A Sterilizing Tuberculosis Treatment Regimen Is Associated with Faster Clearance of Bacteria in Cavitory Lesions in Marmosets

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Shortening the lengthy treatment duration for tuberculosis patients is a major goal of current drug development efforts. The common marmoset develops human-like disease pathology and offers an attractive model to better understand the basis for relapse and test regimens for effective shorter duration therapy. We treated *Mycobacterium tuberculosis*-infected marmosets with two drug regimens known to differ in their relapse rates in human clinical trials: the standard four-drug combination of isoniazid, rifampin, pyrazinamide, and ethambutol (HRZE) that has very low relapse rates and the combination of isoniazid and streptomycin that is associated with higher relapse rates. As early as 2 weeks, the more sterilizing regimen significantly reduced the volume of lung disease by computed tomography ($P = 0.035$) and also significantly reduced uptake of [¹⁸F]-2-fluoro-2-deoxyglucose by positron emission tomography ($P = 0.049$). After 6 weeks of therapy, both treatments caused similar reductions in granuloma bacterial load, but the more sterilizing, four-drug regimen caused greater reduction in bacterial load in cavitory lesions ($P = 0.009$). These findings, combined with the association in humans between cavitory disease and relapse, suggest that the basis for improved sterilizing activity of the four-drug combination is both its faster disease volume resolution and its stronger sterilizing effect on cavitory lesions. Definitive data from relapse experiments are needed to support this observation.

Treatment of drug-sensitive tuberculosis (TB) requires 6 months of chemotherapy with a combination of four agents, isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (EMB), to achieve a durable cure (1). The durability of a regimen is determined by carefully measuring the rate of disease recrudescence over 1 to 2 years in patients after completing chemotherapy. Under controlled conditions in the setting of a clinical trial, the rate of relapse with active disease using this four-drug combination is typically 2 to 4% (2), while under more typical conditions in national TB control programs, this rate can be as high as 10 to 14% (3, 4). Shortening the duration of treatment would greatly simplify the management of this disease by improving adherence, reducing the rate of adverse events, and reducing costs (5–7).

An inverse relationship between the risk of relapse and the killing of bacilli in the sputum of patients (culture conversion after 2 months of treatment) has been proposed. Although some risk of relapse is clearly related to culture positivity at 2 months, this association appears to be dependent on geography and extent of disease (8). An additional risk factor for relapse observed in larger phase 3 clinical trials is the presence of cavitory lesions at the time of initial diagnosis of disease (9). However, a clinical trial to shorten therapy to 4 months for patients who did not have cavitory disease by chest X-ray and were culture negative at 2 months was stopped early because of unacceptably high rates of relapse (10). Thus, while both of these biomarkers appear to be risk factors for relapse, even when combined, they are inadequate to predict therapeutic outcome in individual patients on regimens of shorter duration.

Animal models of experimental TB chemotherapy have been proposed to predict duration of therapy in TB patients (11–13). Standard mouse models develop none of the characteristic pathological features of TB in humans yet have been widely used in an attempt to predict treatment duration of novel combination regimens. For example, data from mouse models supported the idea that treatment duration could be substantially reduced by including a fluoroquinolone as a substitute for one of the weaker components of the four-drug standard regimen (13) and motivated the recent conduct of three large phase 3 studies to test this hypothesis. These trials recently reported full data, and the relapse rates in the 4-month treatment arms including a fluoroquinolone were all found to be unacceptably higher than those in the 6-month standard therapy arms (47–49). All three trials found

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that more patients receiving a fluoroquinolone converted their sputum culture to negative at 2 months (than patients on standard therapy), but despite this, they experienced higher relapse rates. These studies demonstrate that culture conversion is a poor surrogate for relapse and conclusively showed that treatment duration in the traditional mouse model is not predictive of requisite treatment duration in humans. The C3HeB/FeJ mouse model and the guinea pig model develop hypoxic lesions with central necrosis that can infrequently progress to liquefaction and feature high numbers of extracellular bacilli (14–16). The *Mycobacterium tuberculosis* infection in these mice appears significantly more refractory to drug therapy than standard *M. tuberculosis*-infected C57BL6 or BALB/c mice (14, 17). Sterilizing drug treatment of *M. tuberculosis* H37Rv-infected mice and guinea pigs has been achieved (15, 16), although it is not yet known if these models will be more predictive of human outcomes.

