A Dose-Finding Study to Guide Use of Verapamil as an Adjunctive Therapy in Tuberculosis

Chandrasekaran Padmapriyadarsini[1,*,†], John D. Szumowski[2,†], Nabila Akbar[1], Prema Shanmugasundaram[1], Anilkumar Jain[3], Marasamy Bathragiri[4], Manoranjan Pattnaik[5], Jyotirmayee Turuk[6], Ramesh Karunaianantham[1], Senthilkumar Balakrishnan[1], Sanghamitra Pati[6], A. K. Hemanth Kumar[1], Manoj Kumar Rathore[7], Jegadeesh Raja[7], K. Raghu Naidu[7], John Horn[8], Laura Whitworth[9,10], Roger Sewell[11], Lalita Ramakrishnan[9,10], Soumya Swaminathan[1,13,‡] and Paul H. Edelstein[9,12,*,‡]

Induction of mycobacterial efflux pumps is a cause of *Mycobacterium tuberculosis* (Mtb) drug tolerance, a barrier to shortening antitubercular treatment. Verapamil inhibits Mtb efflux pumps that mediate tolerance to rifampin, a cornerstone of tuberculosis (TB) treatment. Verapamil's mycobacterial efflux pump inhibition also limits Mtb growth in macrophages in the absence of antibiotic treatment. These findings suggest that verapamil could be used as an adjunctive therapy for TB treatment shortening. However, verapamil is rapidly and substantially metabolized when co-administered with rifampin. We determined in a dose-escalation clinical trial of persons with pulmonary TB that rifampin-induced clearance of verapamil can be countered without toxicity by the administration of larger than usual doses of verapamil. An oral dosage of 360 mg sustained-release (SR) verapamil given every 12 hours concomitantly with rifampin achieved median verapamil exposures of 903.1 ng.h/mL (area under the curve $(AUC)_{0\text{-}12h}$) in the 18 participants receiving this highest studied verapamil dose; these AUC findings are similar to those in persons receiving daily doses of 240 mg verapamil SR but not rifampin. Moreover, norverapamil:verapamil, R:S verapamil, and R:S norverapamil AUC ratios were all significantly greater than those of historical controls receiving SR verapamil in the absence of rifampin. Thus, rifampin administration favors the less-cardioactive verapamil metabolites and enantiomers that retain similar Mtb efflux inhibitory activity to verapamil, increasing overall benefit. Finally, rifampin exposures were 50% greater after verapamil administration, which may also be advantageous. Our findings suggest that a higher dosage of verapamil can be safely used as adjunctive treatment in rifampin-containing treatment regimens.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ Verapamil metabolism is greatly accelerated during rifampin administration, limiting the potential use of adjunctive verapamil therapy in patients with tuberculosis (TB) being treated with rifampin. Such treatment has the potential to shorten TB therapy and to reduce the potential for emergence of drug resistance, both related to the ability of verapamil to decrease TB drug tolerance.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ This study sought to determine a compensatory dosing strategy of verapamil to offset its increased metabolism when given to patients with TB receiving rifampin-based therapy.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

☑ Verapamil sustained release 360 mg twice daily achieved a similar drug exposure as compared with prior studies of verapamil sustained release 240 mg once daily given to persons without TB. Verapamil co-administration appeared to increase the relative concentrations of the less cardioactive metabolites and enantiomers.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

☑ The results of this study are a proof of concept that compensatory dosing of verapamil can be achieved in the context of rifampin-based TB therapy and is well-tolerated.

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[1]National Institute for Research in Tuberculosis, Chennai, India; [2]Division of HIV, Infectious Diseases and Global Medicine, Department of Medicine, Zuckerberg San Francisco General Hospital and Trauma Center, University of California San Francisco, San Francisco, California, USA; [3]National Institute of Tuberculosis and Respiratory Diseases, New Delhi, India; [4]Government Kilpauk Medical College and Hospital, Chennai, India; [5]Department of Pulmonary Medicine, SCB Medical College, Cuttack, India; [6]Regional Medical Research Centre, Bhubaneswar, India; [7]SITEC Labs, Navi Mumbai, India; [8]Department of Pharmacy, University of Washington, Seattle, Washington, USA; [9]Molecular Immunity Unit, Department of Medicine, Cambridge Institute of Therapeutic Immunology and Infectious Diseases, University of Cambridge, Cambridge, UK; [10]MRC Laboratory of Molecular Biology, Cambridge, UK; [11]Trinity College, Cambridge, UK; [12]Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA; [13]Present address: M.S. Swaminathan Research Foundation, Chennai, India. *Correspondence: Chandrasekaran Padmapriyadarsini (padmapriyadarsi.nic@icmr.gov.in); Paul Edelstein (paul.edelstein@pennmedicine.upenn.edu)

[†]These authors contributed equally to this work.
[‡]These authors should be considered co-senior authors.

