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Comparison of Activities of Rifapentine and Rifampin against *Mycobacterium tuberculosis* Residing in Human Macrophages

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The activities of rifapentine and rifampin against *Mycobacterium tuberculosis* residing in human monocyte-derived macrophages were determined. The MICs and MBCs of rifapentine for intracellular bacteria were two- to fourfold lower than those of rifampin. For extracellular bacteria, this difference was less noticeable. Nevertheless, the more favorable pharmacokinetics of rifapentine over rifampin was addressed in other experimental models. These models showed substantial differences after short pulsed exposures of the infected macrophages to the drugs and when the infected macrophages were exposed to changing drug concentrations that imitated the pharmacokinetic curves observed in blood. Once-a-week exposures to rifapentine concentrations equivalent to those attained in blood after one 600-mg dose resulted during the first week in a dramatic decline in the number of bacteria, and this decline was maintained at a minimal level for a period of four weeks. The results of this study have shown the suitability of rifapentine for intermittent-treatment regimens. The prolonged effect of rifapentine found in this study may be associated with high ratios of intracellular accumulation, which were four- to fivefold higher than those found for rifampin. Further studies on the intracellular distribution of rifamycins and on the sites of actual interaction between the drugs and bacteria residing in macrophages are necessary.

The combined use of isoniazid, rifampin, and pyrazinamide provided the basis for a successful short course of tuberculosis therapy. The discovery of long-lasting rifamycins, particularly of rifapentine (RPT), created the possibility for intermittent drug regimens (5, 7, 18). In the early studies, RPT was reported to have an in vitro activity the same as or higher than that of rifampin (RMP) (19, 20). Subsequent studies have shown that the broth-determined MICs of RPT were two- to threefold lower than those of RMP (8, 10). The elimination half-life of RPT in animals and humans was about five times longer than that of RMP, and the levels of RPT in plasma substantially exceeded the MICs for a period of up to 72 h after a single oral dose (1–3, 12). The advantage of RPT over RMP has been shown in murine models (1, 5, 11, 18). Besides its more favorable pharmacokinetic parameters in blood, RPT accumulates to higher concentrations than does RMP in polymorphonuclear leukocytes and macrophages (6, 9, 16).

In anticipation of a controlled clinical trial for the comparison of RPT and RMP in the therapy of tuberculosis, the present study compares the potential activities of these rifamycins against extracellular and intracellular *Mycobacterium tuberculosis*. Inhibitory and bactericidal activities (MICs and MBCs) against intracellular bacteria in human monocyte-derived macrophages were determined and compared with MICs and MBCs against extracellular bacteria. The actual accumulation of these drugs in human monocytes was also determined. Special attention was given to the evaluation of the long-lasting effect of RPT after pulsed exposures of the infected human macrophages. First, we determined the effect of one 2-h pulsed exposure to either drug in equal concentrations (3.0 μg/ml) and a subsequent 2-week-long cultivation in drug-free media. The second type of experiment was conducted for 3 weeks, and the infected macrophages were exposed for the first 72 h to changing drug concentrations in an attempt to imitate the pharmacokinetics in humans after one dose of RPT compared with one or three daily doses of RMP. Finally, in the third experimental model, we imitated the effect of RPT given once a week for 4 weeks by exposing the infected macrophages to the changing drug concentrations for the first 72 h at the beginning of each week.

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MATERIALS AND METHODS

Antimicrobial agents. RPT and its metabolite, 25-desacetyl-RPT, were obtained from Marion Merrell Dow, Inc. (Kansas City, Mo.), and RMP was obtained from Sigma Chemical (St. Louis, Mo.). The drugs were dissolved in methanol according to the manufacturer's instructions and then were diluted in appropriate culture media.

Test strains. Three strains of *M. tuberculosis* were used in this study: $H_{37}Rv$, Erdman, and a clinical isolate (Atencio), which is one of the strains used in our laboratory for quality controls. All three strains are susceptible to all antituberculosis drugs. Subcultures in 7H9 broth were preserved in aliquots frozen at −70°C. For each experiment, a subculture of frozen stock was made in fresh 7H9 broth. After 10 days of cultivation at 37°C, the culture was forced through a 27-gauge needle and was then centrifuged at 250 × *g* for 5 min to remove large clumps of bacteria.

