Ann. Soc. belge Méd. trop.
1974, **54**, 1, 35-41

RESULTS OF INTERMITTENT TREATMENT WITH DAPSONE AND RIFAMPICIN OF MICE INOCULATED WITH MYCOBACTERIUM LEPRAE

by

S. R. PATTYN and E. J. SAERENS

Summary — Dapsone administered continuously in the food at a 0.01 per cent concentration during 6 months was bactericidal for *M. leprae* in the mouse foot pad test.

When the same dose was given once a week or less frequently the effect was bacteriostatic.

The MID for once weekly regimens of rifampicin is situated between 0.125 mg and 0.062 mg.

In a dose of 1.5 mg once a week and once every 2 weeks this drug had a bactericidal effect.

A dose of 0.5 mg once a week and once every two and four weeks was bactericidal. A dose of 0.25 mg was bactericidal when given once a week and once every four weeks, bacteriostatic only when given once every eight weeks.

Such experiments should provide informations on the possibility to use low doses of rifampicin as an introductory treatment in lepromatous leprosy.

KEYWORDS : Leprosy, treatment; Mycobacterium leprae; Dapsone, Rifampicin.

Introduction

The activity of many drugs on *Mycobacterium leprae* has now been well substantiated in the mouse foot pad model (Rees and Weddell, 1970; Shepard, 1971).

Among these, dapsone has been used for the longest time whereas the more recently introduced rifampicin has proved to be the most rapidly bactericidal.

For both drugs the minimal inhibitory concentration in the mouse model has been determined (Shepard, 1966; Ellard *et al.*, 1971; Holmes and Hilson, 1972) for dapsone this corresponds to 0.01-0.03 mcg/ml and for rifampicin 0.3 mcg/ml.

From a practical standpoint intermittent, surveilled therapy in man can offer many advantages (Pattyn, 1972). Therefore it is important that the activity of intermittently administered drugs be evaluated in the laboratory mouse model.

Material and methods

The experiments were conducted with two *M. leprae* strains n^{os} 6382 and 17547, previously described (Pattyn *et al.*, 1972) and fully sensitive to dapsone.

Groups of 10-15 NMRI mice, weighting 25 g were inoculated in one hind foot pad with 5.10^3 acid fast bacilli (AFB).

Dapsone was either mixed with the food administered in 5 g quantities per mouse per day or injected intraperitoneally from a stock solution in propyleenglycol. Except for the mice receiving rifampicin (RMP) daily this drug was administered through an oro-gastric canula, allowing precise dosage.

Drug administration started one week after the start of the experiment.

From the moment the plateau phase in the control animals was reached (respectively after 6 and 7 months in the two experiments), drug treatment was stopped and the treated animals examined at regular intervals thereafter as indicated in table 1.

This allowed to make a distinction between bactericidal and bacteriostatic activity of the regimens as we have previously done for experimental *M. ulcerans* infections (Pattyn and Rooyackers, 1965 a).

Positivity was evaluated through countings of harvests (Shepard, 1960; Pattyn and Rooyackers, 1965 b) or histological examination of foot pads.

Results

A. *Dapsone*

Table 1 shows the overall results. The 4° column indicates the corresponding multiples of the MIC the drugs should attain in the serum of the test animals receiving the doses indicated in the 2° or 3° columns, as is known from other publications (Shepard, 1966; Ellard *et al.*, 1971; Holmes and Hilson, 1972). Results of continuous dapsone treatment at 0.001 and 0.0001 per cent concentrations are taken from a previous DDS sensitivity determination (Pattyn *et al.*, 1972).

Dapsone administered continuously in the food at 0.01 per cent concentration served as a reference treatment known to inhibit completely the multiplication of *M. leprae* in the mouse foot pad.

This treatment proved also to be bactericidal : none of 3 mice observed for a further 4 to 5 months in the absence of treatment, showed any AFB.

