Weekly Moxifloxacin and Rifapentine Is More Active Than the Denver Regimen in Murine Tuberculosis

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Rationale: Treatment of tuberculosis with an efficacious once-weekly regimen would be a significant achievement in improving patient adherence. Currently, the only recommended once-weekly continuation phase regimen of isoniazid plus rifapentine (10 mg/kg) is inferior to standard twice-weekly therapy with isoniazid plus rifampin and is, therefore, restricted to non-high-risk patients. The substitution of moxifloxacin, a new 8-methoxyfluoroquinolone, for isoniazid and an increase in the dose of rifapentine could augment the activity of once-weekly regimens.

Methods: To test this hypothesis we evaluated the sterilizing activity of improved once-weekly rifapentine-based continuation phase regimens in a murine model that mimics the treatment of high-risk patients with tuberculosis. The bactericidal activity of standard daily therapy and standard intermittent therapy ("Denver" regimen) was also assessed to evaluate the effect of intermittent drug administration during the initial phase of therapy.

Results: After 2 mo of treatment, lung colony-forming unit counts were 1 log₁₀ lower in mice treated with standard daily therapy than with the Denver regimen. During the continuation phase, the sterilizing activity of once-weekly moxifloxacin plus rifapentine (15 mg/kg) was significantly greater than that of the predominantly twice-weekly Denver regimen of isoniazid plus rifampin. No significant difference in sterilizing activity was detected between once-weekly isoniazid plus rifapentine (15 mg/kg) and the Denver regimen.

Conclusions: These results suggest that the efficacy of the once-weekly isoniazid plus rifapentine continuation phase regimen can be increased by substituting moxifloxacin for isoniazid and by increasing the dose of rifapentine to a clinically acceptable level of 15 mg/kg.

Keywords: antibiotics; intermittent therapy; mouse; treatment

Effective control of tuberculosis (TB), especially in the setting of the HIV pandemic, requires the use of long and cumbersome chemotherapeutic regimens (1). Despite implementation of the World Health Organization directly observed therapy short-course strategy, nonadherence remains a serious threat to effective control of TB (2). Effective regimens employing drugs that could be administered once weekly (1 of 7 d) would be expected to facilitate supervised therapy by reducing the number of doses requiring supervision (3), leading to improved completion rates

and raising prospects for expansion of DOTS coverage throughout the developing world.

Rifapentine (RPT), a long-lasting rifamycin, is currently recommended for use at a dose of 10 mg/kg in combination with isoniazid (INH) during once-weekly continuation phase therapy (4). However, treatment restrictions limit the use of INH-RPT to non-high-risk patients (HIV seronegative with noncavitary TB) and are based on higher rates of treatment failure or relapse with rifamycin-monoresistant bacilli in high-risk patients (3, 5). Moreover, once-weekly INH-RPT has been shown to be less active than three times- or twice-weekly (3 of 7 or 2 of 7 d) therapy with rifampin (RIF) and INH (5–7). It has been suggested that the high protein binding (97%) of RPT may be partially responsible for the suboptimal activity observed in once-weekly regimens (8, 9). Improvement of RPT-based regimens might be made by increasing the dose of RPT from 10 to 15 mg/kg, to increase the effective concentration of active drug (10). The 15-mg/kg dose of RPT has been well tolerated in humans (11) and increases in the dose of RPT from 10 to 15 mg/kg have demonstrated enhanced sterilizing activity in the mouse model (12). Furthermore, in an early bactericidal activity study of the rifamycins it has been suggested that the most effective dose of RPT may lie between 15 and 20 mg/kg (13). Additional improvement of RPT-based regimens might come from incorporating moxifloxacin (MXF), a new fluoroquinolone with potent bactericidal activity against *Mycobacterium tuberculosis* and a half-life close to that of RPT (14–16). It has been demonstrated in the murine model that inclusion of MXF during the initial and continuation phases of a largely once-weekly INH-RPT_{10 mg/kg} regimen significantly improves sterilizing activity and prevents the selection of rifamycin monoresistance (14). However, it is unknown whether the addition of MXF to INH-RPT or substitution of MXF for INH would confer the greatest increase in sterilizing activity.

