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Which Aminoglycoside or Fluoroquinolone Is More Active against *Mycobacterium tuberculosis* in Mice?

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To identify the most active aminoglycoside or fluoroquinolone for the treatment of tuberculosis, the in vivo activities of four different aminoglycosides and three different fluoroquinolones were compared with that of isoniazid (INH) in a murine tuberculosis model. Mice were each inoculated intravenously with 2.3×10^7 CFU of *Mycobacterium tuberculosis* H37Rv. Treatment began the next day (D1) after inoculation and continued for 4 weeks, at the frequency of six times weekly with one of the following regimens: INH, 25 mg/kg; ofloxacin, 200 mg/kg; levofloxacin, 100 or 200 mg/kg; sparfloxacin (SPFX), 50 mg/kg; and streptomycin, kanamycin, amikacin (AMIKA), and isepamicin, all at 200 mg/kg. The dosages of the treatments were presumably equivalent to their clinically tolerated dosages. The severity of infection and effectiveness of the treatment were assessed by the survival rate, spleen weights, gross lung lesions, and the numbers of CFU in the spleens. The results indicate that INH is more bactericidal than any of the aminoglycosides or fluoroquinolones tested, that AMIKA is the most active aminoglycoside, and that SPFX at 50 mg/kg is far more bactericidal than the treatment with other fluoroquinolones.

Mycobacterium tuberculosis isolates that are resistant to multiple drugs, especially to isoniazid (INH) and rifampin (RMP), are increasing (7–9, 26, 32). Therefore, new antituberculosis agents with bactericidal mechanisms different from those of available first-line drugs, i.e., INH, RMP, and pyrazinamide (PZA), are urgently needed. Among the other classes of antimicrobial agents which display various degrees of in vitro and/or in vivo activities against *M. tuberculosis*, new antituberculosis drugs are likely to be identified from aminoglycosides and fluoroquinolones, not only because some of their derivatives, e.g., streptomycin (SM) (22, 23) or ofloxacin (OFLO) (28), have already been used for the treatment of pulmonary tuberculosis but also because newer derivatives are continually being developed.

The introduction of SM (22, 23) in the 1940s represented a historical beginning of the era of effective chemotherapy for tuberculosis. Other aminoglycosides such as kanamycin (KANA) and amikacin (AMIKA) are also occasionally employed for treatment of tuberculosis, but SM is still the most commonly used aminoglycoside, especially in the developing countries. In the developed world, although aminoglycoside has become a second-line antituberculosis drug since the introduction of short-course chemotherapy (12), because of the emergence of multidrug-resistant tuberculosis SM or other aminoglycosides are often added for the treatment of patients whose organisms are proved or suspected to be resistant to INH and/or RMP. Isepamicin (SCH 21420 or 1-*N*-HAPA gentamicin B) (ISEPA) is a novel broad-spectrum aminoglycoside which possesses a high level of stability to aminoglycoside-inactivating enzymes and a low level of toxicity to the kidney and inner ear (20). Our preliminary experiments in comparing the MICs of various aminoglycosides against six different strains of drug-susceptible *M. tuberculosis* and the same number of strains of *Mycobacterium avium* complex on 7H11 agar medium indicate that the MICs of ISEPA ranged from 1 to 2 μg/ml against the strains of *M. tuberculosis* and from 8 to 64 μg/ml against the strains of *M. avium* complex (unpublished data). The values were virtually the same as those of AMIKA and lower than those of SM, KANA, and capreomycin (CAPREO) for *M. tuberculosis* and were very similar to those of AMIKA, SM, and KANA and lower than those of CAPREO for *M. avium* complex. However, the in vivo activity of ISEPA against tubercle bacilli has not been evaluated, and the in vivo activities of various aminoglycosides have rarely been compared simultaneously in a single experiment.

