Persistence of viable bacilli in highly bacilliferous multibacillary leprosy patients after the twelve doses of multidrug therapy WHO/MDT

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ABSTRACT

The present study evaluate the efficacy of twelve doses of multidrug therapy, as suggested by World Health Organization, in ten lepromatous leprosy patients with bacilloscopic index equal or higher than 4. The patients have taken the doses in twelve month-doses regularly. At the end of treatment a specimen was collected by punch biopsy and inoculated into mouse hind footpad according to Shepard´s technique. Persistence of viable bacilli was demonstrated in three patients. In spite of the small number of patients, same results have been found by other authors, showing that in highly bacilliferous leprosy patients, at the beginning of treatment more doses may be required to prevent relapses.

Key-words: Leprosy, therapeutic, inoculation.

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INTRODUCTION

Recently, the Ministry of Health (BRASIL/MINISTÉRIO DA SAÚDE, 2000), based on conclusions of World Health Organization – WHO, Expert Comittee on Leprosy (WHO, 1998) recommended that the multidrug therapy (MDT) regimen for multibacillary leprosy (MB) could be shortened to twelve doses. WHO recommendations were based on clinical, laboratorial and operational data summarized in the editorial by Dr. Ji Baohong, published at the *Leprosy Review,* 1998. In that paper, the author affirmed that the changes in operational criteria for classification of patients as MB and paucibacillary (PB) groups led to allocating a great number of smear-negative patients into the MB group. Due to difficulties in smear examination in some endemic regions, classification by clinical criteria based on number of lesions was adopted, placing patients with up to four lesions into PB group and patients with five or more lesions into MB group. At the same time, clinical cure has been verified in patients who interrupted treatment and were afterwards recovered; also the absence of relapses in patients treated with 12 or 24 doses was observed, despite of the short follow-up. (Ganapati et al.1992) Added to that the information obtained from clinical and laboratorial research, demonstrating that the bactericidal effect of the dapsone-clofazimine component of WHO/MDT would be able to kill all rifampicin-resistant mutants in 3-6 months, thus favouring shorter regimens of treatment. Finally, there was the rationale stating that shorter regimens would enhance compliance to treatment and the small number of highly smear-positive patients would not result in significant increase in relapse rates (Ji B, 1998).

OBJECTIVE

To evaluate the efficacy of 12-doses WHO/MDT regimen in the treatment of leprosy patients with BI 4.

PATIENTS AND METHODS

Patients:

Ten previously untreated male patients, with clinical, baciloscopic and histopathologic diagnosis of lepromatous and borderline lepromatous leprosy were studied. All patients had baciloscopic indexes (BI) equal or higher than 4, all of them with viable bacilli (Table 1). Smears were made according to Ministry of Health recommendations (BRASIL/MINISTÉRIO DA SAÚDE), 1989. Specimens for histopathological examination were collected with nº 6 punch, processed and stained with haematoxilin-eosin and Wade.

Table 1. Roll of studied patients with initial bacilloscopic index (BI) and histopathology initial diagnosis.

Patient	Bacilloscopy	Histopathology
JD	5,0	MHV
SCO	6,0	MHV
JRZS	4,0	MHV
LAN	4,0	MHV
JAS	4,3	MHV
NDF	4,5	MHV
JMR	5,25	MHV
LAS	5,25	MHV
JGSB	4,25	MHBV
JLCA	6,0	MHV

Treatment regimens and follow-up:

WHO/MDT for MB patients (Rifampin 600 mg and Clofazimine 300 mg in monthly supervised dose; plus Dapsone 100 mg and Clofazimine 50 mg in daily self-administered dose) was administered in 12 doses. All patients completed the treatment in 12 months. And were clinically evaluated by physicians every month, at the time of the supervised dose.

Inoculation:

At the end of treatment, a fragment from a biopsy was taken for inoculation of into mice foot pads (Shepard's technique). After collection, the biopsy was immediately sent to the Instituto Lauro de Souza Lima, Bauru/SP, to be processed according to the protocol described at the Laboratory Technique for Leprosy (WHO – 1987). Briefly, the biopsy was macerated in a tissue grinder containing Hanks balanced saline. The suspension was observed under a light microscope, 10 µl were pipetted onto three circles of 1 cm of diameter previously drawn on the slide. Bacilli number was estimated by mathematical calculation and adjusted to 10,000 (10^4) bacilli/0.03 ml. Forty young BALB/c mice of both sexes were then intradermally inoculated in both hind foot pads. Dapsone (DDS) at the concentrations 0.01%; 0.001% and 0.0001% were incorporated into the diet and administered daily. Rifampin (10 mg/kg weight) was administered once a week for 6 months by gavage. The control group did not receive any drug.

The animals were sacrificed 8 months after inoculation, time required for the bacilli to reach the maximum growth plateau. The foot pads were excised and macerated as previously described for the human biopsy and a slide was prepared for bacilli counting.

Evaluation of results:

Significant bacillary growth was achieved when = 100.000 ($10^{5)}$ bacilli/foot pad were found. Growth means presence of viable bacilli in the biopsy which originated the inoculum. Bacillary multiplication in the control group and absence of multiplication in groups which received either dapsone or rifampin suggest that these bacilli are sensitive to these drugs; bacilli multiplication in an animal that received the drug indicates resistance. Finally, when there is no multiplication in none of the groups (control and treated), it is not possible to evaluate susceptibility or resistance, because the bacilli may already been dead at the time of inoculation.