In the current experiment, pharmacokinetic studies were conducted to determine the most clinically relevant dosing scheme of MXF and dose of RPT in the mouse necessary for extrapolation to humans. After determination of equipotent doses between mouse and human, we studied the potential of RPT_{15 mg/kg} and/or MXF in combinations to improve once-weekly therapy in a murine model with a high burden of infection to better mimic the treatment of high-risk patients. All test regimens were preceded with a 2-mo initial phase of the standard intermittent Denver regimen that included 2 wk of daily (5 d/wk or 5 of 7 d) RIF-INH-PZA (pyrazinamide) followed by 6 wk of twice-weekly (2 of 7 d) RIF-INH-PZA (17). Primary end points were reduction in lung colony-forming unit counts after 2 mo of treatment and proportion of mice with positive cultures 3 mo after discontinuation of 4, 5, and 6 mo of treatment (Table 1).

Portions of the results of this experiment have been previously reported in abstract form (18).

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TABLE 1. SCHEME OF EXPERIMENT

Treatment Group*	Number of Mice Killed on:					
	Day -18	Day 0	Month 2	Month 4 (+3) [‡]	Month 5 (+3)	Month 6 (+3)
Control						
Infected, untreated	8	8				
2-mo RIF-INH-PZA (5/7) plus RIF-INH (5/7)			10	15	15	24
6-mo Denver regimen [†]			10	15	15	24
Test regimens						
2-mo Denver plus RPT (1/7)				15	15	24
2-mo Denver plus INH-RPT (1/7)				15	15	24
2-mo Denver plus MXF-RPT (1/7)				15	15	24
2-mo Denver plus MXF-INH-RPT (1/7)				15	15	24

Definition of abbreviations: INH = isoniazid; MXF = moxifloxacin; PZA = pyrazinamide; RIF = rifampin; RPT = rifapentine.

* Drugs were given orally at the following doses (in mg/kg): INH, 25 and 75 when administered daily (5/7) and once or twice weekly (1/7 and 2/7), respectively; RIF, 10; PZA, 150 and 300 when administered 5/7 and 2/7, respectively; MXF, 100 twice per day once weekly; RPT, 15.

[†] The Denver regimen is defined as 2 wk of RIF-INH-PZA (5/7) plus 6 wk of RIF-INH-PZA (2/7) plus 4 mo of RIF-INH (2/7). The 2-mo Denver refers to the first 2 mo of the 6-mo Denver regimen.

[‡] Months 4 (+3), 5 (+3), and 6 (+3) indicate that mice were killed 3 mo after completing 4, 5, and 6 mo of treatment (i.e., 2-mo RIF-INH-PZA or Denver followed by 2, 3, and 4 mo of control or test continuation phases, respectively).

METHODS

Antimicrobials

Drugs were obtained and stock solutions were prepared as previously described (16). RPT tablets, donated by Sanofi-Aventis (Kansas City, MO), were ground into a powder and suspended in water before gavage.

M. tuberculosis Strain

Strain H37Rv was passaged in mice to maintain its virulence and then subcultured in 10% oleic acid-albumin-dextrose-catalase (OADC)-enriched Middlebrook 7H9 broth (Fisher, Pittsburgh, PA). This culture was used for aerosol infection when the optical density at 600 nm was 0.73.

Aerosol Infection

Six-week-old female BALB/c mice were purchased from Charles River (Wilmington, MA) and infected as previously described (16). Mice were infected in four consecutive runs. The study protocol was approved by the institutional animal care and use committee.

Chemotherapy

After infection, mice were block randomized into four treatment groups (Table 1). The control groups received the standard daily regimen, 2 mo of RIF-INH-PZA (5 of 7 d) plus 4 mo of RIF-INH (5 of 7 d), or the standard intermittent Denver regimen, 2 wk of RIF-INH-PZA (5 of 7 d) plus 6 wk of RIF-INH-PZA (2 of 7 d) plus 4 mo of RIF-INH (2 of 7 d). Test regimens included an initial phase of 2 mo of the Denver regimen followed by 4 mo of once-weekly (1 of 7 d) RPT, INH-RPT, MXF-RPT, or MXF-INH-RPT. Eight infected, untreated mice were kept to assess mortality from infection.

