been underestimated to a small degree.

These findings are thus very similar to those previously obtained with bacilli recovered

from skin biopsies⁵⁻⁷ and suggest that rifampicin-containing treatment is as effective in killing bacilli in nasal secretions as those harboured in skin lesions. They therefore indicate that the level of infection of multibacillary patients for their contacts will be rapidly reduced once chemotherapy has been initiated with the WHO-recommended multidrug treatment.⁹ Thus the world-wide implementation of such treatment, which has already resulted in the cure and discharge of over 3 million patients and a substantial reduction in the prevalence of the disease,^{10,11} may also make a significant contribution to controlling its transmission.^{12,13}

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Perte de viabilité du *Mycobacterium leprae* isolé des sécrétions nasales de lépreux lépromateux suite à un traitement quotidien de rifampicine et de DDS

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Résumé Nous avons recueilli des sécrétions nasales de quatre sujets multibacillaires (LL) précédemment non traités à l'hôpital ALERT, immédiatement avant et pendant le traitement quotidien consistant en 600 mg de rifampicine et 100 mg de dapsona (DDS). Le *Mycobacterium leprae* recueilli des sécrétions nasales a été dénombré et inoculé dans la semelle de souris normales. Les bacilles recueillis auprès de 2 sujets, n'ont pas réussi à infecter les souris suite au premier jour de traitement tandis que les bacilles provenant des 2 autres sujets avaient perdu leur infectiosité après deux jours de traitement. Ces résultats démontrent la rapidité avec laquelle un traitement médicamenteux combiné contenant de la rifampicine, est susceptible de réduire l'infectiosité des sujets pour les personnes qui les entourent.

Pérdida de viabilidad de los *Mycobacterium leprae* aislados de secreciones nasales de pacientes de lepra lepromatosa luego de terapia diaria con rifampicina y dapsona

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Resumen En el hospital ALERT se recogieron secreciones nasales de cuatro pacientes multibacilares (LL) no previamente tratados, inmediatamente antes y durante el tratamiento con 600 mg de rifampicina y 100 mg de dapsona. Los *Mycobacterium leprae* recuperados de las secreciones nasales fueron enumerados e inoculados en las plantas de las patas de ratones normales. Los bacilos recuperados de 2 de los pacientes no infectaron a los ratones luego del primer día de tratamiento, y el carácter infeccioso de los bacilos de los otros 2 pacientes se perdió luego de 2 días de tratamiento. Estos hallazgos demuestran la rapidez con la cual un tratamiento multidroga que contenga rifampicina puede llegar a reducir la capacidad infecciosa de los pacientes respecto de sus contactos.