Suzana Madeira Diório[1]
Patrícia Sammarco Rosa[2]
Andréa de Faria Fernandes Belone[3]
Beatriz Gomes Carreira Sartori[4]
Lazara Moreira Trino[5]
Ida Maria Foschiani Dias Baptista[6]
Elaine Valim Camarinha Marcos[7]
Jaison Antonio Barreto[8]
Somei Ura[9]

RELAPSE RELATED TO DRUG RESISTANCE IN LEPROSY

ABSTRACT

The multidrugtherapy proposed by the World Health Organization has been effectively implemented in Brazil in 1991. It helped reduce the prevalence and achieve the cure of leprosy. However, its proven efficacy has not prevented the occurrence of relapses in some leprosy patients. Irregular treatment, bacillary persistence or resistance of *Mycobacterium leprae* to drugs are factors that may be associated with relapse. The objective of this study was assess the occurrence of relapse and associate it with the presence of *Mycobacterium leprae* resistant strains. In order to do that, 28 individuals who were clinically diagnosed as relapse after treatment with sulphone monotherapy, the National Division of Sanitary Dermatology scheme or multidrugtherapy. Biopsies from lesions of multibacillary patients attended by spontaneous demand were collected to verify resistance to drugs through the mouse foot pad inoculation technique. Among the samples evaluated 42.8% had bacilli susceptible to dapsone and rifampicin and 10.7% showed resistance to dapsone. No rifampicin resistant bacilli were isolated. The emergence of resistant strains, especially to rifampicin, is a threat to leprosy control programs, therefore, monitoring the spread of these strains is important because resistance pose a serious obstacle to the elimination of disease, particularly in countries where the disease is endemic.

Keywords: *Mycobacterium leprae*, leprosy, relapse, drugs resistance.

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1 Scientific Researcher. Mestre em Doenças Tropicais. Chefe da Equipe Técnica de Microbiologia do Instituto Lauro de Souza Lima/Bauru – SP. micro@ilsl.br
2 Scientific Researcher. Doutora em Doenças Tropicais. Equipe Técnica de Biologia do Instituto Lauro de Souza Lima/Bauru – SP – prosa@ilsl.br
3 Scientific Researcher. Doutora em Patologia. Chefe da Equipe Técnica de Patologia do Instituto Lauro de Souza Lima/Bauru – SP – abelone@ilsl.br
4 Biologist. Equipe Técnica de Microbiologia do Instituto Lauro de Souza Lima/Bauru – SP – beatriz_sartori@yahoo.com.br
5 Biologist. Equipe Técnica de Microbiologia do Instituto Lauro de Souza Lima/Bauru – SP – lazaratrino@ig.com.br
6 Scientific Researcher. Doutora em Biologia Celular e Molecular. Equipe Técnica de Microbiologia do Instituto Lauro de Souza Lima/Bauru – SP – ifoschiani@gmail.com
7 Scientific Researcher. Mestre em Doenças Tropicais. Equipe Técnica de Farmácia e Bioquímica do Instituto Lauro de Souza Lima/Bauru – SP – emarcos@ilsl.br
8 Dermatologist. Mestre em Ciências/Saúde Pública – jaisonbarreto@gmail.com
9 Scientific Researcher. Dermatologist. Diretor da Divisão de Pesquisa e Ensino do Instituto Lauro de Souza Lima/Bauru – SP - pesquisa@ilsl.br
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INTRODUCTION

The multidrugtherapy scheme (MDT) was implemented by the World Health Organization (WHO) in 1981, helping to dramatically reduce the prevalence of leprosy worldwide[1]. WHO recommended the MDT scheme after the frequent reports of resistance to dapsone (DDS) and rifampicin (RFP) following monotherapy treatments aiming at preventing the selection of resistant *Mycobacterium leprae* strains.

In Brazil, the efficacy of MDT was evaluated for a few years; however, it was effectively implemented in 1991[2]. After two decades of its implementation and expansion to the health services, it was possible to demonstrate the decreasing rate of new leprosy cases detection. In 2007, the new cases detection rate reached 21.08/100,000 inhabitants and the prevalence, 21.94/100,000. Although the country registered a significant decrease in the indices, it remains a public health problem, demanding continuous surveillance [3, 4].

