ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1993, p. 407–413
0066-4804/93/030407-07$02.00/0

Vol. 37, No. 3

Powerful Bactericidal Activity of Sparfloxacin (AT-4140) against *Mycobacterium tuberculosis* in Mice

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Received 21 September 1992/Accepted 14 December 1992

The bactericidal activities of various monotherapies and combined regimens were compared in mice at different stages after infection with *Mycobacterium tuberculosis*. These therapies mimicked the initial and continuation phases of chemotherapy for human tuberculosis. As monotherapy, the bactericidal activity of sparfloxacin (SPFX) was dose related; the activity of SPFX at 100 mg/kg of body weight was comparable to that of rifampin (RMP) and was significantly greater than those of isoniazid (INH), pyrazinamide (PZA), or ofloxacin (OFLO) during both the initial and continuation phases of chemotherapy. During the initial phase, the addition of SPFX did not enhance or diminish the activities of the combinations INH-RMP-PZA or RMP-PZA; the combinations SPFX-PZA-streptomycin (SM) and SPFX-PZA-kanamycin (KANA) displayed powerful bactericidal activity. Because the area under the plasma concentration-time curve of SPFX (100 mg/kg) in mice is similar to that of SPFX (400 mg) in humans, the promising bactericidal activity displayed by SPFX in mice might be achieved in humans by administration of the drug in a clinically tolerated dosage. In addition, the combinations SPFX-PZA-SM and SPFX-PZA-KANA may be useful for the treatment of multidrug-resistant tuberculosis.

Pulmonary tuberculosis can be effectively treated by short-course chemotherapy that consists of an initial 2-month phase of daily treatment with isoniazid (INH), rifampin (RMP), and pyrazinamide (PZA), with or without ethambutol (EMB) or streptomycin (SM); this is followed by a 4-month continuation phase of daily treatment with INH-RMP (13). However, *Mycobacterium tuberculosis* isolates that are resistant to multiple drugs, including INH and RMP, have become a serious problem in many parts of the world (6, 26). At present, only a limited number of alternative chemotherapeutic regimens are available, none of them is very effective, and the mortality from multidrug-resistant tuberculosis is high (26). Therefore, effective new antituberculosis drugs with bactericidal mechanisms different from those of the presently available agents are urgently needed.

Among the commercially available fluoroquinolones, only ofloxacin (OFLO) has displayed a modest degree of bactericidal activity against *M. tuberculosis* in mice (23, 24) and humans (20, 25). However, many new fluoroquinolones are developed every year (5). Sparfloxacin (SPFX, AT-4140) is a new derivative that exhibits extremely potent antimicrobial activity against a broad spectrum of both gram-negative and gram-positive microorganisms (19). Our preliminary experiments demonstrated that the MICs of SPFX for 50% and 90% of 18 clinical isolates of *M. tuberculosis* were, respectively, 0.25 and 0.5 μg/ml, 1 to 2 $\log_2$ dilutions less than those of ciprofloxacin and OFLO; on a weight-to-weight basis, SPFX was six- to eightfold more active than OFLO in preventing mortality and the development of gross lesions in mice infected with *M. tuberculosis* (16). Because of these very encouraging results, we studied the pharmacokinetics of SPFX in mice and compared the bactericidal activity of SPFX with those of OFLO and the standard antituberculosis agents during both the initial and the continuation phases of chemotherapy for tuberculosis in mice.

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MATERIALS AND METHODS

Mice. Female outbred Swiss mice (age, 28 days) were purchased from the Janvier Breeding Center, le Genest Saint-Isle, France.

Antimicrobial agents. The following compounds were generously provided by the indicated manufacturers: SPFX, Rhone D.P.C. Europe, Antony, France; INH, Roche, Neuilly, France; RMP and PZA, Merrel-Dow, Neuilly, France; OFLO, Roussel Uclaf, Romainville, France; SM, Diamant, Paris, France; and kanamycin (KANA), Bristol, Puteaux, France. All of the agents except SM and KANA were suspended in 0.05% agar in distilled water at the desired concentrations; the suspensions were prepared weekly and were stored at 4°C.

Pharmacokinetic studies of SPFX in mice. Mice that had been fasted for 12 h were administered a single dose of SPFX (100 mg/kg of body weight) through an esophageal cannula. Before drug administration and at 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12, 16, 24, and 48 h after drug administration, three mice were bled and the plasma samples were pooled. The experiments were repeated in triplicate, and the concentration of SPFX in plasma was determined by a high-pressure liquid chromatographic method (21). The peak level of drug in plasma, the time to peak level of drug in plasma, the terminal half-life, and the area under the concentration-time curve were calculated as described previously (12).

