Ann. Soc. belge Méd. trop.
1977, **57**, 3, 169-173

ACTIVITY OF THREE NEW RIFAMYCIN DERIVATES ON THE EXPERIMENTAL INFECTION BY *MYCOBACTERIUM LEPRAE*

by

S. R. PATTYN & E. J. SAERENS

Summary — Three new rifamycin derivates characterized by longer lasting serumlevels were tested against *M. leprae* in the mouse model. Their minimal effective dose is slightly to moderately lower than that of rifampicin. Intervals of administration can however not be increased over once every 2 weeks.

On a weight basis one of the drugs is 8 times more potent than rifampicin.

KEYWORDS : Leprosy, Experimental; Mycobacterium leprae; Rifamycins.

Rifampicin, a semi-synthetic derivate of rifamycin is a potent antileprosy drug in mice and man (Rees *et al.*, 1970; Shepard *et al.*, 1972; Pattyn *et al.*, 1974, 1975).

We have now investigated the antileprosy activity of 3 new Rifamycin derivates, characterized by long lasting serum levels as demonstrated after administration of a single dose of these drugs to different animal species. These substances are presently only known under their code numbers : L 8492, L 4947 and L 11473.

Materials and Methods

The procedures of inoculation and their evaluation have been described previously (Pattyn & Saerens, 1974, 1975). All experiments were done on the dapsone sensitive strain 6832 (Pattyn *et al.*, 1972). Substances were administered by gastric canula. In some experiments rifampicin was administered as a control drug.

We have investigated these substances in 3 phases : in a first experiment we determined the minimal effective dose 50 per cent (MED_{50}) in once weekly administration (Pattyn & Saerens, 1974).

In a second experiment two drugs were further evaluated by administering a 2 or 4-fold multiple of their MED_{50} as defined above at increasingly spaced intervals.

Finally the drug with the lowest MED_{50} value was administered weekly for different lengths of time to determine the Total Minimal Inhibitory dose (Pattyn & Saerens, 1975).

Results

1. *Minimal effective dose 50 per cent (in a once weekly administration)*

As shown in table 1, the MED_{50} (o/w.) were respectively, for :

L 8492 : 0.31 mg/k body weight.

L 4947 : between 1.25 and 0.62 mg/k body weight.

L 11473 : between 0.31 and 0.15 mg/k body weight.

Compared with rifampicin for which the MED_{50} (o/w.) is approximately 2 mg/k b.w. (table 1 and Pattyn & Saerens, 1974), L 4947 is slightly more potent, while the L 8492 and L 11473, are 6 to 7 times more potent, on a mg/k body weight basis.

TABLE 1

Minimal effective dose against *M. leprae* of 3 rifamycin derivates compared with rifampicin

1/7 (a)	RMP	L 8492	L 11473		L 4947
10 mg/k	0/4 (b)	0/5	0/9		0/6
5 mg/k	1/8	0/9	0/7		0/6
2.5 mg/k	1/8	0/9	0/8		0/9
1.25 mg/k	8/10	0/13	0/10	0/10	1/13
0.62 mg/k		1/13	0/13	0/10	10/10
0.31 mg/k		3/6	0/8	0/10	8/8
0.15 mg/k				8/13	
0.007 mg/k				8/8	

MED_{50} (o.w.) RMP = 2 mg/k.
L 11473 = 0.31 - 0.15 mg/k.
L 4947 = 1.25 - 0.62 mg/k.
L 8492 = 0.31 mg/k.

(a) Frequency of administration.
(b) Mice showing multiplication of *M. leprae*/total number of mice inoculated.

2. *Intermittency*

Two substances were further evaluated by administering them at increasing intervals : L 4947 was administered at twice its MED_{50} (o/w.) and L 11473 at two- and fourfold its corresponding MED_{50}.

Table 2 shows the results.

TABLE 2

Effect of intermittency on inhibition of growth of *M. leprae* by 2 rifamycin derivates, comparison with rifampicin

		Plat (a)	+ 1mo (b)	+ 3mo (c)
L 4947				
2.5 mg/k	1/w (24×)	0/10 (d)	0/4	0/3
	1/2w (12×)	0/10	0/4	0/4
	1/4w (6×)	3/13	0/1	3/4
	1/8w (3×)	9/11	—	4/4
L 11473				
0.62 mg/k	1/w (24×)	0/10	0/5	0/5
	1/2w (12×)	0/9	0/5	0/2
	1/4w (6×)	10/10	4/5	4/4
	1/8w (3×)	10/10	—	—
1.25 mg/k	1/w (24×)	0/9	—	0/5
	1/2w (12×)	0/10	—	0/3
	1/4w (6×)	0/11	—	0/5
	1/8w (3×)	0/9	—	1/2
RMP	1/4w (4×)	1/10	—	—
10 mg/k	1/4w (6×)	0/10	—	0/6

(a) When plateau level of multiplication was attained in control mice.
(b) one month later.
(c) three months later.
(d) Mice showing multiplication of *M. leprae*/total number of mice inoculated.

