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Activity of Ofloxacin Against *Mycobacterium leprae* in the Mouse[1]

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Earlier in the search for new antimicrobial drugs active against *Mycobacterium leprae,* the activity of two fluoroquinolones, pefloxacin (PEFLO) and ciprofloxacin (CIPRO), was examined employing the *M. leprae*-infection of mice described by Shepard ([17]). Administered in a dosage of 150 mg per kg body weight by esophageal cannula (gavage) five times weekly, PEFLO exhibited possible bactericidal activity; whereas CIPRO was virtually inactive ([7]). Although the activity, *in vitro,* of CIPRO against many microorganisms, including mycobacteria ([5]), is greater than that of PEFLO, the pharmacokinetic properties of the latter drug are more favorable ([9]), especially in the mouse, perhaps accounting for the greater activity of PEFLO against *M. leprae.*

Ofloxacin (9-fluoro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-2,3-dihydro-7H-pyrido-(1,2,3-de)1,4-benzoxazine-6-carboxylic acid) (OFLO), a more recently described fluoroquinolone ([15]), has been reported to be more active than PEFLO against a variety of microorganisms ([23]), including mycobacteria ([11, 14, 20]), and to possess rather similar pharmacokinetic properties ([2]). It appeared appropriate, therefore, to assess the activity of OFLO against *M. leprae* in the infection of the mouse foot pad, and to compare it to that of PEFLO, dapsone (DDS), and prothionamide (PTH).

MATERIALS AND METHODS

DDS (batch 2540546; a gift of Specia, Paris) was administered by incorporation into the mouse diet. OFLO (batch 10-167; a gift of Daiichi Seiyaku, Tokyo), PEFLO (batch 9001; a gift of Roger Bellon, Paris), and PTH (batch AQU410; a gift of Theraplix, Paris) were all prepared fresh every fortnight in sterile distilled water containing 0.05% agar, stored in light-proof containers at 4°C, and administered by gavage.

Four-week-old, outbred, female Swiss mice (purchased from the Janvier Breeding Centre, 53680 Le Genest, France) were maintained in conventional animal quarters. Seven-hundred-twenty mice were inoculated in the left hind foot pad, with 4.8×10^3 *M. leprae* of a DDS-susceptible strain (17547), originally supplied by S. R. Pattyn, Antwerp, Belgium. These bacilli had been propagated in mouse passage, and were freshly harvested during logarithmic multiplication. The mice were allocated randomly to six groups of equal size: an untreated control group, and groups administered DDS in a concentration of 0.01 g per 100 g diet, OFLO 50 mg per kg body weight, OFLO 150 mg per kg, PEFLO 150 mg per kg, or PTH 50 mg per kg body weight.

The study was carried out by Shepard's "kinetic" technique ([17]). Drugs were administered during the period 62 to 150 days after inoculation, DDS was given continuously in the diet, and the remaining drugs were given by gavage five times per week. All of the animals were weighed monthly, and the dosages of the drugs administered by gavage were adjusted accordingly. Beginning 4 months after inoculation, seven mice from each group were sacrificed every month, and individual harvests of *M. leprae* were performed from the inoculated foot pads, according to the method of Shepard ([16]).

The numbers of acid-fast bacteria (AFB) per foot pad at each interval were compared by means of the Mann-Whitney *U* test ([4]).

RESULTS

During the 3 months of drug administration, 1% of the control mice and 4% of the

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DDS-treated mice died. Mortality among the mice administered drug by gavage was 9% for mice administered PTH, 1% for mice administered PEFLO and the smaller dose of OFLO, and 4% for mice administered the larger dose of OFLO. Both during and after the period of drug administration, the weight gain among the treated mice was no different from that of the control mice. Thus, with the possible exception of PTH, the drugs appeared well tolerated, and repreated esophageal cannulation was not associated with excessive mortality.

In the untreated control mice 4 months after inoculation with *M. leprae*, the mean number of harvested organisms was 2.6×10^5 (range $1.2–5.4 \times 10^5$), a value significantly greater than the inoculum, indicating that the *M. leprae* were multiplying as expected ([16]). The "plateau" phase was reached by 6 months after inoculation, at which time all of the mice harvested revealed at least 10^6 AFB per foot pad. Based on the increase in the numbers of *M. leprae* between 4 and 5 months after inoculation, i.e., during the phase of logarithmic multiplication, one may calculate the doubling time to be about 15 days, as in the earlier study with the same strain of *M. leprae* ([7]).