The same dose of dapsone administered intermittently once a week and once every two and once every three weeks also inhibited the growth of *M. leprae* but in these groups some mice became positive during the follow-up after treatment was stopped.

These regimens must thus be considered to have only a bacteriostatic activity. The next lower dose of dapsone : 0.001 per cent (0.05 mg/animal or 2 mg/kg body weight), resulting in a serum concentration at least 10 times the MIC if given continuously, still has an inhibitory activity but

TABLE 1

Intermittent treatment dapsone exp. leprosy

Drug	Frequency	Conc. in food	× MIC cont. th.	Results at 6 months	Follow up at months 4	5
Controls	—	—	—	13/14		
DDS p. o.	7/w	0.01 %	100 ×	0/4	0/1	0/2
DDS p. o.	1/w	0.01 %	100 ×	0/4	0/1	1/3
DDS	1/2w	0.01 %	100 ×	0/4	0/1	2/3
DDS	1/3w	0.01 %	100 ×	0/4	4/5	
DDS	7/w	0.001 %	10 ×	0		
DDS	1/w	0.001 %	10 ×	1/8		
DDS	1/2w	0.001 %	10 ×	8/8		
DDS	7/w	0.0001 %	1 ×	0		
DDS	1/w	0.0001 %	1 ×	4/4		
DDS	1/2w	0.0001 %	1 ×	4/4		
DDS i. p.	1/w	0.40 mg		0/4	0/3	1/5
DDS i. p.	1/w	0.06 mg		0/2		0/2
DDS i. p.	1/2w	0.06 mg		0/3	0/1	2/2

7/w : 7 days per week.
w : week; 1/w : once a week; 1/2w : once every 2 weeks etc.
× MIC : multiples of MIC the corresponding drugs should attain in the serum of the animals receiving the doses indicated in the 2nd and 3th columns as is known from the publications of Shepard (1966) Ellard *et al.* (1971) and Holmes and Hilson (1972).

proves insufficient when administered intermittently once a week or once every two weeks.

When the dose is lowered to 0.0001 per cent (0.2 mg/kg body weight) intermittent schedules failed to provide any inhibition.

Levy and coworkers (1972) showed that one of the differences of metabolism of dapsone between mouse and man is that the halflife of the drug is much shorter in mice (2.1-3 hours as compared to 14-53 hours). Drug plasmalevels of mice given drug containing food are rather constant because the animals tend to eat during the whole day. An effort was made to administer a comparable dose of drug by I. P. injection as was taken in by the animals through the food during a 24 hour period.

Because dapsone 0.5 mg I. P. was toxic, the dose was reduced to 0.40 mg I. P. once a week. The results were comparable with the oral administration of 0.5 mg for 24 hours a week.

The results with a dose 6.6 times lower show an irregularity : 0.06 mg once a week would be more effective than 0,40 mg once every week.

This must be the result of an insufficient number of mice examined : indeed only 2 mice were left 5 months after arrest of treatment.

TABLE 2

Intermittent RMP treatment exp. leprosy

Drug	Frequency	Food	mg/kg	× MIC	Results	Follow up			
				cont. th.	6 M	1 M	4 M	5 M	6 M
DDS p. o.	7/w	0.01 %	20	100 ×	0/3		0/2	0/3	0/1
RMP	7/w	0.01 %	20	10 ×	0/4			0/2	0/1
RMP	1/w	can. 0.5 mg	20	10 ×	0/7		0/2	0/4	0/4
RMP	1/2w	can. 0.5 mg	20	10 ×	0/5	0/1	0/2	0/3	0/2
RMP	1/4w	can. 0.5 mg	20	30 ×	0/2	0/1			0/4
RMP	1/8w	can. 0.5 mg	20	30 ×	0/2	0/1			2/3
RMP	1/w	can. 1.5 mg	60	30 ×	0/6		0/2	0/4	0/2
RMP	1/2w	can. 1.5 mg	60	30 ×	0/5		0/2	0/4	0/2
RMP	1/w	can. 0.25 mg	10	5 ×	0/2	0/1			0/2
RMP	1/4w	can. 0.25 mg	10	5 ×	0/3	0/1			0/3
RMP	1/8w	can. 0.25 mg	10	5 ×	2/4	2/3			
RMP	1/w	can. 0.125 mg	5	2.5 ×	0/5	0/1			0/2
RMP	1/w	can. 0.002 mg	2.5	1.25 ×	2/8	1/4			

