LABORATORY EVALUATION OF A NEW LONG-ACTING 3-AZINOMETHYLRIFAMYCIN FCE 22250

C. DELLA BRUNA, D. UNGHERI, G. SEBBEN and A. SANFILIPPO

Farmitalia Carlo Erba S.p.A., Biological Research and Development
Via Giovanni XXIII, 23-20014 Nerviano, Milano, Italy

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FCE 22250 (3-(*N*-piperidinomethylazino)methylrifamycin SV) is a member of the new class of 3-azinomethylrifamycins characterized by a long persistance in animals, a good oral absorption and a broad antibacterial spectrum including mycobacteria.

In the experimental mice infection sustained by *Mycobacterium tuberculosis* H37Rv, FCE 22250 shows an efficacy 14 times higher than rifampicin and is still therapeutic when administered once every three weeks.

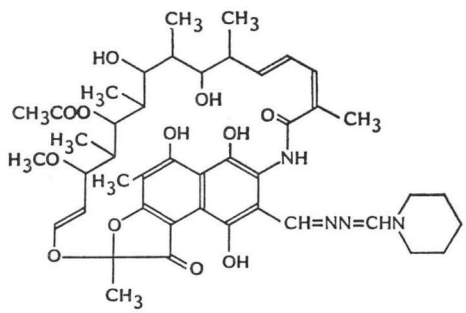
The chemistry and the main biological properties of a new class of 3-azinomethylrifamycins have been described in a previous paper by MARSILI *et al.*[1]. One member of this class, 3-(*N*-piperidinomethylazino)methylrifamycin SV (Fig. 1), whose code number is FCE 22250 (compound No. 1 in the earlier paper) has been selected for its outstanding biological characteristics including antibacterial activity and favorable pharmacokinetic properties. Its activity and pharmacokinetics in laboratory animals are reported in this paper.

Materials and Methods

Compounds

FCE 22250 and rifampicin were used for the *in vitro* tests. Both compounds were dissolved in dimethylformamide (DMF) in a concentration of 1,000 μg/ml and subsequently diluted in 1/15 M phosphate buffer (pH 7.2). For the administration to laboratory animals the compounds were dissolved in phosphate buffer (pH 7.2) plus DMF 5% (mice and rats) or suspended in aqueous solution of 1% lauryl sulfate (dogs).

Fig. 1. Structural formula of FCE 22250.



Minimum Inhibitory Concentration (MIC)

The MIC values were determined on clinical and laboratory strains (see Tables 1 and 2) according to the following methods:

a) Gram-positive and Gram-negative Bacteria: MICs were performed by the serial dilutions technique in Bacto Antibiotic Medium 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 10^4 cells per plate or per ml. Incubation was at 37°C for 1~2 days.

b) *Mycobacterium tuberculosis*, *M. bovis* and *M. ulcerans*: MICs were determined by the serial dilution technique in Bacto Albumin Dubos Medium (Difco) inoculated with about 10^8 cells/ml and incubated for 7 days at 37°C.

c) Other *Mycobacteria*: MICs were performed on Middlebrook 7H10 Medium (Difco)+OADC plates containing the antibiotics at various concentrations. The surface of the plates was inoculated

Table 1. Susceptibility of Gram-positive and Gram-negative bacteria to FCE 22250 and rifampicin (laboratory strains).

Strain (No. tested)	Antibiotic	MIC (μg/ml)	
		Geometric mean	Range
Staphylococcus aureus (5)	FCE 22250	0.024	0.012~0.037
	Rifampicin	0.015	0.012~0.018
S. aureus rifampicin resistant (3)	FCE 22250	>100	12.5~>200
	Rifampicin	>100	6.25~>200
Streptococcus pyogenes (6)	FCE 22250	0.43	0.2~1.25
	Rifampicin	0.48	0.2~2.5
S. faecalis (2)	FCE 22250	0.49	0.4~0.6
	Rifampicin	0.35	0.2~0.6
Micrococcus luteus (1)	FCE 22250	0.037	—
	Rifampicin	0.037	—
Clostridium perfringens (1)	FCE 22250	0.025	—
	Rifampicin	0.025	—
Peptococcus sp. (1)	FCE 22250	0.0075	—
	Rifampicin	0.006	—
Escherichia coli (7)	FCE 22250	6.9	3.12~12.5
	Rifampicin	6.9	3.12~12.5
E. coli rifampicin resistant (3)	FCE 22250	>200	>200
	Rifampicin	>200	>200
Klebsiella sp. (6)	FCE 22250	13.0	6.25~25
	Rifampicin	10.35	6.25~25
Salmonella sp. (5)	FCE 22250	13.7	5~25
	Rifampicin	11.9	6.25~25
Morganella morganii (5)	FCE 22250	9.1	6.25~12.5
	Rifampicin	9.1	6.25~12.5
Pseudomonas sp. (5)	FCE 22250	15.8	5~50
	Rifampicin	13.7	10~25
Neisseria gonorrhoeae (8)	FCE 22250	0.51	0.39~0.78
	Rifampicin	0.15	0.09~0.39
N. meningitidis (1)	FCE 22250	0.39	—
	Rifampicin	0.09	—
Haemophilus influenzae (3)	FCE 22250	1.56	0.78~3.12
	Rifampicin	0.39	0.39
Bacteroides fragilis (3)	FCE 22250	0.99	0.78~1.6
	Rifampicin	0.12	0.05~0.2