RESULTS

Clinical evolution:

Clinical improvements were observed in all patients with flattening, re-pigmentation and reduction in the size of skin lesions (Figure 1 & 2 patient JD; Figure 3 & 4 patient SC).

Bacilloscopic evolution:

At the end of 12 doses there was a reduction of BI in the ten patients. The mean BI was 4.8 and decreased to 3.4 at the end of 12 doses. (Table 2)

Inoculation:

Bacilli multiplication was observed in 3 out of 10 control groups of mice inoculated with specimens from different patients. However, no growth was observed in the treated mice, indicating the bacilli were not resistant to the drugs used. (Table 3)

The papers in which are based the recommendations for shortening of treatment regimens rely on the probability that the new scheme would be efficient in the majority of MB leprosy patients.

Table 2. Comparison between initial and final bacilloscopic indexes showing reduction of the BI.

Patient	Inicial BI	Final BI	
JD	5,0	4,25	
SCO	6,0	5,0	
JRZS	4,0	2,5	
LAN	4,0	0,25	
JAS	4,3	4,0	
NDF	4,5	4,0	
JMR	5,25	NR	*2,5
LAS	5,25	4,0	
JGSB	4,25	4,25	
JLCA	6,0	3,25	
Mean	4,8	3,4	

*done 1 year after release from treatment

Table 3. Results of foot pad inoculation - 2000.

Patient	Control Group	Treated Group
JD	**POSITIVE**	NEGATIVE
SCO	NEGATIVE	NEGATIVE
JRZS	NEGATIVE	NEGATIVE
LAN	NEGATIVE	NEGATIVE
JAS	**POSITIVE**	NEGATIVE
NDF	NEGATIVE	NEGATIVE
JMR	NEGATIVE	NEGATIVE
LAS	NEGATIVE	NEGATIVE
JGSB	NEGATIVE	NEGATIVE
JLCA	**POSITIVE**	NEGATIVE

The rationale strongly depends on the bactericidal power of the dapsone– clofazimine component of MDT to eliminate rifampin–resistant mutants. Although this can be true in small *M. lepræ* populations, it may not occur in patients harboring 10^{10} to 10^{12} bacilli. In the papers reporting recovered 24 doses defaulters, besides no mention to bacilloscopic status at the beginning of treatment, better results were observed in the group that had taken 10 – 20 doses, in comparison with the group that had taken less than 10 doses, thus demonstrating the benefits of longer treatment regimens.

It is indisputable the possibility that, among patients with high bacillary load, a group relapses. Jamet et al.(1995), reported 7 (20%) relapses in 35 patients who had taken 24 doses in a 72.7 ± 17.3 months follow-up. This shows that patients with BI 4 have higher risk of relapse even if treated for longer time. These results were confirmed by Girdhar *et al.,* 2000, who studied relapses in 561 patients. It was also observed that the number of relapses increased proportionally with the time of follow-up. Reports that did not show relapses after 1-5 years of follow-up in 12 and 24 dose regimens need further investigation. (Ji B, 2001)

It may also be added that bacilli grown in mice were sensitive to the drugs of WHO/MDT, suggesting an insufficient number of doses rather than bacillary resistance.

Ji (2001) in recent editorial in the *Leprosy Review* mentions the previous papers and acknowledges the need of prolonged treatment in this group of patients, showing that there is great correlation between bacillary load at the beginning of treatment and relapse, and that BI 4 is considered the most significant risk factor. He still says that these relapses observed in the 24-dose regimens will likely increase in the 12-dose regimens. And finally, if the number of highly bacilliferous MB patients is large, the policies for elimination and shortening of leprosy treatment should be reviewed.

Hence, the question to be answered is if the percentage of highly bacilliferous MB patients is significant. A 10 years retrospective study in the Instituto Estadual de Dermatologia Sanitaria (Hospital Estadual Curupaiti, RJ) including 788 MB patients, showed that the percentage is not negligible, it ranges from 15.5 to 31.8% per year (mean 23.2%). (Table 4)

Because all was said before and supported by our preliminary data, we take with caution the recommendation of interruption of smear examination in the routine of leprosy out-patients units, by Ministry of Health (Regulation MS 200/2000). These data demonstrates that, if new parameters for detection of highly bacilliferous MB patients are not established, as proposed by Lemaster *et al.*, 2001, they will continue at risk of relapse, remaining as a source of contagion, thus endangering all efforts to eliminate leprosy.

Table 4. Number of patients and percentage of newly diagnosed multibacillary cases with bacilloscopic index =4, IEDS 1991. 2000.

Year	BI<4	BI ≥ 4		MB Total
		N	%	
1991	58	16	**21,6**	74
1992	62	29	**31,8**	91
1993	64	25	**28,0**	89
1994	71	17	**19,3**	88
1995	82	31	**27,4**	113
1996	53	18	**25,3**	71
1997	47	09	**16,0**	56
1998	38	07	**15,5**	45
1999	63	15	**19,2**	78
2000	67	16	**19,2**	83
TOTAL	**605**	**183**	**23,2**	**788**

CONCLUSION

The present paper reports 10 highly bacilliferous MB leprosy patients treated with the 12-dose WHO/MDT. By inoculation into mouse footpad, the presence of viable bacilli was demonstrated in 3 patients (30% of the sample). Preliminary result is corroborated by other researchers, indicating that highly bacilliferous patients may require treatment with more than 12 doses.

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