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Activity of Sparfloxacin Against *Mycobacterium leprae* Measured by the Proportional Bactericidal Test[1]

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A new generation of synthetic fluoroquinolones has been developed with broad spectra of activity, including activity against mycobacteria. The new fluoroquinolones have been endowed with the properties of good penetration and concentration inside mammalian cells ([4, 19]), and they have been found to have bactericidal activity against intracellularly growing mycobacteria ([17]). Ofloxacin has been found to be extremely effective against experimental leprosy infection in both normal ([7, 15]) and nude mice ([2]). Ciprofloxcin, an effective quinolone against common fast-growing organisms, failed to show any activity against *Mycobacterium leprae* in mice ([1, 9]). Another active quinolone, pefloxacin, on a weight-to-weight basis was less bactericidal than ofloxacin against *M. leprae* in mouse foot pad studies ([9, 14]). In a preliminary clinical study, ofloxacin has been found to kill a significant proportion of viable organisms in human tissues after 26 days of treatment, as evidenced by subinoculation into mice from biopsies from treated lepromatous leprosy patients ([8]). A further report showed that 22 doses of either drug resulted in a 4-log reduction in viability (99.99%) ([11]). On the basis of their earlier estimation that rifampin resistance in a lepromatous leprosy patient before treatment is unlikely to be more than 4 logs, these authors speculate that given together with rifampin, either pefloxacin or ofloxacin should be able to eliminate *M. leprae* infection within a short period of time. Both pefloxacin and ofloxacin were found to be free from significant side effects and are tolerated extremely well. Further clinical trials are currently in progress.

Sparfloxacin is a newly synthesized difluorinated quinolone which was found to have rapid bactericidal action against *M. tuberculosis* and against many strains of *M. avium* ([16, 17]). It achieves high extravascular levels in most tissues ([13]) in experimental animals. Recent animal studies have shown that it is active against *M. leprae* ([6]). The present study examines further the nature of the activity of this quinolone by the proportional bactericidal test, which provides a method for satisfactory distinction between the bacteriostatic and bactericidal action of a drug ([3]). The study also compares the activity of this compound to that of ofloxacin, the other new compound found active against *M. leprae*. The contribution of sparfloxacin to the already powerful bactericidal action of combinations of rifampin, minocycline and clarithromycin was difficult to assess in the immunocompetent mouse ([12]). This is currently being investigated using the athymic mouse model.

MATERIALS AND METHODS

Animals. Normal CD1 mice (Charles River, U.K.) were maintained in a conventional clean room separately from other *M. leprae*-infected mice.

Drugs. Sparfloxacin was obtained, as a gift, from Rhône D.P.C. Europe (Antony Cedex, France). Ofloxacin was donated by Hoechst UK Limited (Milton Keynes, Bucks, U.K.). The compounds were prepared as suspensions in sterile 0.05% agar in distilled water once a week and kept at 4°C in the dark; they were mixed thoroughly before being administered as a 0.1-ml bolus by gavage (based on a 30-g mouse). The mice received 25 mg or 50 mg sparfloxacin per kg body weight and 25, 50 or 150 mg of ofloxacin per kg by gavage.

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THE TABLE. *Effects of sparfloxacin and ofloxacin on* M. leprae *in mouse foot pads by the proportional bactericidal test.*

Treatment groups	Inoculum size (*M. leprae*/foot pad)				MPN[a]	Percentage survival
	10^4	10^3	10^2	10^1		
Control	10/10[b]	10/10	7/7	6/10	912	—
Sparfloxacin 50 mg/kg	0/10	0/7	0/9	NC[c]	0	0
Sparfloxacin 25 mg/kg	1/10	0/9	0/8	NC	<0.01	<0.001
Ofloxacin 150 mg/kg	0/10	0/10	NC	NC	0	0
Ofloxacin 50 mg/kg	8/10	5/10	1/9	0/10	7.5	0.82
Ofloxacin 25 mg/kg	6/7	7/8	8/9	7/10	100	10.9

[a] Most probable number of viable *M. leprae* per 10,000 bacteria.
[b] Number of mice showing multiplication (>10^3 bacilli per foot pad)/total number of mice inoculated.
[c] NC = Not counted.

Inoculum. The inoculum was obtained from a nude mouse foot pad which had been inoculated 9 months earlier with 10^4 *M. leprae* passaged (5th passage) from a patient biopsy.

The inoculum was prepared by homogenizing the nude mouse foot pad in Dubos' broth using a sealed unit homogenizer (Silverson Machines, Chesham, Buckinghamshire, U.K.). The homogenate was examined microscopically by a method based on that of Shepard and McRae ([18]). The slides were prepared and Formalin and heat-fixed. They were then stained by the cold Ziehl-Neelsen method for 20 min, and the acid-fast bacilli (AFB) were enumerated.

The AFB count in the foot pad was 2×10^8/ml. It was then diluted to give 4×10^5 *M. leprae* per ml. From this suspension, ten-fold dilutions were made and 25 μl of suspension was inoculated into the hindfoot pads of normal CD1 mice for each drug and for the control mice. Treatment began on day 6 postinoculation and continued for 6 days per week for 60 days.

