

Safety and Bactericidal Activity of Rifalazil in Patients with Pulmonary Tuberculosis

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Rifalazil, also known as KRM-1648 or benzoxazinorifamycin, is a new semisynthetic rifamycin with a long half-life of approximately 60 h. Rifalazil has potent bactericidal activity against *Mycobacterium tuberculosis* in vitro and in animal models of tuberculosis (TB). Prior studies in healthy volunteers showed that once-weekly doses of 25 to 50 mg of rifalazil were well tolerated. In this randomized, open-label, active-controlled phase II clinical trial, 65 subjects with sputum smear-positive pulmonary TB received one of the following regimens for the first 2 weeks of therapy: 16 subjects received isoniazid (INH) (5 mg/kg of body weight) daily; 16 received INH (5 mg/kg) and rifampin (10 mg/kg) daily; 17 received INH (5 mg/kg) daily plus 10 mg of rifalazil once weekly; and 16 received INH (5 mg/kg) daily and 25 mg of rifalazil once weekly. All subjects were then put on 6 months of standard TB therapy. Pretreatment and day 15 sputum CFU of *M. tuberculosis* were measured to assess the bactericidal activity of each regimen. The number of drug-related adverse experiences was low and not significantly different among treatment arms. A transient decrease in absolute neutrophil count to less than 2,000 cells/mm³ was detected in 10 to 20% of patients in the rifalazil- and rifampin-containing treatment arms without clinical consequences. Decreases in CFU counts were comparable among the four treatment arms; however, the CFU results were statistically inconclusive due to the variability in the control arms. Acquired drug resistance did not occur in any patient. Studies focused on determining a maximum tolerated dose will help elucidate the full anti-TB effect of rifalazil.

With good patient compliance facilitated by a directly observed therapy (DOT) program with adequate resources, current 6-month, short-course chemotherapy regimens for the treatment of tuberculosis (TB) can achieve cure rates of 90% or more (2, 12). Resources for implementation of DOT are not available in many developing countries where the burden of tuberculosis is high. The World Health Organization estimates that only 21% of all TB cases are currently treated in areas covered by DOT (21). Despite these advances, the global resurgence of TB serves as a reminder that new drugs are needed that could potentially shorten the duration of treatment or reduce the number of doses required during the course of treatment.

Rifalazil, also known as KRM-1648 or benzoxazinorifamycin, is a new rifamycin derivative related to rifampin and rifabutin. Preclinical studies with rifalazil demonstrated significant in vitro activity against *Mycobacterium tuberculosis*. In vitro MIC studies have shown that rifalazil is 64-fold more active than rifampin and 4- to 8-fold more active than rifabutin against many isolates of *M. tuberculosis* (5). In murine models

of TB, rifalazil was more effective than rifampin in reducing the time required for organ sterilization (10). Furthermore, one in vitro study suggested that some *M. tuberculosis* strains resistant to rifampin may not be cross resistant to rifalazil (20). Like rifampin, but unlike rifabutin, the metabolism of rifalazil does not appear to be dependent on hepatic cytochrome P450 enzymes (6, 11). In addition, animal studies show that, unlike both rifampin and rifabutin, rifalazil is not an inducer of hepatic cytochrome P450 (11). Therefore, the potential for clinically significant drug interactions with other drugs, including the human immunodeficiency virus (HIV) protease inhibitors, may be lower for rifalazil than for other rifamycins in use (7). Importantly, its longer terminal half-life can make dosing less frequent.

Clinical studies of rifalazil in normal healthy human volunteers using doses of 25 to 50 mg demonstrated a dose-related incidence in the number and severity of adverse events (AE) (14; PC-KRM-004, a randomized double-blind intermittent-dose study of the safety and pharmacokinetics of KRM-1648 in normal volunteers, study report, 1999 [PathoGenesis Corp., Seattle, Wash.]). The predominant AE were flu-like symptoms, including chills, fever, and myalgias. Additionally, transient dose-dependent decreases in the total white blood cell counts, absolute neutrophil counts (ANC), and platelet count were observed in some patients. Flu-like symptoms and transient neutropenia also have been reported in 4 to 22% and 3 to 12% of patients, respectively, treated with rifampin and rifabutin in some studies (1, 4, 17, 18). The mean terminal half-life ($t_{1/2}$) of rifalazil in six healthy volunteers receiving 25 mg of rifalazil

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once weekly for 4 weeks was approximately 61 h, with a maximum concentration (C_{\max}) of 44 ng/ml (14).