The need for lengthy tuberculosis (TB) treatment is attributed to antimicrobial tolerance of *Mycobacterium tuberculosis* (Mtb),[1] suggesting the potential of therapeutic approaches specifically targeting tolerant Mtb. Within days of infecting macrophages, Mtb subpopulations become tolerant to rifampin and other antitubercular drugs due to the activity of bacterial efflux pumps.[2–4] Accordingly, Mtb strains with mutations in the Tap/Rv1258c efflux pump, which is induced upon macrophage infection,[5] do not develop macrophage-induced rifampin tolerance.[2,4,6] Beyond mediating rifampin tolerance, Tap/Rv1258c also promotes Mtb growth in macrophages in the absence of antibiotics.[4] *Rv1258c* expression is induced in Mtb in sputum from patients being treated with a rifampin-containing regimen, supporting its relevance in TB.[7]

Drugs with known activity against bacterial efflux pumps have also been shown to inhibit Mtb rifampin tolerance as well as growth within macrophages.[3,4,6,8] Among the most active of these is verapamil, which has been in clinical use worldwide for decades and is on the World Health Organization's list of essential medicines.[9] Its pharmacology and adverse effect profile are well-characterized, and it is available as a generic medication.[10] Verapamil has been successfully used for both cardiovascular and non-cardiovascular indications[11,12] and appears not to have a major effect on blood pressure in non-hypertensive populations.[13] Animal studies show that verapamil is highly concentrated in tissue, including the lungs, with concentrations 40-fold or higher than those in plasma.[14,15] Along with inhibiting Mtb drug efflux, verapamil may potentiate Mtb killing through inhibitory effects on mammalian transporters.[8,16] In support of these findings, mice infected with drug-sensitive Mtb and given shorter courses of a rifampin-containing regimen and adjunctive verapamil had increased rates of relapse-free cure.[17] Moreover, calcium channel blocker use in humans has been associated with a reduced incidence of TB.[18]

Although verapamil is useful in the management of cardiovascular disease due to its calcium channel antagonism, it also inhibits P-glycoprotein (P-gp). Verapamil is administered as a racemic mixture; both its major metabolite norverapamil and its R-enantiomer have substantially reduced cardiac activity,[19–21] but similar P-gp inhibitory activity. Prior work has shown that verapamil inhibits Mtb rifampin efflux through its P-gp inhibitory activity and that both R-verapamil and norverapamil have similar efficacy as racemic verapamil in inhibiting Mtb rifampin efflux, macrophage-induced drug tolerance, and intramacrophage growth.[4,6] Notably, norverapamil is present at plasma levels similar to or higher than verapamil, potentially augmenting the effects of verapamil on rifampin efflux.[22–27]

The major barrier to evaluation of verapamil as an adjunctive TB therapy is its greatly increased metabolism induced by rifampin, resulting in very low serum verapamil concentrations.[28] We therefore sought to determine through a dose-finding pharmacokinetic (PK) study of verapamil given to patients with TB receiving rifampin-based therapy whether we could determine a compensatory dose increase of verapamil to offset its increased metabolism caused by rifampin. Our secondary goals were to determine the safety and tolerability of verapamil given in this way to patients with TB without known cardiac disease and to determine concentrations of verapamil enantiomers and norverapamil during rifampin-based TB therapy.

METHODS

The most appropriate PK parameter to consider for an efflux pump inhibitor is uncertain. We chose an area under the curve from zero to 12 h ($AUC_{0\text{-}12h}$) target of 1,000 ng.h/mL, which is comparable to or lower than AUC ranges for human patients taking well-tolerated, moderate doses of sustained-release verapamil (240 mg/day) in the absence of rifampin.[29,30]

Study participants with smear-positive pulmonary TB were enrolled from the National Institute for Research in Tuberculosis (NIRT) and Kilpauk Medical College and Hospital in Chennai, India, along with the Regional Medical Research Center and SCB Cuttack Medical College and Hospital in Bhubaneswar, India (**Figure 1**). All participants were 18 to 55 years old and weighed 45–75 kg. They were in their last week of first-line TB therapy with daily rifampin, isoniazid (INH), and ethambutol per India's National TB Elimination Programme (NTEP), had converted sputum smears to negative, and were clinically improved. TB drug dosing was based on NTEP standardized weight bands. All TB medications were provided by the NTEP. Verapamil was provided as Calaptin sustained-release (SR; Abbott) and obtained from commercial suppliers. We chose the extended-release formulation of verapamil because the less frequent dosing simplifies treatment and provides for more consistent drug levels.[31]

After providing written informed consent, participants underwent physical examination and baseline testing, including complete blood count, renal and liver function testing, HIV serology, urine pregnancy testing, chest radiography, and electrocardiogram (ECG). Exclusion criteria included: systolic blood pressure < 100 mmHg; heart rate less than 60 beats per minute; clinical signs or symptoms of congestive heart failure or cirrhosis; treatment with medications other than INH, rifampin, ethambutol, and pyridoxine; comorbid conditions, including HIV, cardiac arrhythmia or congestive heart failure, diabetes mellitus, active substance use or other uncontrolled medical condition; laboratory abnormalities, including hemoglobin < 9 mg/dL, creatinine clearance < 50 mL/min, aspartate aminotransferase or alanine aminotransferase > 2.5 the upper limits of normal; conduction delay on ECG (PR interval > 200 milliseconds

