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Activities of Pefloxacin and Ciprofloxacin Against *Mycobacterium leprae* in the Mouse[1]

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For the chemotherapy of leprosy in general, only four drugs are useful—dapsone, clofazimine, the thioamides (ethionamide and prothionamide), and rifampin ([27]). Because of the increasing incidence of relapses caused by the emergence of *Mycobacterium leprae* resistant to dapsone ([8]), the skin discoloration provoked by clofazimine ([14, 16, 17, 28]), the liver toxicity of the thioamides ([2, 9, 13]) and the emergence of strains resistant to rifampin ([5, 7]), there is obviously an urgent need for new active drugs against *M. leprae.* Among the newly discovered drugs, the fluoroquinolones, especially pefloxacin and ciprofloxacin, appear promising—pefloxacin because of its favorable pharmacokinetics ([11]), and ciprofloxacin because of its low minimal inhibitory concentration (MIC) against many bacterial species ([1, 12, 23]). Furthermore, both compounds have a definite activity against cultivable mycobacteria ([4]). It was therefore important to test the activity of pefloxacin and ciprofloxacin against *M. leprae.* Because the activity of the quinolones had to be tested in the mouse ([19]), we first investigated the pharmacokinetics of both quinolones in this animal in order to test drug dosages that give blood levels in the mouse as comparable as possible to those levels attained in man. Then, using the classical mouse foot pad system, we tested their activities against *M. leprae.*

MATERIALS AND METHODS

Antimicrobial agents. Pefloxacin (batch no. O.P.3) was supplied by the Roger Bellon Laboratory, Paris, France, and ciprofloxacin (batch no. 907452) by Bayer-Pharma, Paris, France. For both antimicrobial agents, fresh solutions were prepared every fortnight in sterile distilled water and stored at 4°C in light-proof containers.

Animals. Outbred female Swiss mice (purchased from the Janvier Breeding Centre, 53680 Le Genest, France) were maintained in conventional animal quarters.

Pefloxacin and ciprofloxacin serum concentrations. The concentrations of pefloxacin and ciprofloxacin were measured in the serum of mice (3 months old, 30 g body weight) administered the drugs by esophageal canula. Three groups of 35 mice were randomly allocated to receive either pefloxacin, 50 or 150 mg/kg body weight, or ciprofloxacin, 150 mg/kg body weight. Additional mice were employed for measurements of maximal (peak) and minimal (trough) serum levels of pefloxacin on three consecutive days after daily oral administration of 150 mg/kg body weight. In each case, blood samples for assays of pefloxacin and ciprofloxacin were obtained by orbital puncture from three mice and pooled. Blood was taken at 20 min, 30 min, and at 1, 1.5, 2, 4, 6, 8, and 24 hr after a single dose of pefloxacin; at 5, 10, 20, and 30 min and 1, 2, 3, 6, and 24 hr after a single dose of ciprofloxacin; and at 30 min and 24 hr after repeated doses of pefloxacin. Serum quinolone levels were determined by microbiological cup diffusion assay using antibiotic medium 5 (Difco) as the test medium and *Bacillus subtilis* (ATCC 6633) and *Klebsiella pneumoniae* (ATCC 10031) as test organisms for pefloxacin and ciprofloxacin, respectively.

Pharmacokinetic analyses were performed with a rotating iterative procedure modified program ([24]) on an Apple II computer.

Mouse inoculation and assessment of bacillary growth. The continuous and kinetic

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TABLE 1. *Pharmacokinetics of pefloxacin and ciprofloxacin in the mouse.*

Drug	Tmax[a] (hr)	Cmax[b] (μg/ml)	AUC[c] (μg·hr/ml)	T½[d] (hr)
Pefloxacin				
50 mg/kg	0.5	9.2	24.6	2.1
150 mg/kg	0.5	16.9	65.8	2.3
Ciprofloxacin				
150 mg/kg	0.3	3.6	10.8	1.9

[a] Tmax = time of maximum serum concentration.
[b] Cmax = maximum serum concentration.
[c] AUC = area under the serum concentration–time curve.
[d] T½ = half-life.