M. tuberculosis. The virulent H37Rv strain of *M. tuberculosis* was grown on Löwenstein-Jensen (L-J) medium. Colonies were subcultured in Dubos broth (Diagnostics Pasteur, Paris, France) for 7 days at 37°C, and the turbidity of the resulting suspension was adjusted with distilled water to match that of a standard suspension of *Mycobacterium bovis* BCG (1 mg/ml). For inoculation of mice, the suspension was

further diluted 500-fold in experiment I or 5-fold in experiment II. The MICs for strain H37Rv were 0.1 μg/ml for INH, 0.5 μg/ml for RMP, 0.25 μg/ml for SPFX, all in 7H11 agar, and 10 μg/ml for PZA in L-J medium (pH 5.5).

Inoculation of mice. Two hundred twenty mice were inoculated for experiment I, and 170 mice were inoculated for experiment II; each mouse was inoculated intravenously with 0.5 ml of a bacterial suspension containing $10^{4.40}$ CFU of *M. tuberculosis* H37Rv in experiment I and $10^{6.65}$ CFU in experiment II.

Chemotherapy. At 14 days after inoculation, 10 mice were sacrificed and the CFU in the individual spleens of mice of experiment I or in the individual spleens and lungs of mice of experiment II were enumerated. At that time, the remaining mice were allocated randomly to one control group and six treatment groups, and treatment was initiated. Drugs were administered six times weekly through an esophageal cannula, but SM and KANA were injected subcutaneously. The dosages were selected to provide concentrations in serum or areas under the concentration time curve comparable to those achievable in humans, as follows: INH, 25 mg/kg (10); RMP, 10 mg/kg (10); PZA, 150 mg/kg (9, 10); OFLO, 300 mg/kg (23); SPFX, 50 (1, 7) or 100 mg/kg; SM and KANA, 200 mg/kg (10).

In experiment I, groups of 30 mice were administered INH, RMP, PZA, OFLO, or SPFX (50 or 100 mg/kg) for 8 weeks, to mimic the initial phase of chemotherapy of human tuberculosis. Ten mice in each group were sacrificed for enumeration of the CFU in the spleens at 2, 4, and 8 weeks after beginning treatment.

To mimic both the initial and continuation phases of chemotherapy of human tuberculosis, the mice in experiment II were treated for a total of 24 weeks. At the beginning of the treatments, 10 mice were maintained as untreated controls; a group of 110 mice was initially administered the combination INH-RMP-PZA; and five groups, each of 10 mice, were administered one of the following combinations: INH-RMP-PZA-SPFX, RMP-PZA, RMP-PZA-SPFX, SPFX-PZA-SM, or SPFX-PZA-KANA. After 8 weeks, 10 mice of the group that was administered INH-RMP-PZA and all of the surviving mice from all other groups were sacrificed for enumeration of the CFU in spleens and lungs. The remaining 100 mice that were administered INH-RMP-PZA were allocated randomly to five subgroups, each of 20 mice; one group was maintained without further treatment, and the mice in the remaining groups were administered INH, RMP, PZA, or SPFX (100 mg/kg), respectively, six times weekly for an additional 16 weeks. Ten mice in each subgroup were sacrificed at 8 and 16 weeks after beginning the continuation phase of chemotherapy for enumeration of the CFU in the spleens and lungs of individual mice.

Enumeration of CFU. The organs were removed aseptically and homogenized by a standard procedure (14), and the suspensions were made up to 5 ml for each organ suspension. To enumerate the CFU in the organs of mice in experiment I, the mice sacrificed after the initial phase of experiment II, and the untreated controls and PZA-treated mice sacrificed during the continuation phase of experiment II, appropriate dilutions of the suspensions were plated onto L-J medium. To enumerate the CFU in the organs of all of the remaining mice that were treated during the continuation phase in experiment II, the entire volume of the suspension of the individual organs was plated without further dilution onto 10 to 15 tubes of L-J medium. The results of the cultures were recorded after incubation at 37°C for 6 weeks. A culture was considered to be positive if any number of colonies was detected. The numbers of CFU were calculated from the average number of colonies per tube of L-J medium, the dilution of the suspension, and the volume of suspension that was plated or, in the cases in which the entire undiluted suspension was plated, from the total number of colonies observed.