with the aid of a multipoint inoculator yielding approximately 10^4 cfu per spot. Incubation was at 37°C for 3~4 days.

In Vivo Therapeutic Activity

a) Gram-positive and Gram-negative Bacteria: Groups of 8~10 female CD_1 albino mice (Cobs) weighing 20±2 g were infected by intraperitoneal (ip) route with 5 LD_{50} of the tested strains.

The animals were treated by oral (po) or subcutaneous (sc) route (0.1 ml/10 g of body weight) as reported in Table 3. The number of survivors was recorded over 96 hours and the ED_{50} was determined according to LITCHFIELD and WILCOXON[2].

b) *M. tuberculosis*: Groups of 10~12 mice as described above, were infected by intravenous (iv) route with 3 LD_{50} of *M. tuberculosis* H37Rv. The antibiotics were given by oral route according to various schedules as specified in Table 4. The number of survivors was recorded over 6 weeks and ED_{50} was determined as above.

Determination of Plasma and Tissue Levels

a) Mice: Groups of female CD_1 albino mice (Cobs) weighing 22±2 g were treated intravenously

Table 2. *In vitro* activity of FCE 22250 and rifampicin against typical and atypical *Mycobacterium* (clinical isolates).

Strain (No. tested)	Antibiotic	MIC (μg/ml)	
		Geometric mean	Range
Mycobacterium tuberculosis (7)	FCE 22250	0.014	0.01~0.03
	Rifampicin	0.014	0.01~0.04
M. tuberculosis SM-EMB-INH Res (4)	FCE 22250	0.12	0.03~0.5
	Rifampicin	0.07	0.02~0.25
M. tuberculosis Rif Res (10)	FCE 22250	25	6.25~50
	Rifampicin	23.32	12.5~50
M. ulcerans (2)	FCE 22250	0.87	0.6~1.2
	Rifampicin	3.50	1.2~10
M. marinum (6)	FCE 22250	0.35	0.2~0.4
	Rifampicin	0.87	0.8~1.6
M. kansasii (9)	FCE 22250	0.31	0.2~0.4
	Rifampicin	1.14	0.4~1.6
M. scrofulaceum (5)	FCE 22250	2.72	1.6~6.2
	Rifampicin	6.2	6.2
M. avium-intracellulare (6)	FCE 22250	2.2	0.6~10
	Rifampicin	1.0	0.15~10
M. chelonei (4)	FCE 22250	1.75	1.6~2.5
	Rifampicin	4.42	1.6~6.2
M. fortuitum (10)	FCE 22250	4.26	3~100
	Rifampicin	40.6	25~100

SM=Streptomycin, EMB=ethambutol, INH=isoniazid, Rif=rifampicin, Res=resistant.

Table 3. Therapeutic activity of FCE 22250 on experimental Gram-positive and Gram-negative infections of the mouse.

