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Supplementary Materials for

Pharmacokinetics of rifapentine and rifampin in a rabbit model of tuberculosis and correlation with clinical trial data

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Supplementary Materials

Materials and Methods

Preparation of drug Rifampin or rifapentine (Sanofi) was dissolved in a vehicle solution (methods adapted from Sanofi intravenous formulation instructions), which contained 50 ml of 100 mM sodium bicarbonate, 49 ml distilled water, 1 ml ethanol and 0.1 g sodium ascorbate (pH 10.5, adjusted with 10 M sodium hydroxide) (*47*). Drug solution was prepared in a flask covered with aluminum foil and stirred by a magnetic bar on a magnetic platform at 4°C. Drug solutions were filter-sterilized with a 0.22 µM filter before dosing. Rifampin or rifapentine was delivered to rabbits by intravenous administration using a disposable spring driven beeline infusion pump (100 ml) and beeline tubing (50 ml/hours or 100 ml/hours) (Moog Medical devices) at a constant rate over the course of dosing. Briefly, rabbits were anaesthetized with an intramuscular injection of ketamine (17-35 mg/kg), xylazine (5-10 mg/kg), and acepromazine (0.75 mg/kg). A catheter for drug delivery was placed in the rabbit marginal ear vein followed by attachment of microclave to seal the catheter. After fixation with tegaderm tape and “Y” tegaderm patches (Henry Schein), the catheter was flushed with 3 ml of sterile Ringer's lactate to ensure the taping setup was not restricting blood flow, followed by flushing with 300 units of heparin (Henry Schein) and an additional 3 ml of Ringer’s lactate. The catheterized marginal ear vein was connected to an infusion pump coupled with beeline tubing (50 ml/hour for rifampin and 100 ml/hour for rifapentine); the pump was previously filled with the corresponding drug solution. Despite minor differences in rabbit body weight, drug solutions were administered based on a body weight of 4 kg during the study. A central arterial catheter was also placed for blood collection for PK sampling. In order to maintain blood flow, the catheters were flushed with 300 units of heparin and 3 ml of Ringer’s lactate after drug administration and following each blood draw.

Sample collection and processing

For blood collection for PK, 1 ml of blood first collected from the catheterized central ear artery was always discarded. An additional 1 ml of blood was then distributed into a lithium heparin coated microcontainer (Capijet) and then inverted 10 times to ensure proper mixing of blood and lithium heparin prior to placing the sample on ice. Plasma was obtained by centrifugation at 13,000x RCF for 2 min prior to filtering through a 0.22 µm filter and transfer to a clean 2 ml screw cap tube.

Three to six small pieces of tissue from each compartment of diseased lungs, as described previously, were dissected during lung necropsy and deposited into 2 ml screw cap tubes on dry ice. Then, tissue samples were precisely weighed and recorded prior to addition into the 2 ml screw cap tubes of 2 mm glass beads and 70% methanol. Following homogenization on a homogenizer (Precellys) for 3 cycles that consisted of bead beating at 7200 rpm for 30 seconds and resting for 1 min on ice, the homogenates were obtained by centrifugation at 13,000 rpm at 4°C for 5 min and filter-sterilized to clean 2 ml screw cap tubes. All the plasma and lung homogenates were stored at -80°C for LC-MS/MS analysis.

PET-CT imaging of infected rabbits

^{11}C-rifampin was synthesized at the Johns Hopkins PET Center using a modification of the methods of Liu et al (30), as previously described (31). A rabbit that was infected bronchoscopically in a manner intended to produce cavities that were not in the right lower lung (to ensure signal could be measured in the pulmonary TB lesions without interference from liver signal) was used to perform PET/CT.^{11}C has a half-life of 20.4 min, so a catheter system was set up for on-table intravenous (marginal ear vein) administration of the tracer, as described previously (31). A dose of 108.04 MBq of ^{11}C-rifampin was administered intravenous on-table to

the rabbit followed immediately by a dynamic PET performed over 55 min using the Mosaic HP PET small animal imager (Philips, Bothell, WA) following this acquisition protocol: 10 one- min, 5 two-min, and 7 five-min windows post injection. CT images were obtained with the CereTom CT scanner (NeuroLogica, Danvers, MA). Images were reconstructed and co-registered with CT images using Amide 1.0.4 (http://amide.sourceforge.net), and standardized uptake values (SUV) were computed. Using the co-registered CT images as a reference, spherical regions of interest (ROI) were manually drawn in the liver, heart (left ventricle), and lung (healthy and cavitary tissues) and applied to the PET images. Correction for partial volume effects was applied to all imaging data using Amide. Correction was applied to compensate for the decay of ^{11}C-rifampin over the 60 min of imaging. Lung segmentation and three-dimensional visualization were done using VivoQuant 2.5 (inviCRO, Boston, MA, USA).