Drug susceptibility of the organisms isolated from treated mice at the end of the continuation phase. To determine whether the organisms isolated from the organs of the treated mice at the end of the continuation phase were drug-resistant mutants that had been selected in the course of chemotherapy, the susceptibilities of the isolated organisms to the drug that had been administered as monotherapy during the continuation phase were tested by the proportion method (3). Colonies were collected from L-J medium, and the organisms were dispersed by shaking with glass beads. The turbidity of each resulting suspension was adjusted with distilled water to that of a suspension of BCG (1 mg/ml) and was further diluted to 10^{-1}, 10^{-3}, and 10^{-5}. To test the organisms' susceptibilities to INH and RMP, 0.2 ml of each dilution was plated onto the surface of two slants of drug-free L-J medium and two slants of L-J medium containing the appropriate drug. To test susceptibility to SPFX, 7H11 agar was used in place of L-J medium, because the latter has not been used for this purpose. To test susceptibility to PZA, two sets of L-J slants were used: one of standard L-J medium (pH 6.8) and one of L-J medium that was acidified to pH 5.5. The final concentrations of drugs for testing were 0.2 μg/ml for INH (3), 40 μg/ml for RMP (3), 200 μg/ml for PZA, and 1 μg/ml for SPFX. Results were recorded after incubation at 37°C for 6 weeks. The isolate was considered susceptible if the number of colonies on medium containing INH, RMP, or SPFX was less than 1% of that on drug-free medium or if the number of colonies on PZA-containing medium was less than 10% of that on drug-free medium (3, 4).

Statistical analysis. Results were analyzed by the Student *t* test and Fisher's exact probability calculation; differences were considered significant at the 95% level of confidence.

RESULTS

Pharmacokinetic studies of single-dose SPFX (100 mg/kg) in mice. No SPFX could be detected in plasma obtained 24 or 48 h after drug administration, indicating that the concentrations were below the limit of detectability, i.e., <0.05 μg/ml. The major pharmacokinetic parameters of SPFX in mice calculated from these data, together with the results obtained from mice and humans by other investigators (1, 17, 18), are listed in Table 1.

Activities of SPFX and other drugs administered as monotherapy in experiment I and during the continuation phase in experiment II. (i) Mortality, body and spleen weights, and gross pulmonary lesions. During the monotherapy phases, no death resulted from *M. tuberculosis* infection, but six mice in experiment I died by accident during daily gavage.

In experiment I, after infection with 0.001 mg of *M. tuberculosis*, the mean body weight of the mice increased progressively during the course of the experiment and did not differ significantly between the control and the treated groups. Whereas the mean spleen weight increased progressively in control mice, it decreased gradually in treated mice and eventually became significantly less than the pretreatment value ($P < 0.01$) except among those mice treated with OFLO (Table 2). After 8 weeks of treatment, the mean spleen weight of mice treated with SPFX (100 mg/kg) was

TABLE 1. Comparative major pharmacokinetic parameters in mice and humans after administration of a single dose of SPFX[a]

Test animal and dosage (reference)	C_{max} (μg/ml)	T_{max} (h)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (mg · h/liter)
Mice				
5 mg/kg (18)	0.25	0.3	5.0	0.74
50 mg/kg (1)	2.80	0.75	5.0	15.18
100 mg/kg	8.54	0.75	5.5	47.94
Humans				
200 mg (17)	0.70	4	20.8	18.75
400 mg (17)	1.18	5	18.2	32.73

[a] C_{max}, peak level of drug in plasma; T_{max}, time to peak level of drug in plasma; $t_{1/2}$, terminal half-life; $AUC_{0-\infty}$, area under the concentration-time curve from time zero to infinity.