Infection	Route of administration	Interval between treatment and infection (minutes)	ED_{50} (mg/kg) (limits, P 0.05)	
			FCE 22250	Rifampicin
Staphylococcus aureus PV_3	po	15	0.3 (0.2 ~ 0.4)	0.1 (0.06~0.2)
	sc	15	0.1 (0.08~ 0.15)	0.09 (0.06~0.12)
Streptococcus pyogenes ATCC 12384	sc	15	19.5 (10.9~34.9)	7.9 (4.8 ~13)
Salmonella abortivoequina ATCC 9842	po	3 hours	>100	57.5 (47.4 ~70)
	sc	15	>50	35.4 (26.4 ~47.5)
Klebsiella pneumoniae ATCC 8047	sc	15	>80	20 (14.6 ~27.4)
Escherichia coli O26: B6	po	3 hours	81 (57.6 ~113.7)	15.3 (8.6 ~27.5)
	sc	15	>150	10.6 (7.9 ~14.2)

or orally with 10 mg/kg of antibiotic prepared as described for therapeutic activity; at different intervals 6 animals were sacrificed, plasma was collected and tissues were removed and homogenized in phosphate buffer (pH 7). Individual samples were assayed for antibiotic content on *Micrococcus luteus* ATCC 9341 by the agar diffusion technique against standard solutions prepared in blank samples of homologous tissues. The limit of detection was 0.1 μg/ml of plasma and 0.2 μg/g of tissues.

b) Rats: Groups of 3~9 CD albino rats (Cobs) weighing 250±20 g were treated intravenously or orally with 10 mg/kg of an antibiotic; at various times one group was sacrificed and plasma and tissues were assayed for antibiotic content as described above.

c) Dogs: 3 Animals weighing about 10 kg were treated by oral route with 30 mg/kg of FCE 22250. At various times after the administration, plasma samples were collected and the antibiotic content in

Table 4. Therapeutic activity of FCE 22250 in experimental mice infection by *M. tuberculosis* H37Rv.

Compound	Schedule of treatment (oral administration)	Start of treatment (day)	No. of doses	ED_{50} (mg/kg)	
				Daily dose	Cumulative dose
FCE 2250	1 dose every 3 days	+ 3	10	0.9	9
〃	1 〃 〃 week	+ 3	5	2.4	12
〃	1 〃 〃 2 weeks	+ 3	3	2.6	7.8
〃	1 〃 〃 3 weeks	+ 3	2	3.3	6.6
〃	1 〃 〃 week	+10	5	4.5	22.5
Rifampicin	1 〃 〃 3 days	+ 3	10	>12.5	>125
〃	5 doses 〃 week	+ 3	25	3.8	95
〃	5 〃 〃 week	+10	25	5.1	127.5

plasma was determined by microbiological assay as previously described.

Results

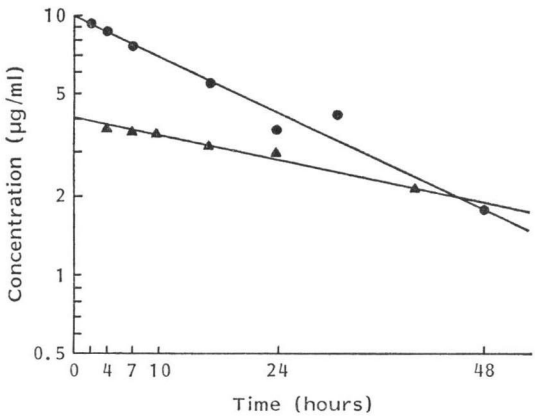
In Vitro and *In Vivo* Activity

The *in vitro* activity against Gram-positive and Gram-negative bacteria is reported in Table 1. Against the majority of the tested bacteria both FCE 22250 and rifampicin have similar activity. Only against *Neisseria*, *Haemophilus* and anaerobes FCE 22250 is 3~10 times less active than rifampicin.

In Table 2 are given the MICs for typical and atypical *Mycobacterium*. FCE 22250 and rifampicin exhibit a similar activity against tubercular *Mycobacterium* with complete cross-resistance. FCE 22250 is somewhat more active than the reference compound against atypical *Mycobacterium* with the exception of *M. avium-intracellulare*; the activity is 9.5 times greater than rifampicin against *M. fortuitum* and 2~4 times against the other tested strains.

The results of the *in vivo* activity are shown in Tables 3 and 4. The effectiveness of FCE 22250 against Gram-positive and Gram-negative bacteria is always inferior to that of rifampicin. A quite different picture is observed when *M. tuberculosis* infection is considered; in this case the therapeutic effectiveness of FCE 22250 is definitely higher than that of rifampicin with an advantage depending on the schedule of treatment. The cumulative ED_{50}, reported in Table 3, are a measure of the protective activity of the two antibiotics in the various experimental conditions.

Fig. 2. Average plasma levels of FCE 22250 following intravenous injection of 10 mg/kg in rats (▲) and in mice (●).