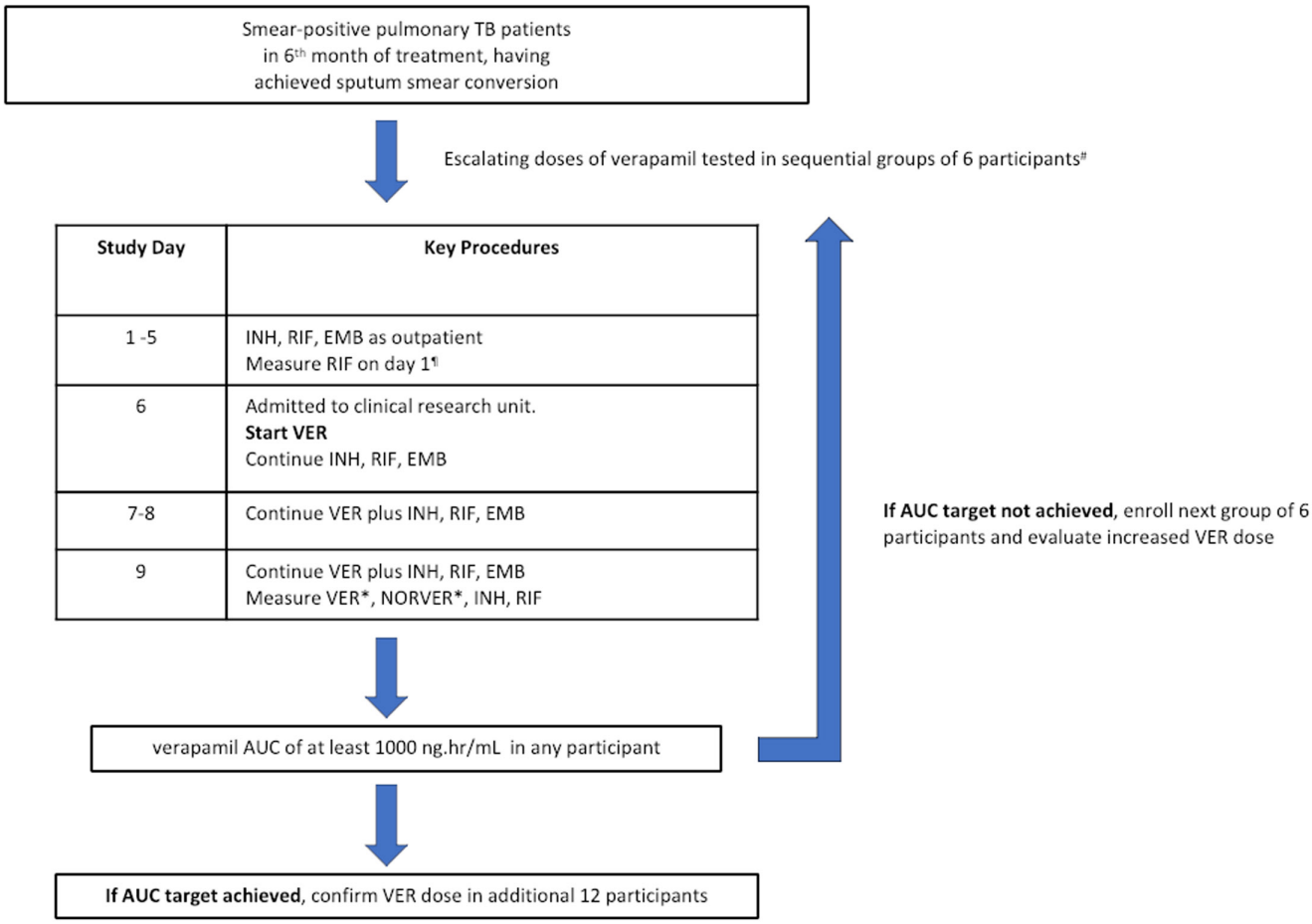

Figure 1 Study procedures. AUC, area under the curve; EMB, ethambutol; INH, isoniazid; NORVER, norverapamil; RIF, rifampin; TB, tuberculosis; VER, verapamil.

or QRS > 120 milliseconds); ejection fracture < 45%; pregnancy, or lactation.

Escalating dosages of sustained release verapamil were studied in sequential groups of six study participants. The first dose group included only three participants, as we anticipated very low verapamil levels in this dose range. Blood was collected for measurement of rifampin levels on study day 1. Participants received rifampin, INH, and ethambutol under direct supervision at the study site outpatient clinic for study days 1–5. Participants were subsequently hospitalized to facilitate intensive PK monitoring. On study day 6, sustained-release verapamil was initiated and co-administered with rifampin, INH, and ethambutol. Verapamil was administered every 12 hours prior to meals. All medications were given under direct supervision. On study day 9, blood was collected for measurement of INH, rifampin, verapamil, and norverapamil levels prior to morning medication administration and then at 1, 2, 4, 8, and 12 hours afterward. Drug levels were measured for each group of six participants and reviewed by the Data Safety and Monitoring Committee before advancing to the next dose in another group of six participants.

Pulse and blood pressure were checked before each verapamil dose (**Figure S1**) and daily ECGs were obtained on study days 6–9. The study protocol dictated that verapamil dose escalation would be halted once an $AUC_{0\text{-}12h}$ of at least 1,000 ng.h/mL was achieved in any participant, or if any participant experienced an adverse effect of grade 3 or higher, as defined by the Division of AIDS, NIAID[32] or developed Mobitz type II or complete heart block. For pulse < 55 beats per minute or systolic blood pressure < 90 mmHg or PR interval > 200 milliseconds, verapamil would be held and the measurements repeated in 1 hour; if the abnormal value resolved, then verapamil would be given but, if not, then dose escalation would be halted.

Once the AUC target had been achieved, the verapamil dose was studied in a confirmatory group of 12 participants.

Laboratory methods

Drug levels were measured in parallel in two separate laboratories, NIRT and SITEC Labs. The NIRT laboratory measured racemic verapamil and norverapamil, as well as INH and rifampin using high pressure liquid chromatography. The SITEC laboratory measured R-verapamil, S-verapamil, R-norverapamil, and S-norverapamil using liquid chromatography tandem mass spectrometry. The SITEC laboratory results are used in this report for verapamil and its metabolites because only that laboratory measured enantiomer concentrations. The NIRT laboratory results are used in this report for INH and rifampin levels. To supplement PK analyses of verapamil, we evaluated a subset of single-nucleotide polymorphisms (SNPs) in genes previously linked to verapamil exposure. Sample collection and processing details, analytical methods, and SNP genotyping methods are described in the **Supplementary Information**.