The lack of adequate predictive animal models and clinical surrogate endpoints for treatment duration in patients led us to develop a small nonhuman primate model for understanding drug action and better predicting treatment duration. We previously demonstrated that common marmosets (*Callithrix jacchus*) are highly susceptible to infection with multiple strains of TB and develop pathology similar to human tuberculosis, including cavity lesions (18). In addition, marmosets display more human-like pharmacology than rodents. Serial positron emission tomography (PET) and computed tomography (CT) scans of *M. tuberculosis*-infected animals and humans have been used to monitor disease progression and response to treatment (17–23), allowing precise quantitation of the extent of disease and inflammatory response of the host. These factors encouraged us to investigate two TB drug regimens known to differ in their ability to produce durable cures in patients. The first regimen is the current standard of care, a combination of four drugs (INH, RIF, PZA, and EMB, or HRZE), which shows very low relapse rates in clinical trials after 6 months and achieves sputum culture conversion rates of 70 to 80% at 2 months. We chose the combination of INH and streptomycin (SM) (combination HS) as the comparator regimen, which showed a nearly 30% relapse rate after 6 months of treatment with only half of patients achieving sputum culture conversion by 2 months (2, 24).

MATERIALS AND METHODS

Animals and ethics assurance. This study was carried out in accordance with the recommendations in the *Guide for the Care and Use of Laboratory Animals* (25). The NIAID Animal Care and Use Committee approved the experiments described herein under protocol LCID-9 (permit issued to NIH as A-4149-01). Common marmosets, bred by NIH, used in this study were between 2 and 5 years of age (median age, 3.3 years), of both genders, with a weight range of ~300 to 500 g. They were housed individually or paired in biocontainment cages in a biological level 3 animal facility approved for the containment of *Mycobacterium tuberculosis*. Efforts were made to provide intellectual and physical enrichment and minimize suffering.

Pharmacokinetic analysis of drugs in marmoset plasma and marmoset dosing. Plasma samples were extracted using methanol-acetonitrile (60:25) and analyzed using metronidazole as an internal standard in liquid chromatography-mass spectrophotometry (LC-MS) methods as described previously (26). Relevant pharmacokinetic (PK) parameters were calculated using a SAAMII (v1.2; University of Washington) two-compartment oral delivery model. The concentration of streptomycin in samples was determined by the Kirby-Bauer disc diffusion method using *Mycobacterium smegmatis* as a biological indicator strain and measuring

the zone of inhibition at 48 h postinoculation. Daily doses of the two regimens were given in compounding solutions as an oral suspension to the back of the throat (27) for ~44 doses. RIF was given 30 min before the combination of HZE. Streptomycin was given intramuscularly in alternating sites just before oral INH.

Bacterial strains and media. *M. tuberculosis* CDC1551 (GenBank accession no. AE000516) cultures were grown to mid-log phase and frozen in aliquots for aerosol infection as previously described (28). *M. tuberculosis*-bearing tissue samples and samples of the aerosol inoculum titrated to deliver 250 CFU/liter aerosol were plated in triplicate onto Middlebrook 7H11 plates as previously described (18). The marmosets were infected, monitored, and PET/CT scanned as previously described (18) in three sets of seven or eight animals. Within each set of animals, individuals were randomly assigned to the control, HRZE, or HS treatment groups.

PET/CT data analysis. PET/CT files were coregistered, and serial scans were deformed to a common volume in MIM Maestro (v. 6.2; MIM Software Inc., Cleveland, OH), and then a lung region of interest (ROI) was defined as previously described (18, 20). The lung ROI for each scan series was inspected and adjusted to manually remove regions of the heart, mediastinum, liver, and other structures revealed in the CT from the ROI. Finally, this common ROI was contracted 0.6 mm from all surfaces to exclude the pleural surface-organ interface, and this ROI was used in all calculations. Hounsfield unit (HU) histograms were analyzed using two ranges that represented primarily soft abnormal densities (−500 to −100 HU range) and hard densities (−100 to +200 HU range). [¹⁸F]2-fluoro-2-deoxyglucose (FDG) uptake or total activity (TA) for the lung voxels with a standardized uptake value (SUV) of ≥2 at baseline was calculated in order to eliminate the background activity of healthy lung parenchyma (1.0 to 1.6 SUV in uninfected animals with less than 2% of voxels having an SUV of >2) (18, 20). This modified ROI was assessed for change in TA in subsequent scans. Three-dimensional projections were reconstructed using Osirix v 5.9 software (Pixmeo, Geneva, Switzerland).