In this report we describe the first experience with rifalazil in human subjects with pulmonary TB. The primary objective of this phase II clinical trial was to evaluate the safety and pharmacokinetics of low doses of rifalazil administered once weekly to HIV-noninfected patients with newly diagnosed sputum smear-positive pulmonary TB. We also assessed the microbiologic activity of rifalazil by comparing the bactericidal activity in sputum of rifalazil (10 mg or 25 mg once weekly) plus isoniazid (INH) (5 mg/kg of body weight/day) for 14 days and the bactericidal activity in sputum of INH (5 mg/kg/day) or INH (5 mg/kg/day) plus rifampicin (10 mg/kg/day) for 14 days.

MATERIALS AND METHODS

This open-label randomized clinical trial was conducted at the Hospital Universitário Cassiano Antonio de Moraes of the Universidade Federal do Espírito Santo (UFES) in Vitória, Brazil. The study protocol was approved by the institutional review boards of UFES and Case Western Reserve University and University Hospitals of Cleveland, Ohio. All patients gave written informed consent for study participation and received pre- and post-HIV test counseling.

Adults who were 18 years of age or older with newly diagnosed initial episodes of sputum smear-positive (acid-fast bacilli detected) pulmonary TB were eligible for study participation. HIV-infected patients; patients with suspected miliary or meningeal TB, severe hemoptysis (greater than 50 ml during the previous week), or suspected drug-resistant TB; and pregnant or lactating women were excluded from the study.

Patients were randomized to one of four treatment groups for the initial 14 days of anti-TB treatment: (i) INH, 300 mg once daily (treatment group H); (ii) INH, 300 mg once daily, plus rifampin (450 mg for patients weighing less than 50 kg and 600 mg for those over 50 kg) daily (treatment group HR); (iii) INH, 300 mg once daily, plus rifalazil, 10 mg on day 1 and day 8 (treatment group HRz10); or (iv) INH, 300 mg once daily, plus rifalazil, 25 mg on day 1 and day 8 (treatment group HRz25). All drugs were administered orally, under DOT, in the early morning one-half hour after breakfast. Patients in the rifalazil treatment arms received a standard lipid-rich meal including a cheeseburger with bacon one-half hour before each rifalazil dose; a lipid-rich meal increases the absorption of rifalazil (PathoGenesis Corp. study report). At the conclusion of the 14-day study period, all patients were treated with 6 months of standard short-course chemotherapy, with 2 months of daily INH, rifampin, pyrazinamide, and ethambutol followed by 4 months of daily INH and rifampin. All patients were hospitalized for the first 14 days of the study for DOT and specimen collection.

Sputum collection and processing. Twelve-hour pooled sputum collections were used for all quantitative studies done during the first 28 days of the study. Two sputum samples were collected before the study drug was started to establish baseline counts of *M. tuberculosis* CFU. Two posttreatment samples were collected, on days 14 and 15, to assure collection of an evaluable endpoint. Samples were collected at baseline (day 1) and on days 3, 4, 8, 11, 14, 15, and 28. Spot sputum specimens were obtained at day 42 and monthly after 2, 3, 4, 5, and 6 months of anti-TB therapy for routine qualitative acid-fast bacillus smear and cultures to assess response to standard treatment (9).