All mice were killed 1 year after the start of treatment, and their foot pads were examined individually for AFB as described above. Each foot pad was scored as positive or negative for growth; positive foot pads were those in which the counts were 10^5 or more. Most probable numbers (MPN) were obtained by computer analysis based on the Halvorson-Ziegler equation ([10]).

RESULTS

The results are shown in the The Table. There were no excessive deaths in the treated group of animals compared to the control group, and no evidence of toxicity was apparent. The inoculum proved to be of good viability; MPN of viable organisms were estimated in the control group to be 912 viable *M. leprae* per 10^4 bacilli or 9.12% viability. Comparing the results of the drug-treated groups, sparfloxacin 50 mg/kg given for 60 days produced killing of a significant proportion of the viable organisms corresponding to killing of more than 99.9%. A similar bactericidal effect also was produced by ofloxacin, but it required 150 mg/kg to produce this effect. Sparfloxacin 25 mg/kg also effected a significant bactericidal action, virtually similar to the effect produced by the higher level of sparfloxacin (50 mg/kg), resulting in more than 99.9% killing. Ofloxacin 25 mg/kg had only a slight effect, giving a MPN of viable bacilli of 100 and a survival of 10.9% after treatment. Ofloxacin 50 mg/kg was more bactericidal, with a MPN of 7.5 and 0.82% survival. This effect, however, was significantly lower than that produced by 50 mg/kg sparfloxacin.

DISCUSSION

Sparfloxacin has a powerful bactericidal action as demonstrated by these results in the proportional bactericidal test. It is active at both 25 mg/kg and 50 mg/kg body weight. These dose levels compare with 150 mg/kg of ofloxacin which also produces a profound bactericidal effect. Ofloxacin, used alone for the treatment of established *M. leprae* infection in nude mice, produced a significant reduction in viability when measured by the proportional bactericidal test. In conjunction with rifampin (single doses of 10 mg/kg on days 0, 28 and 56), no enhanced activity was demonstrated but in combination with dapsone (0.01% daily for 56 days), a

marked additive effect was observed ([2]). Ofloxacin and pefloxacin, on the basis of preliminary animal experimental and clinical studies, have proved to be extremely valuable additions in the fight against leprosy.

Sparfloxacin has shown a remarkable antibacterial effect against a range of fast-growing pathogenic organisms both *in vitro* and also in humans. Initial studies of the effect of sparfloxacin against a number of cultivable mycobacteria has shown promising results ([4, 16, 17]). Sparfloxacin has superior pharmacological properties, such as achieving high intracellular concentration, compared to other fluoroquinolones and superior intrinsic antibacterial activity. Serum levels, in man, of 0.54 μg/kg 2 hr after a single oral dose of 30 mg/kg has been reported, and the C_{max} in plasma and muscle has been reported to be 0.19 μg/ml and 0.42 μg/ml, respectively [Kanamura, *et al.* Pharmacokinetics and safety of a new quinolone AT4140 in healthy volunteers. (Abstract) 28th Interscience Conference, 1988].

It also has been reported that the half-life in normal mice given 5 mg/kg orally was about 3 hr and, in a separate study, single oral doses of 100, 200 and 400 mg of sparfloxacin resulted in serum C_{max} of 0.44, 0.65 and 1.39 μg/ml, respectively, with a half-life of 16.8, 16.3 and 16.0 hr ([13]). It diffuses well and concentrates in the cells of the reticuloendothelial system, and it is more liposoluble than its earlier analogs. In earlier studies sparfloxacin has been found to inhibit growth of *M. leprae* at 15 mg/kg and 30 mg/kg ([6]). In the present experiment, sparfloxacin has proved to be more active than ofloxacin and, hence, there exists a strong case for pursuing this agent as a potential new treatment for leprosy especially in combination with other antileprosy agents.

SUMMARY

The activity of 25 mg/kg and 50 mg/kg sparfloxacin was measured against *Mycobacterium leprae* in normal (immunocompetent) mouse foot pads by the proportional bactericidal test. This was compared with the action of 25, 50, and 150 mg/kg ofloxacin by the same method. Sparfloxacin, at both concentrations, was found to be strongly bactericidal by this method, comparable to 150 mg/kg ofloxacin.

RESUMEN

Se utilizó la prueba bactericida proporcional en el modelo de la almohadilla plantar del ratón para evaluar la actividad bactericida de 25 mg/kg de esparfloxacina contra el *Mycobacterium leprae*. El efecto de la droga se comparó con el de la ofloxacina a las concentraciones de 25, 50, y 150 mg/kg. La actividad bactericida de la esparfloxacina a las dos concentraciones probadas fue tan alta como la de la ofloxacina a la concentración de 150 mg/kg.

RÉSUMÉ

L'activité vis-à-vis de *Mycobacterium leprae* de la sparfloxacine à la dose de 25 mg/kg et 50 mg/kg a été mesurée dans le coussinet plantaire de souris normales (immunocompétentes) par le test bactéricide proportionnel. Cette activité a été comparée à l'action de 25, 50, et 150 mg/kg d'ofloxacine par la même méthode. On a trouvé par cette méthode que la sparfloxacine avait un pouvoir fortement bactéricide aux deux concentrations, pouvoir comparable à 150 mg/kg d'ofloxacine.

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