TABLE 3. Number of CFU of *M. tuberculosis* in the spleens of mice treated with various monotherapies during the initial phase in experiment I

Treatment[a]	Mean ± SD no. of CFU (log_{10}) at the following times (wk) during treatment[b]:			
	0 (wk 2)	2 (wk 4)	4 (wk 6)	8 (wk 10)
Untreated control	6.33 ± 0.18	5.70 ± 0.34	6.03 ± 0.33	6.13 ± 0.46
INH, 25 mg/kg		4.57 ± 0.28	4.11 ± 0.21	3.47 ± 0.29
RMP, 10 mg/kg		5.29 ± 0.17	4.12 ± 0.36	2.42 ± 0.55
PZA, 150 mg/kg		5.20 ± 0.31	4.43 ± 0.36	4.17 ± 0.70
OFLO, 300 mg/kg		5.70 ± 0.26	5.38 ± 0.22	5.31 ± 0.53
SPFX, 50 mg/kg		5.35 ± 0.25	5.14 ± 0.21	3.65 ± 0.54
SPFX, 100 mg/kg		5.15 ± 0.20	4.25 ± 0.13	2.90 ± 0.58

[a] Mice were treated from 14 days after inoculation with *M. tuberculosis*. Drugs were administered by gavage six times weekly.

[b] The results at each time point represents the mean number of CFUs in the spleens of 9 to 10 mice. The numbers in parentheses are weeks after inoculation.

significantly less than that of mice treated with RMP, OFLO, or SPFX (50 mg/kg) ($P < 0.05$ or <0.01). The severity of the gross pulmonary lesions was scored from 0 to 2+ (23). All control mice, including those sacrificed 2 weeks after inoculation, demonstrated pulmonary lesions of 2+ severity, whereas virtually no pulmonary lesions were observed among the mice that were sacrificed after treatment with INH, RMP, and SPFX (100 mg/kg) for 4 weeks or longer. All of the mice to which OFLO was administered demonstrated pulmonary lesions of 1+ severity. Those animals treated with PZA or SPFX (50 mg/kg) demonstrated lesions of 1+ severity 2 or 4 weeks after beginning treatment; however, between one-third and one-half of these mice demonstrated only very tiny lesions (± severity) after treatment for 8 weeks.

At the beginning of the continuation phase of experiment II, among the mice that were inoculated with 0.1 mg of *M. tuberculosis* and treated with the combination INH-RMP-PZA for the initial 8 weeks, the spleens were slightly enlarged (Table 2) and no pulmonary lesions were observed. The mean body weight of all of the mice gradually increased during the 16 weeks of the continuation phase, with no significant difference observed between untreated controls and treated mice. The mean spleen weight increased progressively among the control mice and was significantly greater ($P < 0.01$) than those among the treated mice at the end of continuation phase (Table 2); by that time, the mean spleen weights of all treated mice were not significantly different from those at the beginning of the continuation phase. All control mice sacrificed at 8 and 16 weeks of the continuation phase showed pulmonary lesions of 2+ severity, whereas only very tiny lesions (± severity) were observed among all PZA-treated mice and among a minority of mice treated with other drugs.

(ii) Enumeration of CFUs in spleens of mice in experiment I. At the time that drug administration was begun, the enumeration of the mean number of CFU per spleen indicated that the *M. tuberculosis* isolates multiplied during the 2 weeks after inoculation. As shown in Table 3, there was no further multiplication of isolates among control mice. In fact, because of the natural killing, the mean number of CFU decreased significantly between 2 and 4 weeks after inoculation ($P < 0.01$), returning to the pretreatment level only 10 weeks after inoculation. In the course of treatment, the mean number of CFU decreased in all treated mice; except for the mice administered OFLO for 2 weeks, the mean number of CFU of all treated mice was significantly less than those of the corresponding control mice ($P < 0.05$ or <0.01). The CFU declined continuously among the mice treated with INH, RMP, or SPFX (100 mg/kg) ($P < 0.01$) and decreased significantly between 2 and 4 weeks after treatment with OFLO ($P < 0.05$) or PZA ($P < 0.01$) and between 4 and 8 weeks after treatment with SPFX (50 mg/kg) ($P < 0.01$). By adjusting the differences of the mean number of CFU between control and treated mice by the natural decline among control mice, INH appeared to be the most effective bactericidal agent early during the course of treatment; RMP and SPFX (100 mg/kg) were as bactericidal as INH after 4 weeks

TABLE 2. Spleen weights of mice during experiment I and the continuation phase of experiment II

Treatment[a]	Mean ± SD spleen wt (mg) in the indicated experiments and time (wk) during treatment[b]:						
	Experiment I				Experiment II		
	0	2	4	8	0	8	16
Untreated control	283 ± 63	331 ± 46	384 ± 86	436 ± 73	218 ± 47	278 ± 73	380 ± 96
INH, 25 mg/kg		193 ± 32	225 ± 42	175 ± 28		307 ± 85	264 ± 77
RMP, 10 mg/kg		230 ± 54	210 ± 42	203 ± 36		285 ± 32	207 ± 32
PZA, 150 mg/kg		275 ± 60	223 ± 50	183 ± 36		245 ± 19	228 ± 21
OFLO, 300 mg/kg		271 ± 30	274 ± 57	245 ± 55			
SPFX, 50 mg/kg		268 ± 49	275 ± 53	199 ± 35			
SPFX, 100 mg/kg		283 ± 72	211 ± 45	171 ± 22		230 ± 54	219 ± 53