Necropsy and dissection. A necropsy plan was made using the PET/CT images for each animal so that major lesions, cavities, and samples of apparently uninvolved lung could be located. Not all animals had cavities identified by CT in the pre-necropsy scans (as lesions with very low-density cores), but all cavities identified were sampled. Each organ, lobe of the lung, and lesion in the lung was dissected with separate, sterile instruments. Logs describing the gross pathology observed and collection and division of lesions into matched samples for histology and bacterial burden were prepared as previously described (18, 20, 23). Once histology slides were available, hematoxylin-and-eosin-stained tissue sections were examined to look for eroded bronchioles as well as further classify lesions and regions of involved and uninvolved lung.

Statistical analysis. Longitudinal analyses of response to treatment based on CT and PET images were conducted using random-effect models in Stata 12.0 (to account for clustered observations), as were comparisons of log CFU within cavities (at necropsy). Primary analyses focused on differences between HRZE and HS. Analysis of variance (ANOVA) was used to compare the means of the treated groups to the mean of the control group. *P* values less than 0.05 were considered significant.

RESULTS

Pharmacokinetics of standard antituberculosis drugs in marmosets. First, we determined the pharmacokinetics of the five individual drugs in uninfected, healthy animals, following a single oral dose for the first-line drugs and via intramuscular injection for SM. Peak plasma drug concentrations (C_{max}) and 24-h exposure (area under the curve [AUC]) values measured for each drug are summarized in Table 1, along with published clinical PK parameters as a reference. After several test exposures to the drugs, the following doses were selected for the efficacy trials to match human exposure: 30 mg/kg INH (30 mg of INH per kg of body weight), 10 mg/kg RIF, 50 mg/kg EMB, 125 mg/kg PZA, and 20

TABLE 1 Pharmacokinetic parameters of first-line anti-TB drugs in marmosets in comparison with published clinical parameters^a

Drug	Marmoset data (this study)			Published data for humans				Reference(s)
	Dose (mg/kg)	C _{max} (μg/ml)	AUC (μg · h/ml)	Dose (mg) [mg/kg]	C _{max} range (μg/ml)	AUC range (μg · h/ml)	Normal C _{max} range 2 h postdose (TDM) (μg/ml)	
Isoniazid (INH)	30	5.0 (0.8–11)	11 (6–20)	300 [5]	2–15	11–33	3–6	34, 42
Rifampin (RIF)	15	17 (10–21)	191 (122–254)	600 [10]	1–15	20–60	8–24	36, 42
Ethambutol (EMB)	75	3.9 (2.4–5.2)	13.4 (12–14)	750 [12.5]	2–5	14–25	2–6	34, 43, 44
Pyrazinamide (PZA)	125	80 (37–128)	743 (284–1,237)	1,500 [25]	20–80	381–561	20–60	34, 45
Streptomycin (SM)	20	89 (85–95)	151 (141–158)	1,000 [16.7]	43 (2.9–85)	267 (175–343)	35–45	46

^a Data are median values (ranges shown in parentheses). TDM, therapeutic drug monitoring (37).

mg/kg SM. Due to different elimination kinetics of SM in marmosets and humans, both C_{max} and AUC could not be matched with one daily dose (Table 1). Since the efficacy of aminoglycosides is driven by peak plasma drug concentrations (29), a final SM dose of 20 mg/kg was selected to achieve a higher C_{max} than in humans—despite the lower AUC—to avoid underestimating the efficacy of the HS regimen. To confirm that the targeted exposure was achieved in marmosets on treatment at steady state, peak and trough (approximately 2 and 24 h postdose) plasma samples were collected monthly in a subset of treated animals. Overall, plasma levels of INH, PZA, and EMB were on target in treated marmosets. However, RIF levels were significantly lower at steady state in marmosets on treatment than in the single-dose PK study. At the 10-mg/kg efficacy dose, average peaks ranged from 1.0 to 1.7 μg/ml, while average troughs were 0.05 to 0.2 μg/ml.

