J Antimicrob Chemother 2021; **76**: 710–717
doi:10.1093/jac/dkaa512 Advance Access publication 9 December 2020

Journal of Antimicrobial Chemotherapy

Rifabutin pharmacokinetics and safety among TB/HIV-coinfected children receiving lopinavir/ritonavir-containing second-line ART

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Received 10 July 2020; accepted 9 November 2020

Background: Treatment options are limited for TB/HIV-coinfected children who require PI-based ART. Rifabutin is the preferred rifamycin for adults on PIs, but the one study evaluating rifabutin with PIs among children was stopped early due to severe neutropenia.

Methods: We evaluated rifabutin safety and plasma pharmacokinetics among coinfected children 3–15 years of age receiving rifabutin 2.5 mg/kg daily with standard doses of lopinavir/ritonavir. The AUC_{0-24} at 2, 4 and 8 weeks after rifabutin initiation was described using intensive sampling and non-compartmental analysis. Clinical and laboratory toxicities were intensively monitored at 12 visits throughout the study.

Results: Among 15 children with median (IQR) age 13.1 (10.9–14.0) years and weight 25.5 (22.3–30.5) kg, the median (IQR) rifabutin AUC_{0-24} was 5.21 (4.38–6.60) μg·h/mL. Four participants had AUC_{0-24} below 3.8 μg·h/mL (a target for the population average exposure) at week 2 and all had AUC_{0-24} higher than 3.8 μg·h/mL at the 4 and 8 week visits. Of 506 laboratory evaluations during rifabutin, grade 3 and grade 4 abnormalities occurred in 16 (3%) and 2 (0.4%) instances, respectively, involving 9 (60%) children. Specifically, grade 3 ($n = 4$) and grade 4 ($n = 1$) neutropenia resolved without treatment interruption or clinical sequelae in all patients. One child died at week 4 of HIV-related complications.

Conclusions: In children, rifabutin 2.5 mg/kg daily achieved AUC_{0-24} comparable to adults and favourable HIV and TB treatment outcomes were observed. Severe neutropenia was relatively uncommon and improved with ongoing rifabutin therapy. These data support the use of rifabutin for TB/HIV-coinfected children who require lopinavir/ritonavir.

Introduction

TB is the leading cause of death among children with HIV.[1,2] Compared with those with TB monoinfection, mortality rates are 4-fold higher among coinfected children.[3,4] Yet, current co-treatment strategies for children are limited. Many antiretroviral medications have significant drug interactions with rifampicin, a key component of TB treatment, and paediatric formulations for co-treatment are lacking.

The 2016 WHO guidelines recommend lopinavir/ritonavir-based ART for children under 3 years of age as first-line therapy, as well as for older children who have failed first-line ART.[5] While the 2019 WHO guidelines promote use of dolutegravir-containing ART as a promising universal treatment strategy for all ages, its use in children remains understudied, particularly among those with TB/HIV coinfection, and dosing guidelines and formulations for young children are not yet available.[6] Thus, lopinavir/ritonavir

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remains widely used among children in resource-limited settings. Rifampicin is a potent inducer of cytochrome P450 3A4 and intestinal p-glycoprotein, reducing lopinavir exposure by 90% when given in combination.[7] In children with TB, giving additional ritonavir in combination with lopinavir/ritonavir is recommended to overcome this drug interaction. However, ritonavir is not available in most settings and the oral solution used in young children is unpalatable.[8–10]

For coinfected adults who require PI-based ART, the WHO recommends rifabutin in place of rifampicin.[5] Rifabutin coadministration has minimal effect on lopinavir concentrations and thus standard PI dosing is recommended.[11] However, all ritonavir-boosted PIs increase serum concentrations of rifabutin, so the concurrent rifabutin dosage is reduced. In adults, rifabutin dosed at 150 mg thrice weekly results in rifabutin exposure below that achieved with the recommended dose of rifabutin (300 mg daily) without ART, while rifabutin 150 mg daily with lopinavir/ritonavir results in higher exposure.[12–14]

The combination of rifabutin and lopinavir/ritonavir has not been adequately studied in children. US guidelines recommend rifabutin 10–20 mg/kg once daily for treatment of drug-susceptible TB, but no dosing guidelines exist for children receiving lopinavir/ritonavir-containing ART.[15] In the only published study to evaluate rifabutin pharmacokinetics among children receiving lopinavir/ritonavir, two of six children developed treatment-limiting neutropenia.[16] This safety concern contrasts with our programmatic experience in the PEPFAR-supported AIDS Prevention Initiative in Nigeria (APIN) Public Health Initiatives programme that has supported access to rifabutin for coinfected adults and children since 2009. Severe neutropenia was rare in a retrospective analysis of rifabutin safety among 48 coinfected children (median age of 1.7 years) who received concurrent lopinavir/ritonavir-based ART, with only one instance (2%) of grade 3 neutropenia (no grade 4 neutropenia).[17]

Five of six children achieved adequate rifabutin concentrations when dosed 5 mg/kg thrice weekly in the Moultrie *et al.*[16] study. We evaluated rifabutin 2.5 mg/kg daily along with lopinavir/ritonavir as it provides a similar weekly dosage, while simplifying the treatment regimen by giving all medications once daily.

