LM 427, A NEW SPIROPIPERIDYLRIFAMYCIN: *IN VITRO* AND *IN VIVO* STUDIES

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The spiropiperidylrifamycin LM 427 (4-deoxo-3,4-[2-spiro-*N*-isobutyl-4-piperidyl]-(1*H*)-imidazo-(2,5-dihydro)rifamycin S) displays a broad spectrum of potent antibacterial activity *in vitro*. *In vivo* it is particularly effective in the therapy of experimental tubercular infections of mice.

Three schedules of treatment were employed and the best results were obtained when intermittent administrations were used (ED_{50} of LM 427; 7 times lower than rifampicin).

LM 427 is well distributed in tissues of mice and rats, with lung concentrations 10~20 times higher than plasma levels.

In a previous paper[1] we described the antibacterial activity of a new class of rifamycin S derivatives: the spiropiperidylrifamycins. One member of this class (4-*N*-isobutylspiropiperidylrifamycin S; see structure below) whose code number is LM 427 (compound **11** in the earlier paper), showed remarkable activity against *Mycobacterium tuberculosis in vitro* and *in vivo*, together with an interesting profile of tissue distribution in rats.

Recent research[2] carried out at the Center for Disease Control in Atlanta, Georgia, USA, demonstrated that LM 427 inhibits the growth of a number of clinical strains of rifampicin-resistant *M. tuberculosis* and displays a well documented antimicrobial activity on strains of *M. avium-intracellulare* complex refractory to the known antimycobacterial agents. The latter results are of particular relevance because the therapy of *M. avium-intracellulare* infections is still an open medical problem.

The antimycobacterial properties and the pharmacokinetic characteristics of LM 427 have now been more extensively studied and the experimental results are reported in this paper.

Portions of these data were presented at the 12th International Congress of Chemotherapy (Florence, 1981, Abstracts No. 956 & 960).

Materials and Methods

In Vitro Activity

The minimal inhibitory concentration (MIC) on Gram-positive and Gram-negative bacteria, both standard and clinical isolates, was determined by the serial dilution technique in Bacto Antibiotic Medium No. 3 (Difco) supplemented with 1.5% of Agar (Difco) for aerobic strains and in Bacto FTM (Difco) for the anaerobic strains. The inoculum consisted of about 10^5 cells per plate or per ml. Incubation was at 37°C for 1~2 days. The MIC on *M. tuberculosis* was determined by the serial dilution technique in Bacto Albumin Dubos Medium (Difco) inoculated with about 10^6 cells/ml and incubated for 7~10 days at 37°C.

Rifampicin was taken as a reference compound. The antibiotics were dissolved in dimethylform-

amide at 1 mg/ml and subsequently diluted in 1/15 M phosphate buffer, pH 7.

In Vivo Tests

Therapeutic Activity: Groups of 10~12 female CD1 albino mice (Cobs) weighing 20±2 g were infected by i.v. route with 3 LD_{50} of *M. tuberculosis* H37Rv. The animals were treated by oral route with a solution of antibiotic prepared in phosphate buffer, pH 7 + 5% of dimethylacetamide (0.1 ml/10 g body weight). The treatment was continued for 5 weeks according to three schedules: *a*) 5 consecutive days/week starting 3 days after infection; *b*) 2 days/week (Monday and Thursday) starting 3 days after infection; *c*) as schedule *a*, but starting 10 days after infection. Deaths were recorded daily; ED_{50}s were determined on the basis of the survival rate[3] at the end of the experiments. In one experiment, mice were infected with 1 LD_{50} of *M. tuberculosis* H37Rv and treated according to schedule *a*. At the end, lungs were removed and homogenized, and living mycobacteria counted by plating in Middlebrook Agar (Difco). Rifampicin was tested in comparison.

Plasma and Tissue Levels: 1) Mice: Groups of CD1 female Cobs albino mice were treated orally with 50 mg/kg of antibiotic prepared as described above for therapeutic activity; at different intervals, 3 animals were sacrificed, plasma was collected, and tissues were removed, homogenized in phosphate buffer, pH 7, and assayed for antibiotic content on *Micrococcus luteus* ATCC 9341 by the agar diffusion technique[4] against standard solutions prepared in blank samples of homologous tissues.

2) Rats: Groups of 5 CD Cobs albino rats weighing 250±10 g were treated orally with 50 mg/kg of antibiotic as above; at various times one group was sacrificed and plasma and tissues were assayed for antibiotic content as described above.