Pooled specimens were collected in sterile disposable 50-ml polypropylene centrifuge tubes from 9 p.m. to 9 a.m. at each sampling point and stored at 2 to 8°C prior to processing. Samples were homogenized by incubation with *N*-acetyl L-cysteinesodium citrate (50 mg/ml in *N*-acetyl L-cysteine in 2.9% sodium citrate) for 4 min and vortexing with several 4-mm-diameter glass beads for 30 s. The homogenate was then decontaminated by incubation for 15 min with an equal volume of 2% sodium hydroxide and 1.5% sodium citrate and concentrated by centrifugation at $4,000 \times g$ at 8 to 10°C for 15 min. The sediment was reconstituted to a 2.5-ml volume with phosphate buffer, and the resulting suspension was used to prepare smears and cultures on solid and BACTEC media.

Quantitative culture (CFU assay). Serial 10-fold dilutions were prepared by adding 0.5 ml of the sediment to 4.5 ml of 0.25% Tween 80 (Sigma catalog no. P1754) in 0.9% saline. From each dilution (10^0 to 10^{-5}), 60 μ l was inoculated on selective and nonselective sides of Middlebrook 7H10 agar biplates supplemented with oleic acid albumin-dextrose-catalase. The medium was made selective by the addition of final concentrations of polymyxin B (200 U/ml), carbenicillin (50 mg/ml) trimethoprim (20 mg/ml), and amphotericin B (10 mg/ml). Plates were sealed, incubated at 37°C in 5 to 10% CO₂, and examined after 2, 3,

4, and 6 weeks. Colonies were counted on plates with dilutions yielding 10 to 50 visible colonies and expressed as log₁₀ CFU per milliliter of undiluted sputum.

The selective 7H10S medium was included to compensate for fungal contamination and resulting nonevaluable quantitative cultures. Yajko et al. have shown that *M. tuberculosis* CFU counts may be slightly decreased when agar containing antibiotics is used for cultivation (22). For this reason, changes in CFU counts are usually reported from cultures grown on identical media. In this study, the mean change in CFU from the two different media for each of the treatment groups was compared and found to be similar. Therefore, CFU counts taken from selective plates (7H10S) were used if pretreatment or posttreatment samples grown on nonselective media were nonevaluable. Ultimately, data from 7H10 and 7H10S media were used for 44 and 15 patients, respectively.

Species identification of isolates and drug susceptibility testing. Pretreatment and day 14 or 15 sputum isolates from each patient were confirmed as *M. tuberculosis* by using the BACTEC para-nitro-acetyl amino-hydroxy-propionone susceptibility method (15) Susceptibility testing against isoniazid, rifampin, streptomycin, ethambutol, and pyrazinamide was performed on pretreatment and day 14 or 15 sputum isolates from each patient using standard BACTEC methods (16). The indicated critical concentrations of the following were used: INH, 0.1 μ g/ml; rifampin, 2.0 μ g/ml; streptomycin, 2.0 μ g/ml; ethambutol, 2.5 μ g/ml; and pyrazinamide, 100 μ g/ml.

Pharmacokinetics. Samples for the determination of rifalazil concentrations in plasma were collected and kept at 4°C until processing, which took place within 1 h of collection. Collections were performed at 0 h (predose) and at 3, 6, 8, 12, 24, 48, and 72 h postdose. Plasma samples were divided in aliquots and stored at -70°C, for up to 3 months. Trough levels were measured prior to administration of the second dose on day 8; peak levels were measured 6 h after dosing on days 1 and 8. Plasma samples were analyzed at PathoGenesis Corp. by high-performance liquid chromatography using a Beckman Ultrasphere ODS, column (diameter, 5 μ m; 4.6 by 250 mm), and quantitated by measuring visible absorbance of the column effluent at 600 nm.

The pharmacokinetic analysis of rifalazil involved the determination of the noncompartmental parameters obtained from the first dose and the second dose for the two individual doses studied. Parameters measured included time to C_{\max} , C_{\max} , $t_{1/2}$, and area under the curve (AUC).