Despite there have been no doubts about the effectiveness of MDT, it has not prevented the occurrence of relapses after patients have long been release from treatment. Although considered a rare event, the recurrence is an important indicator of treatment efficacy. According to the National System of Notification of Diseases - Secretaria de Vigilância em Saúde/MS (SINAN/SVS), in 2007 Brazil registered 3.8% of relapses[5] as compared to 1% of the mean relapse percentage worldwide[6]. After 2001 the relapse rate in the country varied (2.7%) and a slight increase in the relapse rate could be observed. It is believed that these indices do not represent the real magnitude of relapses, besides, cases the reactional episodes, which may occur several years after patients are released from treatment, are sometimes diagnosed as relapse[7, 8, 9]. These cases are again treated, returning to the active record, and causing a negative impact on the prevalence of the disease.

Cases of relapse associated with resistance to MDT drugs represent an emerging problem, however, since the 60's different reports about relapses have been described. The DDS was the first drug to show evidence of resistance and this was possible only after the standardization of the mouse foot pad inoculation with *M. leprae* by Shepard, in 1960[10]. The first case of DDS resistance was described in 1964 using this methodology[11]. Because DDS was used for many years as monotherapy, it is the drug most often associated with resistance. Reports of resistance to RFP are less frequent, but such cases are of great concern, because RFP it is the backbone of multidrugtherapy due to its high bactericidal activity[12,13,14].

Currently, in addition to the foot pad inoculation technique, it is also possible to detect resistant bacilli by different molecular methods. The polymerase chain reaction (PCR), analysis of polymorphisms, heteroduplex and sequencing have been the most utilized molecular methods[15]. In this case, the molecular detection of resistance for mycobacteria has been based on the observation of mutations in genes that encode regions involved in the target mechanism of action of drugs or their activation.

Application of molecular techniques has demonstrated that the mechanism of resistance of *M. leprae* to DDS is associated with mutations in the *folP1* gene, which encodes the production of the enzyme dihidropteroate synthase (DHPS). Some strains undergo spontaneous mutations that occur in the cromossomal copy of *folP* gene, while others seem to be the result of translocation. In most cases, the resistant organisms produce a modified form of DHPS, which continue to catalyze the reaction of condensation in dihidropteroate, however they are refractory to inhibition by sulfonamides[16,17].

The genetic basis of resistance to the RFP has been studied since the 90's. A mutation in a small segment of the *rpoβ* gene, which encodes the *β* subunit of DNA-dependent RNA polymerase, was identified among isolates of bacilli that were resistant after inoculation in the footpad of mice[18].

The objective of the present study was to verify the occurrence of leprosy relapses associated with resistance to drugs, after treatment with sulphone monotherapy, the National Division of Sanitary Dermatology (DNDS) scheme or MDT for multibacillary (MB) using the mouse foot pad inoculation technique.

PATIENTS AND METHODS

Patients: between January, 2003 and March, 2005, MB patients were evaluated (n = 28). They were previously treated for lepromatous leprosy (LL) or borderline - lepromatous leprosy (BL), and looked again for medical attention showing clinical sign and/or symptoms of reactivated leprosy. The patients were attended by spontaneous demand at the Dermatology Service of the Institute "Lauro de Souza Lima" - Bauru / SP. They were submitted to dermato-neurological evaluation. All patients had completed treatment at least five years before.

Biopsy: two fragments were collected from the lesion, one was sent for histopathological examination, and the other for inoculation in the footpad for drug susceptibility testing with DDS and RFP.

Inoculation in mouse foot pad – Shepard's technique: the protocol described in the manual Laboratory Techniques for Leprosy[19] was followed to assess the susceptibility of bacilli to drugs. Briefly, the biopsy was macerated in a tissue homogenizer containing 2ml of a Hank's balanced salt solution (Gibco BRL®), to obtain the bacillary suspension. Then, 30 µl of the suspension were deposited on slides for microscopy, fixed and stained

by Ziehl-Neelsen technique. After counting bacilli, 50 BALB/c mice of both sexes were intradermally inoculated in the left rear footpad, with 10,000 bacilli/0.03 ml. The animals were divided into 05 groups: control (diet without drugs), 0.01% g DDS, DDS 0.001% g, 0.0001 g % DDS and RFP 10mg/Kg. The DDS (Sigma®) was added to the feed and RFP (Merck®), administered via gavage once a week for six months. The animals were kept at 22° C controlled temperature and receiving water and feed *ad libitum*. After 10 months of inoculation, the animals were sacrificed and the footpad excised and processed according to the protocol used for the biopsy of the patient, with subsequent counting of the number of bacilli. Significant bacillary multiplication was considered when ≥100,000 bacilli were recovered from each footpad.