Treatment began 19 d postinfection (Day 0). Drugs were administered by gavage either 5 of 7, 2 of 7, or 1 of 7 d at the following doses (mg/kg): INH, 25 and 75 when administered 5 of 7 and 1 of 7 or 2 of 7 d, respectively; RIF, 10; PZA, 150 and 300 when administered 5 of 7 and 2 of 7 d, respectively; MXF, 100 twice per day once weekly; RPT, 15.

Assessment of Treatment Efficacy

Two untreated mice from each of four aerosol runs were killed the day after infection and on Day 0 as pretreatment controls. Ten mice were killed after 2 mo of treatment with the standard daily regimen or the Denver regimen. After 4, 5, and 6 mo of therapy mice from each group went untreated for an additional 3 mo before being killed to assess the proportion of culture-positive mice. Colony-forming unit counts and body and spleen weights were determined as previously described (16). RIF susceptibility testing was performed by the agar proportion method.

Pharmacokinetic Analysis

Uninfected 6-wk-old female Swiss mice (Charles River) were administered a single dose of MXF alone at 100, 200, or 400 mg/kg or RPT alone at 10 or 15 mg/kg. RPT was also administered with INH-MXF to assess for pharmacokinetic interaction. At predetermined time points (0.25, 0.5, 1, 2, 4, and 10 h for MXF; and 4, 6, 8, 14, 24, and 48 h for RPT), three mice were exsanguinated. Serum was harvested and concentrations of MXF and RPT were determined by validated high-performance liquid chromatography. Serum concentration-time curves were generated with determination of relevant pharmacokinetic parameters (mean area under the serum concentration-time curve [AUC_{0-∞}] and peak serum concentration [C_{max}]; Stata version 8.2; StataCorp, College Station, TX).

Statistical Analysis

Colony-forming unit counts were log₁₀ transformed before analysis. Mean colony-forming unit counts were compared by one-way analysis of variance or the two-sample *t* test (Stata version 8.2). The proportions of mice relapsing were compared by Fisher exact test. The Bonferroni procedure was used to adjust the type I error rate for all multiple comparisons.

RESULTS

Pharmacokinetics of RPT and MXF

The main pharmacokinetic parameters of RPT and MXF in mice are shown in Table 2 in comparison with known corresponding parameters in humans (10, 19). After oral administration in mice, the mean ($\pm$ SD) peak serum concentrations (C_{max}) of RPT were 11.1 $\pm$ 0.39 and 16.7 $\pm$ 1.10 μ g/ml for 10 and 15 mg/kg, respectively. Likewise, the areas under the serum concentration-time curve (AUC_{0-∞}) for RPT were 309 $\pm$ 35.5 and 474 $\pm$ 5.80 μ g $\cdot$ h/ml for 10 and 15 mg/kg, respectively. Both C_{max} and AUC_{0-∞} compared favorably between mouse and human at 10 and 15 mg/kg. The mean C_{max} values for orally administered MXF were 14.18 $\pm$ 3.87, 20.04 $\pm$ 3.02, and 30.08 $\pm$ 0.90 μ g/ml for 100, 200, and 400 mg/kg, respectively. The corresponding AUC_{0-∞} values were 23.58 $\pm$ 9.51, 34.84 $\pm$ 7.27, and 59.23 $\pm$ 6.11 μ g $\cdot$ h/ml, respectively. As shown in Table 2, for MXF at 100 mg/kg administered twice daily in mice the estimated AUC_{0-∞} and C_{max} were 47.2 $\pm$ 9.51 μ g $\cdot$ h/ml and 14.18 $\pm$ 3.87 μ g/ml, best approximating the corresponding parameters in humans treated with a 400-mg dose.

RPT serum concentrations were not significantly different when administered alone or in combination with INH-MXF.