Pharmacokinetics

In Fig. 2 are shown the average plasma levels of FCE 22250 in mice and rats following intravenous injection and in Figs. 3~5 the plasma concentrations in mice, rats and dogs following oral treatments in comparison with rifampicin. All the results indicate that FCE 22250 is characterized by a long persistence in the body of the three tested species, while rifampicin shows a remarkably shorter half-life especially in mice and rats.

Fig. 3. Plasma levels of FCE 22250 (■) and rifampicin (●) following oral daministration of 10 mg/kg in mice.

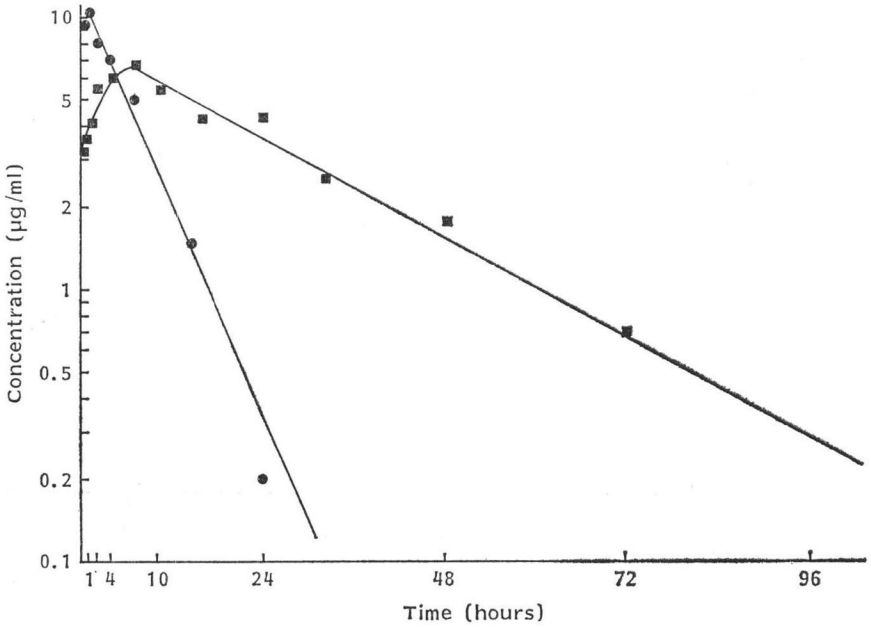
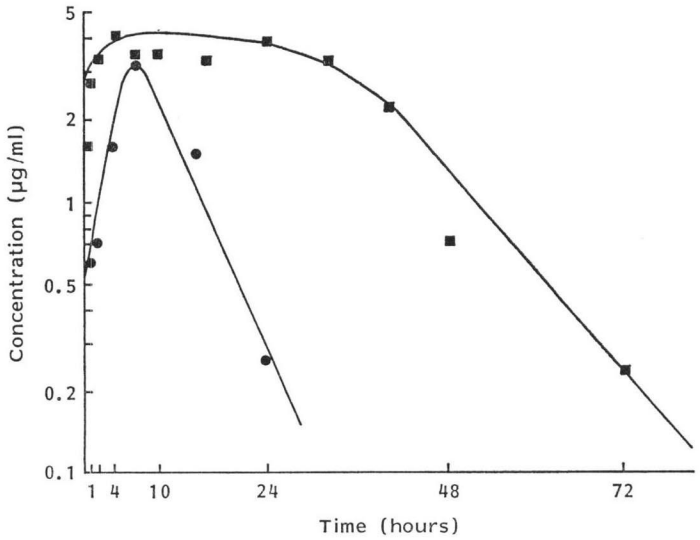


Fig. 4. Plasma levels of FCE 22250 (■) and rifampicin (●) following oral administration of 10 mg/kg in rats.



In mice the plasma half-life can be estimated at 19 hours following either po or iv administration. The plasma levels following gavage of 10 mg/kg reach maximum (7 μg/ml) at about 7 hours after dosing.

In rats the half-life is about 45 hours following iv injection, while following po administration the plasma levels remain almost unchanged until 25 hours after dosing when the concentrations decline with

Fig. 5. Plasma levels of FCE 22250 (■) and rifampicin (●) following oral administration of 30 mg/kg in dogs.

