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Anti-*Mycobacterium leprae* Activity of Several Quinolones Studied in the Mouse[1]

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Fluoroquinolones are basically drugs active against gram-negative bacteria. Numerous derivatives with a broader spectrum of activity and/or more favorable pharmacokinetics have been synthetized ([2]). Some quinolones are active against *Mycobacterium leprae* ([5, 7–9, 16]), and pefloxacin and ofloxacin have already been studied in the human disease ([8, 13]).

We present here results on the activity of several fluoroquinolones against *M. leprae* in the mouse.

MATERIALS AND METHODS

The anti-*M. leprae* activity of the drugs was studied in the mouse foot pad by either the continuous or kinetic method ([19, 20]) or by the proportional bacterial test ([3]) using *M. leprae* strains 6348 and 17547 used in previous studies ([16]). The drugs were gifts from drug companies and were administered by gavage: ofloxacin (OFLO) from Hoechst, Frankfurt, Germany; A-56619 (= difloxacin), A-56620 ([4]) and temafloxacin (TEFLO) from Abbott Laboratories, Chicago, Illinois, U.S.A.; fleroxacin (FLERO) from Roche, Basle, Switzerland; lomefloxacin (LOFLO) from Searle & Co., Skokie, Illinois, U.S.A.; tosufloxacin (TOSU); PD-117596 from Parke-Davis, Ann Arbor, Michigan, U.S.A.

Statistical calculations were done using Fisher's exact test.

RESULTS

As shown in Table 1, at a dosage of 150 mg/kg A-56620 is inactive and A-56619 is active against *M. leprae* but only when administered daily, not when administered once weekly.

FLERO at 150 mg/kg is active when administered both daily and once weekly.

TEFLO, LOFLO, and PD-117596 are all active at 150, 75 and 37.5 mg/kg administered daily.

The activity of the drugs when administered intermittently at dosages of 150 or 300 mg/kg with intervals of 1, 2, and 4 weeks studied by the continuous method is shown in Table 2. At the lower dosage of 150 mg/kg LOFLO and TEFLO were less active than FLERO and PD-117596; at the higher dosage of 300 mg/kg there were no differences among these drugs.

The effects of one to six monthly doses of 300 mg/kg of five quinolones were studied by kinetic tests. Table 3 shows that the most active compounds are FLERO and LOFLO, which are active even after a single dose, followed by PD-117596, active after two monthly doses, while OFLO inhibits the multiplication of *M. leprae* only after six monthly doses. Since all the mice became positive during the next 3 months, the activities of the drugs in these circumstances are bacteriostatic.

The activity of OFLO was also studied by a kinetic test after a single dose and two to five daily doses of 300 mg/kg. Table 4 shows that three daily doses of 300 mg/kg resulted in a limited growth delay of *M. leprae* in the treated mice which increased considerably after four and five daily doses.

In a proportional bactericidal test, OFLO administered daily, 5 days a week for two successive weeks, at 300 mg/kg was bactericidal.

TOSU could only be investigated partially since only a small amount of the drug was available. The drug was inactive when administered either 5 days a week for 8 weeks at 37.5 mg/kg or once every 28 days at 150 mg and 300 mg/kg.

DISCUSSION

The discovery of the fluoroquinolones constitutes a major advance in antibacterial

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TABLE 1. *Effect of six fluoroquinolones on the multiplication of* Mycobacterium leprae *in mouse foot pads (continuous method).*[a]

Drug	Dosage (mg/kg)	Frequency[b]	Results[c]	Significance p[d]
Controls	—	—	5/5	—
A56619	150	5/7	0/5	0.003
A56619	150	1/7	3/5	0.66
A56620	150	5/7	5/5	1
A56620	150	1/7	5/5	1
FLERO	150	5/7	0/5	0.003
FLERO	150	1/7	0/5	0.003
TEFLO	150	5/7	0/5	0.003
LOFLO	75	5/7	0/5	0.003
PD-117596	37.5	5/7	0/5	0.003

[a] Mice were inoculated with 10^3 *M. leprae* per foot pad, treatment started 3 weeks postinoculation, results considered positive when harvests after 8 months reached 5×10^4 or more per foot pad.
[b] Frequency of administration per 7 days.
[c] Number of mice revealing multiplication/number of mice inoculated.
[d] p Value (Fisher's exact test).

therapy, including mycobacteria ([10]). The potential number of derivatives is enormous, and more than 5000 have already been synthesized ([2]). Basically, the fluoroquinolones are active against gram-negative organisms, but some of them have more favorable pharmacokinetic properties and some are active also against gram-positive organisms. They also offer great promise for the treatment of leprosy ([5, 7–9, 13, 16]).

Since the pharmacokinetics of the quinolones is quite different in mice as compared to man and because, in particular, the serum half-life in mice is much shorter, 50–100 mg/kg in the mouse is considered equivalent to 6.6 mg/kg in man ([6]). The 300 mg/kg administered in mice in some of the experiments described in this paper corresponds to the highest dosage that could be administered in man, e.g., 400 mg and more of the drug per day.