Results and Conclusions

The *in vitro* inhibitory activity of LM 427 on Gram-positive and Gram-negative bacteria is reported in Table 1 in comparison with rifampicin; the two antibiotics behave similarly with minor differences

Table 1. Antibacterial activity *in vitro* of LM 427 in comparison with rifampicin on collection (C) and clinical (CL) strains.

Strains	Number of strains	MIC (μg/ml)	
		LM 427	Rifampicin
Staphylococcus aureus 209 P (C)	1	0.005	0.005
Staphylococcus sp. (CL)	10	0.16*	0.113*
Staphylococcus sp. (rifampicin resistant) (CL)	4	>10 *	>10 *
Streptococcus faecalis ATCC 8043 (C)	1	2.5	1.25
S. haemolyticus (CL)	1	0.075	0.037
Streptococcus sp. (CL)	1	0.31	0.075
Clostridium perfringens ATCC 13124 (C)	1	0.007	0.007
Escherichia coli B (C)	1	2.5	5
E. coli (CL)	9	5.94*	7.12 *
Klebsiella pneumoniae ATCC 10031 (C)	1	10	>20
Klebsiella sp. (CL)	9	15.42*	15.37 *
Proteus vulgaris ATCC 27973 (C)	1	5	2.5
Proteus sp. (CL)	9	22.45*	6.97 *
P. inconstans (CL)	1	2.50	2.50
Pseudomonas aeruginosa ATCC 9224 (C)	1	20	10
Pseudomonas sp. (CL)	5	>20 *	14.14 *
Enterobacter sp. (CL)	6	14.10*	12.84 *
Citrobacter sp. (CL)	2	10	12.25
Haemophilus influenzae (CL)	1	0.62	0.62
Bacteroides fragilis ATCC 23745 (C)	1	0.075	0.15

* Geometrical mean

LM 427
Chemical formula; $C_{46}H_{62}N_4O_{11}$
Structural formula:

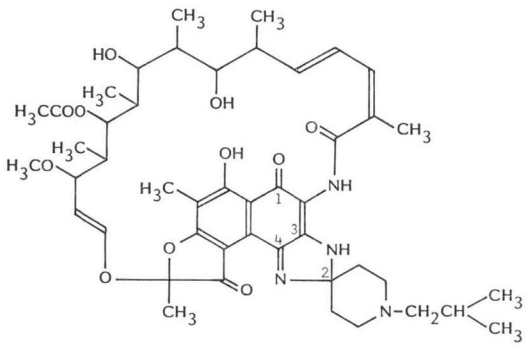


Table 2. *In vitro* activity of LM 427 in comparison with rifampicin on clinically isolated strains of *Mycobacterium tuberculosis*.

Strains	Resistant to	MIC (μg/ml)	
		LM 427	Rifampicin
No. 9	—	0.003	0.006
No. 10	—	0.003	0.006
SMR 1	Streptomycin	0.006	0.006
SMR 4	〃	0.006	0.012
INIR 2	Isoniazide	0.006	0.025
INIR 3	〃	0.006	0.012
No. 5	Rifampicin	10.0	>10.0
No. 6	〃	5.0	>10.0
No. 11	〃	1.25	5.0
H37Rv*	—	0.006	0.012

* Standard strain.

Table 3. Activity of LM 427 and rifampicin on experimental infections of mice with *M. tuberculosis*.

Exp.	Interval between infection and treatment (days)	Number of treatments per week	ED_{50} (mg/kg)				$\frac{ED_{50}\ Rifampicin}{ED_{50}\ LM427}$
			Daily dose		Cumulative dose		
			LM 427	Rifampicin	LM 427	Rifampicin	
1	3	5 (schedule *a*)*	0.6 (0.46~0.78)**	3.48 (2.36~5.13)	15	87	5.8
2	3	2 (schedule *b*)*	0.89 (0.84~1.35)	6.1 (4.07~9.19)	8.9	61	6.9
3	10	5 (schedule *c*)*	0.76 (0.43~1.32)	5.35 (3.13~9.41)	19	133.8	7

* See text.
** In brackets: confidence limits, $P=0.05$.

which are not biologically significant. The activity on *M. tuberculosis*, tested on the standard strain H37Rv and on several clinical isolates, is given in Table 2. LM 427 shows about twice the potency of rifampicin on sensitive strains and a somewhat higher activity on three rifampicin-resistant isolates; streptomycin and isoniazide resistant strains are normally susceptible to both antibiotics. The *in vivo* efficacy against *M. tuberculosis* H37Rv-infected mice is shown in Table 3. LM 427 is more effective than rifampicin under all the experimental conditions tested.

Table 4. Living mycobacteria in mice lungs after 5 weeks of treatment with LM 427 and rifampicin (treatment: schedule *a*; infection: i.v., $1LD_{50}$).