RESULTS

We evaluated 28 cases with clinical signs suggestive of reactivated leprosy and which were considered high-risk group for resistance to drugs. In 12/28 (42.8%) samples of bacillary multiplication was observed in the foot pad, indicating the presence of viable bacilli in the initial biopsy. Among these 12 samples, 09 (75%) had bacilli sensitive to DDS and RFP and 03 (25%) resistant to DDS. Inconclusive results, or those in which there was no bacillary multiplication occurred in 16/28 (57.1%) cases.

Considering the total number of cases evaluated (n=28), 32.1% (09/28) presented bacilli susceptible to DDS and RFP, 10.7% (03/28) were resistant to DDS and 57.1% (16/28) showed inconclusive results. No RFP resistance case was observed (Table 1).

Table 1. Result of drug susceptibility testing for rifampicin and dapsone by mouse footpad inoculation with *Mycobacterium leprae* samples from relapsed patients.

Susceptibility to drugs (n=28)			
Susceptible[1]	DDS resistant	RFP resistant	Inconclusive[2]
09 (32,1%)	03 (10,7%)	0 (0%)	16 (57,1%)

[1] Bacillary multiplication only in the control group.

[2] Absence of bacillary multiplication.

Histopathological examination of relapse cases at the time of clinical evaluation was performed in 20/28 (71.4%) cases evaluated. This included 10 out of 12 cases that showed bacillary multiplication in mouse footpad. All of them were consistent with active disease, with presence of typical bacilli. In the group of patients in which there was no bacillary multiplication (inconclusive result), the histopathological examination was performed in 10/16 (62.5%), 05 (50%) showed histopathology compatible with active disease and presence of solid bacilli, other 05 (50%) cases did not show solid bacilli, resulting in regressive disease. From patients who had active disease, three had been treated with MDT/MB/24 and two with sulphone monotherapy.

From the total of 28 patients presented with relapse, disease reactivation was confirmed in 17 (60.7%) by inoculation and/or histopathological examination.

In respect to previous treatment, 05/28 (17.8%) patients were treated with DDS monotherapy, 01/28 (3.5%) were on DNDS scheme and later MDT/MB/24, 15/28 (53.5%) completed MDT/MB/24 scheme, 04/28 (14.3%) MDT/MB with different number of doses and 03/28 (10.7%) had taken MDT irregularly.

The time between the diagnosis of disease and clinical relapse varied from 09 years to 50 years.

The clinical profile of patients from which foot pad inoculation was positive, and the results of susceptibility testing to DDS and RFP are described in Table 2.

DISCUSSION

The definitions of leprosy "cure" and "relapse" make it a very peculiar disease. The concept of "cure" is closely linked to the proposed scheme of treatment for paucibacillary (PB) or MB cases. According to the Guide to Epidemiological Surveillance, Ministry of Health of Brazil, patients are considered cured after they have completed the number of doses recommended by WHO. In respect to relapse, it is considered as a relapse case that individual who after successfully completed the MDT starts showing new clinical signs and symptoms of leprosy[20].

Although the criteria for diagnosis of relapse leprosy cases can vary according to the author or place, signs of clinical activity of disease in patients after they have been discharged from treatment are suggestive of relapse. Skin smear, histopathological examination and inoculation in mouse foot pad are laboratory test that can be used to confirm the diagnosis of relapse.

Several factors predispose to relapse. Persistent bacilli, high bacillary index at diagnosis, inadequate or irregular treatment, monotherapy, especially with DDS, are often associated with confirmed cases of relapse [21].

Reports of relapse associated with resistance to drugs have been more frequently reported, especially after the molecular mechanisms and genes involved in resistance to drugs became known. Shetty et al[22], studying 37 cases of relapse, showed 21% of resistance to DDS and/or RFP out of 28 samples that presented bacillary multiplication on mouse footpads. Using mice inoculation and molecular biology, Maeda et al[23] found a significant number of strains resistant to DDS and RFP after patients were treated with WHO/MDT/MB scheme. Of the 252 isolates from untreated patients, Matsuoka et al[24] found 3% of resistance to DDS and 2% to RFP, which

Table 2. Clinical profile of relapse patients with positive bacillary multiplication in the footpad of mice. Results of susceptibility testing to dapsone and rifampicin.