Data analysis

Study data were doubly entered into a Promasys database (Omnicomm, Ft. Lauderdale, FL). PK analyses were done with WinNonlin version 8.1 (Certara, Princeton, NJ) using noncompartmental analyses. Data

displays, descriptive statistics, and AUC determinations were performed using Prism 9 (GraphPad, San Diego, CA); validation of the accuracy of the AUC_{0-t} calculations using Prism was by comparison with WinNonlin outputs using Bland Altman testing, that showed a 5% difference (bias 5.5%, SD of bias 4.7%, 95% limits of agreement −3.5 to 14.7%). To allow comparison of the verapamil AUC_{0-12h} with historical controls reporting AUC_{0-24h}, the calculated individual subjects' AUC_{0-12h} were doubled because the subjects were at steady-state and receiving verapamil every 12 hours. The same doubling of the AUC_{0-12h} to estimate the AUC_{0-24h} was performed for data from Mattila *et al.*,[23] for identical reasons. Subjects were not included in the final analyses if there were missing data.

Because the published data for historical controls did not include raw data to allow the calculation of the variance of reported ratios and attempts to obtain these data from study authors were unsuccessful, we used Bayesian methods to estimate the population distributions and ratios of both historical data and our data. The Bayesian analyses were conducted using MATLAB (Mathworks, Natick, MA) as detailed in the Supplementary Information **Statistical Appendix 1** and elsewhere.[33]

Ethics review and approval

This study underwent ethics review and approval by the human subjects research committees at the study sites and was authorized by the Office of Drugs Controller General (India). This trial was registered at the Clinical Trials Registry – India (CTRI/2016/05/006928).[34]

RESULTS

No serious adverse events attributable to verapamil were observed in the study, including in the highest dosage group. No study participant developed hypotension or bradycardia (**Figure S1**). No patient developed ECG abnormalities. One individual in the 360 mg twice daily verapamil group had a seizure 6 hours after receiving verapamil. The participant was assessed by a neurologist who determined that the seizure was due to alcohol withdrawal, not verapamil. This individual's ECG was unremarkable.

The detailed NIRT and SITEC data are shown in the Supplement (**Figures S2**, **S3**); there was good agreement between the NIRT and SITEC results for total verapamil and norverapamil. Verapamil exposures in the first two verapamil dosage groups were low, with a median AUC_{0-12h} of 60.5 ng.h/mL (interquartile range (IQR): 38.5–102) in the 120 mg twice daily group and a median AUC_{0-12h} of 349 ng.h/mL (IQR: 319.5–445.5) in the 240 mg twice daily group (**Table S2**). At a verapamil dosage of 360 mg twice daily, we attained the prespecified AUC_{0-12h} target (> 1,000 ng.h/mL) among 2 of 6 participants and subsequently advanced this dosing into a larger confirmatory group of 12 participants. The 18 participants receiving the 360 mg twice daily verapamil had a median age of 30 years (IQR: 24–42), median body mass index of 19.2 kg/m^2 (IQR: 17.7–21.9) and received a median of 9.65 mg/kg (IQR: 9.3–10) of rifampin daily (**Table S1**). The median, arithmetic mean, and geometric mean of the verapamil AUC_{0-12h} for the 16 subjects with complete data were 903, 1,020, and 835 ng.h/mL, respectively, with an IQR of 443 to 1,298. Results for other analytes are shown in **Table 1**. When adjusted to AUC_{0-24h} to allow for comparisons with previously published data, these values were comparable to steady-state values reported for individuals taking 240 mg verapamil SR once daily or 120 mg twice daily in the absence of rifampin (**Figure 2**).[23,25,26,29,35] Bayesian analysis confirmed that the current study verapamil AUCs were not significantly different from those of each of these historical controls (**Figure 2b**, **Table S4a**). For statistical definitions and precise meanings of abbreviated phraseology for these and all other Bayesian analyses, see **Statistical Appendix 2**.

Table 1 Summary PK data among participants receiving verapamil SR 360 mg twice daily

	Median C_{max} (IQR), ng/mL (μg/mL for RIF, INH)	Geometric mean C_{max} (95% CI), ng/mL (μg/mL for RIF, INH)	Median AUC (IQR) ng.h/mL (μg.h/mL for RIF, INH)	Geometric mean AUC (95% CI) ng.h/mL (μg.h/mL for RIF, INH)
RIF	11.0 (10.0–13.7)	11.0 (9.6–12.6)	52.7 (46.6–66.0)	54.3 (48.2–61.3)
INH[a]	4.6 (4.0–5.5)	4.7 (4.1–5.4)	15.6 (13.2–24.9)	17.6 (14.4–21.5)
Verapamil[b]	81.9 (60.7–205.2)	106.7 (76.4–148.9)	903.1 (443.4–1,298.0)	835.1 (587.7–1,187.0)
Norverapamil	191.3 (139.4–298.8)	202.3 (163.5–250.4)	1,629.0 (1,072.0–2,425.0)	1,652.0 (1,300.0–2,097.0)
R-verapamil	72.2 (54.6–182.3)	94.2 (67.6–131.4)	800.7 (390.0–1143.0)	737.7 (519.4–1,048.0)
S-verapamil	10.7 (7.0–22.3)	12.2 (8.6–17.5)	106.2 (55.13–140.2)	95.7 (66.1–138.8)
R-norverapamil	147.0 (104.8–226.5)	154.4 (125.1–190.6)	1,212.0 (807.7–1954.0)	1,265.0 (995.0–1,607.0)
S-norverapamil	46.2 (30.9–77.1)	47.0 (36.8–59.9)	388.3 (244.8–621.4)	380.0 (294.9–489.8)