Infection of human macrophages. This model has been described in detail in our previous publications (13, 14). The human mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation and placed in 35-mm-diameter plastic petri plates. After incubation at 37°C for 1 h, the nonadherent cells were washed away and the plates were cultivated for 7 days at 37°C in RPMI 1640 containing 5% unheated human serum. At this point, the monocytes had matured into macrophages and formed a monolayer. The medium was removed from the plates and was replaced with a suspension containing about 10^6 bacterial cells per ml in order to have an average of 5 bacteria per macrophage. The number of bacterial cells in the inoculum (in CFU per milliliter) was estimated by plating samples of the bacterial suspension on the 7H11 agar plates and subsequently performing a colony count. After incubation for 1 h, the plates were washed twice to remove the extracellular bacteria. The infected macrophages were incubated in RPMI 1640 supplemented with 1% unheated human serum at 37°C in the presence of 7% CO_2.

MIC and MBC determinations. The MICs and MBCs for extra- and intracellular bacteria were determined. The MIC was defined as the lowest drug concentration that inhibited more than 99% of the bacterial population within 8 days of observation, and the MBC was defined as the lowest drug concentration in the medium that decreased the bacterial population by 2 or more $\log_{10}$ units within the same period of incubation. For the extracellular bacteria, the MIC and MBC were determined in 7H12 broth (12B BACTEC vials; Becton Dickinson Diag-

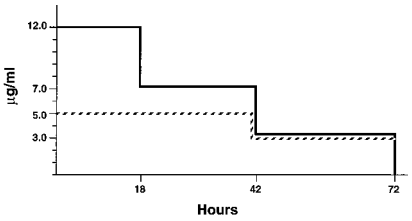

FIG. 1. This graph shows the changing concentrations of RPT and its metabolite to which the infected macrophages were exposed in an attempt to imitate the drug concentrations attainable in the blood after one dose of 600 mg. The solid line indicates RPT, and the broken line indicates the metabolite (25-desacetyl-RPT).

nostic Instrument Systems, Sparks, Md.). The vials containing 4.0 ml of broth were supplemented with various concentrations of the drugs, ranging in twofold fashion from 4.0 to 0.015 μg/ml. Drug-containing and drug-free vials were inoculated with a bacterial suspension to produce 10^4 to 10^5 CFU/ml. During the subsequent 8 days of cultivation, samples from alternate vials were taken to determine the number of CFU per milliliter by plating the samples on 7H11 agar. To determine the MIC and MBC for intracellular bacteria, the infected macrophages were exposed to various drug concentrations for a period of 8 days. The viable counts on days 0, 4, and 8 were determined by colony counting on 7H11 agar plates inoculated with serial dilutions of the macrophage lysates.

Pulsed exposure of the infected macrophages. While other observations were designed to imitate the differences between RPT and RMP in pharmacokinetics (see below), the short pulsed-exposure experiments targeted the potential activities per se, testing both drugs at equal concentrations and at equal times of exposure. For a single pulsed exposure of the infected macrophages, drug solutions were added to achieve a concentration of 3.0 μg/ml of either drug in the culture. After a 2-h incubation, the medium was removed and replaced with a drug-free medium and the monolayers were incubated for 2 weeks. The samples for determining the number of CFU were taken on days 0, 4, 7, and 14. In the second experimental model, we simulated the potential effect of one dose of RPT and of one or three daily doses of RMP. To imitate the effect of one 600-mg dose of RPT, we exposed the infected macrophages to the changing concentrations of the drug and its metabolite as shown in Fig. 1, which is a simplified model of the pharmacokinetic curve in humans with the following parameters: maximum concentration, 17.25 μg/ml for RPT and 5.73 μg/ml for its metabolite; area under the curve, 474 μg · h/ml for RPT and 257 μg · h/ml for the metabolite; and elimination half-life, 14.54 h for RPT and 13.23 h for the metabolite (12). For experiments with RMP, our schedule was based on a maximum concentration of 12 μg/ml, an area under the curve of 32.48 μg · h/ml, and a half-life time of 2 h (17). In experiments with RMP, the infected macrophages were exposed daily to concentrations of 8.0 μg/ml for 2 h followed by exposure to concentrations of 3.7 μg/ml for 4 h only once or during the first 3 consecutive days. After that, the drug-containing medium was replaced with drug-free medium and the macrophages were incubated for 3 weeks. The samples for CFU determination were taken on days 0, 3, 7, 14, and 21. The third experimental model was similar, but the exposures were repeated weekly for 4 weeks, and the samples for the CFU counts were taken on days 0, 3, 7, 14, 21, and 28.