With respect to the fluoroquinolones, within the last decade we have tested the in vivo activities against *M. tuberculosis* of pefloxacin (27), OFLO (17, 19, 21, 27), sparfloxacin (SPFX) (17, 19, 21), and levofloxacin (LVFX) (19) in mice. In terms of CFU counts in the mice (19), the ranking of the anti-*M. tuberculosis* activities of the treatments by various dosages of fluoroquinolones and INH (as positive control) ran in the following order: LVFX (300 mg/kg of body weight) = SPFX (100 mg/kg) > INH (25 mg/kg) > SPFX (50 mg/kg) > OFLO (300 mg/kg) = LVFX (150 mg/kg) > OFLO (150 mg/kg) = LVFX (50 mg/kg). In other words, on a weight-to-weight basis SPFX is more active than other fluoroquinolones, and LVFX is about twice as active as OFLO. However, the ranking is insufficient to identify the most active derivative for clinical application because the clinically tolerated dosages of these derivatives vary widely: while patients tolerate OFLO well at 800 mg daily (18), the manufacturer of SPFX recommends that patients be treated with only 200 mg daily, which is equivalent to the dosage of SPFX at 50 mg/kg in mice (21); thus, the greater activity of SPFX may be offset by its lower clinically tolerated dosage. It may be more informative if the in vivo activities of all fluoroquinolones are compared in mice at dosages equivalent to their maximal clinically tolerated dosages. Up to now, the pharmacokinetic and chronic toxicological data for fluoroquinolones in mice have been very limited; it is difficult to define precisely the dosages in mice which are equivalent to the maximal clinically tolerated dosages in humans. Because the results of a randomized, double-blind trial indicated that human volunteers tolerated well the multiple, oral once-daily

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750-mg and 1-g doses of LVFX (6), it is reasonable to assume that the maximal clinically tolerated dosage of LVFX is similar to that of OFLO. Therefore, we tentatively estimate that OFLO and LVFX at 200 mg/kg and SPFX at 50 mg/kg in mice are equivalent to the maximal clinically tolerated dosages of these derivatives in humans.

To identify the most active aminoglycoside or fluoroquinolone against *M. tuberculosis* infection in mice at dosages relevant to clinical application, we compared in vivo activities among four different aminoglycosides (SM, KANA, AMIKA, and ISEPA) and three different fluoroquinolones (OFLO, SPFX, and LVFX) and used INH as the positive control. CAPREO, one of the injectable antituberculosis drugs, was excluded from the comparison; not only is it not an aminoglycoside, but it has been well documented that its in vivo activity against *M. tuberculosis* is considerably less effective than that of SM in mice (14).

MATERIALS AND METHODS

Antimicrobial agents. The following compounds were generously provided by the indicated manufacturers: LVFX and OFLO, Roussel Uclaf, Romainville, France; SPFX, Rhone D.P.C. Europe, Antony, France; SM, Laboratoire Diamant, Paris, France; KANA and AMIKA, Bristol-Myers Squibb, la Défense, France; ISEPA, Schering-Plough, Levallois, France; and INH, Roche, Neuilly, France. For mouse experiments, all the fluoroquinolones were suspended in 0.05% agar in distilled water, and the aminoglycosides were diluted by normal saline at the desired concentrations. The suspensions or solutions were prepared weekly and stored at 4°C.

In vivo experiment. Two hundred fifty female outbred Swiss mice (age 4 to 6 weeks) were each inoculated intravenously with 0.5 ml of freshly prepared suspension containing 2.3×10^7 CFU of *M. tuberculosis* H37Rv. For strain H37Rv, the MICs, as determined on 10% oleic acid-albumin-dextrose-catalase-enriched 7H11 agar medium, of INH, OFLO, LVFX, SPFX, SM, KANA, AMIKA, and ISEPA were 0.1, 1.0, 0.5, 0.12, 2.0, 4.0, 2.0, and 2.0 μg/ml, respectively.