LC/MS-MS analytical methods.

Plasma and lung tissue were collected from healthy rabbits for assay development and validation. Healthy rabbits that had not received study drug were necropsied, and lung tissue was harvested, and homogenized in 70% methanol as described above. Tissue homogenates were spiked with rifampin and rifapentine to generate QC materials for drug quantification in various tissue compartments. The analytical methods employed did not differ by tissue compartment; rifampin and rifapentine were quantified from a plasma standard curve. QCs were tissue-specific, but did reflect each subcompartment analyzed.

LC-MS/MS was used for rifampin and rifampentine quantification in plasma and tissue. Briefly, 0.020 ml of plasma or tissue homogenate was combined with 0.1 ml of the structural analog $^{2}H_{6}$-rifamixin (RIX-d6) and 0.5 ml of acetonitrile containing 0.5 mg/ml ascorbic acid. Samples were then processed using Captiva protein precipitation plates (Agilent Technologies, Santa Clara, CA). Following elution, 0.5 ml water containing 5 mM ammonium formate and 0.5 mg/ml

ascorbic acid was added to the eluent; 1 µl of material was then introduced in an API QTRAP 5500 (SCIEX, Foster City, CA) mass analyzer interfaced with a Waters Acquity UPLC system (Waters Corporation, Milford, MA). The mass analyzer was operated in positive ionization and selective reaction monitoring (SRM) modes. Transitions monitored were as follows: rifampin: 823.3 → 791.4 *m/z*; RPT 877.6 → 845.5 *m/z*; RIX-d6: 792.2 → 760.3 *m/z*. The analytical measuring range for both analytes in both matrices is 50 – 80,000 ng/ml. Samples below the lowest calibrator (50 ng/ml) were reported as below the quantitative limit for analysis. Notably, tissue samples, which were quantified from a plasma calibration curve (ng/ml), were converted to ng/mg by dividing the observed value by the tissue weight per volume lysed. For example, 25 mg tissue lysed in 0.4 ml of 70% methanol, which was optimized in this study to extract drug from healthy and diseased lung tissue, would yield a concentration of 62.5 mg/ml. If a sample contained 10,000 ng/ml rifampin, the value would be divided by 62.5 mg/ml, resulting in a final concentration of 160 ng rifampin/mg tissue. Rifampin or rifapentine yield was the similar among the tissues collected from different locations in a healthy rabbit lung (data not shown).

MALDI-MSI

MALDI-MSI was performed as previously described (2). In brief, rabbit lung biopsies were sectioned at 12 µm thickness using a Leica CM1850 cryostat (details) and thaw mounted onto stainless steel slides (MALDI-MSI) or standard glass microscope slides (histology). 3 ml 50% methanol containing 3 pmol/µl rifampin-D3 (TRC, Toronto) was applied to the surface by airspray deposition at 40 p.s.i., followed by 15 mg/ml 2′ ,4′ ,6′ -trihydroxyacetophenone monohydrate (Acros Organics, Morris Plains, NJ) (50% methanol). The airbrush was positioned at a distance of 30 cm from the tissue, and 20 passes over the tissue were performed, with the tissue being allowed to dry for 30 seconds (s) between coatings.

MALDI-MSI acquisition was performed using a MALDI LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) with a resolution of 60,000 at *m/z* 400, full width at half maximum. Imaging data were acquired in full scan mode to maximize sensitivity, and drug peak identities were confirmed by acquiring several MS/MS spectra directly from the dosed tissues. Limits of detection (LOD) for rifampin and rifapentine were 410 ng/g and 380 ng/g calculated as described previously (6). Spectra were acquired across the mass range *m/z* 500-950 with a laser energy of 20 μJ and 20 shots per position (1 microscan per position). The laser step size was set between 75-100 μm (based upon the dimensions of the tissue), and total analysis time for each tissue section was between 9-22 hour.