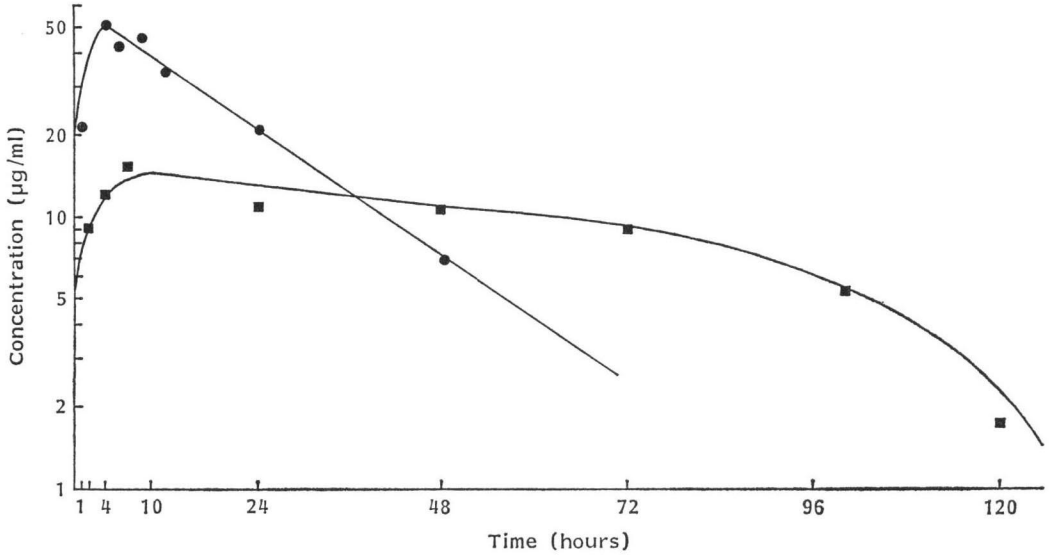


Fig. 6. Tissue levels of FCE 22250 and rifampicin following oral administration of 10 mg/kg in mice.

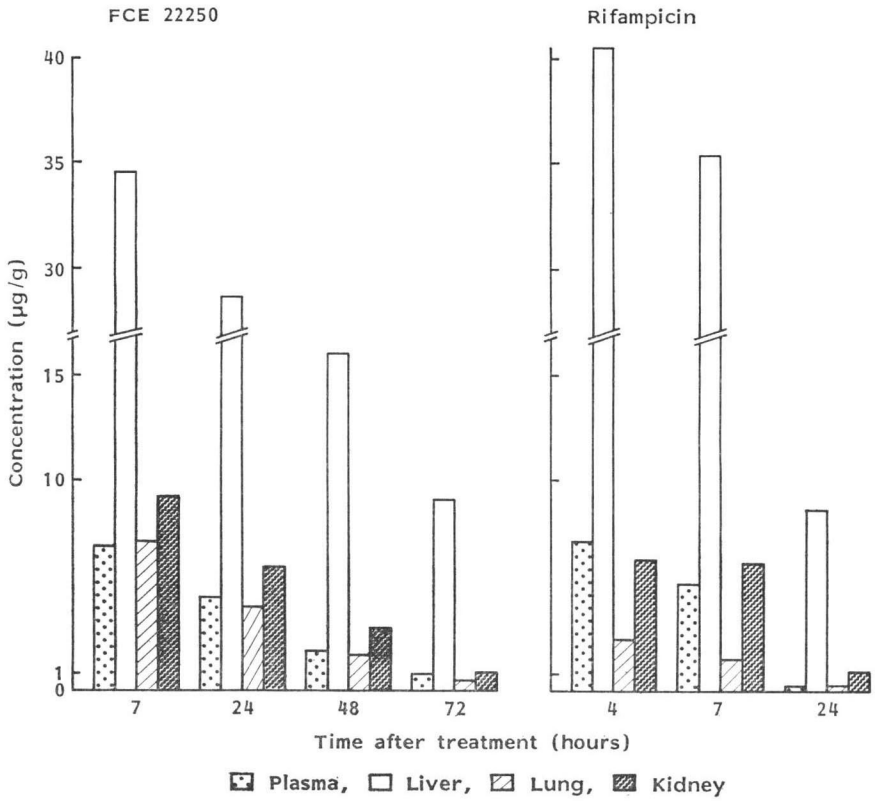
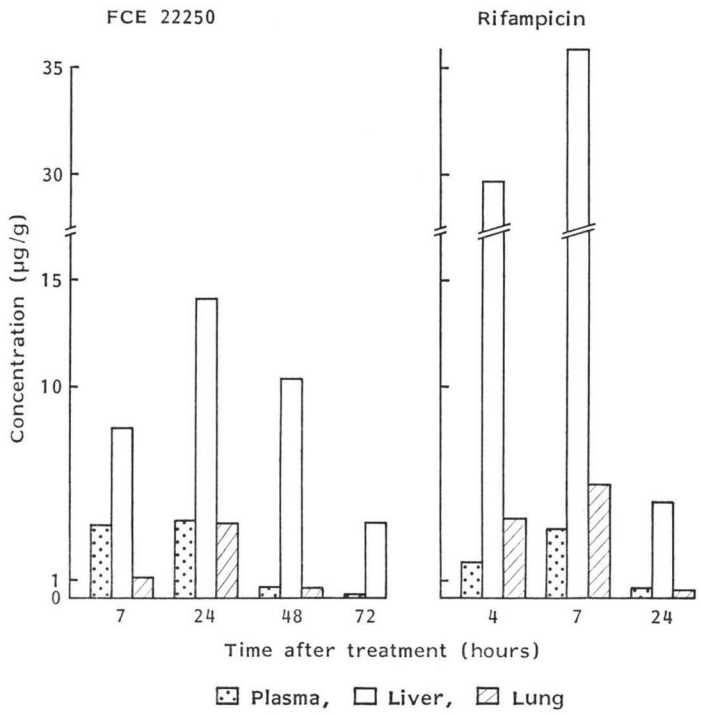