TABLE 2. PHARMACOKINETICS OF RIFAPENTINE AND MOXIFLOXACIN IN MOUSE AND HUMAN

Mouse			Human		
Regimen*	AUC _{0-∞} (μg · h/ml)	Cmax (μg/ml)	Regimen	AUC _{0-∞} (μg · h/ml)	Cmax (μg/ml)
RPT ₁₀ mg/kg	309 ± 35.5	11.1 ± 0.39	RPT ₁₀ mg/kg [§]	296	12.2
RPT ₁₅ mg/kg	474 ± 5.80	16.7 ± 1.10	RPT ₁₅ mg/kg [§]	410	14.6
MXF ₁₀₀ mg/kg 2× [†]	47.2 ± 9.51	14.18 ± 3.87	MXF _{6.6} mg/kg (400 mg)	45.5 ± 4.68	4.98 ± 1.01

Definition of abbreviations: 2× = twice daily; AUC_{0-∞} = mean area under the serum concentration–time curve (± SD); Cmax = mean peak serum concentration (± SD); MXF = moxifloxacin; RPT = rifapentine.

* Mice were administered a single oral dose of RPT at 10 or 15 mg/kg; mice also received a single oral dose of MXF at 100, 200, or 400 mg/kg.

[†] The AUC of 100 mg/kg was doubled in order to simulate a dosing scheme with twice-daily dosing.

[‡] Equipotency is achieved when the RPT or MXF AUCs of mouse and human are approximately equivalent.

[§] Data from Reference 10.

^{||} Data from Reference 19.

The AUC_{0-∞} values for RPT_{7.5} mg/kg alone and in combination with INH–MXF were 179.5 ± 20.99 and 177.6 ± 18.87 μg · h/ml, respectively (*p* = 0.91).

Body and Spleen Weights

On the day of infection, the mean body weight of mice was 17.18 ± 0.16 g. Nineteen days later when treatment began (Day 0), mice weighed 16.96 ± 0.52 g. After 1 wk of treatment, mice lost an average of 8.7% of their Day 0 body weight as a result of stress from gavage and burden of infection. No difference in body weight was observed in mice receiving the standard daily regimen or the Denver regimen at any time point. After 4, 5, and 6 mo of treatment no difference was detected in mean body weights between mice in any of the treatment groups. The mean body weights of all surviving mice at 4, 5, and 6 mo were 21.14 ± 0.35, 21.77 ± 0.43, and 21.89 ± 0.38 g, respectively.

At initiation of treatment (Day 0) the mean spleen weight was 150 ± 30.7 g. After 2 mo of treatment, the mean spleen weights of mice receiving the standard daily regimen and the Denver regimen were 119 ± 34.8 and 130 ± 40.8 g, respectively (*p* = 0.52). Spleen weights were not assessed at the remaining time points.

Organ Colony-forming Unit Counts before and during the Initial Phase of Treatment

The day after infection (Day –18), mean log₁₀ colony-forming unit counts in the lungs were 4.17 ± 0.04, 4.17 ± 0.03, 3.81 ± 0.01, and 4.10 ± 0.14 for mice infected during runs 1 through 4, respectively. On Day 0, mean log₁₀ colony-forming unit counts in the lungs were 8.90 ± 0.17, 8.50 ± 0.18, 7.95 ± 0.11, and 8.02 ± 0.21 for mice infected during runs 1 through 4, respectively. Mean log₁₀ colony-forming units of spleens were 4.75 ± 0.36. After the initial 2 mo of therapy, colony-forming unit counts in the lung were 4.91 ± 0.16 and 3.99 ± 0.06 in mice treated according to the Denver regimen and the standard daily regimen, respectively (Figure 1). Although both regimens demonstrated bactericidal activity, treatment with the standard daily regimen resulted in lung colony-forming unit counts that were 1 log₁₀ lower than those observed after treatment with the Denver regimen (*p* < 0.0001). In spleens, the mean log₁₀ counts were 1.51 ± 0.18 and 2.05 ± 0.30, a difference of 0.54 log₁₀ (*p* < 0.0001). Infected, untreated mice kept for mortality assessment were not cultured as all eight animals died by 4 wk postinfection.