Endpoints. Safety was assessed by measuring the incidence of AE and changes in serum chemistry and hematology measurements. The microbiological endpoint was the bactericidal activity of rifalazil as assessed by comparing the mean change in CFU per milliliter (log₁₀) of *M. tuberculosis* in sputum from baseline (day 1) to day 15 (day 14 was used if the day 15 sample was nonevaluable) between treatment groups. The H treatment arm was used as a control for comparison with the rifalazil treatment groups. The higher CFU-per-milliliter count from the two pretreatment samples for each patient was used as the baseline count.

Statistical analysis. All patients who received at least one dose of study drug were included in the safety analysis. An AE was defined as treatment emergent if (i) it was not present at baseline and occurred after the start of medication, (ii) it was present at baseline but increased in severity after the start of study medication, or (iii) it was considered by the investigator to be related to study medication.

The study was powered to detect a 1.0-log₁₀ or greater mean decrease in CFU per milliliter from baseline to day 15 among the two rifalazil treatment groups compared to the INH-alone treatment group, assuming a standard deviation of 1.0 log₁₀ CFU/ml for the mean change for each treatment group. The statistical test used was analysis of variance. Patients were excluded from the analysis of microbiologic activity if nontuberculous mycobacteria were isolated from any sputum culture, overgrowth of contaminating flora occurred on Middlebrook 7H10S plates, fewer than 10 CFU/ml were measured from prescreening and baseline sputum samples, more than 48 h elapsed between sputum collection and processing, or less than 5 ml of sputum was collected.

RESULTS

Patient disposition. Sixty-five patients received at least one dose of study medication and were included in the safety evaluation. Six patients were not evaluable for the microbiologic activity analysis for the following reasons: withdrawal on day 5 for treatment of concomitant bacterial pneumonia ($n = 1$), contamination of sputum specimens ($n = 4$), and initial INH mono-resistance ($n = 1$). These six patients were similarly distributed among the four treatment groups.

TABLE 1. Most frequent^a drug-related AE

AE	No. (%) of patients in treatment group experiencing AE			
	H (n = 16)	HR (n = 16)	HRz10 (n = 17)	HRz25 ^b (n = 16)
Somnolence	5 (31)	2 (13)	4 (24)	7 (44)
Abdominal pain	5 (31)	2 (13)	1 (6)	6 (38)
Arthralgia	1 (6)	2 (13)	2 (12)	5 (31)
Fever	0 (0)	0 (0)	0 (0)	3 (19)
Myalgia	1 (6)	0 (0)	0 (0)	3 (19)
Taste perversion	1 (6)	0 (0)	0 (0)	3 (19)
Nausea	3 (19)	3 (19)	1 (6)	2 (13)
Dizziness	3 (19)	2 (13)	2 (12)	2 (13)
Pruritus	4 (25)	5 (31)	5 (29)	1 (6)

^a Defined as >15% of patients in any treatment arm.

^b All differences between the HRz25 and the H treatment groups were not statistically significant ($P > 0.05$) according to Fisher's exact test.

AE. AE attributed to study drug treatment with either INH, rifampin, or rifalazil and occurring in 15% or more of patients in any study arm are presented in Table 1. Arthralgias, fever, myalgias, and taste perversion occurred among more patients in either rifalazil treatment arm than among those in treatment arms not receiving rifalazil. Although not statistically significant, the overall incidence of these AE was higher in the HRz25 treatment group than in the HRz10 treatment group, suggesting that the AE could be dose dependent. Other AE were distributed similarly among the four treatment groups. The difference between the proportion of patients with AE in the HRz25 and H treatment groups was not statistically significant ($P > 0.05$) using Fisher's exact test.

Specific dosing data and AE were reviewed further to determine the incidence of drug-related AE occurring within 24 h after receiving rifalazil. These criteria were applied to determine whether patients developed drug-related flu-like symptoms similar to those observed in earlier studies of rifalazil in healthy volunteers. From this review, 1 of 16 (6%) patients in HRz25 developed fever, myalgias, arthralgias, and headache within 24 h after receiving the second dose of rifalazil. These symptoms resolved within 4 days.