2D ion images were generated using Thermo ImageQuest software (v1.01). Normalized ion images of rifampin were generated by dividing rifampin $[M-H]^-$ signal (*m/z* 821.397 ± 0.003) by rifampin-D3 $[M–H]^-$ signal (*m/z* 824.416 ± 0.003). Normalized ion images of RPT were generated by dividing RPT $[M-H]^-$ signal (*m/z* 875.444 ± 0.003) by rifampin-D3 $[M–H]^-$ signal (*m/z* 824.416 ± 0.003).

Fine alignment of the optical tissue image (prior to matrix acquisition) and the MS image was performed using the total ion chromatogram (TIC) MS image. To prevent bias, caseum and cellular lesion areas were drawn using the optical tissue image as a mask without exposure to the extracted ion images of rifampin or rifapentine.

Mathematical modeling

The model-building process was detailed as below:

(i) Rabbit plasma PK model**:** One- and two-compartment models with a first-order elimination, parameterized in terms of clearance (CL), volume of distribution (Vc),

intercompartmental clearance (Q), and peripheral volume of distribution (Vp), were fitted to the data. All doses were given per kg of body weight and were modeled as such, given the narrow weight range in this experimental animal population. Between-subject variability was allowed on plasma clearance and assumed to be log-normally distributed.

(ii) Residual error model. Several models describing the residual variability were investigated: additive and proportional error models and a slope-intercept model. Different residual errors were evaluated for plasma and lung lesions.

(iii) Tissue penetration model. Lesion penetration was described using effect compartment models similar to those of Sheiner (Fig. S5), with the following equation:

$$\frac{dC_{lesion}}{dt} = k_{plasma-lesion} \times (PC_{lesion} \times \frac{A_{plasma}}{V_{plasma}} - c_{lesion})$$

where C is concentration, $k_{plasma\text{-}lesion}$ is the rate constant for the transfer of drug from the plasma to lung lesion, PC_{lesion} is the penetration coefficient between plasma and lung lesion and A_{plasma}/V_{plasma} is the concentration of drug predicted in the plasma compartment at time t with A_{plasma} being the amount of drug in plasma and V_{plasma} the apparent volume of the plasma compartment. Between-subject variability was investigated on the parameters belonging to the lesion penetration model.

(iv) Model evaluation. Standard goodness of fit plots and visual predictive checks (VPCs) were performed to evaluate the appropriateness of the final model.

(v) Translational model. Site-of-action PK derived from the animal model was substituted into a previously developed PK/PD model comprising clinical data from Tuberculosis Trials Consortium (TBTC) studies 29 and 29X, with PK/PD analyses performed as previously described (4,5,8). In brief, to describe the time to stable

sputum culture conversion in patients with pulmonary TB, a parametric survival function was used, according to the equation:

$$S_t = e^{-\int_0^t h(t)d(t)}$$

The hazard was h_t, and the survival S_t was a function of the cumulative hazard from time 0 to time t describing the probability of not converting the culture to negative within this time interval. The base model was developed by exploring different functions for the hazard h_t, starting from a simple time-independent constant hazard and gradually progressing to more complex functions, including Weibull function according to the equation:

$$h_t = h_0 \gamma (h_0 t)^{\gamma - 1}$$

where h_0 was baseline hazard at time 0 and γ was a shape parameter.

Model building was guided by the likelihood ratio test, diagnostic plots, and internal model validation techniques, including visual and numeric predictive checks. Data were analyzed using a nonlinear mixed-effects approach with software (NONMEM, version 7, ICON, Dublin, Ireland).

Individual post hoc Bayesian estimates of PK parameters, including AUC and C_{max}, were derived from the population PK model of rifapentine developed earlier. For subsequent PK modeling, individual AUC from participants in Study 29 were adjusted to account for drug administration on 5 of 7 days per week, compared with participants in Study 29X in which drugs were administered 7 days per week. Site of action RPT PK variables were derived combining penetration ratios obtained from the rabbit experiments and patients' individual plasma PK measurements (e.g. if patient had cavities on the chest radiograph, his/her individual exposure was multiplied by the cavity/plasma penetration ratio). In the next step, individual PK

parameters were incorporated in the survival model parameters (baseline hazard). Increase in site of action PK was significantly (P value= $<10^{-7}$) associated with increased baseline hazard of culture conversion to negative (e.g. shorter time to stable culture conversion) and this relationship was best described by an Emax model.