Treatment of infected marmosets arrests the progression of TB disease. We infected 22 adult marmosets with *M. tuberculosis* strain CDC1551 via aerosol delivery as previously described (18). As expected, weight loss began in these animals about 4 weeks postinfection. On the basis of previous experience, we anticipated that the animals would lose 15 to 20% of their body weight by 8 weeks postinfection, so at 6 weeks, we initiated treatment in the animals randomized to receive chemotherapy and oral supportive care, and the remaining animals were given oral supportive care and handled similarly. The animal groups had similar mean numbers of primary TB lesions in the thoracic cavity (32 to 37 lesions, bilaterally distributed) and similar total disease volumes (3 to 4 ml), determined by PET/CT scan (see Table S1 in the supplemental material). At 6 weeks, CT did not usually reveal cavitory lesions; these became apparent as the necrotic tissue was cleared from the lesions as treatment progressed.

Within 2 weeks of starting treatment with either drug regimen, animals generally stopped losing weight and began regaining weight (see Fig. S1 in the supplemental material). Signs of illness, including listlessness, anorexia, and dehydration, also rapidly resolved. In contrast, control animals continued losing weight and by 9 to 10 weeks postinfection had to be humanely sacrificed. Chemotherapy was administered daily for 6 weeks (a total of 44 doses) after which the animals were humanely sacrificed and necropsied.

Monitoring response to treatment by computed tomography (CT). Animals received FDG-PET/CT scans prior to infection, 4 weeks after infection, immediately prior to treatment initiation, and every 2 weeks thereafter. An example of disease progression and resolution in the thoracic cavity is shown in Fig. 1. Prior to infection, the thoracic cavity contains very little material in the

radiodensity range of −200 to +200 Hounsfield units (HU) (a radiodensity scale benchmarked to physiological structures in the body, with air at −1,000 HU, water near 0 HU, and bone at about +1,000 HU). An animal before infection is shown in Fig. 1A. The top left panel shows voxels between −200 and +200 HU in the thoracic cavity (in red) that represents the larger vessels of the pulmonary vasculature. This figure also shows air present in the trachea and major bronchi in the left and right lungs at less than −950 HU (in blue) as well as the rib cage and spinal cord at >500 HU (in black). After 6 weeks of infection before starting chemotherapy, extensive pulmonary pathology due to TB infection is easily visible (Fig. 1A, bottom left panel). At this time, nearly half of the animal's lungs contain lesions resulting from infection. After 6 weeks of treatment, these lesions have extensively resolved (Fig. 1A, bottom right panel). The large region on the left in this scan represents the middle lobe of the right lung, which experienced a collapse resulting in significantly reduced volume.

These data can be analyzed quantitatively by coregistering the CT scans and then using the preinfection healthy lung as a template to isolate the entire volume of the lung of an infected animal. This analysis results in a histogram of voxel volumes by HU density (Fig. 1B). In the preinfection histogram, there is only a small amount of density above 0 HU, and this volume progressively increases until the pretreatment scan at day 42. Successive scans show the resolution of these lesions and provide a quantitative estimate of the rate of treatment resolution. We have previously correlated radiodensity between −100 and +200 HU (“hard density”) with specific radiological abnormalities, such as large nodules, consolidations, and cavities in human CT scans and shown that the resolution of these features correlates with treatment success (19). This analysis was extended to the entire cohort of animals, and the results are summarized in Fig. 2. Figure 2A shows the distribution of disease volumes in animals in each group at the time of therapy initiation ranging from 1 ml to 5 ml of lesion volume between −100 and +200 HU. The plots in Fig. 2B show the changes in these volumes during treatment for the entire group of animals on a log₂ scale. Treatment group and time were both statistically significant, indicating that there is a mean difference by treatment and a mean decline over time ($P = 0.035$) (Fig. 2C). A test for different slopes for HRZE and HS, which evaluated whether there was a different rate of change over time, was not significant. In contrast, control animals demonstrated considerable increases in hard volume that accelerated over time relative to both HRZE- and HS-treated animals ($P < 0.001$ for both).