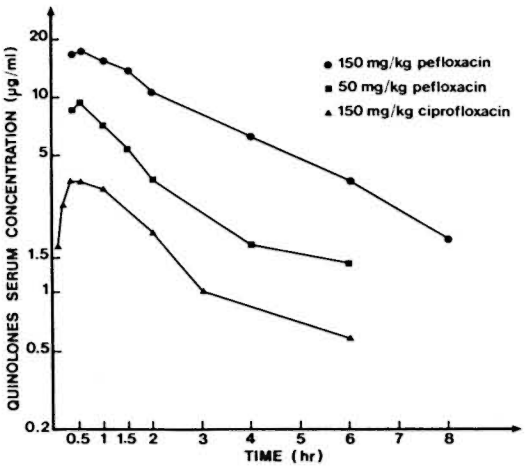

FIG. 1. Serum concentrations of pefloxacin and ciprofloxacin in the mouse after a single oral dose.

methods described by Shepard ([20, 21]) were used. For the continuous method, 210 mice were infected in the left hindfoot pad with 5×10^3 *M. leprae* of a dapsone-sensitive mouse-passage strain (no. 17547) supplied by S. R. Pattyn ([14]). The mice were randomly allocated to three groups; an untreated control group and the two groups treated with either 50 mg/kg or 150 mg/kg of pefloxacin. The same experiment was then repeated with ciprofloxacin. Drug treatment, given 5 days a week by esophageal canula (gavage), began 1 month after infection and was given continuously. Multiplication of *M. leprae* in the foot pad was followed by means of individual harvests, performed monthly from eight mice from each of the control and treated groups, beginning 4 months after infection. The infected foot pads were dissected and homogenized according to the standard method described by Shepard ([19]). Counts of acid-fast bacilli (AFB) were made by the method of Shepard and McRae ([22]). Bacillary growth was considered to have occurred when more than 2×10^4 AFB were harvested.

For the kinetic method, two additional groups of 120 mice were also infected with 5×10^3 *M. leprae,* randomly allocated to two groups, and treated 5 days a week. The first group was administered 50 mg/kg and the second, 150 mg/kg of either pefloxacin or ciprofloxacin. Drug treatment, given 5 days a week by gavage, began 2 months after infection and was given for 3 months only. The bacillary growth following withdrawal of the drug was assessed by means of monthly harvests from eight mice as for the continuous method.

Statistical analysis. Differences among the results of harvests were analyzed statistically by means of the Mann-Whitney *U* test, a nonparametric technique ([30]). The null hypothesis, that treatment was without effect, was rejected at a probability level of 0.05. It was assumed that after the population of *M. leprae* had attained the plateau level of about 10^6 AFB per foot pad, *M. leprae* persist in food pad tissues at the same level, and that, subsequently, the number of AFB harvested remains stable ([15, 18, 25]).

RESULTS

Pefloxacin and ciprofloxacin serum concentrations. The serum concentrations and the principal pharmacokinetic parameters of pefloxacin and ciprofloxacin in the mouse are given in Table 1 and Figure 1. Pefloxacin appears to be rapidly absorbed from the intestinal tract of the mouse, because peak serum concentrations were obtained 0.5 hr after oral administration. The peak serum concentration after a single dose of 150 mg/kg (16.9 μg/ml) was less than twice that attained after 50 mg/kg (9.2 μg/ml). The half-lives for the elimination of 150 mg/kg and 50 mg/kg pefloxacin were, respectively, 2.3 and 2.1 hr. A similar half-life (1.9 hr) was observed for the elimination of ciprofloxacin after a single oral dose of 150 mg/kg. However, after a single 150 mg/kg dose of ciprofloxacin, the peak serum concentration was 3.6 μg/ml and the area under the serum concentration–time curve was 10.8 μg·hr/

TABLE 2. *Activity of 50 mg/kg and 150 mg/kg pefloxacin given by gavage 5 days a week against* M. leprae *in the mouse as measured by the continuous method.*