[a] In experiment I, treatments were begun at 14 days (week 0) after inoculation; in experiment II, week 0 of the continuation phase of chemotherapy refers to the results at the end of the initial 8 weeks of treatment with the combination INH-RMP-PZA. Drugs were administered by gavage six times weekly.

[b] The mean value from 9 or 10 mice per group at each sacrifice.

TABLE 4. Positive cultures of organs from mice during the continuation phase of experiment II[a]

Treatment[b]	% Positive culture at wk[c]:					
	0		8		16	
	Spleen	Lung	Spleen	Lung	Spleen	Lung
Untreated control	100	100	100	100	100	100
INH, 25 mg/kg			100	90	90	40
RMP, 10 mg/kg			60	20	20	20
PZA, 150 mg/kg			100	100	100	100
SPFX, 100 mg/kg			100	80	60	10

[a] A culture was considered positive if any number of colonies was detected after incubation at 37°C for 6 weeks. Organs from 10 mice were tested.
[b] All mice were treated initially 8 weeks with the combination of INH-RMP-PZA six times weekly. During the continuation phase, drugs were administered by gavage six times weekly for 16 weeks.
[c] Times are weeks during the continuation phase.

of treatment and were more actively bactericidal than INH by the end of 8 weeks of treatment ($P < 0.01$). After 8 weeks of treatment with either RMP or SPFX (100 mg/kg), fewer than 10^3 of the *M. tuberculosis* isolates were found to have survived and no significant difference between the bactericidal activities of these two drugs at any interval could be discerned. Beginning at 4 weeks after the start of treatment, SPFX (100 mg/kg) demonstrated more potent bactericidal activity than SPFX (50 mg/kg) ($P < 0.01$). PZA was less active than INH, RMP, and SPFX (100 mg/kg) and displayed activity similar to that of SPFX (50 mg/kg). OFLO demonstrated the weakest bactericidal activity of all of the drugs tested.

(iii) Enumeration of CFU in spleens and lungs during the continuation phase of experiment II. All of the organs were culture positive at the beginning of the continuation phase and remained so among untreated control mice and mice treated with PZA up to the end of the continuation phase (Table 4). After treatment for 8 weeks, the frequency of culture-positive organs did not differ significantly among the groups, with the exception that the frequency of positive cultures among the RMP-treated mice was lower than that among mice from other groups ($P < 0.05$). At the end of the continuation phase, the frequency of positive cultures among animals treated with SPFX was lower than that among the control mice and those administered PZA ($P < 0.05$), but did not differ significantly from that among the mice treated with INH or RMP. At the end of the continuation phase, all the organisms isolated from treated mice remained susceptible to the drug that was administered as monotherapy, with the exception that the organisms from one mouse each were resistant to INH or RMP.

At the beginning of the continuation phase of therapy, the mean numbers of CFU were $10^{3.12 \pm 0.46}$ in the spleens and $10^{2.59 \pm 0.29}$ in the lungs. During the course of the continuation phase, the mean numbers of CFU per spleen and lung increased progressively among the control mice and were always significantly greater ($P < 0.01$) than those among treated animals (Fig. 1). At both 8 and 16 weeks of treatment, the mean numbers of CFU were significantly greater among the mice treated with PZA than among the mice treated with the remaining drugs ($P < 0.01$); among the PZA-treated mice, the mean number of CFU per spleen decreased slightly during the first 8 weeks of treatment ($P < 0.05$) and remained unchanged thereafter; the mean number of CFU per lung increased significantly between 8 and 16 weeks of treatment ($P = 0.01$), reaching a level greater than the pretreatment value ($P < 0.01$). Among the mice treated with the remaining drugs, the mean numbers of CFU per organ declined dramatically ($P < 0.01$) during the first 8 weeks of continuation therapy; the mean numbers of CFU in the organs of the mice treated with RMP were significantly less than those in the organs of all of the other treated mice ($P < 0.01$), whereas the values in the mice treated with INH or SPFX were very similar. At the end of the continuation phase, the mean number of CFU in RMP-treated mice remained unchanged, whereas those per spleen of the INH- and SPFX-treated mice declined further to the levels observed among the mice administered RMP; at that time, the