Methods

Study design and population

We designed a prospective, open-label study to evaluate rifabutin pharmacokinetics and safety among 15 TB/HIV-coinfected children 3–15 years of age receiving lopinavir/ritonavir-based ART. All children were ART experienced, recently diagnosed with active TB infection and required second-line lopinavir/ritonavir-based ART due to either current or prior failure of NNRTI-based ART. Participants were recruited at two large paediatric ART clinics in Nigeria: University College Hospital (UCH) in Ibadan and Jos University Teaching Hospital (JUTH).

Eligible children either continued on or were switched to lopinavir/ritonavir-based ART the same day that rifabutin-containing TB treatment was started. Participants also received WHO-recommended daily dosages of isoniazid (10 mg/kg), pyrazinamide (35 mg/kg) and ethambutol (20 mg/kg).[18] Lopinavir/ritonavir and the nucleoside backbone were dosed according to WHO weight bands. All medication dosages were adjusted for weight gain at each clinical visit.

Rifabutin formulation and dosing

Currently there is no manufactured paediatric formulation; however, an oral suspension of rifabutin 20 mg/mL compounded from capsules (Mycobutin®, Lupin Pharmaceuticals) in sweetening vehicles is stable for at least 12 weeks at temperatures up to 40°C.[19] APIN pharmacists compounded the suspension with Ora-Plus (suspending vehicle, Perrigo®) and Ora-Sweet (flavoured sugar-free vehicle, Perrigo®). Rifabutin was dosed 2.5 mg/kg daily based on extrapolation from adult data, consideration of the unique developmental pharmacology of infants and children, programmatic experience from the APIN programme and adequate concentrations reported by Moultrie *et al.* using a similar total weekly dose.[16,17,20,21]

Rifabutin pharmacokinetic target

While the optimal therapeutic target for rifabutin exposure is not known, our primary outcome measure was rifabutin AUC_{0-24} with a target average exposure of 3.8 μg·h/mL for the population. This is based on data from three studies of adults receiving rifabutin 300 mg daily without ART, in which median AUC_{0-24} values were 2.71, 5.64 and 3.05 μg·h/mL, resulting in a mean AUC_{0-24} of 3.8 μg·h/mL.[12–14] Moreover, AUC_{0-24} of 3.2 μg·h/mL is designated a minimum threshold as the Tuberculosis Trials Consortium 23A study found that rifabutin AUC_{0-24} <3.2 μg·h/mL was associated with failure or relapse due to acquired rifamycin resistance.[22]

Clinical monitoring and toxicity grading

Clinical and laboratory toxicity were monitored at 11 visits over 48 weeks: 1 week after starting TB treatment, every 2 weeks for the first 2 months of therapy, monthly through completion of TB treatment at 24 weeks, then at 36 and 48 weeks. At each visit, a physical examination was performed, clinical toxicities recorded and blood samples collected for determination of total WBC count, absolute neutrophil count (ANC), haemoglobin, platelet count, ALT and creatinine. Participants were monitored clinically for uveitis, a rare complication of rifabutin, and any child with evidence of eye redness, pain, photophobia or visual changes underwent ophthalmic examination.[23,24] An adverse event (AE) was defined as any grade 1–4 event, while a severe AE (SAE) was defined as a grade 3–4 event.[25] Only those laboratory AEs with a severity grade at least one grade higher than the baseline value were considered AEs.

Pharmacokinetic sampling and processing

Steady-state rifabutin pharmacokinetic sampling was performed at three visits: 2, 4 and 8 weeks after starting rifabutin. At each visit, serial rifabutin concentrations were measured pre-dose (0) and at 2, 4, 8, 12 and 24 h after the observed morning dose. Blood samples were processed to separate plasma within 60 min after collection, then stored at −80°C until analysed. Plasma concentrations of rifabutin and the primary metabolite, 25-hydroxy-desacetyl rifabutin (des-rifabutin), were quantified simultaneously at the University of Cape Town utilizing a validated LC-MS/MS assay as previously described.[16] Rifaximin was the internal standard at a concentration of 100 ng/mL. The mean accuracy for rifabutin and des-rifabutin was between 95% and 102% at low, medium and high concentrations during inter-run validation. The validated range for rifabutin was between 3.91 ng/mL [the lower limit of quantification (LLOQ)] and 1000 ng/mL and for des-rifabutin was between 0.780 ng/mL (LLOQ) and 200 ng/mL.