Proportion of Mice with Positive Cultures 3 Mo after Treatment Completion

In mice that received the standard daily regimen the proportion of mice that were lung culture positive 3 mo after completing

4, 5, and 6 mo of treatment was 12 of 12 (100%), 9 of 15 (60%), and 6 of 24 (25%), respectively.

After 4 mo of therapy and 3 mo of follow-up, the proportion of mice that were lung culture positive was 100% in the Denver regimen and all RPT-containing groups.

After 5 mo of therapy and 3 mo of follow-up, the proportion of mice that were lung culture positive was 15 of 15 (100%) in the Denver regimen; in mice that received the Denver regimen for the initial 2-mo phase and once-weekly RPT-containing therapy during the continuation phase, the proportion of mice that was lung culture positive was 23 of 24 (96%) in the MXF–RPT group, and 15 of 15 (100%) in all remaining once-weekly RPT-containing groups. This difference was not statistically significant (*p* = 1.0).

After 6 mo of therapy and 3 mo of follow-up (Figure 2), the proportion of mice that were lung culture positive was 23 of 24 (96%), 21 of 24 (88%), 18 of 24 (75%), 15 of 24 (63%), and 14 of 24 (58%) in the following groups: 6-mo standard Denver regimen, and the Denver regimen completed with MXF–INH–RPT, INH–RPT, RPT alone, or MXF–RPT during the 4-mo continuation phase, respectively. The difference in the proportion of mice that were lung culture positive after treatment with MXF–RPT or RPT alone and the Denver regimen was statistically significant both before and after adjustment for multiple comparisons (*p* = 0.016 and *p* = 0.04 for the former and latter comparisons, respectively). No difference in the proportion of mice with positive lung culture was detected between the Denver regimen and INH–RPT (*p* = 0.10).

Figure 1. Change in log₁₀ lung colony-forming unit (CFU) counts after 2 mo of treatment with standard daily therapy (rifampin–isoniazid–pyrazinamide) or the intermittent Denver regimen. * Significant difference when compared with the Denver regimen (*p* < 0.0001).

Figure 2. Proportion of mice with culture-positive lungs 3 mo after completing treatment with 4, 5, and 6 mo of the Denver regimen (rifampin plus isoniazid, 2/7) or once-weekly (1/7) rifapentine-based continuation regimens. Mice were considered to be culture positive if one or more colony-forming units was detected when the entire organ was plated. * One of five lung homogenates harbored rifamycin-resistant mutants. Significant differences compared with the Denver regimen (rifampin plus isoniazid, 2/7) are shown as $^{\dagger}p = 0.04$, $^{\ddagger}p = 0.016$. RH = rifampin plus isoniazid; MHP = moxifloxacin plus isoniazid plus rifapentine; HP = isoniazid plus rifapentine; P = rifapentine; MP = moxifloxacin plus rifapentine.

After 6 mo of treatment the distribution of the proportion of mice with culture-positive spleens was similar to that of mice with culture-positive lungs, although the magnitude of the colony-forming unit counts substantially decreased (data not shown).

Resistance to RIF

After 6 mo of treatment, isolates from the five mice with the highest burden of colony-forming units from each treatment group were selected to undergo RIF susceptibility testing. One of five mice (20%) receiving RPT_{15 mg/kg} alone had isolates that were resistant to rifampin. Colonies isolated from mice in all other treatment groups were fully susceptible to rifampin.

DISCUSSION

This study reports for the first time on a direct comparison between standard daily therapy and a well-accepted alternative for intermittent therapy, the so-called Denver regimen. The predominantly twice-weekly Denver regimen is currently used throughout the United States by numerous public health departments to facilitate supervision and adherence to therapy. After 2 mo of treatment with the standard daily regimen and the Denver regimen in the experimental mouse model, we observed a 4.35 and 3.43 $\log_{10}$ kill of *M. tuberculosis* in the lungs, respectively (Figure 1). Although both regimens were effective in decreasing the initial bacillary burden, standard daily therapy was approximately 1 $\log_{10}$ more bactericidal. These results suggest that daily RIF–INH–PZA during the first 2 wk followed by twice-weekly RIF–INH–PZA during the next 6 wk is less active than 8 wk of daily RIF–INH–PZA in killing the population of bacilli that predominate during the initial phase of therapy. Furthermore, 3 mo after completion of 6 mo of therapy, the proportion of mice with culture-positive lungs was significantly greater in the Denver regimen as compared with the standard