7/w : 7 days per week.
w : week; 1/w : once a week; 1/2w : once every 2 weeks etc.
× MIC : multiples of MIC the corresponding drugs should attain in the serum of the animals receiving the doses indicated in the third and fourth columns as is known from the publications of Shepard (1966) Ellard *et al.* (1971) and Holmes and Hilson (1972).

TABLE 3

Results of experimental intermittent therapy extrapolated to human treatment

Drug	Corresponding human dosage	Schedules	Result during treatment	Long term result
Dapsone	100 mg	Daily	A	A
	100 mg	1/w 1/2w 1/3w	A	I
	10 mg	Daily	A	A
	10 mg	1/w	I	I
	1 mg	Daily	A	
	1 mg	1/w 1/2w	I	
Rifampicin	3,600 mg	Daily	A	A
	3,600 mg	1/w 1/2w	A	A
	1,200 mg	1/w 1/2w 1/4w	A	A
	1,200 mg	1/8w	A	I
	600 mg	1/w 1/4w	A	A
	600 mg	1/8w	I	
	300 mg	1/w	A	A
	150 mg	1/w	I	

A = active
I = inactive

B. *Rifampicin*

Dapsone and RMP administered continuously in the food at a 0.01 per cent concentration served as a reference treatment producing complete inhibition as shown by previous work (Shepard, 1966; Pattyn and Rooyackers, 1965 b; Rees and Weddell, 1970; Holmes and Hilson, 1972).

Rifampicin 60 mg/kg given to mice intermittently once every week, and once every 2 weeks was entirely bactericidal. Rifampicin 0.5 mg given once a week, once every two weeks and once every 4 weeks was also completely inhibitory.

The same dosage administered once every 8 weeks was insufficient.

This compares with the results published by Shepard *et al.*, (1972) who found that 25 mg/kg body weight of rifampicin (corresponding to 0.62 mg per animal) given twice on the 70th and 140th day of the experiment, completely inhibited the multiplication of *M. leprae.*

The still lower dose of 0.25 mg corresponding to 10 mg/kg body weight was bactericidal when administered once a week and even when administered once every 4 weeks. The same dose administered once every 8 weeks inhibited only partially the growth of *M. leprae.*

In terms of intermittent once weekly administration, the MID (minimal inhibitory dose) does appear to be situated between 0.125 mg and 0.062 mg corresponding respectively to 5 and 2.5 mg/kg body weight.

In continuous treatment Holmes and Hilson (1972) found 2 mg/kg body weight as the M. I. D.

The regimen 0.5 mg once a week and once every 2 weeks appears as efficient as the same dose given 7 days a week. 0.5 mg given once every 2 weeks gives identical results as 0.25 mg once a week. 0.25 mg can be given once every 4 weeks but a fourth of the same dose given weekly is inactive. This illustrates the importance of the higher, albeit less frequent, blood peaks realized by rifampicin. On the other hand a time interval of 8 weeks with a dosage of 0.5 mg or 20 mg/kg body weight appears to be insufficient.

Discussion

Table 3 gives a summary of the results and their extrapolation to human treatment schedules.

For dapsone it has been shown that administration of the drug in the food at a 0.01 per cent concentration produces drugplasmalevels in mice comparable to those obtained in man after administration of 100 mg per day (Shepard, 1966; Ellard *et al.*, 1971). The dosages of rifampicin for man have been calculated in mg per kg body weight as for the mouse.