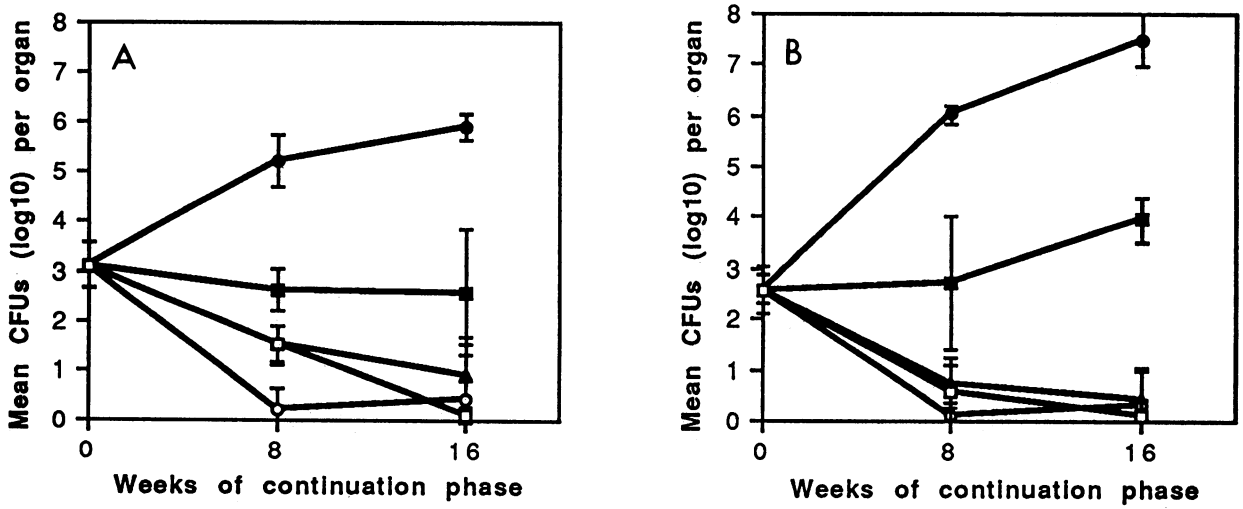

FIG. 1. Spleen (A) and lung (B) cultures during the continuation phase of chemotherapy in experiment II. Mice were inoculated intravenously with 0.1 mg of *M. tuberculosis* H37Rv and were initially treated with INH-RMP-PZA for 8 weeks. Then the mice were randomly allocated to an untreated control group (●) or were treated with INH (25 mg/kg) (▲), RMP (10 mg/kg) (○), PZA (150 mg/kg) (■), or SPFX (100 mg/kg) (□) six times a week for 16 weeks. Each point represents the mean number of CFU for 10 mice. Error bars represent standard deviations.