Mos. after infection with 5×10^3 *M. leprae*	Controls		Pefloxacin			
			50 mg/kg		150 mg/kg	
	No. of positive mice[a]	AFB median value (range)[b]	No. of positive mice	AFB median value (range)	No. of positive mice	AFB median value (range)
4	7/8	7.9×10^4 (3.9×10^4–3.2×10^5)	3/8	$<2 \times 10^4$[c] (3.9×10^4–7.9×10^4)	0/8	$<2 \times 10^4$[c]
5	8/8	2.8×10^5 (2.4×10^5–1.3×10^6)	5/8	7.9×10^4[c] (7.9×10^4–3.5×10^5)	0/8	$<2 \times 10^4$[c]
6	8/8	1.2×10^6 (7.5×10^5–1.3×10^6)	7/8	1.2×10^5[c] (7.9×10^4–4.3×10^6)	0/8	$<2 \times 10^4$[c]
7	ND[d]	—	8/8	1.6×10^5[c] (7.9×10^4–6.7×10^5)	1/8	$<2 \times 10^4$[c] (-5.4×10^4)
8	ND	—	6/8	2.4×10^5[c] (2×10^5–5.5×10^5)	0/8	$<2 \times 10^4$[c]
10	ND	—	ND	—	0/8	$<2 \times 10^4$[c]
12	ND	—	ND	—	1/8	$<2 \times 10^4$[c] (3.9×10^4)

[a] $>2 \times 10^4$ AFB per foot pad.
[b] Positive harvests.
[c] Significantly different from controls ($p < 0.05$).
[d] ND = not done.

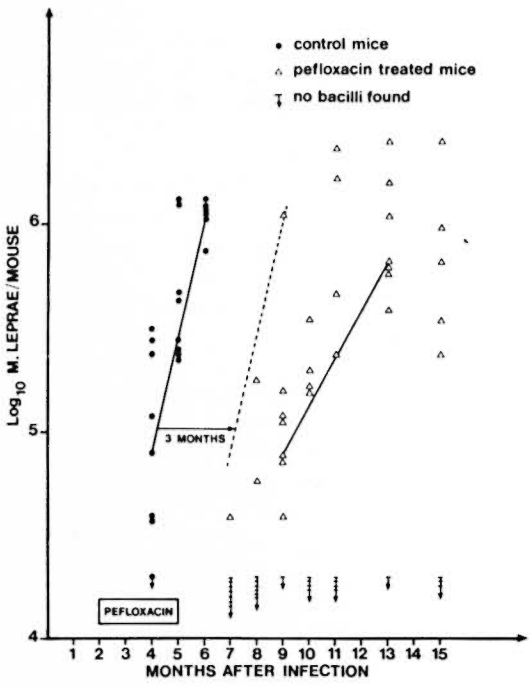

FIG. 2. Activity of daily 150 mg/kg pefloxacin against *M. leprae* as measured by the kinetic method in the mouse foot pad.

ml less than the values obtained after 150 mg/kg and 50 mg/kg pefloxacin at 65.8 and 24.4 μg·hr/ml, respectively. With both drugs, peak serum concentrations remained in a similar range after repeated daily doses, and the 24-hr concentrations were below detectable values, indicating that there was no apparent accumulation of drug.

Activity of pefloxacin against *M. leprae*. The survival and the body weight gain of the mice were not affected by treatment with pefloxacin or ciprofloxacin. During treatment, the mortality rate was about 0.1%/month, and the mean body weight of the mice increased, at a similar rate to that of the controls, from 20 g at week 4 to 25 g at week 7, 30 g at week 13, and 35 g at week 23, and required regular increases in the daily drug doses.

The results obtained with the continuous method are given in Table 2. Four months after infection with 5×10^3 *M. leprae*, 7 of the 8 control mice showed the beginning of bacillary growth in their foot pads. The plateau phase was obtained at 6 months, when all of the mice demonstrated about 10^6 AFB per foot pad. Taking into account the ob-

TABLE 3. *Activity of 50 mg/kg and 150 mg/kg pefloxacin given by gavage 5 days a week against* M. leprae *in the mouse as measured by the kinetic method.*