$$h_0 = h_0 \times (1 + \frac{E_{max} \times AUC_{site-of-action}}{AUC_{site-of-action} + AUC_{50}})$$

(vi) Software. Data were analyzed using the first-order conditional estimation method as implemented in the software NONMEM, version 7.3 (ICON Development Solutions, Ellicott City, MD). Graphical, statistical, and exploratory analyses were conducted using the R package (version 2.11.1), while Xpose (version 4.0) was used for data set checkout and graphical evaluations of the modeling output. Visual predictive check (VPC) were performed using PsN software (version 3.2.12), and scripts were created using R.

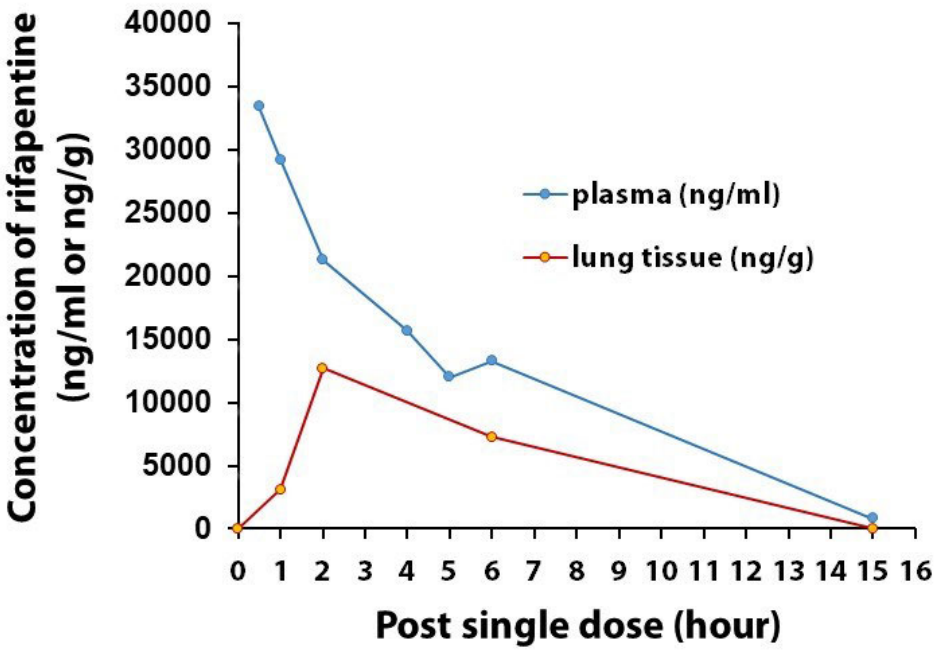

Fig. S1. Concentration-time curve in plasma and lung tissue after a single dose of 30 mg/kg rifapentine administered to healthy rabbits. Blood was collected at different time points for rifapentine concentrations in plasma. Healthy rabbits were sacrificed at 1, 2, 6 and 15 hours post intravenous injection, respectively followed by dissecting 4-6 pieces of lung tissues to measure rifapentine concentrations. Concentrations of rifapentine were measured by LC-MS/MS prior to calculation of the means of the drug concentrations at each time point. In plasma C_{max}, $T_{1/2}$ and AUC_{0-15} were 33 µg/ml (0.5 hour), 4 hours and 170 µg hour/ml. In lung tissue C_{max}, $T_{1/2}$ and AUC_{0-15} were 13 µg/ml (2 hours), 6.7 hours and 82 µg hour/ml.