daily regimen ($p = 0.0005$). Similar findings have been observed in humans in a large nested case-control study that compared the risk of relapse in patients receiving daily or three-times-weekly therapy (20, 21). In that study treatment with three-times-weekly therapy significantly increased the odds of relapse as compared with treatment with daily therapy. The authors suggested that the increased risk of relapse in three-times-weekly treatment was partially due to more rapid sterilization with daily treatment. It is also worth mentioning that in the Tuberculosis Trials Consortium Study 22, HIV-negative patients with either cavitation and/or positive sputum culture at 2 mo were at increased risk of failure/relapse when administered intermittent therapy (either once-weekly INH–RPT or twice-weekly RIF–INH) during the continuation phase of therapy (5). Finally, initiation of intermittent dosing during the first 2 mo of therapy has been shown to be an independent risk factor of relapse and development of rifamycin resistance in HIV-infected patients (22). Even though intermittent therapy is efficacious and has been used successfully, there is a clear need to improve its activity, especially in the setting of high-risk patients. Other studies have examined the efficacy of intermittent initial phase therapy but lacked comparative control groups and statistical power to detect differences of clinical importance (17).

A once-weekly continuation phase regimen could reduce the number of supervised doses necessary to complete a course of directly observed therapy by about 30% (5). However, the once-weekly INH–RPT_{10 mg/kg} continuation phase regimen was less efficacious than twice-weekly RIF–INH in HIV-infected and other high-risk patients (3, 5). In the final portion of our experiment we examined the sterilizing activity of improved once-weekly RPT-based continuation phase regimens by assessing the number of culture-positive mice 3 mo after completing 4, 5, and 6 mo of treatment. All RPT-containing continuation phase regimens were preceded by an initial 2-mo phase of the Denver regimen to directly compare the activity of once-weekly RPT, INH–RPT, MXF–RPT, and MXF–INH–RPT with twice-weekly RIF–INH. After completing 4 or 5 mo of treatment no significant differences in the proportion of culture-positive mice were detected among the groups (Figure 2). However, after completing 6 mo of treatment 58 and 63% of mice were lung culture positive after treatment with once-weekly MXF–RPT and RPT, respectively, as compared with 96% of mice treated with twice-weekly RIF–INH. It is interesting to note that it was not the addition of MXF to INH–RPT but rather the substitution of MXF for INH in the once-weekly INH–RPT regimen that resulted in an increase in sterilizing activity. These results complement those described in studies examining the role of MXF in the initial phase of therapy, in which the substitution of MXF for INH in the daily RIF–INH–PZA regimen conferred the greatest increase in potency (15). The precise mechanism of antagonism between INH and MXF–RPT or INH and RIF–MXF–PZA is unknown and deserves extensive study.

There are several caveats that must be considered in the interpretation of these data. Clearly, the high proportion of culture-positive mice receiving the Denver regimen and other intermittent regimens cannot be extrapolated directly to the treatment of human disease. The Denver regimen has been employed successfully in the treatment of TB for more than a decade since its introduction and is an approved regimen for supervised therapy (4). The expected relapse rate is 6% or less (4, 17). However, it is clear that the relapse rate is significantly higher in persons with cavitory disease and other risk factors (17), implying a slim margin for optimal treatment outcomes in the presence of severe disease or complicating host factors. Moreover, the Denver regimen has never been compared head-to-head with a daily treatment regimen. Perhaps the most useful

interpretation of the data presented here is to consider these inbred mice, with a high bacillary burden at initiation of treatment, to represent patients at the highest risk of relapse. What is important is not the absolute proportion of culture-positive mice after discontinuation of therapy, but the relative differences between the Denver control group and the experimental groups. In this light, the once-weekly MXF–RPT continuation phase regimen offers an alternative regimen that is, at the same time, more convenient and more efficacious than twice-weekly RIF–INH. Moreover, it is interesting to note that the same increase in risk of culture positivity after discontinuation of therapy, comparing intermittent with daily therapy during the initial phase of therapy, was observed in our mouse study and in an observational study in Hong Kong (20). In this study, the increase in risk of relapse was 3.92; in our mouse study the increase in risk of culture positivity was 96/25 (or 3.84), comparing intermittent to daily therapy. This further emphasizes that, although, the absolute proportion of culture positivity might be higher than would be expected in humans, the relative differences are comparable.