Mos. after infection with 5×10^3 *M. leprae*	Controls		Pefloxacin			
			50 mg/kg		150 mg/kg	
	No. of positive mice[a]	AFB median value (range)[b]	No. of positive mice	AFB median value (range)	No. of positive mice	AFB median value (range)
4	7/8	7.9×10^4 (3.9×10^4–3.2×10^5)	ND[c]	—	ND	—
5	8/8	2.8×10^5 (2.4×10^5–1.3×10^6)	ND	—	ND	—
6	8/8	1.2×10^6 (7.5×10^5–1.3×10^6)	ND	—	ND	—
7	ND	—	8/8	8.3×10^5[d] (3.2×10^5–1.7×10^6)	1/8	$<2 \times 10^4$[e] (3.9×10^4)
8	ND	—	ND	—	2/8	$<2 \times 10^4$[e] (5.9×10^4–1.8×10^5)
9	ND	—	ND	—	7/8	7.9×10^4[e] (3.9×10^4–1.1×10^6)
10	ND	—	ND	—	4/8	$<2 \times 10^4$[e] (1.6×10^5–2×10^5)
11	ND	—	ND	—	4/8	$<2 \times 10^4$[e] (2.4×10^5–2.3×10^6)
13	ND	—	ND	—	7/8	6.3×10^5[d] (3.9×10^5–2.5×10^6)
15	ND	—	ND	—	5/8	2.4×10^5[d] (2.4×10^5–2.5×10^6)

[a] $>2 \times 10^4$ AFB per foot pad.
[b] Positive harvests.
[c] ND = not done.
[d] Not significantly different from controls ($p > 0.05$).
[e] Significantly different from controls ($p < 0.05$).

served AFB values and the time needed to reach the plateau level, the curve of logarithmic growth has been drawn on Figure 2; the doubling time of *M. leprae* in the control mice has been calculated as 15.3 days. In the treated groups 4 months after infection, only 3 of the 8 mice of the 50 mg/kg-group and none in the 150 mg/kg-group showed bacillary growth. Subsequent harvests demonstrated that growth had occurred in the 50 mg/kg-mice, but in only two mice treated with 150 mg/kg. However, during the first 8 months of the experiment, the number of AFB harvested per foot pad of the mice treated with 50 mg/kg remained less than that of the control mice, the difference being statistically significant. Therefore, the 50 mg/kg daily oral dose was definitely although weakly bacteriostatic against *M. leprae* in the mouse foot pad system.

Using the kinetic method, the treatment with the 50 mg/kg daily (5 times a week) oral dose of pefloxacin demonstrated no growth delay; whereas treatment with the 150 mg/kg dose demonstrated definite growth delay of *M. leprae* following withdrawal of the drug. As shown in Table 3 and Figure 2, the administration of 150 mg/kg over a period of 3 months resulted in no bacillary growth at all in several mice and delayed bacillary growth in the others. Ten months after infection and 5 months after withdrawal of the drug, the difference between the median numbers of AFB harvested per foot pad from treated and control mice was still statistically significant. In Figure 2, it is possible to draw the line representing logarithmic bacillary growth in the treated mice between the median value corresponding to the first bacillary growth, i.e., 7.9×10^4 AFB at month 9, and that corresponding to the plateau level, i.e.,

TABLE 4. *Activity of 50 mg/kg and 150 mg/kg ciprofloxacin given by gavage 5 days a week against* M. leprae *in the mouse as measured by the continuous method.*

Mos. after infection with 5×10^3 *M. leprae*	Controls		Ciprofloxacin			
			50 mg/kg		150 mg/kg	
	No. of positive mice[a]	AFB median value (range)[b]	No. positive mice	AFB median value (range)	No. positive mice	AFB median value (range)
4	7/8	3.1×10^5 (7.9×10^4–3.5×10^5)	7/8	7.9×10^4[c] (3.9×10^4–3.1×10^5)	7/8	7.9×10^5[c] (5.9×10^4–3.5×10^5)
5	8/8	7.9×10^5 (3.2×10^5–1.8×10^6)	8/8	3.9×10^5[c] (2×10^5–1.1×10^6)	7/8	1.9×10^5[d] (7.9×10^4–5.1×10^5)
6	8/8	1.1×10^6 (8.3×10^5–1.9×10^6)	8/8	1.3×10^6[c] (9.8×10^5–1.9×10^6)	8/8	4×10^5[d] (3.2×10^5–9.1×10^5)

[a] $>2 \times 10^4$ AFB.
[b] Positive harvests.
[c] Not significantly different from controls ($p > 0.05$).
[d] Significantly different from controls ($p < 0.05$).

6.3×10^5 at month 13. The bacillary growth in the treated mice began much later than if the activity of pefloxacin had been only sustained during the administration of the drug (broken line). It may be calculated that the excess of growth delay induced by 3 months of pefloxacin was about 126 days, equivalent to 8.2 doublings (128/15.3 = 8.2). This excess of growth delay suggests that approximately 99% of the *M. leprae* could have been killed by 3 months' treatment with 150 mg/kg pefloxacin.

