Activity of sparfloxacin against *Mycobacterium leprae* inoculated into footpads of nude mice

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Summary The antileprosy activity of a new quinolone, sparfloxacin, was examined in the nude mouse footpad model. By serial dosing (once a day, 5 or 6 times per week, during the 3rd–5th months postinoculation), 10 mg/kg of sparfloxacin displayed bactericidal-type activity and bacteriostatic activity was present at daily doses of 5 and 2 mg/kg. By intermittent dosing (once a day, twice weekly at daily doses of 10 and 20 mg/kg or once weekly at a daily dose of 30 mg/kg, during the 3rd–5th months postinoculation), sparfloxacin markedly inhibited the growth of leprosy bacilli with slight remultiplication at later stages. Sparfloxacin seems to be worth studying clinically as a novel antileprosy drug.

Introduction

At present, the most reliable chemotherapy for the treatment of leprosy is the multidrug therapy that uses dapsone, clofazimine and rifampicin. However, resistance of *Mycobacterium leprae* to each of these agents has been reported,¹ and the inhibitory action of dapsone is bacteriostatic, and clofazimine has an unfavourable side-effect, namely, skin pigmentation. Rifampicin is a reliable bactericidal drug, but expensive. Therefore it is necessary to develop new agents which are not cross-resistant with existing antileprosy drugs, are bactericidally effective, less toxic and less expensive than rifampicin.

Sparfloxacin² (Figure 1) is a novel quinolone with broad and potent antibacterial activity *in vitro* and *in vivo*.³ Previously we found that sparfloxacin was more potent than ofloxacin in growth-inhibitory activity against *M. leprae* in the nude mouse footpad model, and its activity seemed to be the bactericidal-type when it was given orally once daily, 6 times a week, 3–5 months postinoculation at doses of 15 and 30 mg/kg.⁴ Such an encouraging result caused us to examine the antileprosy activity of sparfloxacin in more detail.

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Sparfloxacin (SPFX)

Figure 1. Chemical structure of sparfloxacin.

Materials and methods

DRUG ADMINISTRATION

Sparfloxacin was provided by Research Laboratories, Dainippon Pharmaceutical Co. Ltd, Osaka, Japan. It was homogenized in distilled water containing 0.001% Tween 80, sterilized for 25 min at 121°C, and kept at -80°C until use. The drug suspension, in doses of $\frac{1}{10}$ ml, was given orally to each nude mouse through a mouse catheter. The treatment followed the Shepard's kinetic method.⁵ In the first experiment, sparfloxacin was given continuously, 5 or 6 times a week, at doses of 2, 5 and 10 mg/kg, and in the second experiment it was given intermittently, 2 times a week at doses of 10 and 20 mg/kg, or once a week at a dose of 30 mg/kg.

MICE

BALB/c (nu/nu) female mice, aged 5 weeks, were purchased from Clea Japan Inc., Tokyo, Japan. They were randomly grouped into 10 mice per group (5 mice per cage) in a vinyl isolator (Sanki Scientific Arts Co., Tokyo, Japan) and kept at $22 \pm 1^{\circ}\text{C}$, being fed on a sterilized heat-stable pellet form diet, MB-6E (Funabashi Farm Co., Chiba, Japan), and sterilized drinking water.

M. LEPRAE INFECTION

We used the *M. leprae* strain Thai-53, which had been isolated from a subcutaneous leproma of a Thai lepromatous patient in 1980⁶ and passed through the nude mouse footpads 7 or 8 times. Inocula were prepared according to the method of Nakayama *et al.*⁷ Several infected swollen footpads were aseptically homogenized with chilled physiological saline (PS), and centrifuged at $330 \times g$ for 3 min at 4°C . The supernatant was treated with alkali and centrifuged. The precipitated bacilli were suspended in 0.1% Tween 80-containing PS (pH 3)⁷ and washed. The washed bacilli were suspended in PS at a cell density $> 2 \times 10^8$ bacilli/ml. A 0.05-ml portion was inoculated into each of both hind footpads of nude mice.

COUNTING OF ACID-FAST BACILLI

We took 4 or 6 footpads of 2 or 3 mice at specified time points, homogenized with PS, centrifuged at $330 \times g$ as described above, and diluted the supernatant appropriately with PS. Acid-fast bacilli (AFBs) were counted in duplication according to the method of Shepard and McRae.⁸ AFBs were counted in more than 40 microscopic fields with a Nikon microscope, Model Optiphot XF-21 (Nikon Corp., Tokyo, Japan).

DETERMINATION OF HIND FOOTPAD VOLUME

The weight of water removed by the soaking from below the *malleolus lateralis* of the hind footpad was measured by a digital volume meter, Model MK-550 (Muromachi Kikai Co., Tokyo, Japan).