Clinical form	Date diagnosis	Date relapse	Treatment	Histopathology	Result of inoculation
L	1985	2003	Mono DDS MDT/14	Active disease	Resistant DDS
L	1982	2003	Mono DDS MDT/24	_____	Susceptible
L	1990	2003	MDT/24	_____	Susceptible
L	1991	2003	MDT/24	Active disease	Susceptible
BL	1986	2004	Mono DDS	Active disease	Susceptible
L	1959	2004	Mono DDS	Active disease	Resistant DDS
BL	_____	2004	DNDS MDT/24	Active disease	Susceptible
L	1954	2004	MDT/24 irregular	Active disease	Resistant DDS
L	1984	2004	RFP + DDS	Active disease	Susceptible
L	1990	2004	MDT/24	Active disease	Susceptible
BL	1964	2004	Mono DDS	Active disease	Susceptible
L	1994	2005	MDT/24	Active disease	Susceptible

shows the circulation of resistant strains among patients; on the other hand, resistance rates were higher in patients who relapsed showing 15% resistance to DDS and 8% to RFP.

In the present study we found that 12/28 (42.8%) samples resulted in bacillary multiplication in the foot pad, indicating the presence of viable bacilli in biopsies used for confirmation of relapse. Among these 3/12 (25%) cases showed resistance to DDS and none to RFP. The first reported case of resistance was from a monotherapy treated patient (DDS for 16 years) who also had also taken 14 doses of MDT/MB. The second had been treated with DDS for 19 years and the third case of resistance used MDT/MB/24 regular and other doses irregularly, and despite not having any record of monotherapy his leprosy diagnosis was done before the implementation of MDT in the country.

Relapse was associated with drug resistance in 10.7% (3/28) of samples evaluated. Despite RFP resistance have not been detected the finding of DDS resistance can not be neglected. The emergence of organisms resistant to drugs is always a concern and threat for infectious diseases control programs, and leprosy is not different, because it is a chronic disease, the emergence of resistant strains represents a potential risk for its control.

After more than 20 years of implementation of MDT, reports of relapse associated with the resistance among patients with DDS monotherapy, have been described in the literature. Matsuoka et al[25] isolated bacilli with resistance to DDS, RFP and Ofloxacin for a patient who did monotherapy with different drugs, but not with to standard MDT/MB. Zhang et al[26] investigated the occurrence of multiple resistance to DDS and RFP in a patient who had been treated with monotherapy with DDS and RFP. Madeira-Diorio *et al.*[27] observed 12.5% resistance to DDS (55% were from monotherapy) and 5% to RFP among 40 patients who showed clinical signs of relapse.

Another important result to be considered is the samples sensitive to drugs (9/12). In such cases, factors other than the resistance have contributed to appearance of clinical signs of disease. It seems that inadequate or irregular treatment was not a risk factor because the majority of patients reported regular treatment with PQT/24. However, in two cases who received monotherapy with DDS, the treatment may have constituted a risk for relapse, because the bacteriostatic mechanism of DDS. In such cases, it would be expected to find more strains resistant to DDS, which more commonly happens. As the susceptibility testing was performed using the footpad inoculation, it is possible that resistance has not been detected because it is a less sensitive method when compared to genetic polymorphism. Persistent bacilli may also be associated with relapse. Bacilli have been identified in immunologically favorable conditions for its survival such as dermal nerves, smooth muscles, lymph nodes, bone marrow and liver. These organisms are present in about 10% of MB patients, and

their proportion may be higher in cases with high bacillary index[21].

According to WHO, a study where a large number of patients were evaluated after completion of treatment, showed that rates of relapse are very low, with cumulative risk less than 1% during follow-up of nine years[28]. This percentage, however, has not been reported in other studies[29]. Currently, Brazil has the highest relapse rate (4% in 2008), notified in the world. However, we know that these figures do not indicate the real magnitude of relapses in the country, since there only a few studies have been undertaken with the objective of evaluating relapse in leprosy patients.

We can not discard the high number of relapses described in the present study. Despite 42.8% of the cases evaluated have been confirmed as relapse by Shepard's technique, when the results of inoculation are evaluated together with results of histological examination that were consistent with relapse (n=5), the percentage increases to 60.7%. In cases where the inoculation showed no bacillary multiplication, the result of the hispathological examination was very important for the diagnosis of relapse, emphasizing the importance of performing additional tests in cases suspected of reactivation of leprosy.

Another aspect to be considered is shortening the treatment of MB patients from 24 to 12 doses, or even six, may cause in future in increased number of relapse cases, also with increasing number of bacilli resistant to drugs. Our finding of confirmed relapse in patients who have irregular or monotherapy treatment and less than 24 doses of MDT supports this assertion. The follow up of patients for long periods is necessary for early detection of relapse, because they are a source of new infections. Particularly, it is essential the monitoring of patients in high endemic areas, and the possibility of patients in this study have been re-infected can not be discarded since the contacts of these patients were not re-evaluated.

An important perspective for further studies is the development and validation of rapid methods for detection of strains of viable *M. leprae*, also resistant to drugs of the MDT scheme.

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