Statistical analyses

Statistical analyses were performed using Stata version 13.1 (StataCorp). Pharmacokinetic data were analysed using non-compartmental methods, with AUC_{0-24} calculated using the linear trapezoidal rule. Concentrations below the LLOQ (BLQ) were given a value of half LLOQ or, in the case of serial BLQ concentrations after maximum drug concentration (C_{max}), the first

timepoint was assigned a value half the LLOQ and subsequent timepoints were given a value of 0 ng/mL. The C_{max} and time to C_{max} (T_{max}) were determined by visual inspection. To account for repeated measures, median (IQR) values were calculated as the population median of each participant's mean AUC (or other pharmacokinetic parameter) at weeks 2, 4 and 8 (for one participant who had a single pharmacokinetic visit at week 2, these values were used as the mean). Similarly, median laboratory values during rifabutin treatment were calculated as the population median of the mean laboratory value during weeks 1 through 24 for each individual. Mean AUC change (95% CI) was calculated as the population mean change from the week 2 value to week 4 or 8, as indicated, for each participant.

The non-parametric Wilcoxon signed rank test was used to compare paired samples due to non-normal distribution of continuous variables. Mixed-effect longitudinal models were utilized to evaluate clinical or laboratory predictors of rifabutin AUC_{0-24} [including study week, age, sex, weight (kg), BMI (kg/m^2), baseline CD4+ cell count and viral load, and rifabutin dosage (total mg and mg/kg dosage)]. Finally, since neutropenia is a potential dose-limiting toxicity of rifabutin use in children, an association between AUC and ANC was explored by Spearman rank based correlation at each of weeks 2, 4 and 8. *P* values <0.05 were considered significant.

Ethics

All patients/caregivers enrolled in the APIN programme provided consent for care and were given the option to allow their de-identified data to be used for future evaluations (Harvard Data Repository protocol 16506). The Partners Institutional Review Board (2014P001768) and Ethics Committees of UCH (UI/EC/15/0072) and JUTH (JUTH/DCS/ADM/127) approved the current study. Since treatment was consistent with standard of care in Nigeria, the primary study intervention involved only sample collection for concentration determination. For study participation, a parent/guardian signed a written consent and children 7 years of age or older signed an assent form. A data safety and monitoring board reviewed results after half of participants were enrolled.

Results

Study population

Fifteen participants were enrolled in this study between January 2017 and March 2018. Baseline patient demographics are summarized in Table 1. The median (range) age of participants was 13.1 (10.2–15.0) years, the median (range) CD4+ cell count was 156 (24–652) cells/mm^3 and the median (range) CD4 percentage was 10% (1%–26%). All children were underweight with a median (range) weight of 25.5 (18.0–45.0) kg and BMI z-score of −1.9 (<−3 to −1.3).

The median (range) duration of first-line ART prior to rifabutin initiation was 3.8 (1.5–8.9) years. Fourteen children were switched from a failing first-line ART regimen to second-line, lopinavir/ritonavir-based ART the same day rifabutin-containing TB treatment was started. However, one child received lopinavir/ritonavir-based ART for 5.9 years prior to TB diagnosis, at which point rifabutin-containing TB treatment was initiated and lopinavir/ritonavir-based ART continued. In addition to lopinavir/ritonavir, 14 children received abacavir plus lamivudine and 1 child initially received abacavir, lamivudine and tenofovir (abacavir was later discontinued).

Clinical response to TB/HIV co-treatment

Most participants (14 of 15, 93%) completed the 6 month TB treatment course with resolution of TB-associated symptoms and weight gain; all 14 children remained free of symptoms at 12 months, suggesting no TB relapse or recurrence (Table 2). One child died just prior to the week 4 study visit after presenting with 5 days of fever, diarrhoea and vomiting. This child had a baseline CD4+ cell count of 24 cells/mm,[3] was the only participant who was lopinavir/ritonavir experienced at entry and HIV genotyping performed retrospectively revealed extensive protease resistance, conferring high-level lopinavir resistance, at study baseline.

Regarding HIV outcomes, the median (IQR) CD4 count increase from baseline to 12 months was 184 (73–316) cells/mm^3. Of 14 participants with viral load measurements at the week 24 visit, 13 (92%) achieved virological suppression (HIV-1 RNA <1000 copies/mL). However, at 48 weeks only nine (64%) were suppressed. HIV genotyping revealed no protease mutations among the five samples with detectable viral load at 12 months.