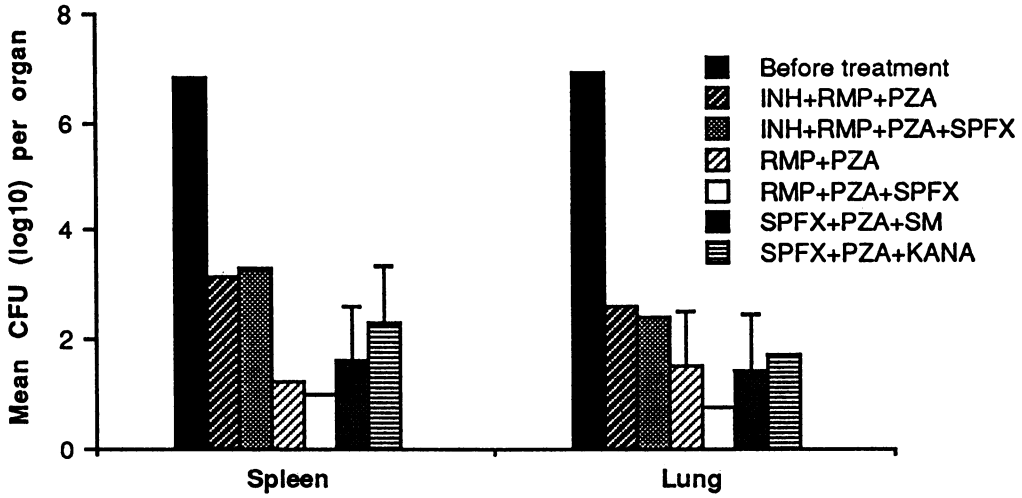

FIG. 2. Enumeration of CFU in spleens and lungs during the initial phase of chemotherapy in experiment II. Mice were inoculated intravenously with 0.1 mg of *M. tuberculosis* H37Rv, and treatments were begun 14 days after inoculation. The following drugs were administered six times weekly for 8 weeks at the indicated dosages: INH, 25 mg/kg; RMP, 10 mg/kg; PZA, 150 mg/kg; SPFX, 100 mg/kg by gavage; and SM and KANA, 200 mg/kg each by subcutaneous injection. Each bar represents the mean number of CFU for 10 mice; error bars represent standard deviations of $\geq 10^{0.5}$.

mean number of CFU per spleen among the SPFX-treated mice was significantly less than that among the INH-treated mice ($P < 0.01$).

Activities of the combinations of drugs administered during the initial phase of experiment II. (i) Mortality. As expected (23), all 10 untreated control mice died within 10 weeks (range, 27 to 65 days) after the inoculation of 0.1 mg of *M. tuberculosis* H37Rv, whereas no mortality was observed among the treated mice during the 8 weeks of the initial phase, a highly significant difference ($P < 0.01$).

(ii) Enumeration of CFUs in spleens and lungs. By 14 days after inoculation, the mean number of CFU per spleen or lung was close to 10^7. As shown in Fig. 2, after 8 weeks of treatment the CFU in all treated groups was significantly less than the pretreatment values ($P < 0.01$), indicating that all of the drug combinations possessed various degrees of bactericidal activity. The mean numbers of CFU per spleen and lung were significantly greater among the mice administered INH-RMP-PZA than among those administered RMP-PZA ($P < 0.01$ in spleens, $P < 0.05$ in lungs) and are consistent with the antagonism previously demonstrated (12) between INH and the combination RMP-PZA. No significant differences were observed between the mean numbers of CFU among the mice treated with INH-RMP-PZA and the corresponding values derived from the mice treated with INH-RMP-PZA-SPFX, indicating that the addition of SPFX did not overcome the antagonism between INH and RMP-PZA. The mean numbers of CFU appeared lowest among the mice administered RMP-PZA-SPFX, but these values were not significantly less than the corresponding values derived from the mice administered RMP-PZA, indicating that SPFX neither enhanced nor diminished the activity of the combination RMP-PZA. The combination SPFX-PZA-SM displayed very powerful bactericidal activity: fewer than 10^{-5} of the *M. tuberculosis* isolates survived 8 weeks of treatment, results that were only slightly inferior to those observed among mice administered RMP-PZA-SPFX ($P < 0.05$) but that did not differ significantly from those observed among the mice treated with RMP-PZA. The combination SPFX-PZA-KANA, which was less actively bactericidal than the combination SPFX-PZA-SM, RMP-PZA-SPFX, or RMP-PZA ($P < 0.05$), nevertheless reduced the mean numbers of CFU in the spleens and lungs to $10^{-4.5}$ and $10^{-5.2}$ of the corresponding pretreatment values, respectively.

Comparison of spleen and lung cultures in experiment II. Altogether, the results of 170 pairs of cultures were available for analysis: 70 pairs from mice sacrificed during the initial phase and 100 pairs from mice sacrificed during the continuation phase. Negative cultures and discrepancies between the results of spleen and lung cultures were observed only among the 60 mice sacrificed after treatment with INH, RMP, and SPFX during the continuation phase; 43 (71.7%) of the spleen cultures and 26 (43.3%) of the lung cultures were positive; positive spleen cultures and negative lung cultures were observed among 18 pairs, whereas negative spleen cultures and positive lung cultures were observed in only 1 pair. The frequency of positive spleen cultures was significantly greater than that of positive lung cultures ($P < 0.05$). This difference in the frequency of positive cultures was most apparent after 16 weeks of treatment with INH or SPFX (Table 4).

The mean numbers of CFU per spleen were similar to those per lung in 9 (52.9%) of 17 sets of results; each set was based on measurements from 10 mice. The mean numbers of CFU per spleen were significantly greater than those per lung in five (29.4%) sets of results (after initial treatment with INH-RMP-PZA, INH-RMP-PZA-SPFX, and SPFX-PZA-KANA and after 8 weeks of continuation treatment with INH and SPFX), whereas the mean numbers of CFU per lung were significantly greater in three (17.7%) sets of results (untreated control mice after 8 and 16 weeks of the continuation phase and PZA-treated mice after 16 weeks of the continuation phase).