Both L 4947 and L 11473 were effective when administered once every two weeks but L 4947 appeared ineffective at once every 4 weeks; L 11473 however at fourfold it's MED_{50} (o/w.) remained active when administered once a month. This is comparable with rifampicin that is also effective when administered once every 4 weeks at 5 times it's MED_{50} (o/w.).

In this experiment L 11473 remains fully active at 1.25 mg/k (4 times its MED_{50}) when administered every four weeks whereas for rifampicin under similar conditions a 10 mg/k (5 times its MED_{50}) is required. This points to a potency ratio of 8 to 1 for L 11473 versus Rifampicin on a mg/k body weight basis under the outlined experimental conditions.

3. *Total minimal effective dose*

This test was only carried out with substance L 11473, at 1.25 mg/k b.w. (4-6 times the MED_{50} o/w.).

As shown in table 3, complete sterilisation of the mice foot pads was obtained when the drug was administered at weekly or two weekly intervals, even after so short a total administration time as 4 weeks (either once a week or two times every two weeks).

None of the administrations at 4 weeks intervals was able to sterilize the infection completely : in the groups of monthly treated mice and kept under observation for 6 months after the controls had become positive, *M. leprae* started to multiply again.

TABLE 3

Total minimal inhibitory dose of L 11473 against *M. leprae* at 1.25 mg/k body weight

		At plat.	+ 6mo
1w (a)	24w (b)	0/3 (c)	0/10
	20	0/3	0/10
	16	0/3	0/10
	12	0/8	0/8
	8	0/7	0/8
	4	0/6	0/6
1/2w (a)	24w	0/3	0/10
	20	0/3	0/8
	16	0/3	0/9
	12	0/6	0/6
	8	0/5	0/6
	4	0/6	0/7
1/4w (a)	24w	0/3	1/10
	20	0/2	4/9
	16	1/3	10/10
	12	1/7	2/6
	8	0/9	7/10
	4	3/9	5/9

(a) Frequency of administration.

(b) Duration of therapy.
At plat : multiplication of *M. leprae* in treated mice when this multiplication had reached plateau level in the controls.
+ 6 months : results 6 months later.

(c) Numerator number of positives. Denominator number of mice examined.

Discussion

It is clear from these experiments that in comparison with rifampicin the newer rifamycin derivatives with long lasting serum levels do not allow a substantial greater intermittency of drug administration in the experimental *M. leprae* infection. This may be related to the long generation time of *M. leprae*. Another possible reason might be that these new drugs do not have a greater bacteriopausal effect than rifampicin.

L 11473 however could be of potential interest since 6 to 8-fold smaller doses than rifampicin proved to be effective in the mouse foot pad (and) provided that toxicological and kinetic data justify its further study in man.

Samenvatting — **Aktiviteit van drie nieuwe Rifamycine derivaten op de experimentale infectie door** *Mycobacterium leprae.*

De aktiviteit tegen *M. leprae* van drie nieuwe semi-synthetische derivaten van rifamycine werd onderzocht bij de muis.

Deze derivaten produceren langdurige serumspiegels. Hun minimale werkzame dosis ligt lichtjes tot matig lager dan die van rifampicine. Bij intermitterende behandeling kan men nochtans de 2 weken intermittentie niet overschrijden.

Eén der derivaten is even actief als rifampicine bij een dosis die evenwel acht maal kleiner is.

Résumé — **Activité de trois nouveaux dérivés de la Rifamycine sur l'infection expérimentale par** *Mycobacterium leprae.*

Trois nouveaux dérivés de la rifamycine, caractérisés par des taux sériques prolongés, ont été examinés quant à leur activité vis-à-vis de *M. leprae* chez la souris.

Leur dose minimale effective est inférieure à celle de la rifampicine. Toutefois en administration intermittente on ne peut guère prolonger l'intermittence au delà de 2 semaines.

Un des produits a une activité égale à celle de la rifampicine à une dose huit fois moindre.

S. R. Pattyn : University of Antwerp & Institute of Tropical Medecine, Nationalestraat 155, B-2000 Antwerpen, Belgium.
E. J. Saerens : Dow Lepetit, Brussels, Belgium.
Received for publication on December 22, 1976.

REFERENCES

Pattyn, S. R., Rollier, R., Rollier, M. R., De Muynck, A. Janssens, P. G. & 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. & Saerens, E. J. (1974) : Results of intermittent treatment with dapsone and rifampicin of mice inoculated with *Mycobacterium Leprae*. Ann. Soc. belge Méd. trop., **54**, 1, 35-41.

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