TABLE 2. *Activity against* M. leprae *in the mouse of OFLO, FLERO, LOFLO, TEFLO, and PD-117596 administered at intervals of 1, 2, and 4 weeks (continuous method).*[a]

Drug	Dosage (mg/kg)	Frequency[b]	Results[c]	Significance p[d]	Interpretation[e]
Controls	—	—	5/5	—	
OFLO	150	1/7	0/5	0.003	A
	300	1/7	0/5	0.003	A
		1/14	0/5	0.003	A
		1/28	0/5	0.003	A
FLERO	150	1/7	0/5	0.003	A
		1/28	1/5	0.002	A
	300	1/28	0/5	0.003	A
LOFLO	150	1/7	0/6	0.003	A
		1/14	3/6	0.12	I
		1/28	3/6	0.12	I
	300	1/7	0/6	0.002	A
		1/14	0/6	0.002	A
		1/28	0/6	0.002	A
TEFLO	150	1/7	0/8	0.003	A
		1/14	3/8	0.045	X
		1/28	3/8	0.045	X
	300	1/7	0/5	0.003	A
		1/14	0/5	0.003	A
		1/28	0/6	0.003	A
PD-117596	150	1/7	0/4	0.007	A
		1/14	0/4	0.007	A
		1/28	0/4	0.007	A
	300	1/7	0/5	0.003	A
		1/14	0/5	0.003	A
		1/28	0/5	0.003	A

[a] Mice were inoculated with 10^3 *M. leprae* per foot pad, treatment started 3 weeks postinoculation, results considered positive when harvests after 10 months were 5×10^4 per foot pad.
[b] Frequency of administration per 7 days.
[c] Number of mice revealing multiplication/number of mice inoculated.
[d] p Value, (Fisher's exact test).
[e] A = active, significantly ($p < 0.05$) different from controls; I = inactive, not significantly ($p > 0.05$) different from controls; X = difference from controls p = 0.04–0.05.

TABLE 3. *Activity of a single dose and 1 to 5 monthly doses OFLO, FLERO, LOFLO, TEFLO, PD-117596 against* M. leprae *in the mouse (continuous method).*[a]

Dosage 300 mg/kg	No. monthly doses					
	1	2	3	4	5	6
OFLO	4/4[b,c]	4/4	1/4 (0.05)	1/4	2/4 (0.16)	0/4 (0.007)
FLERO	1/5 (0.02)	0/5 (0.003)	0/5	0/5	0/5	0/5
LOFLO	0/6 (0.002)	1/6 (0.01)	0/6 (0.002)	0/6	0/6	0/6
TEFLO	3/4 (0.5)	4/4 (1)	2/4 (0.13)	2/4 (0.13)	0/4 (0.02)	0/4
PD117596	2/5 (0.08)	0/5 (0.02)	0/5	0/5	0/5	0/5

[a] Mice were inoculated with 10^3 *M. leprae* per foot pad, treatment started 3 weeks postinoculation, results considered positive when harvests after 10 months were 5×10^4 per foot pad.
[b] Number of mice revealing multiplication/number of mice inoculated.
[c] p Value, (Fisher's exact test).

Not all fluoroquinolones are equally active against *M. leprae* as illustrated by the first quinolones studied in the mouse—OFLO, pefloxacin and ciprofloxacin, the former being the most active and the latter inactive ([1,9] and own unpublished results)—and the present results with A-56619 and A-56620.

The anti-*M. leprae* activity of quinolones in the mouse can be compared by their minimal effective dose (MED) in the continuous method and their activity after intermittent administration. The MEDs of LOFLO, TEFLO and PD-117596 is less than 37.5 mg/kg, considerably lower than the MEDs of OFLO and PEFLO which are 50 mg/kg and 150 mg/kg, respectively ([6,8,15]). The limited information obtained with TOSU shows that this drug is less active than the other quinolones included in the present study.

On the basis of their activity after intermittent administration from once weekly to once every 2 weeks and once every 4 weeks in the continuous test, FLERO and LOFLO are the most active followed by PD-117596, TEFLO, and OFLO. However, after monthly administration even those quinolones with a long serum half-life (Table 6) are only bacteriostatic.

OFLO administered once monthly is inactive but is highly bactericidal when administered daily. Whereas Grosset, *et al.* ([7]) showed that OFLO administered daily at 150 mg/kg for 3 months is bactericidal for the mouse foot pad inoculum, it is shown here that the same result is obtained when the drug is administered daily at 300 mg/kg for 2 weeks (Table 5) and even 1 week (Table 4). In the meantime a killing rate of 99%–99.9% of viable *M. leprae* has been measured in man after 22 daily doses of 400 mg OFLO ([8]).

The drugs most active in intermittent administration are the two closely related drugs FLERO and LOFLO ([2]) which have long serum half-lives (Table 6). The greater activity of PD-117596 against *M. leprae* may be related to its greater activity against gram-positive organisms ([17]). The important activity of OFLO when administered daily may be due to some accumulation of the drug ([6]).