In long-term experiments (3 or 4 weeks), the number of macrophages in the monolayers gradually decreased in drug-free controls because of the intensive intracellular multiplication of the tubercle bacilli. This did not occur in cultures treated with RPT or RMP. The decrease in the number of macrophages in drug-free cultures started after 2 weeks of cultivation, and the loss was about 50% at the end of the third week and up to 90% at the end of the fourth week. Therefore, to estimate the actual number of bacteria in the cultures, the samples taken for CFU-per-milliliter counts at any time point included the total number of bacteria per plate, including those from both the still-intact remaining monolayers and the supernate containing floating cells. In parallel experiments, we previously confirmed the known fact that *M. tuberculosis* does not grow in cell-free RPMI 1640 with 1% human serum. This gave us confidence that the total number of bacteria recovered from macrophage cultures represented the outcome of intracellular growth only.

Methods for determining intracellular concentrations. Mononuclear cells obtained through the Ficoll-Hypaque gradient centrifugation were purified by adherence to the surface of the plastic petri plates. After incubation for 10 min at 37°C, the nonadherent cells were removed by washing the plates three times with cold saline, and the adherent cells were detached by vigorous pipetting and scraping with a rubber policeman. The suspension in RPMI 1640 was adjusted to 2×10^7 cells per ml. The drugs in various concentrations (10, 20, and 40 μg/ml) were added to the monocyte suspension. After incubation at 37°C for 2 h, the cells were separated from the extracellular fluid by velocity gradient centrifugation. The gradient was prepared in 1.8-ml microcentrifuge tubes (Sarstedt, Inc., Newton, N.C.) with two oil products: five parts of DC550 with a specific gravity of 1.07 (Dow Corning, Midland, Mich.) plus 1.1 parts of paraffin oil with a specific gravity of 0.838 (Aldrich Chemical, Milwaukee, Wis.). The gradient (1.0 ml) was layered in the bottom of each tube and overlaid with 0.5 ml of the monocyte suspension. After centrifugation at 14,000 × *g* for 3 min in a microcentrifuge (Eppendorf, Netheler, Germany), the cells had passed through the gradient to form a pellet in the bottom of the tube. After the cell-free supernate was removed, the oil was removed and the pellet was resuspended in 0.4 ml of Hanks balanced salt solution. The number of cells was counted with a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.), and the cells were lysed by sonication with a sonicator cell disrupter for 15 s at a power control setting of 2.5 (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.). Twofold serial dilutions from the cell lysate as well as from the supernate were made.

To determine the concentrations of the drugs, a bioassay was performed in 100-mm-diameter petri dishes filled with 10 ml of antibiotic agar no. 5 (Difco Laboratories, Detroit, Mich.) inoculated with *Micrococcus luteus* (ATCC 9341). Four 8-mm-diameter wells per plate were filled with the various dilutions of the cell lysate or supernate (0.04 ml/well). For controls, the solutions of drugs were made in RPMI 1640. In addition, the same drug solutions were made in drug-free macrophage lysate. Plates were incubated for 18 h at 37°C, after which the diameters of the growth inhibition zones were measured. The results from the controls were used to determine a standard curve by regression analysis. The drug concentrations in experimental samples were calculated with this standard dose-response curve. The results were expressed in micrograms per milliliter of the original (undiluted) sample. The volume of one monocyte was reported as 421 μm^3 (15), and therefore the number of cells in a sample multiplied by 421 μm^3 represented the total volume of cells before lysing. The ratio of cellular volume to sample volume was used to translate the results obtained in the bioassay into the intracellular concentration of the drug in micrograms per milliliter of the cell mass. The extracellular fluid was diluted for the bioassay at the same ratio as that for the cellular volume and sample volume of the cellular samples.