During the 3 months of drug administration, as shown in The Table, all five treatments appeared to inhibit multiplication of *M. leprae*. During the month (the sixth following inoculation) after the termination of drug administration, multiplication appeared to have resumed in two mice that had been administered the smaller dosage of OFLO, as demonstrated by the numbers of AFB harvested at the end of this month. Harvests performed after 7 months showed that multiplication of the organisms had resumed in mice in all of the treated groups, with the exception of the mice that had been administered the larger dosage of OFLO. In fact, in all but this last group the organisms had multiplied to levels indistinguishable from those of the control mice by the end of the ninth month. On the other hand, harvests of *M. leprae* performed 15 and 18 months after inoculation from the mice that had been administered the larger dosage of OFLO failed to reveal evidence of resumption of multiplication.

DISCUSSION

The results of this study demonstrate that in mice infected with *M. leprae* and treated for 3 months with 0.01 g % DDS in the diet, or with OFLO at 50 or 150 mg per kg body weight, or PEFLO at 150 mg per kg, or PTH at 50 mg per kg administered five times weekly, the organisms did not multiply during treatment but multiplication resumed during the fourth month after termination of treatment in all groups of mice, except in those administered OFLO in the dosage of 150 mg per kg. According to Shepard's "kinetic" technique ([17]), the antimicrobial activity of a drug may be considered only bacteriostatic when the growth of *M. leprae* in the treated mice is delayed by no more than 3 months (the duration of treatment) in comparison with their multiplication in the control mice. Antimicrobial activity may be considered bactericidal or bacteriopausal (that resulting from prolonged bacteriostasis) when the delay of multiplication exceeds 3 months; the longer the delay, the more potent is the bactericidal activity. Because the excess of growth-delay after termination of treatment with DDS, PEFLO, PTH, and the smaller dosage of OFLO was uniformly 1 month, all of these treatments may be considered to have exerted a similar degree of bacteriopausal or bactericidal activity. If one assumes that the *M. leprae* that survive treatment multiply at the same rate as in untreated mice, then the excess of growth-delay is directly related to the proportion of organisms that survived treatment. If the doubling time of the organisms is 15 days, then a delay of 1 month represents two doublings, and the proportion of *M. leprae* that survived treatment may be calculated to be 25%, i.e., 75% of the organisms were killed. Such a conclusion is in agreement with the known activities of DDS ([3]), PTH ([3, 18]), and PEFLO ([7, 11]).

The most important result of this study is the total failure of resumption of multiplication of the organisms in the mice that had been administered OFLO in the dosage of 150 mg per kg body weight for 3 months. Because as few as five viable *M. leprae* can give rise to multiplication in the mouse foot pad ([21]), this treatment must have killed all

THE TABLE. *Numbers of* M. leprae *harvested from left hind foot pads of mice infected with 5 × 10³* M. leprae *and treated from day 62 to day 150 after infection.*

Mos. after infection	Median (range) nos. *M. leprae* per foot pad × 10^4 in mouse groups[a]					
	Controls	DDS[b,c] 0.01	PTH[b,c] 50	PEFLO[b,c] 150	OFLO[b,c] 50	OFLO[b,c] 150
4	26 (12–51)	<2[d] (<2–3.9)	<2[d] (<2–5.9)	<2[d]	<2[d] (<2–2)	<2[d] (<2–5.9)
5	110 (80–170)	2[d] (<2–3.9)	<2[d] (<2–3.9)	<2[d] (<2–3.9)	<2[d] (<2–2)	<2[d] (<2–2)
6	340 (100–520)	<2[d] (<2–3.9)	2[d] (2–3.9)	<2[d]	<2[d] (<2–14)	<2[d] (<2–2)
7	250 (79–390)	2[d] (<2–5.9)	7.9[d] (<2–33)	3.9[d] (<2–26)	7.9[d] (2–26)	<2[d]
8	230 (65–610)	5.9[d] (<2–33)	55[d] (26–83)	16[d] (<2–51)	9.9[d] (2–22)	<2[d] (<2–2)
9	63 (<2–220)	43 (7.9–130)	130 (65–250)	98 (26–350)	87 (28–120)	<2[d]
10	210 (75–470)	210 (43–330)	200 (53–460)	120 (33–220)	240 (<2–360)	<2[d]
11	330 (34–500)	190 (32–430)	160 (110–330)	200 (39–510)	310 (150–660)	<2[d]
13	240 (85–380)	260 (250–780)	200 (120–540)	200 (110–1200)	280 (81–590)	<2[d]
15	220 (87–370)	ND[e]	ND	ND	ND	<2[d] (<2–2)
18	310 (230–590)	ND	ND	ND	ND	<2[d]