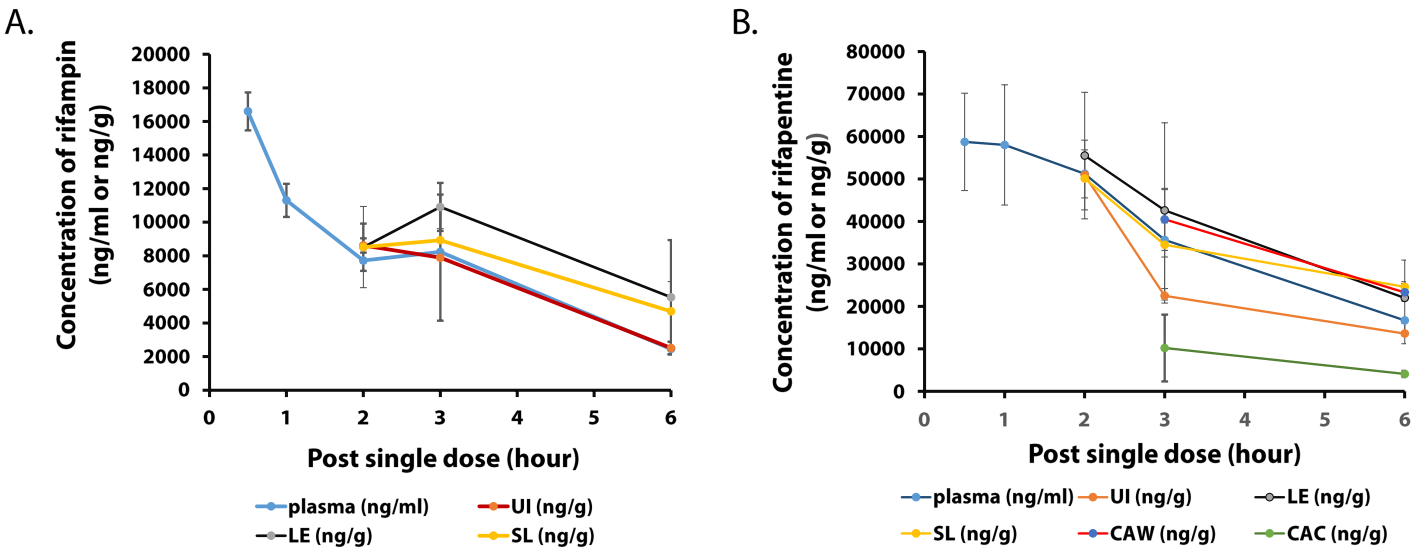

Fig. S2. Concentration-time curve for rifampin and rifapentine in plasma and lung lesions after a single drug dose in rabbits with pulmonary cavitary TB. A single dose of 10 mg/kg of rifampin (A) and 30 mg/kg of rifapentine (B) was intravenously injected into rabbits with cavitary lung disease, respectively, followed by collection of blood at different time points and sacrifice at 2, 3 and 6 hours post injection. Concentrations of rifampin and rifapentine were measured by LC-MS/MS in plasma and different compartments of the lung tissues including uninvolved lung tissues (UI), cellular lesions (LE) and tissues surrounding the lesions (SL), cavity wall (CAW) and cavity caseum necrotic center (CAC). C_{max}, $T_{1/2}$ and AUC_{0-6} in plasma and lung tissues were shown in Table S2, except of that in CAW and CAC due to incomplete data.

