Ann. Soc. belge Méd. trop.
1977, **57**, 3, 175-179

THE EFFECT ON THE MULTIPLICATION OF *MYCOBACTERIUM LEPRAE* OF IRREGULAR ADMINISTRATION OF DAPSONE TO MICE. RESULTS OF THE TOTAL MINIMAL INHIBITORY TEST

by

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Summary — Dapsone in a 0.01 per cent concentration in the food was administered to mice for 1 to 6 days a week every week, and every 2, 3 and 4 weeks. It was further administered daily for periods ranging from 4 to 28 weeks after infection. In all drug regimens dapsone was purely bacteriostatic, since multiplication started in some of the animals sometime after stopping treatment. It is concluded that human paucibacillary leprosy should preferably be treated with a more bactericidal drug and multibacillary cases during an initial phase with drug combinations.

KEYWORDS : Leprosy, Experimental; Mycobacterium leprae; Dapsone; Drug Administration Schedule.

Introduction

The most widespread treatment of leprosy is still dapsone 50 or 100 mg daily in monotherapy and autotreatment. In many cases patients are handed at each visit a number of dapsone tablets sufficient for daily treatment during a number of weeks until the next visit. It is a well known fact in the field of tuberculosis treatment that drug intake without supervision becomes frequently very irregular, leading either to relapse or the development of drug resistance. Recent investigations in East Africa (Ellard *et al.*, 1974; Low and Pearson, 1974) have shown that leprosy is no exception and that a considerable proportion of leprosy patients who claim to take DDS regularly in fact do not.

It was therefore decided to study the effect of irregular dapsone treatment on the experimental infection in mice, and furthermore apply the minimal inhibitory test (Pattyn and Saerens, 1975) to the same experimental infection.

Materials and methods

These were as described previously (Pattyn and Saerens, 1974, 1975). Except for the control mice, in which counts were performed, all foot pads were examined histologically. Dapsone was administered in the

food in a 0.01 per cent concentration, producing serum levels in the mouse comparable to those obtained in man after 100 mg dapsone intake per day. Treatment was stopped when the multiplication of *M. leprae* in the control mice had reached plateau level. Intermittency of administration is shown in the tables.

TABLE 1

Irregular administration of dapsone to mice

Every 4 weeks	At plateau	+ 3 months (c)	+ 4 months (c)
2/7 (a)	0/10 (b)	4/10	
3/7	0/10	0/4	2/6
4/7	0/9	0/3	3/5
5/7	0/10	1/10	
6/7	0/10	0/4	2/6 Pos.
Every 3 weeks			
2/7	0/9	0/4	2/4
3/7	0/10	1/4	3/6
4/7	0/10	1/4	3/5
5/7	0/10	0/4	1/5
6/7	0/10	1/4	1/6 Pos.
Every 2 weeks			
2/7	0/10	1/4	2/5
3/7	0/10	0/4	2/5
4/7	0/10	0/4	1/6
5/7	0/18	9/20	11/16
6/7	1/19	6/14	1/10 Pos.
Every week			
1/7	0/9	0/3	1/5
2/7	0/3	0/2	0/3
3/7	0/16	3/10	2/9
4/7	0/16	5/11	3/14
5/7	1/16	3/8	1/8 Pos.
6/7	0/10	0/2	0/6 Neg.

(a) Number of days a week dapsone was administered.

(b) Nominator : number of mice showing multiplication of *M. leprae*. Denominator : number of mice inoculated.

(c) Results in mice examined respectively 3 and 4 months after plateau level of multiplication was reached in the control group, and treatment stopped.

Results

It can be seen from table 1 that dapsone had a bacteriostatic effect on the multiplication of *M. leprae* in all therapeutic regimens, even when administered as infrequently as only 2 days every 4 weeks. However when mice were examined 3 and 4 months later multiplication was noted in some animals of all groups, except two. The first of these had been treated for two days every week, but due to accidental deaths too small a number of mice could be examined. The second group in which no delayed multiplication of *M. leprae* occurred was the one treated for 6 days a week.

TABLE 2

Total minimal inhibitory dosage of dapsone in the experimental *M. leprae* infection

Duration of treatment (*)	At plateau (+)	+ 12 w	+ 16 w	+ 18 w	+ 21 w	+ 24 w	+ 26 w	+ 30 w	+ 34 w	+ 42 w
4 weeks	0/10 (**)	12/12								
8 weeks	0/11	15/15								
12 weeks	0/10		5/6				5/6			
16 weeks	0/10		2/5			1/2		2/2		
20 weeks				0/5			4/6	3/6		
24 weeks				2/6	1/3	2/7			4/4	
28 weeks					0/6				0/6	7/7

(*) 0.01 per cent DDS in the food, continuously.
(**) Number of mice showing multiplication of *M. leprae*/number of mice inoculated.
(+) Plateau phase in controls was reached after 20 weeks.

Table 2 shows the results of the total minimal inhibitory dosage of dapsone. In this experiment plateau phase was reached in the control group after 20 weeks. Thus in the mice treated for 24 and 28 weeks, treatment exceeded the incubation time for 4 and 8 weeks respectively. It is evident from this experiment that even continuous dapsone administration at 0.01 per cent in the food for more than half a year is unable to sterilise the infection.

Discussion

The present experiments illustrate the purely bacteriostatic effect of dapsone on *M. leprae*, even when administered in quantities producing serum levels 100 times higher than the minimal inhibitory concentration (Shepard *et al.*, 1966; Ellard *et al.*, 1971). Bacterial multiplication in the mouse is inhibited even when the drug is administered very infrequently (twice a week every 4 weeks).