Results

Efficacy with serial treatment. Antileprosy activity of sparfloracin was examined in nude mice inoculated with 10^7 leprosy bacilli per footpad and given sparfloracin orally, once a day, 5 or 6 times a week, between 60 and 152 days postinoculation on daily doses of 2, 5, and 10 mg/kg. As shown in Figure 2, the average AFBs in 4 footpads of 2 untreated nude mice reached above 10^9 AFBs per footpad on day 1 of month 8 and 9 postinoculation, gradually increased thereafter and reached nearly 10^{10} bacilli per footpad 11 months postinoculation. The average numbers of AFBs in the sparfloracin 2-mg/kg group were 3×10^7 and above 10^9 AFBs per footpad at 8 and 11 months after inoculation, respectively, suggesting that growth of leprosy bacilli was inhibited by sparfloracin but that the regrowth occurred when medication was stopped. The average numbers of AFBs in the sparfloracin 5-mg/kg group were below the inoculated level (10^7 bacilli per footpad) until 9 months but increased about 10 times at both 10 and 11 months after inoculation, suggesting a delayed growth of leprosy bacilli. In contrast, no increase in the average numbers of AFBs was observed throughout the observation period in the sparfloracin 10-mg/kg group, demonstrating that the drug action was bactericidal-type against leprosy bacilli at this dosage. The average mouse footpad volume of the untreated control markedly increased with time, while that of the sparfloracin 2-mg/kg group showed a slight swelling only at 10 and 11 months postinoculation and no swelling was detected in the sparfloracin 5- and 10-mg/kg groups (Figure 3).

Efficacy with intermittent treatment. In order to assess the influence of dose regimens upon the efficacy of sparfloracin, nude mice were inoculated with 10^7 leprosy bacilli per footpad and orally treated with sparfloracin, once a day, twice a week, with doses of 10 and 20 mg/kg, or once a week with 30 mg/kg, between 61 and 154 days postinoculation. The untreated control showed a marked increase of 2 or 3 orders of magnitude in the average numbers of AFBs from 8 months after inoculation (Figure 4). The average numbers of AFBs in the sparfloracin 10-mg/kg-twice-a-week group increased about 10 times at 10 and 11 months postinoculation and those of the sparfloracin 20-mg/kg-twice-a-week and 30-mg/kg-once-a-week groups rose several times only at 11 months postinoculation. All the medicated groups showed only slight mouse footpad swelling at

Figure 2. Growth inhibition of leprosy bacilli inoculated into nude mouse footpads by serial medication with sparfloxacin. Groups of 10 nude mice were infected with the strain Thai-53 of *M. leprae* by injecting 10^7 bacilli into each of both hind footpads, and orally treated with sparfloxacin (SPFX) once a day, 5 or 6 times a week, between 60 and 152 days postinfection at daily doses of 0, 2, 5 and 10 mg/kg. In total, 4 or 6 footpads of 2 or 3 mice were taken at indicated months postinfection and acid-fast bacilli (AFBs) in the footpads were counted.

10 and 11 months postinoculation (Figure 5). These results suggested that sparfloxacin did not kill all the leprosy bacilli by these 3 intermittent regimens and allowed some of them to remultiply when medication was stopped.

Discussion

In a previous paper,⁴ we found that sparfloxacin orally given at doses of 15 and 30 mg/kg once a day, 5 or 6 times a week, for 3 months starting from month 3 postinfection completely inhibited the growth of *M. leprae* in the footpads of nude mice throughout the

Figure 3. Inhibition of footpad swelling of nude mice infected with *M. leprae* by serial medication with sparfloxacin (SPFX). Mouse footpad volumes were measured in the mice used in the experiment shown in Figure 2; mean $\pm$ SD.

11 months postinfection, while ofloxacin at the same dosages showed only slight temporary growth inhibition. This result was of interest because ofloxacin had been considered to be the most effective among quinolones on the *M. leprae* infection in mice.⁹ So, we repeated similar experiments with lower dosages of sparfloxacin to confirm the present result.

As shown in the Results section, 10 mg/kg of sparfloxacin given orally once a day, 5 or 6 times a week, for 3 months starting from month 3 postinfection completely inhibited the growth of *M. leprae* throughout the 11 months postinfection. This suggests that in this case the drug action was the bactericidal type. On a dose of 5 mg/kg, administered as above, sparfloxacin completely inhibited the bacterial growth till month 9 postinoculation, but a slight remultiplication occurred at 10 and 11 months, suggesting that the drug action was no more than bacteriostatic at this dosage. A suppressed but continued growth of *M. leprae* was observed in mice given 2 mg/kg of sparfloxacin indicating that it has less bacteriostatic drug action at this dosage. These results, when combined with the previous ones,⁴ show that sparfloxacin was more potent than ofloxacin⁹ in activity against *M. leprae* in nude mice, and are consistent with the findings of Franzblau and White¹⁰ that sparfloxacin is more potent than ofloxacin in activity against *M. leprae* *in vitro*.

Figure 4. Growth inhibition of leprosy bacilli inoculated into nude mouse footpads by intermittent medication with sparfloxacin. Groups of 10 nude mice were infected with the strain Thai-53 of *M. leprae* by injecting 10^7 bacilli into each of both hind footpads, and orally treated with sparfloxacin (SPFX) once a day, twice a week, at a daily dose of 10 and 20 mg/kg, or once a week at a dose of 30 mg/kg, between 61 and 154 days postinfection. In total 4 or 6 footpads of 2 or 3 mice were taken at indicated months postinfection and acid-fast bacilli (AFBs) in the footpads were counted.