The next day (D1) after inoculation, 10 inoculated mice were sacrificed to provide the baseline values of spleen weight, lung lesion, and the number of CFU in the spleen; the remaining mice were allocated randomly to an untreated control group with 20 mice and nine treated groups. Treatments began on the same day and were carried out six times weekly for 4 weeks. Each treated group received one of the following nine treatments: INH, 25 mg/kg; OFLO, 200 mg/kg; SPFX, 50 mg/kg; LVFX, 100 or 200 mg/kg; SM, KANA, AMIKA, or ISEPA, 200 mg/kg per dose. At the beginning of treatment, there were 20 mice each among groups treated with INH or fluoroquinolones, and 30 mice each among groups treated with aminoglycosides. INH and fluoroquinolones were given through an esophageal cannula, and aminoglycosides were administered by subcutaneous injection. The last dose of treatment was given on D28, and all surviving mice were sacrificed on D30. The severity of infection and the effectiveness of the treatment were assessed by the survival rate, the spleen weights, the gross lung lesions, and the numbers of CFU in the spleens. The severity of gross lung lesions was scored from 0 to 2+, with the latter referring to a lung extensively infiltrated with tubercles (17, 27).

Enumeration of CFU. During sacrifice, the spleens were removed aseptically and homogenized (11); the suspension was made up to 3.5 ml for each spleen. At least three serial 10-fold dilutions of the suspension were plated onto Löwenstein-Jensen medium with three to five tubes per dilution. The results of the cultures were recorded after incubation at 37°C for 6 weeks. The bactericidal effect of the treatment was defined as a significant decrease in the mean number of CFU in the treated group from the pretreatment value.

Statistical analysis. Multiple comparisons among pairs of group means were performed by Scheffé's method (10, 30). Differences were considered significant at the 95% level of confidence.

RESULTS

Survival rates of mice for 30 days after inoculation. As expected (17, 27), after inoculation with $\geq 10^6$ CFU (0.1 mg [wet weight]) of virulent H37Rv per mouse, all untreated controls died by D21 after inoculation, and the first deaths occurred at D14 (Fig. 1). On the other hand, except for a few deaths occurring in mice treated with OFLO at 200 mg/kg or LVFX at 100 mg/kg, no mortality was observed in mice treated with other regimens. At D30, the survival rate in mice treated with LVFX at 100 mg/kg or OFLO at 200 mg/kg was 80 or 90%, respectively, which was higher than that in the untreated control group ($P < 0.01$) and did not differ significantly from those of mice treated with other regimens.

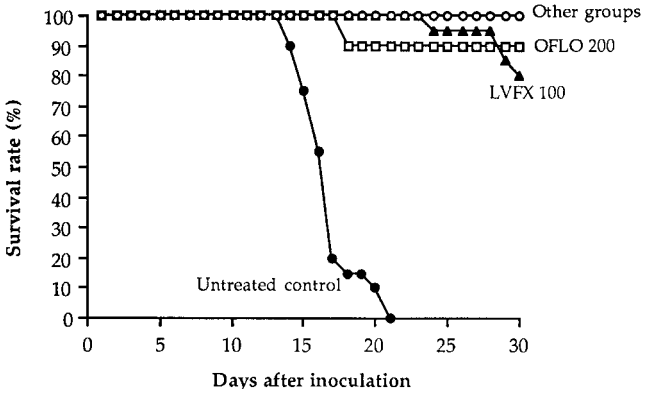

FIG. 1. Survival rates of mice within 30 days after infection intravenously with 2.3×10^7 CFU of *M. tuberculosis* H37Rv. By the time (day 1) the treatments were begun, there were 20 mice in the control group and 20 to 30 mice in each treated group. The numbers after the abbreviations for the drugs indicate the doses (in milligrams per kilogram of body weight) of each administration. "Other groups" refers to those treated with INH at 25 mg/kg; SM, KANA, AMIKA, and ISEPA at 200 mg/kg; SPFX at 50 mg/kg; and LVFX at 200 mg/kg per dose.