Fig. 7. Tissue levels of FCE 22250 and rifampicin following oral administration of 10 mg/kg in rats.



a half-life of about 13 hours. After the dose of 10 mg/kg maximum plasma concentration of 4 μg/ml is reached at 5~6 hours.

In dogs following oral treatment with 30 mg/kg, the plasma peak of 15 μg/ml is reached at 7 hours. Until 72 hours the levels decrease slowly and after that time the rate of elimination increases; in this phase the half-life can be estimated as 24 hours.

Increasing in the rate of elimination is observed in rats and dogs respectively at 36 and 72 hours following oral administration; metabolic activation can be hypothesized.

The bioavailability following oral administration has been also determined; the ratio of plasma AUCs (0~48 hours) following oral and intravenous treatment is 82% in mice and 87% in rats.

The tissue levels of FCE 22250 and rifampicin in mice and rats are reported in Figs. 6 and 7. The penetration of FCE 22250 is good in both species; the lung, kidney and plasma concentrations are similar at almost all the times considered while higher levels are reached in liver. The ratio liver/plasma ranges from 4.9 (7 hours) to 12.7 (72 hours) in mice and from 2.5 (7 hours) to 15.4 (72 hours) in rats. At the earlier times the levels and the pattern of distribution of rifampicin are similar to FCE 22250, but after 24 hours the concentrations are lower and similar to those observed with FCE 22250 after 72 hours.

Discussion and Conclusions

FCE 22250 is a new rifamycin characterized by a unique pharmacokinetics in the laboratory animals which includes an excellent bioavailability following oral administration and a long persistence in the body. These properties make the compound very interesting mainly for the therapy of tubercular infections; in fact the therapeutic effectiveness against murine tuberculosis is 14 times superior to that

of rifampicin even though their intrinsic *in vitro* activities are similar. This high *in vivo* efficacy is very probably due to the more prolonged blood and tissue levels observed than those of rifampicin.

The activity against atypical mycobacteria is also of some interest, especially against *M. fortuitum*, but more investigations are needed to determine if the observed inhibiting concentrations are of therapeutic value. The *in vitro* activity of FCE 22250 against Gram-positive and Gram-negative bacteria is similar to that of rifampicin but its ability to cure mice septicemias is lower than expected.

For many years after the development of rifampicin, no antibiotic of this class with clinical potential has been presented, but recently two new rifamycins, LM 427[4] and DL 473[5], with remarkable activity have been synthesized and are now in clinical trials. In comparison with these compounds, FCE 22250 strongly differs from LM 427, which is a drug with an outstanding activity on atypical mycobacteria and many rifampicin-resistant *M. tuberculosis*[6,7], but can be compared to DL 473 which is characterized by a long half-life and a spectrum on mycobacteria very similar to that of rifampicin[5].

Studies aimed at determining the pharmacokinetics of FCE 22250 in humans are under way. If the data are in agreement with those observed in animals, this compound will be an excellent candidate for tubercular and leprosy therapy.

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