Hematologic effects. Transient decreases in ANC to fewer than 2,000 cells/mm³ occurred in subjects in all treatment groups except H (Table 2). Of the seven patients in the three rifamycin-receiving treatment arms who experienced decreases in ANC to fewer than 2.00×10^3 cells/mm³, the fall in ANC occurred by study day 8 in four subjects. One patient in the HRz10 group had a decrease in ANC to fewer than 1,000 cells/mm³. This decrease occurred on day 13 of short-course chemotherapy, 20 days after the patient's last dose of rifalazil,

and was felt by the investigator to be related to standard short-course chemotherapy. The ANC in this patient increased to more than 1,000/mm³ by day 15 of short-course chemotherapy. Decreases in ANC were transient in all patients.

Elevated absolute eosinophil counts (AEC) above 500 cells/mm³ were observed among patients in all four treatment groups, ranging from 8 (50%) patients in the HRz25 group to 12 (75%) patients in the HR group. Of the 41 patients with elevated AEC observed at any time during the study, 28 (68%) had either confirmed or suspected intestinal parasitic infections, including giardiasis, strongyloidiasis, ascariasis, or schistosomiasis. Four patients (10%) had elevated AEC attributed to standard short-course chemotherapy as the eosinophilia initially occurred after patients completed the study and had begun standard TB treatment. The remaining nine patients (22%) with elevated AEC were in the H or HR treatment groups.

Clinically significant thrombocytopenia did not occur in any patient. Effects on erythropoiesis were more difficult to discern, as many patients (33 of 65 [51%]) had anemia at baseline prior to receiving study drug, likely due to TB, malnutrition, concomitant intestinal parasitic infection, or a combination of these factors.

Hepatic, renal, and electrolyte effects. Significant increases in serum aspartate and alanine aminotransferase levels occurred in only one patient (HRz25 treatment group). The increases were felt unlikely to be related to rifalazil, as the patient's elevated transaminases were first detected on study day 21, 13 days after the last dose of rifalazil and 7 days after beginning standard short-course chemotherapy. No significant abnormalities in serum electrolytes, renal function, or other biochemical parameters occurred in any treatment group.

Pharmacokinetics. Pharmacokinetic parameters were determined following the first dose of rifalazil administered to the patients. Approximate dose proportionality is evident as illustrated by the C_{\max} values (13.5 ± 4.6 and 26.4 ± 11.0 ng/ml for the 10- and 25-mg doses, respectively) and the AUC values (280.1 ± 119.7 and 610.3 ± 253.4 for the 10- and 25-mg doses, respectively). The $t_{1/2}$ values are approximately the same as well (8.7 ± 2.7 and 8.6 ± 3.6), indicating no saturation of drug elimination at these doses.

Although the variability in the high-performance liquid chromatography assay was very low as estimated by WinNonLin software, in previous studies (14) the peak drug level after one dose of 25 mg of rifalazil, as measured by the C_{\max} level, appeared to be higher in normal volunteers than that observed for TB patients in our study. The C_{\max} value after the 25-mg dose from this study (26.4 ng/ml) was one-third lower than that observed in normal volunteers (39.3 ng/ml) (14)—a statistically

TABLE 2. ANC during the study

Treatment group	n	Baseline ANC (10^3 cells/mm ³)		No. (%) of patients with drop to:		Lowest ANC (10^3 cells/mm ³) during study
		Mean (SD)	Range	< 2.00×10^3 cells/mm ³	$\geq 50\%$ of baseline	
H	16	6.0 (1.8)	3.9–9.0	0 (0)	0 (0)	2.0
HR	16	6.5 (3.2)	2.6–13.4	3 (19)	7 (44)	1.2
HRz10	17	7.1 (3.8)	2.6–15.5	2 (12)	8 (47)	0.9
HRz25	16	7.2 (3.9)	2.7–18.7	2 (13)	7 (44)	1.6

TABLE 3. Decrease in sputum bacillary load of *M. tuberculosis* by quantitative culture from pretreatment to day 15 of study drug treatment.