Frequentist confidence intervals reported here are based on log-Gaussian distributions.
N = 18 unless otherwise specified. All measurements were obtained at study day 9.
CI, confidence interval; C_{max}, maximum plasma concentration; INH, isoniazid; IQR, interquartile range; PK, pharmacokinetic; RIF, rifampin.
[a]*N* = 12 for INH as it was only measured for the confirmatory group of participants.
[b]*N* = 16 for verapamil AUCs due to missing data.

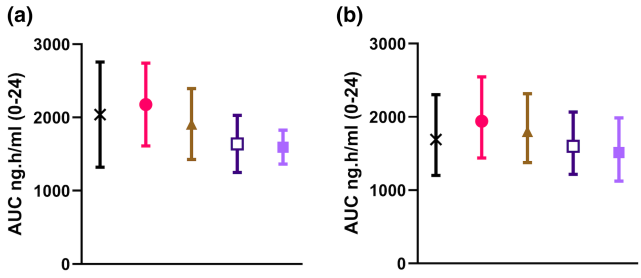

Figure 2 (**a**) Verapamil $AUC_{0\text{-}24h}$ for current study and historical comparators. Observed data are displayed as mean, and 95% frequentist CI of the mean assuming a Gaussian distribution, with the current study and Mattila *et al.*[23] data adjusted from the respectively measured or reported $AUC_{0\text{-}12h}$ to estimate $AUC_{0\text{-}24h}$. Reading from left to right: current study ($n=16$), black with X; Mattila *et al.* ($n=12$), filled red circle; Hla *et al.*[29] ($n=10$), brown triangle; Abernethy *et al.*[25] ($n=8$), dark purple hollow square, Lemma *et al.*[26] ($n=12$), light purple filled square. This is provided for easy comparison with the literature, acknowledging that the reported 95% CI values are potentially incorrect because of the assumption of a Gaussian data distribution. Details of comparison studies are provided in **Table S3**. (**b**) Verapamil $AUC_{0\text{-}24h}$ Bayesian population estimates. The posterior arithmetic mean of the geometric mean over the population is shown, along with the 95% Bayesian confidence interval for the geometric mean over the population, under the assumption (justified in **Statistical Appendix 1** section 4.1) that the population distribution is log-Gaussian. The current study value is not significantly different from that of each of the other studies (<0.70; see **Statistical Appendix 2** item 6, **Table S4a**). Details of comparison studies are provided in **Table S3**. $AUC_{0\text{-}12h}$, area under the curve from zero to 12 hours; CI, confidence interval.

We measured levels of verapamil enantiomers and norverapamil and determined their relative concentrations by calculating the ratios of the AUCs of norverapamil to verapamil, R-verapamil to S-verapamil, and R-norverapamil to S-norverapamil (**Table 1**, **Figure 3**). These data are reported as described in **Statistical Appendix 2** (item 11). The norverapamil:verapamil AUC ratio of our study participants (2.11, 1.89–2.35) was significantly greater (> 0.97) than the ratio in each study conducted among participants receiving 240 mg SR verapamil at steady-state not taking rifampin[23–26] (**Figure 3a**, **Table S4b**). We note that Barbarash *et al.*[28] observed an even larger norverapamil:verapamil ratio when verapamil was co-administered with rifampin, perhaps because their measurements were made after a single dose of 120 mg immediate-release verapamil when steady-state levels would not have been achieved. Together, these findings suggest that rifampin co-administration increases the relative concentrations of norverapamil to verapamil, even before steady-state levels are achieved and maintains these higher relative levels under steady-state concentrations.

The ratio of the AUC of R-verapamil to S-verapamil (7.70, 6.92–8.54) was also significantly higher than that reported for persons receiving verapamil in the absence of rifampin (**Figure 3b**, **Table S4c**).[26,36] These results were different from those reported by Fromm *et al.*,[36] where the R:S ratio was significantly (0.999) lower among persons taking verapamil with rifampin than in those not taking rifampin. This difference could reflect differences in the study population. We note that the results in our study were internally consistent: R-norverapamil to S-norverapamil ratios (3.34, 2.98–3.73) were also significantly greater than those reported in participants receiving verapamil in the absence of rifampin (**Figure 3c**, **Table S4d**).[26] As previously reported,[27] the R:S ratios for both verapamil and norverapamil decreased over time following administration of the last verapamil dose, while always remaining greater than 1 (**Figure 4**).