Spleen weights. As shown in Table 1, at D30 the mean spleen weights of all treated groups were significantly greater than the pretreatment level, i.e., those of mice sacrificed at D1 ($P < 0.01$), suggesting that none of the treatments were able to prevent completely the development of splenomegaly caused by tuberculosis infection. Among the treated groups, the mean weight was the lowest in mice treated with INH and the highest in mice treated with OFLO at 200 mg/kg or LVFX at 100 mg/kg, which was about sixfold greater than the pretreatment value. The values were virtually the same among the four groups that had been treated with the same dosage of aminoglycosides. Among the groups treated with fluoroquinolones, the weights were indistinguishable in mice treated with SPFX at 50 mg/kg or with LVFX at 200 mg/kg; both were in the range of those in mice treated with aminoglycosides and much lower than those in mice treated with OFLO at 200 mg/kg or LVFX at 100 mg/kg.

TABLE 1. Mean spleen weights and spleen CFU of *M. tuberculosis* in mice surviving at 30 days after intravenous inoculation with 2.3×10^7 CFU of H37Rv[a]

Drug (mg/kg)	Mean spleen wt (mg)	Mean spleen CFU ($\log_{10}$)
INH (25)	213 ± 31[b]	3.29 ± 0.25[b]
SM (200)	477 ± 95	5.25 ± 0.35
KANA (200)	444 ± 94	5.93 ± 0.29
AMIKA (200)	451 ± 107	4.88 ± 0.38
ISEPA (200)	471 ± 120	5.32 ± 0.31
SPFX (50)	482 ± 120	3.85 ± 0.37
OFLO (200)	644 ± 113[c]	6.51 ± 0.37[c]
LVFX (100)	709 ± 129[c]	6.52 ± 0.33[c]
LVFX (200)	483 ± 137	4.97 ± 0.42

[a] Drugs were administered six times weekly for 4 weeks. Values are shown as means ± standard deviations. The pretreatment values (i.e., the values for mice sacrificed the day after inoculation and just before the treatment was begun) are as follows: mean spleen weight, 112 ± 13 mg; mean spleen CFU, 6.16 ± 0.10 ($\log_{10}$).

[b] Significantly lower than the other treated groups.

[c] Significantly higher than the other treated groups.

Lung lesions. No lung lesions were detected either in untreated mice sacrificed at D1 or in mice that had been treated with INH, aminoglycosides, SPFX at 50 mg/kg, or LVFX at 200 mg/kg and sacrificed at D30. Severe (2+) lesions were observed in all untreated control mice by the time they died, and also in one of the four dead mice from the group that had been treated with LVFX at 100 mg/kg. Moderate (+) lesions were encountered in about one-third of mice that had been treated with OFLO at 200 mg/kg or LVFX at 100 mg/kg and survived by D30.

Enumeration of CFU in the spleens. By the time the treatment was begun, i.e., D1 after inoculation, the spleens of 10 mice were culture positive for *M. tuberculosis* and the mean number ($\log_{10}$) of CFU per spleen was 6.16 ± 0.10 (Table 1). As compared among the treated groups on D30, the mean CFU was the lowest in mice that had been treated with INH and was the highest in mice that had been treated with LVFX at 100 mg/kg or OFLO at 200 mg/kg; those in the latter two groups were virtually identical. Except in mice treated with the latter two regimens and with KANA, the mean CFU of other treated groups was significantly lower than the pretreatment value, with the reduction in mean number of CFU ranging from $10^{0.84}$ to $10^{2.87}$. These results indicate that, except for OFLO at 200, LVFX at 100, or KANA at 200 mg/kg, all the treatments displayed various degrees of bactericidal activities against *M. tuberculosis*. In mice treated with aminoglycosides, the CFU count was the highest in mice treated with KANA and lowest in mice treated with AMIKA, while those of mice treated with SM or ISEPA were indistinguishable; in other words, the ranking of bactericidal activities of aminoglycosides was as follows: AMIKA > SM = ISEPA > KANA. The mean numbers of CFU in mice that had been treated with fluoroquinolones varied widely: the value was the lowest in mice treated with SPFX at 50 mg/kg, which was also significantly lower than those in mice treated with any of the four aminoglycosides ($P < 0.01$); the value in mice treated with LVFX at 200 mg/kg was lower than those in mice treated with LVFX at 100 or OFLO at 200 mg/kg and was very similar to that in mice treated with AMIKA, which is the most active aminoglycoside against tubercle bacilli in the current experiment.