Parameter	Value in treatment group			
	H	HR	HRz10	HRz25
<i>n</i>	15	15	14 ^a	15
Mean (SD) ^b	2.65 (1.54)	3.23 (1.54)	2.03 (0.96)	2.68 (1.02)
Median	2.28	3.22	1.92	2.65
Maximum	6.79	6.37	3.44	4.59
Minimum	0.92	0.98	0.25	0.92
<i>P</i> ^c			0.39	0.59

^a One patient not evaluable due to fungal contamination of sputum specimens.

^b Mean average and standard deviation of log₁₀ CFU.

^c Wilcoxon rank-sum test comparing the difference in the mean change in CFU for treatment group HRz10 with treatment group H and treatment group HRz25 with treatment group H.

significant difference. The time to C_{\max} value was also somewhat delayed in the TB patients. The corresponding AUC values were comparable between TB patients and normal subjects.

Microbiologic activity. Microbiologic activity was assessed by measuring the decrease in sputum bacillary load, measured as CFU per milliliter of sputum at the end of 2 weeks of study drug treatment. Differences in the mean decrease of CFU per milliliter for treatment groups HR, HRz10, and HRz25 were not statistically significant from those observed for the control group (H) due to higher-than-expected standard deviation; however, the HR group showed the greatest mean decrease (Table 3). For groups HR, HRz10, and HRz25 the mean decrease in CFU per milliliter and the median decrease were similar; for treatment group H, the median decrease was lower than the mean decrease, suggesting greater variability in results among patients in this group.

Two patients did not undergo drug susceptibility testing at day 15. No acquired drug resistance was detected in the remaining 57 patients at the end of the 14 day study treatment period.

DISCUSSION

Rifalazil at dosages of 10 and 25 mg once weekly was well tolerated by patients with smear-positive pulmonary TB. The rates of transient neutropenia in the two treatment groups receiving rifalazil were comparable to that in the group receiving daily rifampin. Additionally, flu-like symptoms clearly developed in only one (6.3%) patient in the HRz25 treatment group. Flu-like symptoms were observed at a three- to fourfold higher rate in healthy volunteers at this same dose (14; PathoGenesis Corp. study report). The low rate of significant AE in the present study suggests that patients with active TB may be able to tolerate doses higher than 25 mg weekly.

An interesting finding suggested by this study is that TB patients may not absorb rifalazil as rapidly as normal subjects. The mean C_{\max} value for TB patients from this study was significantly lower than that for healthy volunteers (14). Food effects are well documented for rifamycins in general, and it is possible that absorption was not optimized in this study (13). We attempted to optimize rifalazil absorption in our study patients by administering the drug on scheduled dosing days

one-half hour after a standard meal containing a lipid-rich sandwich. Many patients in all treatment groups presented intestinal parasitic infections. Although concomitant specific treatment was provided it is not clear whether this could influence drug absorption. In future studies of rifalazil in TB patients, this issue will require further investigation.

The unexpectedly high variance of the CFU counts for the H and HR treatment groups makes it difficult to fully interpret the results for bactericidal activity. Given the variance in CFU counts, the study had only a 59% power to detect a 1.0-log₁₀ difference in CFU per milliliter between the rifalazil-containing treatment groups and the H treatment group, which was lower than the 88% power used to calculate the sample size for the original study design.

In this 2-week study, we were unable to demonstrate a significant "rifampin" effect on sputum CFU counts. Neither rifampin nor rifalazil significantly decreased sputum CFUs when compared to INH. In a study by Jindani et al. the bactericidal activities of INH and rifampin were additive, although this was demonstrated in a small number of patients ($n = 4$) and the standard deviation was not provided (8). Based on our data from a larger study, we conclude that short-term quantitative sputum culture may not be the best indicator of the antimycobacterial activity of rifamycins. Though not yet fully validated, changes in sputum levels of *M. tuberculosis* antigens such as 85B and mRNA may be more sensitive as early indicators of rifampin efficacy and warrant further exploration (3, 19). Further studies are needed to determine the maximal tolerated dose of rifalazil and to explore its microbicidal activity at higher doses.

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