We asked if verapamil AUCs were altered by genetic variants in P-gp (encoded by *ABCB1*) which are associated with alterations in verapamil transport.[37,38] Individuals homozygous for both minor alleles of two closely linked SNPs in *ABCB1* are reported to have increased serum verapamil levels.[38] One of these, rs1045642, is a synonymous change (NM_001348944.2:c.3435T>C) in exon 26, and the other, rs2032582, is a nonsynonymous change (NM_001348946.2:c.2677T>G), in exon 21 (Ser893Ala). We examined the effect of the two SNPs individually on verapamil AUC, as described.[33] The rs1045642 was associated with differences; both CC individuals had higher verapamil AUCs than did the TT and TC subjects (**Figure 5a**, **Table S4e**). For rs2032582, only one of the two GG homozygotes had elevated verapamil AUCs; this individual was also CC homozygous at rs1045642 (**Figure 5b**). Together, these findings suggest that ABCB1 rs1045642 may be the primary driver of the differences in verapamil metabolism observed in the combined haplotype.[38] A genetic variant in the cytochrome P450 enzyme encoded by *CYP3A5*, which metabolizes verapamil, is also reported to influence the verapamil AUC.[39] *CYP3A5* rs776746 (NM000777.5(CYP3A5):c.219-237A>G) in intron 3 encodes the nonfunctional CYP3A5*3 allele.[39] Minor allele GG homozygotes expressing the truncated form are reported to be non-expressors and are associated with higher verapamil levels.[37] However, we did not find significant differences in verapamil AUCs based on rs776746 genotype in this study (**Figure 5c**). This may be due to small sample size, differences in study populations, and/or the inductive effects of rifampin on verapamil that outweigh any differences that would otherwise be seen due to variable CYP3A5 activity.

Because verapamil is reported to increase rifampin exposure in mice,[40,41] we examined the effect of verapamil on rifampin AUCs in those 10 subjects who had both pre- and post-verapamil data. We found that there was a significant increase (1.5-fold) in rifampin AUCs after 3 days of verapamil treatment (**Figure 6**). In contrast, INH AUCs were not significantly increased by verapamil.

DISCUSSION

In this investigation, we studied escalating doses of verapamil given to participants receiving rifampin-based TB therapy to determine whether increased verapamil clearance can be offset by higher verapamil doses. We found that treatment with verapamil 360 mg SR twice daily can attain verapamil exposures similar to those among persons taking moderate doses of verapamil (240 mg SR daily) in the absence of rifampin.[29,30]

As in previous studies, we found that R-verapamil is the predominant verapamil enantiomer in blood. Moreover, norverapamil to verapamil ratios were significantly increased in the presence of rifampin. This is likely due to the selectively increased metabolism of verapamil vs. norverapamil.[28] Thus, verapamil metabolites and

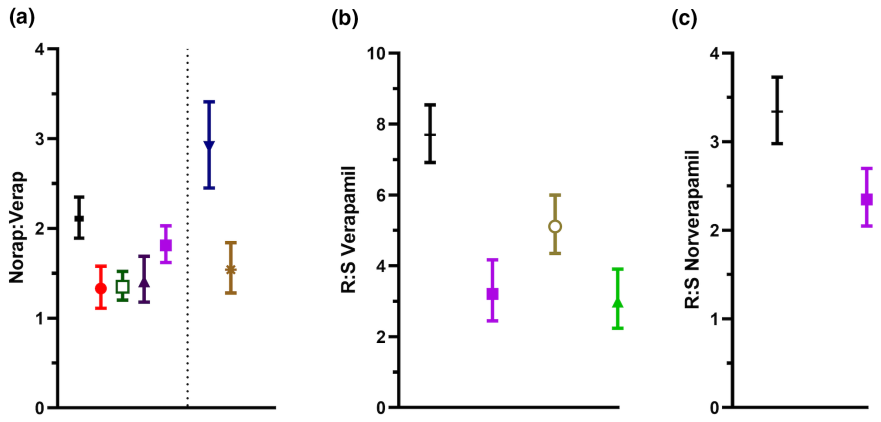

Figure 3 (**a**) Norverapamil:verapamil AUC ratios for current study and historical comparators. Reading from left to right, current study, black horizontal line ($N=16$); Mattila *et al*.[23] ($n=12$), filled red circle; Norris *et al*.[24] ($n=22$), green hollow square; Abernethy *et al*.[25] ($n=8$), dark purple triangle; Lemma *et al*.[26] ($n=12$), light purple solid square. Separated from these by a vertical dotted line are Barbarash *et al*.[28] post rifampin ($n=6$), dark blue downward triangle; and Barbarash *et al*. pre-rifampin, brown asterisk. The Barbarash data are separated because this was a single immediate release verapamil dose study, in contrast to the other studies which were all steady-state delayed-release verapamil studies. Results are reported as described in the **Statistical Appendix 2** (item 11). The probabilities that the present study value is greater than that of each other study are >0.999 except for Lemma *et al*. (0.973), Barbarash post-rifampin (0.003) and pre-rifampin (0.996). The probability that Barbarash *et al*. post-rifampin is greater than pre-rifampin is 0.9999. Details of comparison studies are provided in **Table S3**. (**b**) R:S verapamil AUC ratios for current study and historical comparators. Reading from left to right, current study, black horizontal line ($N=16$); Lemma *et al*.[26] ($n=12$), purple solid square; Fromm *et al*.[36] ($n=8$) pre-rifampin, beige hollow circle; Fromm *et al*. post-rifampin, green triangle. Results are reported as described in the **Statistical Appendix 2** (item 11). The present study value is significantly greater than that of each other study's value (>0.999). The probability that Fromm *et al*. pre-rifampin is greater than post-rifampin is 0.999. Details of comparison studies are provided in **Table S3**. (**c**) R:S norverapamil AUC ratios. Reading from left to right, current study, black horizontal line ($N=18$) and Lemma *et al*.,[26] purple solid square. Results are reported as described in the **Statistical Appendix 2** (item 11). The Bayesian probability that the current study value is greater than that of Lemma *et al*. is 0.999. Details of comparison studies are provided in **Table S3**. AUC, area under the curve; Norap, norverapamil; Verap, verapamil.

enantiomers may contribute significantly to the effects of verapamil in inhibiting efflux and may potentially allow for further increasing verapamil doses, without increasing cardiotoxic potential, in rifampin-containing regimens.