DISCUSSION

The bactericidal activity of SPFX, administered six times weekly at a dosage of 100 mg/kg, was less than that of INH early during the initial phase and was also less than that of RMP early during the continuation phase. On the other

hand, the activity of SPFX was found to be greater than that of INH and similar to that of RMP at the end of the initial and continuation phase and was also significantly greater than that of PZA. Thus, SPFX displayed very powerful bactericidal activity against *M. tuberculosis* in mice during both the initial and the continuation phases of chemotherapy and therefore represents the first potentially important antituberculosis agent in many years since the introduction of RMP.

In the present study, because SPFX (100 mg/kg) displayed greater bactericidal activity against *M. tuberculosis* than SPFX (50 mg/kg), the bactericidal activity of SPFX appeared to be dose related. In terms of the area under the concentration-time curve (Table 1), SPFX administered to mice at a dosage of 100 mg/kg appears to be the equivalent of a 400-mg dose in humans. Therefore, the promising bactericidal activity of SPFX (100 mg/kg) in mice might be achieved in humans by the administration of 400 mg daily. SPFX at 300 mg once daily has been proved to be safe in multicenter double-blind trials (15, 22); it is likely that the 400-mg daily dose may also be well tolerated, although more information about the tolerance to daily treatment with this dose should be accumulated in human trials. Because there is no cross-resistance between INH or RMP and the fluoroquinolones (23), SPFX should also be effective in the treatment of patients whose *M. tuberculosis* isolates are resistant to multiple drugs other than fluoroquinolones.

Although the addition of SPFX did not significantly enhance the activities of the combinations INH-RMP-PZA or RMP-PZA during the initial phase of chemotherapy, neither was antagonism observed. Even more important is the observation that the combinations SPFX-PZA-SM and SPFX-PZA-KANA displayed considerable bactericidal activity. Therefore, these combinations may be useful for the treatment of patients whose *M. tuberculosis* isolates are resistant to both INH and RMP. Because of the widespread use of SM as monotherapy in the 1950s and the more recent inadequate treatment with SM-containing regimens in many countries in which tuberculosis is endemic, a significant proportion of resistant strains of *M. tuberculosis* are also resistant to SM. For example, a recent survey of secondary resistance in France demonstrated that among the strains which were resistant to at least one drug, about 80% were resistant to INH, 50% were resistant to RMP, and 50% were resistant to SM (11). On the other hand, KANA has not been widely used for the treatment of tuberculosis, and the frequency of resistance to KANA is much less than that to SM (2). Therefore, SPFX-PZA-KANA may be an effective alternative regimen for the treatment of patients whose *M. tuberculosis* isolates are resistant to multiple drugs including SM but still susceptible to KANA. Further studies of the activities of the combinations SPFX-PZA-SM or SPFX-PZA-KANA during the continuation phase and the bioavailability of SPFX in these combinations are indicated.

Because PZA alone displayed significant bactericidal activity against *M. tuberculosis* only during the first 4 weeks of treatment (Table 3 and Fig. 1), it appears to be an important component of the combined regimens during the initial phase of chemotherapy but may not be very useful during the continuation phase. Therefore, at least one, and preferably two, new antituberculosis agents are required in addition to SPFX, and the screening of new antimicrobial agents for their activities against *M. tuberculosis* should be intensified.

To assess chemotherapeutic effects in experimental murine tuberculosis, the numbers of CFU were enumerated in spleens (12, 23) or lungs (10), or both (8). To identify the organ which facilitates the most efficient assessment of the bactericidal activity, we compared the results of spleen and lung cultures performed in the course of experiment II. Our results suggest a small advantage in favor of the spleen, although the overall results of the culture of spleens and lungs were similar. It appears, therefore, that either organ is appropriate for the assessment of bactericidal activity and that there is no need to enumerate the CFU in both organs.

ACKNOWLEDGMENTS

This study was supported by the Tuberculosis Unit of the World Health Organization, Geneva, Switzerland.

We are grateful to Rhone D.P.C. Europe for the supply of SPFX and to Eric Gregoire of Pitié-Salpêtrière for his excellent technical assistance.

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