[a] Monthly harvests from seven mice of each treatment group.
[b] DDS = dapsone; PTH = prothionamide; PEFLO = pefloxacin; OFLO = ofloxacin.
[c] DDS 0.01 = DDS 0.01% continuously in the diet; PTH 50 = PTH 50 mg per kg body weight 5 × weekly; PEFLO 150 = PEFLO 150 mg per kg body weight 5 × weekly; OFLO 50 = OFLO 50 mg per kg body weight 5 × weekly; OFLO 150 = OFLO 150 mg per kg body weight 5 × weekly.
[d] Values different from the values for control mice ($p < 0.05$), Mann-Whitney *U* test.
[e] ND = not done.

of the viable organisms. That profound a bactericidal activity against *M. leprae* in the mouse is characteristic only of rifampin ([3, 19]). This suggests that OFLO may prove a very useful drug for the treatment of multibacillary leprosy. The drug appeared nontoxic to mice in the dosages administered. A more critical issue, however, is the degree to which the findings in the mouse may be extrapolated to man. In fact, comparing the pharmacokinetic properties of the drug in mice to those in man, it is apparent that OFLO in the daily dosage of 150 mg per kg body weight to mice is equivalent to a daily dose of 400 mg, the usual therapeutic dosage, in man ([6]). OFLO may therefore prove to be very active in the chemotherapy of leprosy. This is not pure speculation, because PEFLO, which is less active than OFLO in the mouse and which possesses pharmacokinetic properties very similar to those of OFLO, has recently been demonstrated to be strongly active in the treatment of patients with previously untreated lepromatous leprosy (N'Deli and Grosset, unpublished data).

An interesting aspect of the activity of OFLO against *M. leprae* in mice is that, in a dosage of 50 mg per kg body weight, the drug was bacteriopausal or possibly bactericidal, although not profoundly so; whereas bactericidal activity was marked when OFLO was administered in a dosage only threefold larger. Norrby and Jonsson ([10]) have called attention to the small differences between the minimal inhibitory and minimal bactericidal concentrations of the fluoroquinolones. The steep slope of the

dose-response curve observed in this experiment is consistent with this observation.

The introduction into programs of leprosy control of combined drug regimens (World Health Organization Study Group regimen [22]), which include rifampin as the major component, has emphasized the need for new drugs which are active against *M. leprae*. Because of the limited bactericidal activity of DDS, PTH, and clofazimine, the objectionable pigmentation of the skin by clofazimine ([13, 24]), and the liver toxicity of PTH ([1, 8, 12]), an additional drug, which is well accepted by the patient and which possesses strong bactericidal activity, would greatly strengthen the treatment of leprosy. Except for its cost, OFLO seems to possess all of the necessary qualities and, therefore, appears to be a very promising drug for the control of leprosy.

SUMMARY

Mice inoculated with 4800 *Mycobacterium leprae* in the left hind foot pad were treated from day 62 to day 150 after infection with 50 mg or 150 mg of ofloxacin per kg body weight, 150 mg pefloxacin per kg, or 50 mg prothionamide per kg. These drugs were administered by esophageal cannula 5 days weekly with dapsone (0.01 g per 100 g diet). Multiplication of *M. leprae* in the treated and in untreated control mice was assessed by monthly harvests.

The treatment of mice with the smaller dosage ofloxacin, with pefloxacin, prothionamide, or dapsone uniformly resulted in a delay of multiplication of 4 months, compared to the multiplication of *M. leprae* in the untreated controls. The delay of multiplication (4 months) being 1 month longer than the duration of drug administration (3 months), all of the treatments may be considered as bacteriopausal or moderately bactericidal. In contast with these results, treatment of mice with 150 mg ofloxacin per kg resulted in no growth of the organisms whatever as late as 18 months after inoculation, strongly suggesting that, in that dosage, ofloxacin had killed all of the *M. leprae*. Such a profound killing activity has been observed only with rifampin. Although the pharmacokinetic characteristics of ofloxacin are different in man from those in the mouse, the daily dosage of 150 mg ofloxacin per kg body weight in the mouse is equivalent to 400 mg per day in man which is the usual therapeutic dosage; thus, the results obtained in the mouse may be extrapolated to man. Therefore, ofloxacin appears a very promising drug for the chemotherapy of leprosy.