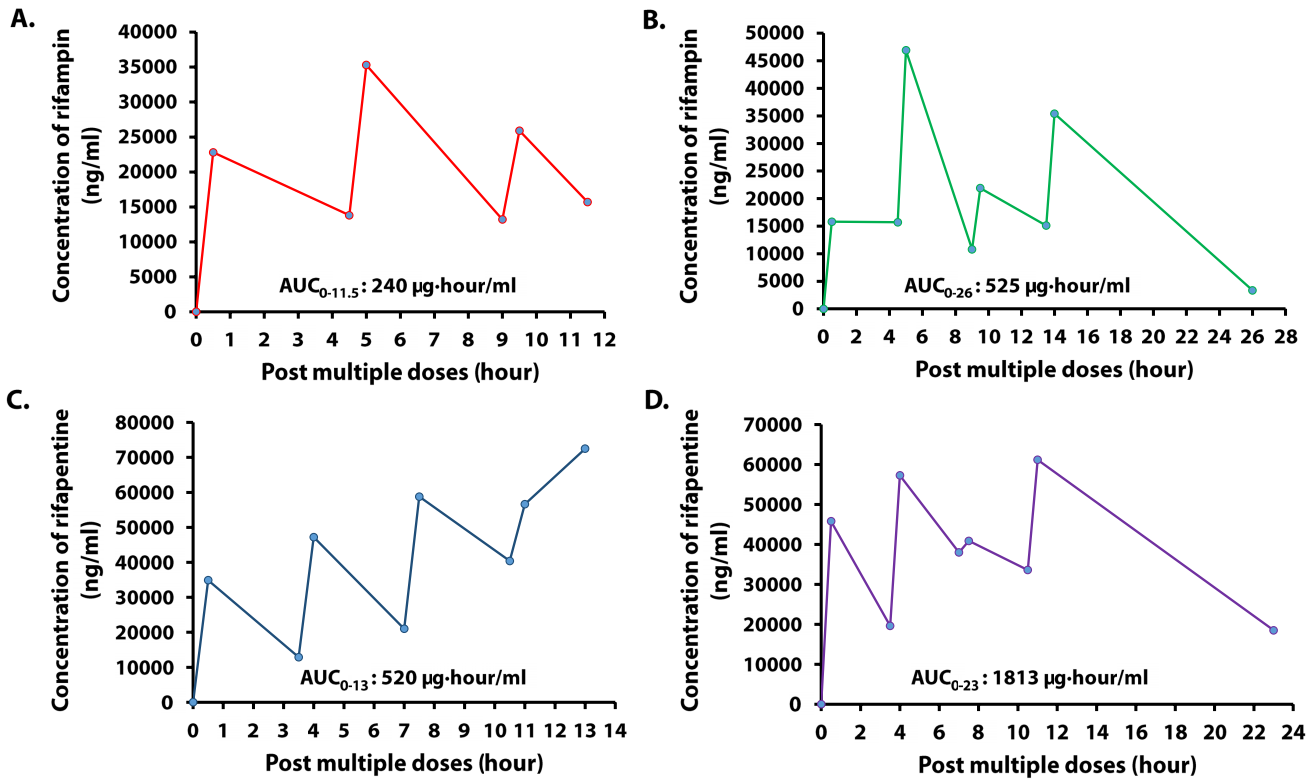

Fig. S3. Concentration-time curve for rifampin or rifapentine in plasma after multiple doses in rabbits with cavitary TB sacrificed 2 hours or 12 hours after the final drug dose.

A. 10 mg/kg of rifampin was given to rabbits three times with an interval of 4 hours followed by sacrifice at 2 hours after the final dose; B. 10mg/kg of rifampin was given to rabbits four times with an interval of 4 hours followed by sacrifice at 12 hours after the final dose; C and D. 20mg/kg of rifapentine was given to rabbits four times with an interval of 3 hours followed by sacrifice at 2 hours and 12 hours, respectively, after the final dose. Blood was collected at 30 min. post dosing and at the end point of each interval prior to next dose. The concentrations of rifampin and rifapentine in plasma were measured by LC-MS/MS followed by calculation of AUC.