TABLE 4. *Growth delay of* M. leprae *in mouse foot pads after 5 daily administrations of 300 mg/kg ofloxacin.*

Drug administrations on days after inoculation of *M. leprae*	Growth delay of *M. leprae* in treated mice as compared with controls (days)
79 only	None
79–80	None
79–81	46
79–82	101
79–83	101

TABLE 5. *Anti-*M. leprae *activity of OFLO in a proportional bactericidal test.*

	Inoculum				p[a]
	10^4	10^3	10^2	10	
Controls	5/6	8/10	2/10	0/10	
Ofloxacin 300 mg 5 days weekly for 2 weeks	0/5	0/5			0.001
Ofloxacin 150 mg 5 days weekly for 2 weeks	0/5	0/5			0.001

[a] p Value, (Fisher's exact test).

TABLE 6. *Comparison of main pharmacokinetic parameters of different fluoroquinolones in man after oral administration of 400 mg.*

Drug	C max (g/l)	T 1/2 (hr)	AUC (μg × hr/ml)	Reference
OFLO	3.5	4.9	28	6, 11
FLERO	6.1	12	78.3	15, 23
LOFLO	4.66	6.20	39.6	12, 21
TEFLO	3.3	6.8	28.4	14
PD117596	5.4	4.25	10.6	17

It is interesting to compare the present results with those obtained by the *in vitro* screening of the activity against *M. leprae* of fluoroquinolones by Franzblau and White ([5]). In the latter study, PD-117596, OFLO, TEFLO, and FLERO were also found to be among the most active drugs. It would be worthwhile to test the *M. leprae* activity in mice of several of the fluoroquinolones found by Franzblau and White to be even more active than those investigated in the present study.

It seems at present that for optimal bactericidal effect against *M. leprae* the fluoroquinolones, when active, have to be administered daily, and that none of these drugs, even those with longer serum half-lives (FLERO, LOFLO) or with greater intrinsic activity (PD-117596), are suitable for monthly administration. This is important when the eventual addition of a fluoroquinolone to the WHO regimen for the treatment of multibacillary leprosy is considered ([22]).

Future evaluations of other new fluoroquinolones against *M. leprae* in mice should be directed to their MEDs in continuous administration and their bactericidal effect after a small number of daily doses.

SUMMARY

The anti-*Mycobacterium leprae* activity of several fluoroquinolones (A-56619, A-56620, ofloxacin, fleroxacin, lomefloxacin, temafloxacin, tosufloxacin, and PD-117596) was studied in the mouse. In a dosage of 150 mg/kg administered daily, A-56619 is active and A-56620 is inactive against *M. leprae*. Ofloxacin administered daily for 2 weeks at 300 mg/kg is bactericidal. The minimal effective dose of PD-117596, lomefloxacin and temafloxacin is less than 37.5 mg/kg. When administered at 300 mg/kg at monthly intervals temafloxacin, PD-117596, and ofloxacin are bacteriostatic; while fleroxacin and lomefloxacin are bactericidal. Tosufloxacin is less active than the other quinolones included in the present study.

RESUMEN

Se estudió la actividad de varias fluoroquinolonas (A-56619, A-56620, ofloxacina, fleroxacina, lomefloxacina, temafloxacina, y PD-117596) en contra del *Mycobacterium leprae* en el modelo del ratón. Se encontró que mientras que una dosis diaria de 150 mg/kg de A-56619 es activa contra el *M. leprae,* una dosis equivalente de A6620 no lo es. La ofloxacina, administrada diariamente durante 2 semanas a una dosis de 300 mg por kg, resultó bactericida. Las dosis efectivas mínimas de PD-117596, lomefloxacina y temafloxacina, fueron menores de 37.5 mg/kg. La temafloxacina, el PD-117596, y la ofloxacina, administrados a 300 mg por kg a intervalos mensuales, resultaron bacteriostáticos, mientras que la fleroxacina y la lomefloxacina fueron bactericidas. La tosufloxacina fue menos activa que las otras quinolonas incluídas en el estudio.

RÉSUMÉ

L'activité anti-*Mycobacterium leprae* de diverses fluoroquinolones (A-56619, A-56620, ofloxacine, fleroxacine, lomefloxacine, temafloxacine, tosufloxacine, et PD-117596) a été étudiée chez la souris. A la dose de 150 mg/kg administrée quotidiennement, A-56619 est active, et A-56620 est inactive vis-à-vis de *M. leprae.* L'ofloxacine est bactéricide à la dose de 300 mg/kg administrée quotidiennement pendant 2 semaines. La dose minimale efficace de PD-117596, lomefloxacine et temafloxacine est inférieure à 37, 5 mg/kg. A la dose de 300 mg/kg administrée mensuellement, la témafloxacine, PD-117596 et l'ofloxacine sont bactériostatiques, tandis que la fleroxacine et la lomefloxacine sont bactéricides. La tosufloxacine est moins active que les autres quinolones prises en compte dans l'étude présente.

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