FDG-PET monitoring of the response to treatment. Qualitative responses to treatment with the quadruple-drug regimen

FIG 1 Computed tomography (CT) can be used to monitor disease development and response to treatment. (A) The top left panel depicts the distribution of voxels in the -200 to 200 HU range in the thoracic cavity of marmoset BG22 (treated with HRZE) (red) excluding the heart and other obvious mediastinal structures, as well as the low-density voxels representing air at less than -950 HU (blue) and the high-density rib cage at >500 HU (transparent black). In the top right panel, the densities are subdivided to those below 0 (i.e., -200 to 0 HU in green) and those above 0 (0 to $+200$ HU in black) in the animal prior to infection. Both density ranges increase by 6 weeks postinfection (bottom left) and partially resolve after treatment for 6 weeks with HRZE (bottom right) (colored by the same HU scale as in the top right panel). (B) Histogram of whole-lung voxels (y axis, frequency of voxels where the whole lung = 1) distributed into bins by density (HU) in the same animal shown in panel A. Each trace represents the distribution of voxels at the time point indicated by the number of days postinfection that the scan was collected (numbers to the right of the traces). Treatment was initiated at day 42 and was indicated by an arrow labeled Rx. A peak appeared at about 100 HU at the time lesions become apparent in the CT scan and resolved upon treatment.

were obvious as early as 2 weeks after administration of the first dose. Figure 3 provides an illustration of this difference in twin animals. The top twin shows a dramatic reduction in the total amount of FDG retained in the lesions at 2 weeks of therapy that continues on at 4 weeks with little remaining inflammation still visible at 6 weeks. In contrast, this animal's sibling was treated with HS resulting in little change in FDG avidity at 2 weeks, modest changes at 4 weeks and more significant reduction only after 6 weeks of treatment.

FIG 2 Treatment of *M. tuberculosis*-infected marmosets with HRZE reversed accumulation of hard (-100 to 200 HU) lung volume more rapidly than HS. (A) The animals began treatment with a range of disease volumes, but the groups had similar lung volumes (HRZE, gray bars; HS, black bars; control, white bars). (B) Change in hard disease (-100 to 200 HU) volume ($\log_2$) was computed for each animal after 2, 4, and 6 weeks of treatment. Some individual animals responded to treatment more slowly than others. (C) The mean $\log_2$ hard disease volume and 95% confidence interval (95% CI) for each group and time point were determined. Starting at week 2, the treatment groups were significantly different ($P = 0.035$ for HRZE versus HS [*]; longitudinal random-effect model). Changes accelerated between untreated and treated animals ($P < 0.001$; for control versus HRZE and HS [***], longitudinal random-effect model).

FIG 3 HRZE treatment reduces FDG uptake more rapidly than HS treatment in *M. tuberculosis*-infected marmosets. The three-dimensional (3D) reconstructions of twin marmosets, marmoset BG22 on HRZE (top panels) and marmoset BG21 on HS (bottom panels), are shown. Projections used a standardized scale with a maximum SUV (a time- and weight-corrected radiation intensity) of 10 for each image. In the chest, the heart (H) uptake is variable, but both lymph nodes (white arrowheads) and lung lesions (white arrows) are visible and traceable over time. WK PI, weeks postinfection.

FDG-PET uptake was quantified in these animals by identifying the lung ROI voxels with an SUV of ≥ 2 at baseline and computing the total activity in these voxels in subsequent aligned scans (Fig. 4A). A cutoff of 2.0 was employed, as less than 2% of voxels in segmented lungs in uninfected animals have an SUV of > 2 (background lung uptake ranges from 1.0 to 1.6). Similar to the CT findings, the TA in affected lung showed a range of FDG uptake at baseline but with a similar distribution across the three groups. Significant decreases in TA were observed for both HRZE- and HS-treated animals ($P < 0.001$), with HRZE demonstrating the greatest initial decline ($P = 0.049$) (Fig. 4C). In contrast, control animals demonstrated considerable increases in TA that accelerated over time relative to both HRZE- and HS-treated animals ($P < 0.001$ for both).