We next examined what the effect of sparfloxacin was when administered intermittently. The growth of *M. leprae* was completely inhibited till 10 months postinfection when sparfloxacin was given orally once a day, twice a week at doses of 10 and 20 mg/kg, or once a week at a dose of 30 mg/kg for 3 months starting from month 3 postinfection, but slight remultiplication was observed in all of the groups at month 11 postinfection. Therefore, the growth-inhibitory activity of sparfloxacin seems to be bacteriostatic when used intermittently in these dosing regimens.

We have reported that the level of sparfloxacin in a pooled serum specimen taken from 10 nude mice by the puncture of their carotid arteries at 2 hours after a single oral

Figure 5. Inhibition of footpad swelling of nude mice infected with *M. leprae* by intermittent medication with sparfloxacin (SPFX). Mouse footpad volumes were measured in the mice used in the experiment shown in Figure 4; mean $\pm$ SD.

administration of 30 mg/kg sparfloxacin was 0.54 $\mu\text{g}/\text{ml}$, when it was measured by an HPLC system.⁴ Nakamura *et al.*¹¹ have reported that C_{max} of sparfloxacin in plasma and muscle are 0.19 $\mu\text{g}/\text{ml}$ and 0.42 $\mu\text{g}/\text{g}$, respectively, with half-lives of about 3 hours in normal mice orally given 5 mg/kg. Kanamaru *et al.*¹² disclosed in the phase I study that the C_{max} of sparfloxacin were 0.44, 0.65 and 1.39 $\mu\text{g}/\text{ml}$ of serum at a single oral dose of 100, 200 or 400 mg per person (1.5, 3.1 and 6.3 mg/kg, respectively), with $t_{1/2}$ of 16.8, 16.3 or 16.0 h.¹² These results show that sparfloxacin might be applicable to leprosy in humans.

The strain Thai-53 of *M. leprae* was derived from a virgin case and susceptible to the present main antileprosy drugs. Taking the risk of monotherapy into consideration, activities by combined regimens with sparfloxacin against this strain are now being examined in the same nude mouse footpad model. The use of the infected nude mouse as an LL-animal model^{13,14} greatly reduced the hinderance due to stained tissue debris in microscopic counting of AFBs and the volumetry of the footpad swelling made a palpable onset semi-quantitatively realizable.

Sparfloxacin is an antibacterial agent under development in Europe, the USA and Japan. Clinical efficacies of sparfloxacin on various bacterial infections have been reported to be excellent with acceptable side-effects in Japan (New Drug Symposium for sparfloxacin, The 38th General Meeting of the West Branch of Japan Society of Chemotherapy, 7 December 1990, Gifu, Japan). Therefore, based on our ongoing

research it seems reasonable to suggest that sparfloxacin is worth studying clinically as a novel antileprosy drug.

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Activité de la sparfloxacine contre *Mycobacterium leprae* inoculée dans la plante du pied de la souris 'nude'

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Résumé Nous avons examiné l'activité antilépreuse d'une nouvelle quinolone, la sparfloxacine, sur la plante du pied de la souris 'nude'. Administrée en traitement en série (une fois par jour, 5 ou 6 fois par semaine, du 3^{ème} au 5^{ème} mois suivant l'inoculation) la sparfloxacine a présenté une activité de type bactéricide à la dose de 10 mg/kg, et une activité bactériostatique aux doses journalières de 5 et 2 mg/kg. En traitement intermittent (une fois par jour, deux fois par semaine aux doses de 10 et 20 mg/kg, ou bien une fois par semaine à la dose journalière de 30 mg/kg du 3^{ème} au 5^{ème} mois suivant l'inoculation), la sparfloxacine a nettement inhibé la croissance du bacille de la lèpre avec une légère reprise de la multiplication vers la fin du traitement. L'étude clinique de la sparfloxacine en tant que nouveau médicament contre la lèpre paraît justifiée.

La actividad de sparfloxacina contra *Mycobacterium leprae* inoculada en las almohadillas de los pies de ratones desnudos

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Resumen Se examinó la actividad contra la lepra de una nueva quinolona, sparfloxacina, en el modelo de la almohadilla del ratón desnudo. Por administración en serie (una vez por día, 5 o 6 veces por semana, durante el 3o hasta 5o mes postinoculación), 10 mg/kg de sparfloxacina presentó actividad antibacteriana, y presentaba bactividad bacteriostática con dosis diarias de 5 y 2 mg/kg. Por medio de dosis intermitentes (una vez por día, dos veces por semana con dosis diaries de 10 y 20 mg/kg, o una vez por semana con una dosis diaria de 30 mg/kg, durante el 3o hasta 5o mes postinoculación), sparfloxacina inhibió positivamente el crecimiento de los bacilos de la lepra con leve multiplicación durante las fases posteriores. Parece que la sparfloxacina merece un estudio clínico como una droga nueva contra la lepra.