That the once-weekly MXF–RPT regimen was not as effective as the standard daily regimen was unlikely to be determined solely by diminished activity of the continuation phase component because the former regimen also included twice-weekly therapy with RIF–INH during the last 6 wk of the initial phase, whereas the latter included daily treatment throughout. Observational data in HIV-infected patients with TB suggest that receipt of intermittent therapy during the initial phase is associated with relapse and development of rifamycin resistance (22). The data presented here lead to the logical hypothesis that the efficacy of intermittent regimens could also be improved by substituting twice-weekly RPT (15 mg/kg) for twice-weekly RIF in the last 6 wk of the initial 8-wk phase before transitioning to once-weekly continuation phase therapy. We are currently evaluating this hypothesis in the murine model.

Finally, before extrapolating the activity of drug regimens from the mouse model of TB to humans, it is essential to demonstrate that drug doses used in the mouse are equipotent to those used in humans (23). In the present experiment, the pharmacokinetics of MXF and RPT were studied to determine the most clinically relevant doses in the mouse. MXF was orally administered to mice in single doses at 100, 200, and 400 mg/kg. The C_{max} of MXF at all tested doses was higher than that obtained with a standard 6.6 mg/kg (400 mg) dose in humans (4.98 µg/ml) (19) and dose linear with increasing concentrations. Furthermore, the calculated half-life of MXF in mice was 2.32 ± 0.14 h as compared with 9 to 12 h in humans (19, 24). To compensate for these pharmacokinetic differences we used a 100-mg/kg dose of MXF administered twice daily in the chemotherapeutic study. This dosing scheme results in a dose equipotent to the standard human dose of MXF (on the basis of the AUC_{0–∞}) without substantially elevated C_{max} values (Table 2). In addition, twice-daily dosing with MXF enabled us to model the longer half-life of MXF in humans relative to mice, which should afford greater protection against the selection of rifamycin-monoresistant mutants during once-weekly therapy. Unlike MXF, the pharmacokinetics of RPT administered at 10 and 15 mg/kg were remarkably similar to that of the human (Table 2). Both the AUC_{0–∞} and C_{max} were comparable at 10 and 15 mg/kg between mouse and human and are consistent with the comparative pharmacokinetics of a single dose of RIF between the two species (23). We also assessed whether RPT could be administered concurrently with regimens containing INH–MXF without antagonistic pharmacokinetic interaction. Previously, it was shown that when INH was concomitantly given with RIF and PZA the AUC_{0–∞} for RIF was significantly reduced by 39% when compared with

treatment with RIF and PZA (25). As might be predicted by the substantial difference in the time to peak serum level (T_{max}) between INH (0.24 h) and RPT (10.7 h), no difference was observed in the AUC_{0–∞} when RPT was administered alone (179.5 µg · h/ml) or in combination with INH–MXF (177.6 µg · h/ml) (23, 26).

In conclusion, we have demonstrated that the standard intermittent Denver regimen is not as active as the standard daily regimen under conditions of overwhelming infection in the murine model of TB. Together with clinical findings suggesting that regimens based on RIF–INH with intermittent intensive phase components may be associated with a higher risk of relapse or acquired rifamycin monoresistance in high-risk patients (20, 22), new intermittent regimens should be sought that fully capitalize on the long half-life and potent bactericidal activity of RPT administered at 15 mg/kg and MXF. The current study provides a step in that direction in demonstrating that a once-weekly continuation phase regimen of RPT and MXF was significantly more active than a twice-weekly regimen of RIF and INH.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has interest in the subject matter of this manuscript.

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