DISCUSSION

As invariably demonstrated by our previous experiments, after infection with a high inoculum ($\geq 10^6$ CFU per mouse) of virulent H37Rv, normal mice developed an acute and fatal infection from which all or the great majority of untreated control mice died within 4 weeks, whereas the survival rate up to 4 weeks after inoculation was high or almost 100% in mice that had been treated with regimens with various degrees of activity against tubercle bacilli (17, 19, 27). Therefore, survival rate is a simple and rapid indicator for identifying the regimens which may effectively prevent the deaths of mice from fatal tubercular infection. However, it is not sensitive enough to distinguish the in vivo activities of various effective regimens. In our earlier experiments, while INH displayed a very promising bactericidal effect as measured by CFU counts and OFLO at 300 mg/kg exhibited only a bacteriostatic or modest bactericidal effect (17, 19), the survival rate was always 100% in mice treated with either regimen (17, 19, 27). As shown in our current and previous studies, the mean spleen weight is a more sensitive indicator than the survival rate in distinguishing the weaker activities of treatment from the stronger ones: the mean spleen weight was always greater in mice treated with OFLO at 300 mg/kg than in mice treated with INH (17, 19); in addition, the weights were OFLO dose dependent (17). Nonetheless, splenomegaly is the outcome of very complicated tissue responses to the tubercular infection and does not exclusively represent the consequence of antimicrobial treatment; the inability to use the mean spleen weight in distinguishing the anti-*M. tuberculosis* activities of various aminoglycosides is a good example of its constraint in assessing the effectiveness of antimicrobial therapy. The results of the current experiment demonstrate that the enumeration of CFU remains the most sensitive indicator in measuring antimicrobial activity in experimental chemotherapy of tuberculosis.

For the treatment of tuberculosis, aminoglycosides have two major disadvantages: first, the parenteral administration causes operational problems in delivery of the treatment and also increases the potential risk in transmission of human immunodeficiency virus infection; and second, the potential toxicities, especially ototoxicity and nephrotoxicity, are dose related, and the total dose of treatment should not be given beyond a certain limit: e.g., 120 g for SM (24) and 25 g for KANA (25). It is, therefore, very unlikely that any of the available aminoglycosides will play an important role for the treatment of human tuberculosis in patients with organisms susceptible to the first-line drugs; however, they may be crucial for the treatment of multidrug-resistant tuberculosis patients because of very limited choice.