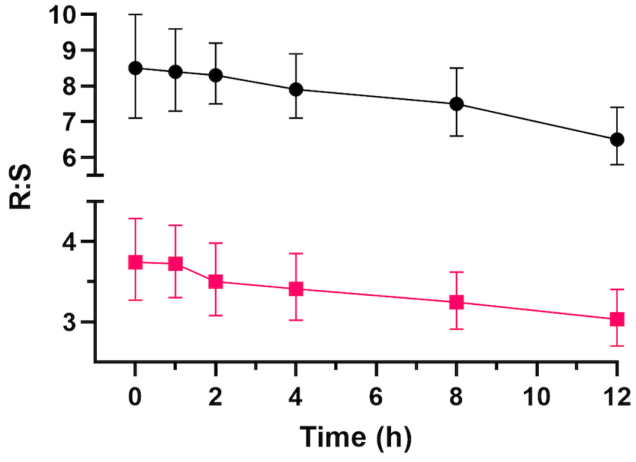

Figure 4 Time trends in R:S enantiomer ratios of verapamil and norverapamil. Verapamil and norverapamil R:S ratios over the time of administration of the seventh oral dose of verapamil SR 360 mg given every 12 hours to subjects who were also receiving rifampin. Sample geometric means and 95% CI of the geometric means of the ratios of the plasma levels of the drugs are shown. Red squares, R:S norverapamil, $N=18$; black circles R:S verapamil, $N=16$; the lower N for verapamil is because of undetectable S enantiomers or missing values. CI, confidence interval; SR, sustained release.

We also considered the potential of verapamil to increase rifampin levels, as increased rifampin levels are associated with therapeutic benefit in TB.[42] It is difficult to predict the effects of verapamil on rifampin levels, because verapamil not only inhibits CYP3A4, but also inhibits gut P-gp with short-term administration and induces P-gp with longer term administration.[26,43] The rifampin dosing in this study was consistent with current practice and achieved rifampin exposures similar to those reported in other studies where verapamil was not administered.[44] We observed a statistically significant, but probably not a clinically significant, increase in rifampin exposures after verapamil treatment, suggesting that verapamil enhances rifampin absorption similar to what is seen with rifampin-piperine co-administration.[45] It is possible that longer term verapamil administration could alter these results if gut P-gp was not fully induced by less than a week of verapamil administration.

Verapamil remains of interest as an adjunctive therapy even with new TB regimens coming into use. Although rifapentine and fluoroquinolone-based therapy permits treatment shortening to 4 months in drug-susceptible TB,[46] adjunctive efflux inhibitory treatment might permit further shortening given macrophage-induced fluroquinolone and rifamycin tolerance.[3] Verapamil inhibits macrophage-induced rifabutin tolerance and is likely to inhibit rifapentine tolerance as well (Ref. 3 and K. N. Adams, unpublished data 2023).

No major adverse events were noted even in those subjects with the highest measured verapamil exposures with the notable absence of hypotension, bradycardia, and PR prolongation. At the

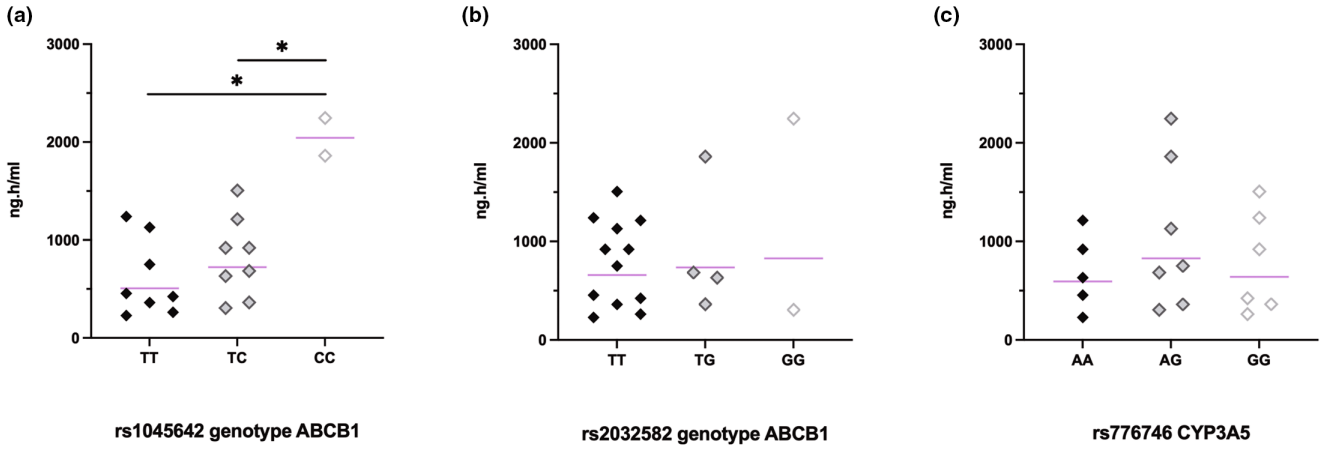

Figure 5 Comparison of verapamil AUC grouped by SNP genotype. Verapamil $AUC_{0\text{-}12h}$, ng.h/mL, for each study participant, grouped by genotype at (**a**) rs1045642, ABCB1 3435T>C; (**b**) rs2032582, ABCB1 2677T>G; and (**c**) rs776746, CYP3A5 6986A>G. Asterisks indicate that the restricted geometric mean of the CC allele in panel (**a**) is significantly greater than each of the two alternative alleles (see **Statistical Appendix 2**, item 13). Unspecified comparisons have probabilities of ≤0.91. Bars indicate geometric means. $AUC_{0\text{-}12h}$, area under the curve from zero to 12 hours; SNP, single-nucleotide polymorphism.