Drug	Dose (mg/kg)	No. of survivors / No. of treated	*M. tbc** $\times 10^3$ (X±SE)
LM 427	5	12 / 12	2.9±0.64
	2.5	12 / 12	5.2±1.6
Rifampicin	10	12 / 12	2.4±1.1
	5	11 / 12	100.0±56.0
Control	—	8 / 17	1600.0±680.0

* *M. tuberculosis* H37Rv.

The ratio of the ED_{50} of rifampicin to that of LM 427 is about 6~7 within the same schedule. The number of living mycobacteria in lungs of mice infected with 1 LD_{50} of *M. tuberculosis* H37Rv and treated according to schedule *a* is reported in Table 4 and documents a stronger *in vivo* bactericidal activity of LM 427 in comparison to rifampicin at the same dosage.

Fig. 1. Plasma and tissue level/time curves for LM 427 after oral administration of 50 mg/kg in mice.

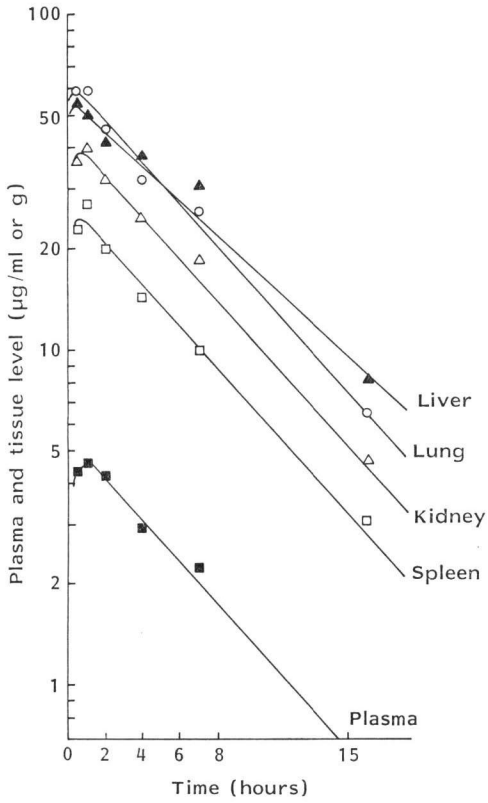
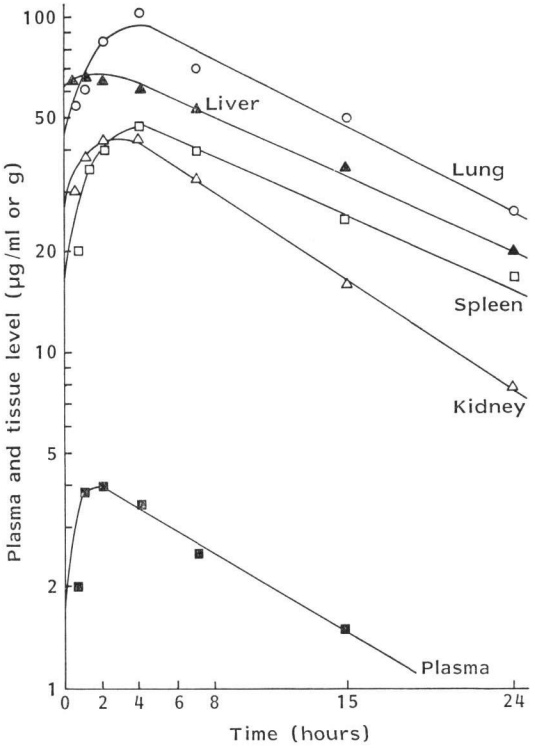


Fig. 2. Plasma and tissue level/time curves for LM 427 after oral administration of 50 mg/kg in rats.



Tissue and plasma levels in mice and rats, after a single oral dose, are depicted in Figs. 1 and 2; LM 427 displays a very good tissue distribution, with maximal levels in lungs of rats and in lungs and livers of mice; very low plasma levels are observed in both species.

In conclusion, LM 427 is a very active antibiotic particularly interesting as an antimycobacterial agent, its activity being directed towards tubercular as well as nontubercular[2] mycobacteria. Its very high potency is optimized *in vivo* by the remarkable tissue tropism and distribution profile which strongly differ from those of rifampicin[5]. The outstanding results of experimental therapy, with different schedules of treatment in mice infected with *M. tuberculosis*, support the possibility of reduced dosage or of intermittent administrations.

The novel structure and the unusual pharmacokinetic behavior of this rifamycin derivative make the product worthy of further study.

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