Bacteriologic responses to treatment. Following a predetermined necropsy plan based on the CT scans, the lungs, spleen, liver, and lymph nodes of the animals were dissected, and bacterial burden was measured. The lung samples were grouped for analysis into cavitory or granulomatous lesions or apparently uninvolved lung. Both treatment regimens resulted in statistically significant 3-log-unit reductions in the burden of organisms in granulomas and uninvolved lung tissue (Fig. 5A and B). Compared to controls, both regimens effectively reduced bacterial burden in the liver, spleen, and lymph nodes (data not shown) with the majority of these samples being sterile. Only in the spleen was HS significantly better than HRZE in reducing bacterial numbers ($P < 0.05$).

The major difference between the two regimens was on organisms in cavitory lesions; 12/13 (92%) of cavities from five animals treated with HS still had culturable organisms, while 10/18 (55%) of cavities from six HRZE-treated animals were nonsterile, and the total bacterial burden within the cavities of these animals was significantly lower than in the animals treated with the two-drug regimen ($P = 0.009$). Cavities were visible radiographically and by histology (Fig. 5B and D) and displayed a characteristic pattern of radiodensity by CT in cross section with hard walls surrounding

an area of low-density air (Fig. 5C). Most cavities were not identifiable by CT at the time treatment was initiated; however, our previous studies of untreated animals revealed that early in cavity formation, liquefaction can occur without drainage of the central necrosis, and we have previously observed the same phenomenon in rabbits and humans with TB (23).

DISCUSSION

Our results demonstrate that marmosets with TB can be treated effectively with combination chemotherapy regimens used in human clinical trials and that such treatment arrests and reverses disease progression in this species. Radiographic surrogate markers are being actively explored for use in human clinical trials of new agents and regimens. In small cohorts of multidrug-resistant (MDR) patients, these markers have shown considerable potential in predicting treatment outcome as early as 2 months after the start of multidrug therapy (19), and parallel studies of cynomolgus macaques and humans have shown comparable rates of radiological response to linezolid (20). In this study, we show that as early as 2 weeks after the initiation of therapy, we can distinguish a regimen that is highly effective in achieving relapse-free cure (the four-drug combination that is the standard of care for drug-sensitive disease) from one that is much less effective (the two-drug combination of INH and SM) based upon a more rapid resolution of diseased lung volume on CT scan and a similar, more rapid decrease in disease-associated inflammation in FDG-PET within the thoracic cavities of infected marmosets.

Since the shortest chemotherapy studies in humans (3 months of SM, INH, RIF, and PZA) showed 20% relapse rates (but 91% sputum culture conversion at 2 months) (30), it is not surprising that none of the animals in this study were cured by 6 weeks of chemotherapy. Our results demonstrate a greater radiological clearance of both pathology and disease-related inflammation in the group treated with the quadruple-drug regimen. We treated animals for 6 weeks to avoid sterilizing animals completely so that we could look for an association between the types of pathology

FIG 4 FDG total activity in treated animals. (A) TA of each animal's lung ROI at baseline is presented; baseline TA values were not different between groups (HRZE, gray bars; HS, black bars; control, white bars). (B) Log₂ change in TA above SUV 2 measured at baseline was calculated for each animal at 2, 4, and 6 weeks. (C) Mean log₂ change and 95% CI for each group and time point were determined. Reductions differed between HRZE and HS starting at week 2 ($P = 0.049$ for HRZE versus HS; longitudinal random-effect model). There was no evidence of a different slope for HRZE and HS, as indicated by a lack of statistical significance for a test of interaction between time and treatment group (HRZE or HS). Changes accelerated between untreated and treated animals ($P < 0.001$; control versus HRZE and HS; longitudinal random-effect model).