RESULTS

MICs and MBCs. The MICs and MBCs of any of the three tested compounds for intracellular bacteria were within the same ranges as those found in experiments with extracellular bacteria (Table 1). For RMP, the MICs ranged from 0.12 to 0.25 μg/ml and the MBCs ranged from 0.25 to 1.0 μg/ml, resulting in an MBC/MIC ratio of from 1 to 4. For RPT, the MICs were from 0.015 to 0.06 μg/ml and the MBCs were from 0.06 to 0.5 μg/ml, giving an MBC/MIC ratio of from 4 to 8. The inhibitory and bactericidal activities of the metabolite, 25-desacetyl-RPT, were in the same ranges as those of the parent compound or were 1 twofold dilution higher (Table 1). The MICs and MBCs of RPT for intracellular bacteria were two- to eightfold lower than those of RMP. The difference between these two agents was much less noticeable against extracellular

TABLE 1. MICs and MBCs of RPT, its metabolite, and RMP for *M. tuberculosis*

Strain	MIC (μg/ml)						MBC (μg/ml)					
	For extracellular bacteria			For intracellular bacteria			For extracellular bacteria			For intracellular bacteria		
	RPT	Metabolite[a]	RMP	RPT	Metabolite	RMP	RPT	Metabolite	RMP	RPT	Metabolite	RMP
$H_{37}Rv$	0.06	0.06	0.12	0.015	0.03	0.25	0.25	0.5	0.5	0.06	0.06	1.0
Erdman	0.06	0.06	0.12	0.06	0.12	0.12	0.5	1.0	0.5	0.25	0.25	0.5
Atencio	0.06	0.12	0.25	0.03	0.06	0.25	0.5	1.0	0.25	0.12	0.25	0.5

[a] The metabolite of RPT is 25-desacetyl-RPT.

TABLE 2. MICs and MBCs of RPT and its metabolite in combination for intracellular bacteria

Strain	MIC (μg/ml)		MBC (μg/ml)		Fractional inhibitory concn coefficient[b]	Fractional bactericidal concn coefficient[b]
	RPT	Metabolite[a]	RPT	Metabolite		
$H_{37}Rv$	0.007	0.015	0.015	0.6	1	0.75
Erdman	0.03	0.06	0.125	0.25	1	1
Atencio	0.015	0.03	0.125	0.25	1	1.5

[a] The metabolite of RPT is 25-desacetyl-RPT.
[b] These coefficients were calculated from MICs and MBCs presented in this table and in Table 1.

bacteria: the difference in MICs was only two- to fourfold, and there was no difference in the MBCs. A combination of RPT and its metabolite has been tested against the same three *M. tuberculosis* strains in human macrophages. The MICs and MBCs of these compounds in combination were twofold lower than the values found for each agent tested alone (Tables 1 and 2). These data indicate the potential for an additive effect of RPT and its metabolite.

Intracellular concentrations. The concentrations of tested compounds in macrophages were greater than those in the extracellular medium. The intracellular accumulation of each agent did not depend on the drug concentration to which the macrophages were exposed, and the intracellular/extracellular ratios were within the same ranges in the presence of concentrations of 10.0, 20.0, or 40.0 μg/ml (Table 3). The mean intracellular/extracellular ratio was 24.0 ± 3.9 for RPT, 7.2 ± 2.4 for its metabolite, and only 4.4 ± 1.9 for RMP (Table 3). These data indicate that RPT accumulates to significantly higher levels in monocytes than does RMP. Despite this intracellular accumulation, the concentrations of any of these three agents in the medium required for complete inhibition of growth of the intracellular bacteria (MIC) or for a 100-fold or greater decrease in the number by killing (MBC) were the same as the MICs and MBCs for extracellular bacteria (Table 1). The difference between RPT and RMP in their abilities to accumulate intracellularly could have been extrapolated to the results obtained in other experimental models described below.