The results show that the daily administration of 100 mg (1.5 mg/kg) dapsone in man is fully active, at least in terms of the number of bacilli that can be followed in this experimental model and which is admittedly low.

They also show that this high dosage — as was expected previously (Pattyn, 1972) — even when taken very infrequently — in this model down to once every 3 weeks — is still bacteriostatic.

Reduction of this dosage to the equivalent of 10 mg (0.15 mg/kg) per day for man is still effective. Shepard (1966) showed this in mice, Waters and Rees (1971) confirmed this in man — but if this dosage is taken infrequently for one reason or another — be it irregular drug supply or insufficient motivation of the patient — it becomes rapidly insufficient. Furthermore the possibility of the selection of drug resistant mutants, by such a treatment is not taken into consideration, because this problem cannot be studied in the conventional mouse model.

Rifampicin lends itself much better to intermittent therapy. This probably results from the very rapid killing effect of this drug on *M. leprae* (Rees *et al.*, 1970; *Shepard* et al., 1972; Pattyn *et al.*, 1972).

The very high dosage of 60 mg/kg could be safely administered in mice once every 8 weeks with a definite bactericidal activity. There are no data in man available at this dose level, that may however not be necessary.

On the basis of the present data it would appear interesting to explore the efficiency of treatment schedules in man with doses of 10 to 15 mg/kg once a week to once every 4 weeks. Even the dose of 5 mg/kg once a week might be sufficient, although this would be close to the MID and thus could favour the selection of drug resistant mutants (unless combined therapy were considered).

A controlled clinical trial comparing daily DDS therapy (1.5 mg/kg) as a control group with rifampicin 10 mg/kg daily, rifampicin 15 mg/kg once weekly and clofazimine 5 mg/kg once weekly is now in progress and preliminary data will be reported in the near future.

On the basis of the present laboratory data further studies with even further reduction of total RMP dosage and taking into consideration both changes in dose and in interval could be considered such as :

— 600 mg once a week for 6 months (total dose administered 15.6 g)

— 600 mg once every 4 weeks for 6 months (total dose 3.6 g)
provided that safety of intermittent RMP administration can be ascertained in man.

We want to stress once more that all these RMP schedules are thought of as introductory treatments in lepromatous leprosy (Pattyn, 1972) and should be followed by the entirely supervisable intermittent administration of acedapsone (Shepard *et al.*, 1968).

Samenvatting — **Resultaten van intermitterende behandeling met dapsone en rifampicine van muizen geënt met** *Mycobacterium leprae.*

In de muisvoetzooltest werkt dapsone bactericied wanneer continu toegediend aan een concentratie van 0,01 ten honderd in het voedsel.

Wordt dezelfde dosis éénmaal per week toegediend dan is de uitwerking slechts bacteriostatisch.

De minimale inhibitoire dosis (MID) voor rifampicine, éénmaal per week toegediend ligt tussen 0,125 mg en 0,062 mg.

Een dosis van 1,5 mg toegediend eens per week, en eens per 2 weken werkt bactericied.

Een dosis van 0,5 mg eens per week, eens per 2 en 4 weken is bactericied. 0,25 mg werkt bactericied wanneer toegediend eens per week en eens per 4 weken; eens per 8 weken is deze dosis slechts bacteriostatisch.

Dergelijk onderzoek moet aanwijzingen geven over de mogelijkheid lage doses rifampicine als inleidingstherapie bij lepra toe te dienen.

Résumé — **Résultats du traitement intermittent à la dapsone et la rifampicine chez la souris inoculée avec** *Mycobacterium leprae.*

Dans le modèle du coussinet plantaire de la souris, l'administration continue de dapsone dans la nourriture à la concentration de 0,01 p. cent est effective.

L'administration de la même dose une fois par semaine a seulement un effet bactériostatique.

La dose minimale inhibitrice de la rifampicine administrée hebdomadairement se situe entre 0,125 et 0,062 mg.