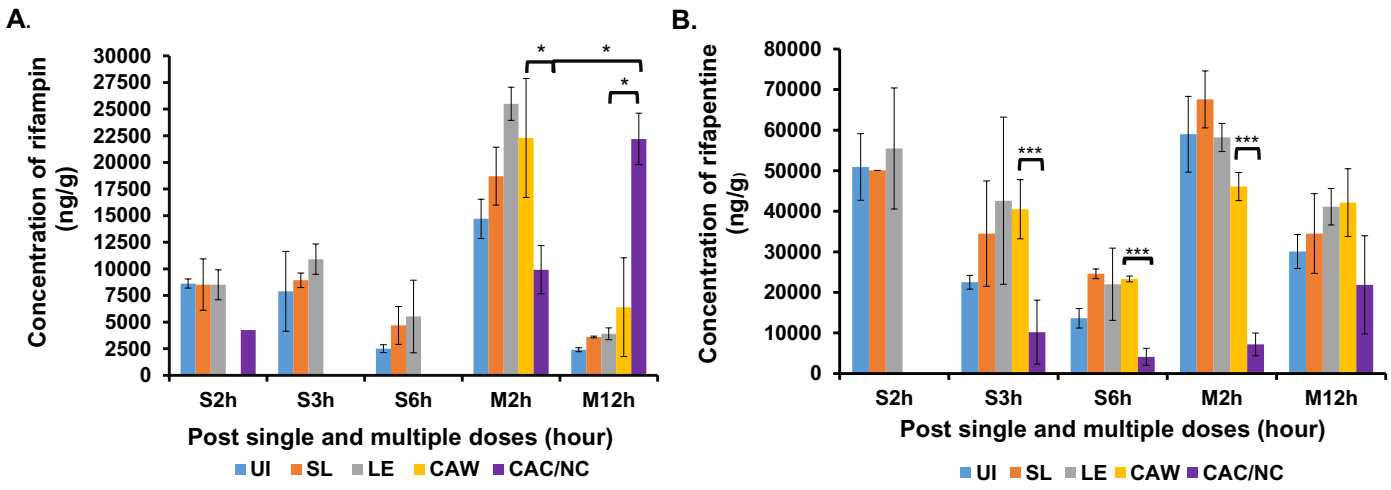

Fig. S4. Absolute drug concentrations of rifampin or rifapentine in different compartments of lung lesions after single or multiple doses in rabbits with pulmonary cavitary TB. A. 10mg/kg of rifampin was given to diseased rabbits one time followed by sacrifice at 2, 3 and 6 hours post dose (denoted as S2h, S3h and S6h), three times with an interval of 4 hours followed sacrifice at 2 hours after the final dose (M2h), and four times with an interval of 4 hours followed by sacrifice at 12 hours after the final dose (M12h). B. 30 mg/kg of rifapentine was given to diseased rabbits one time followed by sacrifice at 2, 3 and 6 hours post dose (denoted as S2h, S3h and S6h); 20 mg/kg was given to diseased rabbits four times with an interval of 3 hours followed by sacrifice at 2 and 12 hours post the final dose (M2h and M12h). 3-6 pieces of lung tissues were subjected to measure absolute concentrations of rifampin and rifapentine by LC-MS/MS, dissected in different compartments of diseased lungs including uninvolved lung tissues (UI), tissues surrounding the lesions (SL), cellular lesions (LE), wall of cavitary lesions (CAW) and cavitary caseum and necrotic center (CAC/NC). The means of the drug concentrations were calculated followed by student's *t*-test for statistical analysis (two-tailed). * $P < 0.05$; *** $P < 0.001$.

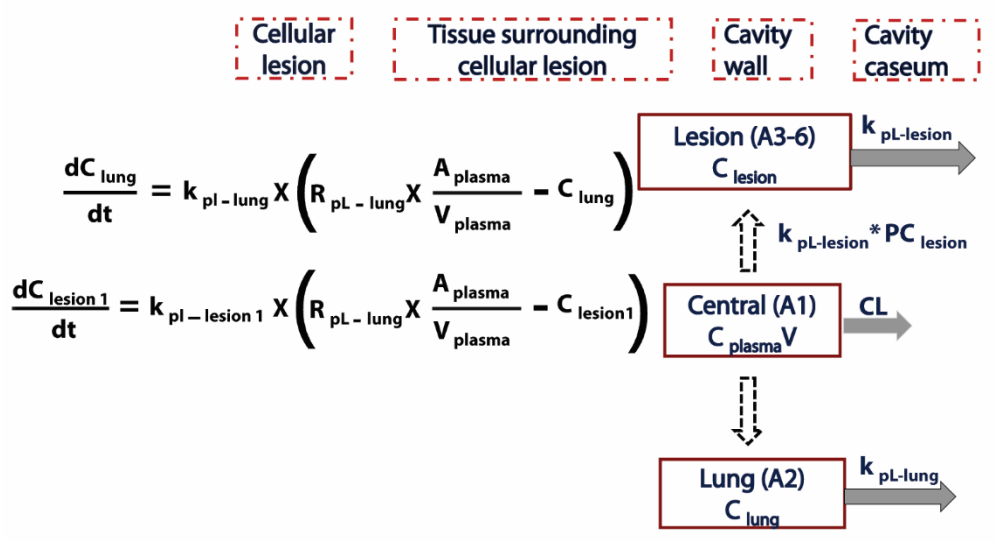

Fig. S5. PK model describing tissue penetration of rifampin and rifapentine.

Table S1. PK parameters of rifampin and rifapentine in plasma and in lung lesions after a single drug dose.

Plasma and lung tissue	Rifampin			Rifapentine		
	C_{max} (μg/ml or μg/g)	$T_{1/2}$ (hour)	AUC_{0-6} (μg·hour/ml)	C_{max} (μg/ml or μg/g)	$T_{1/2}$ (hour)	AUC_{0-6} (μg·hour/ml)
Plasma	17	1.8	45	59	4.2	220
UI	8.6	5.2	32	51	2.8	140
LE	11	6	43	56	4	201
SL	8.9	6.1	38	50	6	181