The last treatment regimen of table 1 (6 days out of 7, every week) is almost comparable with 20 weeks continuous treatment (7 days out of 7) in table 2. In the first case no late multiplication was detected, but follow-

up was only for 5 months. In the second experiment multiplication was not detected after 18 weeks follow-up, but was after 6 months and more. This leads to the conclusion that in all cases, once the drug is withheld, some surviving organisms start to multiply again.

These results confirm and extent those obtained by Hilson and Banerjee (1974) who showed in the proportional bactericidal test, that 60 days administration of dapsone in full dosage to mice kills only 82 per cent of the bacilli.

In all the experiments discussed, mice were inoculated with 5.10^3 bacilli. Extrapolated to the human disease this means a very limited infection, probably polar TT and BT cases and as far as the second experiment is concerned, a rigoreously regular drug intake corresponding to 50-100 mg per day. The present results illustrate the necessity for dapsone to be administered for prolonged periods even in non lepromatous forms of the disease.

The mouse model unfortunately does hardly allow experimentation on the appearance of drug resistant bacilli. However the poor bactericidal effect of dapsone allows the hypothesis that irregular drug intake or low dosage must frequently lead to the selection of drug resistant organisms in multibacillary patients. This problem has indeed recently been shown by Pearson (1975) to be of great concern. After all it is rather astonishing that dapsone, certainly in monotherapy, has given « satisfactory » results during so many years, for clearly, paucibacillary cases of the disease should be treated, whenever possible, with a more bactericidal drug, and multibacillary cases with combinations of drugs, at least during an introductory phase (Pattyn, 1972; Ellard, 1975).

Acknowledgement — *This work was supported by the Damien Foundation, Brussels.*

Samenvatting — **De invloed van onregelmatige toediening van dapsone op de vermenigvuldiging van** *M. leprae* **bij de muis. Resultaat van de totale minimale inhibitie test.**

Dapsone werd toegediend aan muizen aan een concentratie van 0,01 ten honderd in het voedsel gedurende 1 tot 6 dagen per week, om de week, en om de 2, 3 en 4 weken en dagelijks gedurende periodes van 4 tot 28 weken na de infektie. Alle behandelingsschema's waren zuiver bacteriostatisch, gezien in alle behandelde groepen na het stoppen van de behandeling bakteriën zich opnieuw vermenigvuldigden tenminste bij enkele dieren uit elke groep. Het besluit is dat menselijke paucibacillaire vormen van de ziekte met een meer bactericied middel moeten worden behandeld en multibacillaire vormen tenminste gedurende een aanvangsfaze met een combinatie van produkten.

Résumé — **Effet de l'administration irrégulière de dapsone sur la multiplication de** *M. leprae* **chez la souris. Résultats du test d'inhibition minimale totale.**

Des souris furent traitées à la dapsone (0,01 p. cent dans la nourriture) pendant 1 à 6 jours de la semaine, toutes les semaines et toutes les 2, 3 et 4 semaines; en outre d'une façon continue pendant des périodes de 4 à 28 semaines. Dans tous les cas l'effet de la dapsone était bactériostatique puisque dans tous les groupes de souris quelques-unes au moins devenaient positives après l'arrêt du traitement. On en conclut que les cas humains d'infection paucibacillaire devraient être traités si possible par des produits plus bactéricides et les cas multibacillaires, au moins pendant une phase introductoire, par une combinaison médicamenteuse.

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Received for publication on January 13, 1977.

REFERENCES

Ellard, G. A., Gammon, P. T., Rees, R. J. W. & Waters, M. F. R. (1971) : Studies on the determination of the minimal inhibitory concentration of 4,4' - diaminodiphenylsulfone against *Mycobacterium leprae*. Lepr. Rev.,**42**, 101-117.

Ellard, G. A., Gammon, P. T., Helmy, H. S. & Rees, R. J. W. (1974) : Urine tests to monitor the self administration of dapsone by leprosy patients. Am. J. Trop. Med. Hyg., **23**, 464-470.

Ellard, G. A., Gammon, P. T. & Harris, J. M. (1974) : The application of urine tests to monitor the regularity of dapsone self administration. Lepr. Rev., 224-234.

Ellard, G. A. (1975) : Pharmacological aspects of the chemotherapy of leprosy. Lepr. Rev., **46** (suppl.), 41-50.

Hilson, G. R. F. & Banerjee, D. K. (1974) : The proportional bactericidal test : a method for testing in vivo bactericidal action of a persistant drug. I. R. C. S. I., 1037.

Low, S. J. M. & Pearson, J. M. H. (1974) : Do leprosy patients take dapsone regularly ? Leprosy Rev., **45**, 218-223.

Pattyn, S. R. (1972) : Comments on the chemotherapy of leprosy as influenced by the present knowledge on *Mycobacterium leprae.* Lepr. Rev., **43**, 126-136.

Pattyn, S. R. & Saerens, E. J. (1974) : Results of intermittent treatment with dapsone and rifampicin of mice inoculated with *Mycobacterium leprae.* Ann. Soc. belge Méd. trop., **54**, 35-41.

Pattyn, S. R. & Saerens, E. J. (1975) : Minimal inhibitory dosage of rifampicin in intermittent treatment of *Mycobacterium leprae* infection in mice. Zentralbl. Bakt. Hyg. I Abt. Orig. A., **231**, 503-507.

Pearson, J. M. H., Ross, W. F. & Rees, R. J. W. (1976) : DDS resistance in Ethiopia. A progress Report. Internat. J. Lepr., **44**, 140-142.

Shepard, C. C., McRae, D. H. & Habas, J. A. (1966) : Sensitivity of *Mycobacterium leprae* to low levels of 4,4' - diaminodiphenylsulfone. Proc. Soc. Exp. Biol. Med., 893-896.