Une dose de 1,5 mg administrée une fois par semaine et une fois toutes les 2 semaines a un effet bactéricide.

La dose de 0,5 mg administrée une fois par semaine et une fois toutes les 2 et 4 semaines a un effet bactéricide.

La dose de 0,25 mg est bactéricide lorsque administrée une fois par semaine, et une fois toutes les 4 semaines, à la fréquence de une fois par 8 semaines la dose est bactériostatique seulement.

De telles recherches doivent fournir des données intéressantes concernant la possibilité d'administrer de faibles quantités de rifampicine dans le traitement introductoire de la lèpre.

This work was supported by a grant from the Damiaanfonds, Brussels, Belgium.

S. R. Pattyn : Universiteit Antwerpen (U. I. A.) and Department of Bacteriology and Virology, Instituut voor Tropische Geneeskunde, Nationalestraat 155, B-2000 Antwerpen, Belgium.

E. J. Saerens : Universiteit Antwerpen (U. I. A.), Universiteitsplein 1, B-2610 Wilrijk, Belgium.

Received for publication on July 30, 1973.

REFERENCES

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

Holmes I. B. and Hilson G. R. F. (1972) : The effect of rifampicin and dapsone on experimental *Mycobacterium leprae* infections : minimal inhibitory concentrations and bactericidal action. J. Med. Microbiol. **5,** 251-261.

Levy L., Biggs J. T., Gordon G. R. and Peters J. H. (1972) : Disposition of the antileprosy drug, dapsone, in the mouse. Proc. Soc. Exp. Biol. Med. **140,** 937-943.

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

Pattyn S. R., Rollier R., Rollier M. R., De Muynck A., Janssens P. G. and Verdoolaeghe-Van Loo G. (1972) : Correlation of laboratory and clinical data during treatment of leprosy. Ann. Soc. belge Méd. trop. **52,** 537-548.

Pattyn S. R. and Rooyackers J. (1965a) : Traitement de l'infection expérimentale de la souris par *Mycobacterium ulcerans* et *balnei.* Ann. Soc. belge Méd. trop. **45,** 31-38.

Pattyn S. R. and Rooyackers J. (1965 b) : Traitement de l'infection expérimentale à *Mycobacterium leprae* chez la souris. Ann. Soc. belge Méd. trop. **45,** 27-30.

Rees R. J. W., Pearson J. M. H. and Waters M. F. R. (1970) : Experimental and clinical studies on rifampicin in the treatment of leprosy. Brit. Med. J. **I,** 89-92.

Rees R. J. W. and Weddell A. G. M. (1970) : Transmission of human leprosy to the mouse and its clinical implications. Trans. Royal Soc. Med. Hyg. **64,** 31-42.

Shepard C. C. (1966) : Sensitivity of *Mycobacterium leprae* to low levels of DDS. Proc. Soc. **112**, 893-896.

Shepard C. C. (1971) : A survey of the drugs with activity against *Mycobacterium leprae* in mice. Internat. J. Leprosy **39,** 340-348.

Shepard C. C., Levy L. and Fasal P. (1969) : The sensitivity to dapsone of *M. leprae* from patients with and without previous treatment. Am. J. Trop. Med. Hyg. **18,** 258-263.

Shepard C. C., Levy L. and Fasal P. (1972) : Rapid bactericidal effect of rifampicin on *Mycobacterium leprae.* Am. J. Trop. Med. Hyg. **21**, 446-449.

Shepard C. C., Tolentino J. G. and MC Rae D. H. (1968) : The therapeutic effect of 4-4'-diacetyldiaminodiphenylsulfone (DADDS) in leprosy. Am. J. Trop. Med. Hyg. **17,** 192-201.

Waters M. F. R., Rees R. J. W. and Ellard G. A. (1968) : Pilot trial of dapsone 1 mg daily in the treatment of lepromatous leprosy. Int. J. Leprosy **36,** 651.