Activity of ciprofloxacin against *M. leprae*. The results obtained when ciprofloxacin was tested by the kinetic method are presented in Table 4. This table shows that bacillary growth in the control mice was detected as early as month 4 after infection, and reached a plateau level at month 6. Similar bacillary growth occurred in the mice treated with 50 mg/kg ciprofloxacin, indicating that ciprofloxacin at this dosage had no activity against *M. leprae* in the mouse. In mice treated with 150 mg/kg ciprofloxacin, bacillary growth at month 4 was not significantly different from that in the control mice, and was slightly but significantly less at months 5 and 6, indicating a weak bacteriostatic activity. Obviously, this correlated with an absence of growth delay as measured by the kinetic method.

DISCUSSION

The results presented show that pefloxacin given by gavage to mice at a dosage of 150 mg/kg 5 days a week inhibited *M. leprae* growth by the continuous method and demonstrated an excess of growth delay by the kinetic method. They indicate that at the dosage used pefloxacin has a bacteriostatic activity against *M. leprae* and, possibly, a bactericidal activity. In contrast, ciprofloxacin with lower MICs than pefloxacin against many bacterial species, including mycobacteria ([1, 23]), was inactive against *M. leprae* in the mouse at dosages of 50 and 150 mg/kg. Despite the similar half-lives of the two drugs in the mouse, the differences of peak serum concentrations and areas under the serum concentration–time curves probably account for the activity of pefloxacin and the inactivity of ciprofloxacin. But we do not know whether the unfavorable blood levels of ciprofloxacin in the mouse are related to the poor absorption of the drug in the intestinal tract or to the metabolism of the drug. In any case, the inactivity of ciprofloxacin in the mouse cannot be considered as a definite indication of its inactivity in man, especially when the drugs were given to the mouse by gavage 5 days a week and not continuously in the diet. This is especially true when the pharmacokinetics of both fluoroquinolones are much more favorable in man than in the mouse ([6, 11]).

For these very reasons, we can predict that pefloxacin will be active in man. Actually, the serum half-life of pefloxacin is about 10 hr in man ([11]) and about 2 hr in the mouse, and after repeated doses of 400

mg pefloxacin twice a day in man, the peak levels of pefloxacin are about 10 μg/ml and the trough levels are about 3 μg/ml ([26]). Because of the narrow margin between MICs and minimal bactericidal concentrations (MBCs) of quinolones ([12]), it is possible that pefloxacin will have bactericidal activity against *M. leprae* at the serum concentrations obtained in man. In view of the excellent acceptability of pefloxacin ([10]), these experimental findings suggest the importance of initiating controlled pilot clinical trials to study the potential efficacy of the drug in the treatment of lepromatous leprosy.

SUMMARY

Because ciprofloxacin and pefloxacin are fluoroquinolones active against many mycobacterial species, both drugs were tested against *Mycobacterium leprae* in the mouse foot-pad system. Preliminary pharmacokinetic studies in the mouse showed that after a single oral dose of 150 mg/kg ciprofloxacin the peak serum concentration was 3.6 μg/ml, and after 50 mg/kg or 150 mg/kg pefloxacin peak serum concentrations were, respectively, 9.2 μg/ml and 16.9 μg/ml, the half-lives for serum elimination being about 2 hr for both drugs. The activity of daily 50 mg/kg and 150 mg/kg ciprofloxacin and pefloxacin against *M. leprae* was then tested in mice infected with 5×10^3 *M. leprae*. The growth of *M. leprae* was not prevented in mice treated continuously with either 50 mg/kg or 150 mg/kg ciprofloxacin, indicating that this drug had no or a limited bacteriostatic effect at the dosages used. In mice treated continuously with 50 mg/kg pefloxacin, growth of *M. leprae* was not prevented, but at monthly harvests the number of bacilli in the foot pads remained less than those of control mice ($p < 0.05$). No growth of *M. leprae* occurred in mice treated continuously with 150 mg/kg pefloxacin. In mice treated for only 3 months with daily 150 mg/kg pefloxacin, the growth-delay that followed the stopping of the drug was 126 days, suggesting that approximately 99% of the *M. leprae* were killed. The pharmacokinetics of pefloxacin being more favorable in man than in the mouse, pefloxacin appears a possible drug for the chemotherapy of leprosy.