SM is the least toxic derivative of the aminoglycosides that have been used for clinical therapy (16). In the current experiment, 4 weeks of treatment with SM reduced the mean number of CFU by $10^{0.91}$ from the pretreatment value, indicating that SM displayed a moderate degree of bactericidal effect against *M. tuberculosis*, and confirmed the earlier observation that SM is considerably less effective than INH in the murine tuberculosis model (13). However, due to the widespread use of SM as monotherapy in the early days and the more recent inadequate treatment with SM-containing regimens, at present the resistance of tubercle bacilli to SM is rather common: among tuberculosis patients who had never been treated, 10% had isolates resistant to SM (8); the frequency of SM resistance is far higher among multidrug-resistant tuberculosis patients (9, 29) and could be as high as 80% (9). Therefore, a significant proportion of re-treated cases, especially those from the developing countries, may not benefit from treatment with SM. It is well known that no cross-resistance has been found between SM and KANA (1, 15), and the frequency of acquired resistance of *M. tuberculosis* to KANA is much less than that to SM (3). Nonetheless, the current experiment shows that the anti-*M. tuberculosis* effect of KANA is the weakest among those of the four tested aminoglycosides, and 4 weeks of treatment did not have significant bactericidal activity, which also explains why the combination of SPFX-PZA-KANA was less bactericidal than that of SPFX-PZA-SM during the initial phase of treatment in the mouse experiment (21). In addition, the adverse effects of KANA are more serious than those caused by SM (24, 25, 31). Thus, the relatively weak antimicrobial effect and the potential serious adverse effects do not favor the use of KANA for antituberculosis therapy. The current experiment is probably the first attempt to compare the in vivo activity against *M. tuberculosis* between ISEPA and other aminoglycosides at the same dosage. Because 4 weeks of treatment by ISEPA reduced the mean number of CFU by $10^{0.84}$ from the pretreatment level, its bactericidal activity was very similar to that of SM but less effective than that of AMIKA; the pharmacokinetic properties of ISEPA were also very similar to those of other aminoglycosides (2); in the phase III clinical trial, which covered a wide range of infections, the efficacy (5) and safety profile (4) of ISEPA did not differ significantly from those of AMIKA; thus, ISEPA has no particular advantage

over other aminoglycosides for the treatment of human tuberculosis. AMIKA is a semisynthetic derivative of KANA, but its bactericidal effect against *M. tuberculosis* is the strongest among those of the four tested aminoglycosides; 4 weeks of treatment reduced the mean number of CFU by $10^{1.28}$ from the pretreatment value. Therefore, AMIKA seems to be the first choice of aminoglycoside for the treatment of multidrug-resistant tuberculosis cases, especially when the strains are suspected to be resistant to SM. Although the authors of the study of an earlier trial among multiple drug-resistant tuberculosis patients claimed that the activity of AMIKA in the treatment of human disease was very low (1), the sample size of the trial was small (only four patients), and the dosages were relatively low (mostly 500 mg daily); the facts that resistance to AMIKA and KANA emerged after treatment and that the numbers of bacilli were slightly reduced in smears and cultures in some of these patients indicate that AMIKA had certain activity. Because there is complete cross-resistance between AMIKA and KANA and probably incomplete cross-resistance between AMIKA and CAPREO (1), patients may not benefit from AMIKA if their isolates are resistant to KANA or CAPREO. Due to its expense and toxicity, AMIKA has not been used extensively for the treatment of human tuberculosis, and the experience of tolerance of the patients to AMIKA is very limited. The major concern in its clinical application is the greater potential of nephrotoxicity and ototoxicity (16, 31); its adverse effects must be monitored carefully during treatment.

In terms of the CFU counts, the bactericidal activities of fluoroquinolones varied widely: the greatest bactericidal effect was observed in mice treated with SPFX at 50 mg/kg, which was also significantly more bactericidal than any of the four aminoglycosides tested although less bactericidal than that of INH at 25 mg/kg; the activity was significantly less in mice that had been treated with LVFX at 200 mg/kg, as its mean CFU count was $10^{1.12}$ greater than that in mice treated with SPFX at 50 mg/kg; no bactericidal effects were observed in mice treated with LVFX at 100 or OFLO at 200 mg/kg. These results indicate that, at dosages equivalent to the clinically tolerated dosages, SPFX is the most bactericidal fluoroquinolone. Therefore, until a more active new fluoroquinolone is developed, future studies on experimental chemotherapy of tuberculosis should focus on SPFX. To develop an effective regimen for the treatment of multidrug-resistant tuberculosis, the bactericidal effects of various combinations consisting of SPFX at 50 mg/kg and AMIKA at 200 mg/kg should be tested in the murine tuberculosis model.

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