same time, rifampin and verapamil combination regimens carry the risk of substantial increases in verapamil levels if rifampin alone is discontinued. This risk is mitigated by two findings: (i) verapamil doses of 960 mg daily or greater have been safely used in the absence of rifampin, suggesting a wide therapeutic window with verapamil[11,12]; (ii) the delayed timing of CYP3A4 recovery provides a buffer against the effects of missed rifampin doses.[36] Prolonged discontinuation of rifampin, but not verapamil, would result in substantially higher verapamil exposures and potential toxicity. Use of a combined rifampin/verapamil formulation would mitigate this, as well as careful monitoring and counseling to ensure medication adherence. If verapamil is added to a rifamycin-containing treatment regimen then it should only be added after full CYP3A4 induction has occurred, around a week after starting the rifamycin.[36]

This study has limitations that affect its generalizability. Study participants did not have major medical comorbidities or concomitant medication use. Women were under-represented, despite enrollment efforts. The number of subjects studied was relatively small, especially for the before-after rifampin and INH exposure studies and genetic association studies, requiring confirmatory studies in other populations. The use of historical controls of verapamil, and verapamil metabolite exposures, rather than comprehensive before-after studies in the same population requires confirmation of these results in future studies where verapamil levels are measured in the same individuals on and off rifampin. Our study population was carefully selected and intensively monitored during drug administration, limiting generalizability about safety in a less well-selected and monitored population. Whereas intensive cardiac screening with echocardiology and ECGs would be difficult to implement in less-resourced settings, careful clinical examination might be adequate to identify persons who should not receive adjunctive verapamil. We note that verapamil has been widely used for noncardiac indications without intensive cardiac monitoring and has been well-tolerated. Nevertheless, the clinical effect of verapamil combined with other TB drugs on cardiac conduction will need study prior to regulatory agency clearance.

In summary, verapamil is appealing to study for adjunctive TB treatment given the multiple potential pathways through which it may increase anti-tubercular drug efficacy and counter TB

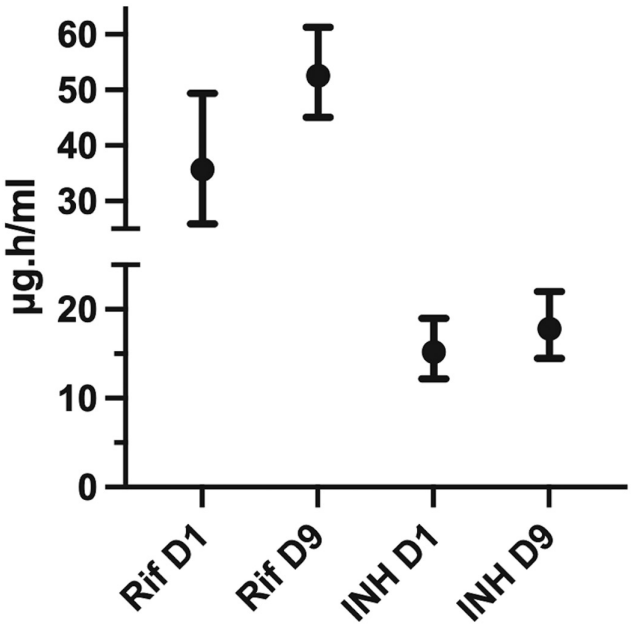

Figure 6 Effect of verapamil on RIF and INH levels. Comparison of INH and rifampin $AUC_{0\text{-}8h}$ in paired subjects ($N=10$) before and after receiving seven doses (84 hours) of twice daily verapamil (360 mg SR q 12 hours), showing the effect of verapamil administration on INH and rifampin exposures. Rif D1, rifampin AUC prior to receiving verapamil; Rif D9, rifampin AUC after receiving 7 doses of verapamil; INH D1, INH AUC prior to receiving verapamil; INH D9, INH AUC after receiving 7 doses of verapamil. Results are reported as described in **Statistical Appendix 2**, item 11. The Bayesian probabilities of the geometric mean drug exposures while receiving verapamil being greater than the geometric mean drug exposures prior to receiving verapamil in these paired subject analyses are 0.98 for rifampin and 0.90 for INH. $AUC_{0\text{-}8h}$, area under the curve from zero to 8 hours; INH, isoniazid; RIF, rifampin; SR, sustained release.

pathogenesis, and its well-characterized pharmacology and decades of clinical experience. We have established a well-tolerated, compensatory dose of verapamil to help inform future studies of adjunctive verapamil in the context of rifampin-based TB therapy.

SUPPORTING INFORMATION

Supplementary information accompanies this paper on the *Clinical Pharmacology & Therapeutics* website (www.cpt-journal.com).

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CONFLICTS OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

C.P., J.D.S., L.W., L.R., and P.H.E. wrote the manuscript. J.D.S., K.R.N., L.R., S.S., and P.H.E. designed the research. C.P., N.A., P.S., A.J., M.B., M.P., J.T., R.K., S.B., S.P., A.K.H.K., M.K.R., J.R., and K.R.N. performed the research. C.P., J.D.S., K.R.N, J.H., L.W., R.S., L.R., and P.H.E. analyzed data.

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