RESUMEN

Usando el sistema de la almohadilla plantar del ratón se probó el efecto de la ciprofloxacina y de la pefloxacina contra el *Mycobacterium leprae*. Las drogas son fluoroquinolonas activas contra muchas especies micobacterianas. Los estudios farmacocinéticos preliminares en el ratón mostraron que después de una sola dosis oral de ciprofloxacina de 150 mg/kg, la concentración máxima en suero fue de 3.6 μg/ml en tanto que después de administrar 50 mg/kg o 150 mg/kg, las concentraciones séricas máximas fueron 9.2 μg/ml y 16.9 μg/ml, respectivamente; los tiempos para la eliminación media en suero fueron de aproximadamente 2 horas para ambas drogas. Después se probó la actividad de 50 y 150 mg/kg/día de la ciprofloxacina y de la pefloxacina sobre el *M. leprae* en ratones infectados con 5×10^3 bacilos. La ciprofloxacina a dosis de 50 y 150 mg/kg/día no inhibió el crecimiento del *M. leprae*. Esto indicó que la droga a las dosis usadas tiene un limitado efecto bacteriostático o carece de él. En los ratones tratados continuamente con 50 mg/kg de pefloxacina, el crecimiento del *M. leprae* tampoco se vió inhibido pero el número de bacilos en las almohadillas plantares de los animales tratados siempre fue menor que el encontrado en los ratones control ($p < 0.05$). No hubo crecimiento del *M. leprae* en los ratones tratados continuamente con 150 mg/kg de pefloxacina. En los ratones tratados por sólo 3 meses con dosis diarias de 150 mg/kg de pefloxacina, el retardo en el crecimiento bacilar observado después de suspender la droga fue de 126 días, sugiriendo que aproximadamente el 99% de los *M. leprae* estuvieron muertos. Siendo la farmacocinética de la pefloxacina más favorable en el hombre que en el ratón, esta droga podría ser usada en la quimioterapia de la lepra.

RÉSUMÉ

La ciprofloxacine et la pefloxacine sont l'une et l'autre des fluoroquinolones actives contre de nombreuses espèces mycobactériennes. Les deux produits ont dès lors été essayés contre *Mycobacterium leprae* dans le système du coussinet plantaire de la souris. Les études pharmacocinétiques préliminaires menées chez la souris ont montré qu'après une dose orale unique de 150 mg/kg, la concentration maximale du sérum atteignait 3,6 μg/ml; après administration de pefloxacine à raison de 50 mg/kg ou de 150 mg/kg, les concentrations sériques étaient respectivement 9,2 mg/ml et de 16,9 μg/ml; la demi-période pour l'élimination dans le sérum était environ de deux heures pour ces deux produits. On a étudié l'activité de doses de 50 mg/kg et de 150 mg/kg de ciprofloxacine et de pefloxacine contre *M. leprae*, chez des souris infectées avec 5×10^3 *M. leprae*. La croissance de *M. leprae* n'était pas empêchée chez des souris traitées de façon continue avec la ciprofloxacine à l'un ou l'autre de ces dosages, ce qui indique que le médicament n'a pas d'effet bactériostatique aux doses utilisées, ou seulement un effet bactériostatique limité. Chez des souris traitées conti-

nuellement par 50 mg/kg de pefloxacine, la croissance de *M. leprae* n'était pas non plus empêchée, mais lors des collectes mensuelles de bacilles, le nombre de ceux-ci, dans les coussinets plantaires, était inférieur au nombre mis en évidence chez des souris témoins ($p < 0.05$). Aucune croissance de *M. leprae* n'a été constatée chez des souris traitées sans discontinuité par 150 mg/kg de pefloxacine. Chez des souris traitées pendant trois mois seulement, par des doses quotidiennes de 150 mg/kg de pefloxacine, le retard de croissance observé après l'interruption du médicament était de 126 jours, ce qui suggère qu'environ 99% des *M. leprae* avaient été tués. La pharmacocinétique de la pefloxacine est plus favorable chez l'homme que chez la souris. Ce produit paraît dès lors pouvoir être envisagé comme médicament pour la chimiothérapie de la lèpre.

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