Effect of one 2-h pulsed exposure. We recognize, on the basis of experiments described in other sections of this report, that the equal concentrations of RPT and RMP would have different effects in vivo because of the differences in their pharmacokinetics. In experiments described here and whose results are shown in Table 4, we have attempted to address only one feature related to the differences in intracellular accumulation in macrophages of the two agents. This difference could be detected only when the infected cells were exposed to equal drug concentrations. In these experiments, the number of viable bacteria in the drug-free controls increased during the 14-day period of incubation from an average of 6.6×10^3 to 1.0×10^4 to 3.9×10^6 to 1.8×10^7 CFU per 2×10^5 macrophages (Table 4). During the same period, the number of viable bacteria in macrophages exposed for 2 h to a concentration of 3.0 μg/ml of RPT or RMP had declined by 1 to 2 $\log_{10}$ units after 7 days of incubation and declined further during the second week. This effect was more noticeable with macrophages treated with RPT, at least for two strains (Table 4).

Effect of 72-h exposure of the infected macrophages. Macrophages infected with *M. tuberculosis* were exposed for 72 h to the changing concentrations of RPT and its metabolite in an attempt to imitate the pharmacokinetic curve that occurs in blood after one 600-mg dose of RPT, as shown schematically in Fig. 1. In this setting, we tested RPT and its metabolite separately and in combination. For the purpose of comparison, we also conducted parallel studies with RMP changing concentrations to imitate the pharmacokinetics that occur within 72 h of three daily 600-mg doses of RMP. For this purpose, the macrophages were exposed to a concentration of 8.0 μg/ml for 2 h and then to a concentration of 3.7 μg/ml for 4 h, and then they were placed in drug-free medium. The procedure was repeated for 3 consecutive days. In addition, we assessed the potential effect of only one dose of RMP by exposing the infected macrophages to concentrations of 8.0 and 3.7 μg/ml of RMP only once, on the first day. After exposure to the drugs, the macrophages were kept at 37°C for 3 weeks and samples were taken at days 0, 3, 7, 14, and 21 to determine the number of CFU. Figure 2 shows that in experiments with all three strains tested, the number of viable bacteria in untreated macrophages increased by 2 to 3 $\log_{10}$ units during the 3-week period. At the same time, the number of viable bacteria in macrophages treated with RPT was dramatically decreased by 2 to 3 $\log_{10}$ units during the first 7 days, but this was followed by some rebound on days 14 and 21 (Fig. 2A). The effect of the metabolite was also substantial but less dramatic. There was no difference in the activity of RPT alone compared with its activity in combination with the metabolite in experiments with

TABLE 3. Accumulation of the agents in human monocytes

Concn (μg/ml) of drug in medium	Intracellular/extracellular ratio[a]		
	RPT	Metabolite[b]	RMP
10.0	26.8 ± 4.0	6.5 ± 2.2	4.2 ± 1.9
20.0	24.8 ± 3.1	7.7 ± 3.8	4.5 ± 2.5
40.0	20.5 ± 2.1	7.5 ± 0.3	4.5 ± 1.7
Overall mean	24.0 ± 3.9	7.2 ± 2.4	4.4 ± 1.9

[a] Values are means ± standard deviations for three or four experiments.
[b] Metabolite of RPT (25-desacetyl-RPT).

TABLE 4. Effect of one 2-h pulsed exposure of infected macrophages to RPT and RMP

Drug[a]	Strain	Average CFU per 2×10^5 macrophages at day:			
		0	4	7	14
RPT	$H_{37}Rv$	6.6×10^3	1.0×10^2	4.3×10^1	3.0×10^1
	Erdman	1.0×10^4	2.1×10^2	4.5×10^2	3.6×10^2
	Atencio	9.7×10^3	1.2×10^2	2.6×10^1	7.9×10^2
RMP	$H_{37}Rv$	6.6×10^3	3.0×10^2	1.1×10^2	2.3×10^2
	Erdman	1.0×10^4	7.4×10^2	3.7×10^2	1.2×10^3
	Atencio	9.7×10^3	1.9×10^2	1.2×10^2	6.4×10^2
None	$H_{37}Rv$	6.6×10^3	5.3×10^4	9.8×10^5	1.8×10^7
	Erdman	1.0×10^4	3.4×10^4	8.2×10^5	8.3×10^6
	Atencio	9.7×10^3	9.5×10^3	6.0×10^4	3.9×10^6

[a] The concentration of the drugs was 3.0 μg/ml.