observed and the rates of sterilization between the two drug regimens. In fact, we observed that both regimens treated simple granulomatous lesions equally well. In extrapulmonary sites of infection, the SM-containing regimen showed greater reductions in bacterial burden in the spleen, although the significance of this is not clear. In contrast, the quadruple-drug regimen sterilized cavitory lesions significantly better. Nearly half of the cavities in HRZE-treated animals were sterile after 6 weeks of treatment compared to only 8% of cavities in animals treated with HS. This finding is critically important because cavities are an independent risk factor for relapse disease in large phase 3 studies (9, 31), and the sterilization of cavities, therefore, may be the reason why HRZE cures patients better than HS. Other important risk factors include bilateral abnormalities and failure to convert sputum culture by 2 or 3 months. Combined cavitation and sputum culture conversion can significantly predict relapse in retrospective analyses, although prospectively they have had limited value thus far (10, 32). Cavitory disease, of course, does not occur alone and tends to be associated with more severe disease manifestations. Human TB patients show highly variable initial extents of disease and varied predispositions to form cavities. These considerations reveal the complexity of predicting sterile cure in TB patients, and while it may be true that 80% of TB patients are cured within the first 3 months (33), identifying the 20% who are not cured has proven challenging.

Pharmacokinetic monitoring of marmosets on treatment revealed low peak and trough concentrations of RIF. Low RIF levels are commonly observed in close to half of TB patients (34–37), where the normal peak range is 8 to 24 $\mu\text{g/ml}$ (38). While the phenomenon is not fully understood and likely multifactorial, formulation, autoinduction of cytochrome P450 (CYP)-mediated metabolism, and complex drug-drug interactions are thought to contribute to decreased plasma levels of RIF at steady state (39–41). Thus, lower-than-normal RIF levels during treatment may have led us to underestimate the effects of HRZE treatment and the superiority of HRZE over HS, reinforcing the conclusion that HRZE achieves better outcomes than HS in marmosets as it does in humans. We considered whether this was simply due to the failure of SM to penetrate cavitory lesions. Two rabbits with active cavitory TB received a single 20-mg/kg dose of intramuscular SM 3 h prior to measuring drug levels in uninvolved lung, closed nodules, whole cavities, liquefied cavity caseum, and cavity wall. Peak plasma drug concentrations were reached 45 min postdose. At 3 h postdose, the SM concentrations in fibrotic/necrotic lesions, cavity walls, and small cavities reached 25 to 60% of plasma SM concentrations. Interestingly, the highest SM concentrations were measured in the liquefied caseum of two large cavities, with caseum/plasma ratios of 1.2 to 1.5. The results indicate that SM distributes favorably into the lesions types sampled here.

Marmosets offer an attractive model to identify new combination chemotherapies that may shorten the duration of antituberculosis chemotherapy. Their small size, disease susceptibility, and the pathology of the underlying disease all suggest that this animal could be a predictive model of the expected response in humans, although definitive experiments to measure the corresponding relapse rates are necessary. The results presented here show that marmosets treated with a sterilizing regimen resolve disease faster as measured by both pathological (CT) and immunologic (PET) responses compared to marmosets treated with a nonsterilizing regimen. In addition to this higher rate of disease resolution, the

FIG 5 HRZE treatment was more effective in reducing the *M. tuberculosis* bacterial load in cavities than HS. (A) Both regimens significantly reduced the bacterial burden in individually dissected lesions in all treated animals compared to control (****, $P < 0.001$ by ANOVA), but HRZE sterilized cavities more effectively than HS (***, $P = 0.009$; random-effect model). In both panels A and B, symbols in the graph indicate that measurable CFU were present and are real values, × symbols on the graph indicates samples for which no CFU were obtained and were assigned a value based on the limit of detection for that particular sample. Each symbol represents the value for an individual animal or lesion, and the black bar shows the mean value for the group of measurements. (B) Both regimens significantly reduced the bacterial burden in apparently unaffected lung, spleen, and liver (****, $P < 0.001$ by ANOVA), although HS appeared to be more effective in killing bacteria in the spleen than HRZE (**, $P < 0.01$ by ANOVA). (C) Axial CT image of a cavity in an HRZE-treated marmoset after 6 weeks of treatment. (D and E) Line profile through the center of the cavity (shown in panel C) depicting the HU density (D) and a cavity lesion (E) from the same HRZE-treated marmoset stained with hematoxylin and eosin (10×). The cavities in panels C and E are indicated by a black arrow.

sterilizing regimen is associated with improved clearance of viable organisms from cavitory lesions. It remains to be seen whether this model is predictive of human therapeutic responses to other classes of agents, but these results suggest that marmosets may offer an attractive intermediate step prior to initiating expensive phase 3 clinical trials for TB drug development.

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We declare that we have no conflicts of interest.

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