RESUMEN

Un grupo de ratones inoculados con 4800 *Mycobacterium leprae* en la almohadilla plantar izquierda trasera se trató del día 62 al día 150 después de la infección, con 50 mg o 150 mg de ofloxacina por kg de peso corporal, 150 mg de pefloxacina por kg, ó 50 mg de protionamida por kg. Estas drogas fueron administradas por cánula esofageal 5 días a la semana junto con dapsona (0.01 g por 100 g de alimento). La multiplicación de *M. leprae* en los ratones tratados y en los controles no tratados se estableció por cosechas mensuales.

El tratamiento de los ratones con las dósis más pequeñas de ofloxacina, pefloxacina, protionamida o dapsona, dió como resultado un retardo uniforme en la multiplicación del *M. leprae*. El retardo en la multiplicación (de 4 meses) fue un mes más largo que la duración del tiempo de administración de la droga (3 meses). Todos los tratamientos pueden considerarse como bacteriopausales o como moderadamente bactericidas. En contraste, el tratamiento de los ratones con 150 mg de ofloxacina por kg no permitió el crecimiento del organismo incluso a los 18 meses después de la inoculación, sugiriendo fuertemente que esa dosis de ofloxacina mata a todos los *M. leprae*. Tal actividad marcadamente bactericida sólo se ha observado con la rifampina. Aunque las características farmacocinéticas de la ofloxacina son diferentes en el hombre y en el ratón, la dosis diaria de 150 mg por kg de peso corporal en el ratón es equivalente a la dosis terapéutica usual en el humano de 400 mg por día. Así, los resultados obtenidos en el ratón, podrían ser extrapolados al humano. La ofloxacina parece ser una droga muy prometedora en la quimioterapia de la lepra.

RÉSUMÉ

Des souris inoculées avec 4800 *Mycobacterium leprae* dans le coussinet plantaire de la patte avant gauche ont été ensuite traitées entre le 62ème et le 150ème jour après l'infection par 50 à 150 mg d'ofloxacine par kg de poids corporel, 150 mg de pefloxacine par kg, ou 50 mg de prothionamide par kg. Ces médicaments ont été administrés par une canule oesophagienne 5 jours par semaine, avec accompagnement de dapsone dans le régime à raison de 0,01 g par 100 g de nourriture. On a évalué la multiplication de *M. leprae* chez les animaux traités et chez les souris-témoins non traitées lors de récoltes successives de bacilles.

Le traitement des souris tant par les dosages les plus faibles d'ofloxacine, que par la pefloxacine, la prothionamide, ou la dapsone, a entraîné uniformément un retard de multiplication de 4 mois, par rapport à la multiplication de *M. leprae* chez les animaux non traités. Ce retard de multiplication, c'est-à-dire 4 mois, est d'un mois plus long que la durée d'administration du médicament (3 mois). Tous ces traitements peuvent dès lors être considérés comme bactériostatiques ou modérément bactéricides. Contrastant avec ces résultats, le traitement des souris avec 150 mg d'ofloxacine par kg a résulté en une absence de croissance des organismes, et ceci pendant une période aussi longue que 18 mois après l'inoculation. Ces dernières observations suggèrent que, à ce dosage, l'ofloxacine a tué tous les bacilles de la lèpre. Une activité bactériocide tellement prononcée n'a été observée qu'avec la rifampicine. Quoique les caractéristiques pharmacocinétiques de l'ofloxacine soient différentes chez l'homme et chez la souris, le dosage quotidien de 150 mg d'ofloxacine par kg de poids corporel chez la souris est égal à 400 mg par jour, ce qui est la dose thérapeutique habituelle chez l'homme. Dès lors les résultats obtenus chez la souris peuvent être extrapolés à l'homme. L'ofloxacine semble être un médicament extrêmement prometteur pour la chimiothérapie de la lèpre.

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