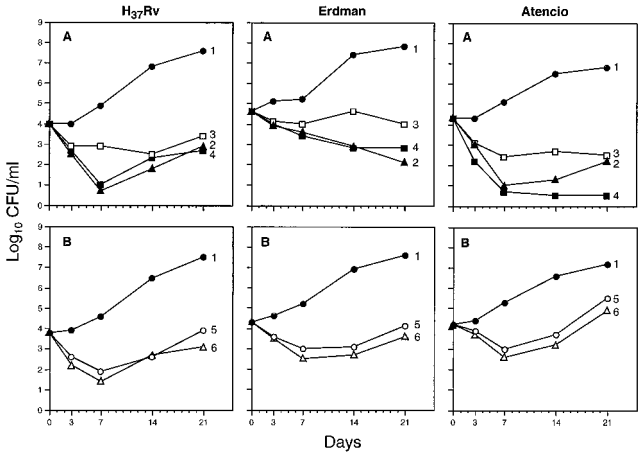

FIG. 2. Number of viable bacteria (CFU) in infected macrophages during a 3-week period of observation after the infected cells were exposed only once to the antimicrobial agents. (A) Effect of RPT and its metabolite as used in changing concentrations for 72 h as shown in Fig. 1. (B) Effect of RMP as used at a concentration of 8.0 μg/ml for 2 h and then 3.7 μg/ml for 4 h for either one or three daily exposures. 1, drug-free control; 2, RPT; 3, RPT metabolite; 4, RPT and metabolite combined; 5, RMP (one exposure); 6, RMP (three daily exposures). The standard error for all experiments shown in these graphs was less than 5%, and the confidence limits are not shown graphically because of their small values.

two strains, and there was a very slight, insignificant difference in observations with the Atencio strain. Therefore, we concluded that the metabolite did not enhance the effect of RPT when the agents were combined in the concentrations shown in Fig. 1.

The initial first-week effect of either one or three doses of RMP was similar to the effect of RPT, but the rebound in the number of viable bacteria during the following 2 weeks was substantially higher, especially when the macrophages were exposed to the imitated 1-day dose of RMP (Fig. 2B).

These experiments clearly indicated that one dose of RPT has a greater potential for a long-lasting effect against intracellular tubercle bacilli than does one to three doses of RMP. At the same time, exposure to changing RPT concentrations imitating those found in the blood after one dose could not sterilize the macrophages, a finding which led to the evaluation of the potential effects of four doses of RPT given once a week for a period of 4 weeks.

Effect of changing RPT concentrations over 4 weeks. The infected macrophages were exposed to RPT at the beginning of each week to evaluate the potential effect of one dose given weekly for 4 weeks. To imitate the pharmacokinetics of the drug in the blood, the macrophages were exposed weekly to a concentration of 12.0 μg/ml for 18 h, then to one of 7.0 μg/ml for 24 h and finally to a concentration of 3.0 μg/ml for 30 h. The metabolite, either alone or in combination with RPT, was used in a concentration of 5.0 μg/ml for 42 h and then in one of 3.0 μg/ml for 30 h. This schedule produced an area under the curve close to those observed with blood. After this 72-h exposure, the macrophages were kept in a drug-free medium for 4 days. Samples for determining the number of viable bacteria were taken on days 0, 3, 7, 14, 21, and 28. In contrast to the previous series of experiments with one 72-h exposure, experiments with weekly exposures to the drug excluded any rebound in the number of viable bacteria after the initial decline by 2 or 3 $\log_{10}$ units seen during the first week of observation (Fig. 3). In fact, the number of viable bacteria continued to decline during the remaining period, and only a few colonies were isolated from some samples. The effects of RPT and its metabolite were about the same, but in combination, the metabolite did not enhance the effect of RPT, since the number of bacteria surviving in macrophages treated with the combination was no different from that of bacteria in macrophages treated with RPT alone (Fig. 3).

DISCUSSION

RPT has shown higher bacteriostatic and bactericidal activities (MICs and MBCs) than RMP, especially against intracellular bacteria growing in human monocyte-derived macrophages, which is in agreement with our previous data (10). The difference between RPT and RMP was especially noticeable when the ratios between the peak concentrations attainable in blood and the MICs were taken into account (about 300 for RPT and 100 for RMP). But the major advantage of RPT over RMP that is not taken into account by this calculation is a substantial difference in parameters other than maximum concentrations, particularly in the area under the curve.

The intracellular accumulation of RPT in human monocytes was much higher than that of RMP, which is in agreement with the findings with other cell models (6, 9, 16). Despite average extracellular/intracellular ratios of 24 for RPT and 4.4 for RMP, the lowest extracellular concentrations of these agents required to induce complete inhibition of growth of the intracellular bacterial population were no different from the MIC ranges found with cell-free liquid medium, which were found in both cases by assessing 8-day exposures. The MBCs for intracellular bacteria were also in the same range as the MBCs for extracellular bacteria. The paradox of these data and the evidence of substantial intracellular accumulation, especially of RPT, may be explained by the possibility that only a portion

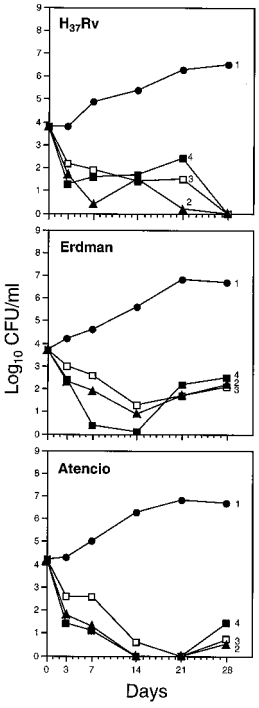

FIG. 3. Number of viable bacteria (CFU) in infected macrophages during a 4-week observation of drug-free control macrophages (1) and macrophages exposed weekly for 72 h to changing concentrations of RPT (2), its metabolite (3), and a combination of both (4).

of the cell-associated drug interacted with the intracellular bacteria. The solution to the problem requires further studies, particularly on the intracellular traffic and distribution of these rifamycins in different cellular compartments.

Nevertheless, the difference between the activities of RPT and RMP in pulsed exposures may have been associated with the different ratios of intracellular accumulation. The inhibitory effect during the 14-day incubation in drug-free medium after one 2-h pulsed exposure of the infected macrophages to equal concentrations (3.0 μg/ml) of RPT and RMP was greater for RPT in experiments with two of three *M. tuberculosis* strains. The most important difference between these two agents is in their pharmacokinetics, which suggested that administration of RPT once or twice a week would be necessary to achieve at least the same effect as the daily administration of RMP. To assess this potential against *M. tuberculosis* residing in human macrophages, we conducted two types of experiments. In one of them, the infected macrophages were exposed to changing concentrations imitating the pharmacokinetic curves in humans given one dose of RPT or one or three doses of RMP. In these experiments, the potential effect of one dose of RPT was greater than the potential effect of one or three daily doses of RMP. In another model, exposure to RPT, its metabolite, and a combination of the two was repeated weekly for 4 weeks, and this resulted in a dramatic initial decline of the bacterial population to a level at which it remained during the 4-week period of observation. Technical difficulties prevented us from imitating the two weekly doses in 4-week experiments and from longer periods of observation. Nevertheless, we conclude from the observations presented in this report that RPT has an obvious potential for effective use in intermittent drug regimens. In the murine model, use of RPT in combination with isoniazid resulted in the complete sterilization of the organs within 2 months (4). Therefore, a treatment regimen including RPT may be the subject of clinical evaluation of the efficacy not only of the intermittent protocol but of an ultrashort therapy schedule as well.

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