Laboratory abnormalities at baseline

Prior to initiating rifabutin, most participants (87%; 13 patients) had one or more laboratory abnormalities and three (20%) had asymptomatic grade 3 or 4 abnormalities (Table 3). Anaemia was present in 12 (80%) children (grade 1, $n = 4$; grade 2, $n = 5$; grade 3, $n = 2$; grade 4, $n = 1$). Other abnormalities included: three participants (20%) with baseline neutropenia (grade 1, $n = 2$; grade 2, $n = 1$), one (grade 1) with thrombocytopenia and two with elevated ALT (grade 1).

AEs during rifabutin

Among the 617 individual laboratory evaluations following initiation of rifabutin, compliance with laboratory checks was excellent; excluding the participant who died, all laboratory values were available for 97% (i.e. 150 of 154) of planned visits.

Of the 506 laboratory values obtained during the rifabutin course, there were 56 (11%) AEs (any grade 1–4 events) and 18 (4%) SAEs (grade 3–4 events). After rifabutin initiation, haemoglobin was evaluated 155 times with 83 (54%) meeting criteria for anaemia, but only 16 (13%) instances among seven children were of increased severity from baseline (Table 3). Frequency of severe anaemia decreased over time: seven of eight instances of severe anaemia (six children) occurred at or before the week 6 visit. Two participants exhibited three instances of grade 1 thrombocytopenia and one patient had seven instances of grade 1 ALT elevation (resolved after week 16 despite rifabutin continuation).

During rifabutin therapy, seven (47%) participants had 30 (24%) instances of neutropenia (Table 3). Most (70%; 7 of 10) instances of severe neutropenia fell within the first 8 weeks, during the intensive phase of TB treatment. In all cases, neutropenia resolved or improved despite rifabutin continuation, with no associated adverse clinical events. Of the four participants with grade 3 or 4 neutropenia, it only persisted for two or more consecutive laboratory evaluations once. The proportion of participants experiencing neutropenia by severity grade across study weeks is displayed in Figure 1(a). The intra-participant mean change (95% CI) in ANC from baseline values is notable for a trend toward decreased ANC after treatment initiation that is non-significant with wide inter-participant variability (Figure 1b).

There were no discontinuations of TB or ART medications and no severe clinical toxicities reported. In the one instance of grade 4 neutropenia at the week 4 visit, rifabutin discontinuation was

Table 1. Patient characteristics prior to rifabutin initiation; $N = 15$

Age (years), median (IQR)	13.1 (10.9–14.0)
Female, *n* (%)	8 (53)
Anthropometrics, median (IQR)	
weight (kg)	25.5 (22.3–30.5)
BMI z-score	−1.9 (<−3 to −1.5)
WHO clinical stage, *n* (%)	
3	11 (73)
4	4 (27)
CD4+ cell count (cells/mm^3), median (IQR)	156 (52–294)
CD4%age (%), median (IQR)	10 (4–18)
HIV RNA PCR (copies/mL), median (IQR)	51 530 (22 620–159 241)
Duration of first-line ART prior to rifabutin start (years), median (IQR)	3.8 (2.9–8.1)
Patients with lopinavir/ritonavir exposure prior to rifabutin, *n* (%)	1 (7)
Rifabutin dosage at start, total (mg), median (IQR)	75 (58–82)
Rifabutin mg/kg dosage at start (mg/kg), median (IQR)	2.6 (2.5–2.8)
ART regimen	
NRTI backbone in addition to lopinavir/ritonavir, *n* (%)	
abacavir+lamivudine	14 (93)
abacavir+lamivudine+tenofovir	1 (7)

Table 2. TB and HIV treatment outcomes among children who received rifabutin-containing TB treatment in combination with lopinavir/ritonavir-based ART

TB and HIV treatment outcomes	Six months after rifabutin+lopinavir/ritonavir initiation	Twelve months after rifabutin+lopinavir/ritonavir initiation
TB treatment outcomes, *n* (%)		
completed rifabutin course with no TB symptoms	14 (93)	14 (93)
TB relapse or recurrence	not applicable	0
died	1 (7)	1 (7)
lost to follow-up	0	0
HIV treatment outcomes		
change in CD4+ cell count (cells/mm^3)[a], median (IQR)	71 (−2–155)	184 (73–316)
change in CD4%[a], median (IQR)	6.1 (3.6–22.7)	5.0 (0.6–8.7)
HIV RNA PCR <1000 copies/mL, *n* (%)	13 (92)	9 (64)
Clinical outcomes		
change in weight (kg)[a], median (IQR)	4.5 (0–6.5)	4.5 (3.0–7.6)

[a]Denotes change from baseline to 6 or 12 months after rifabutin+lopinavir/ritonavir initiation.

considered, but continued based on lack of alternative treatment, clinical improvement from baseline, weight gain and repeat ANC improvement and then resolution at week 16 despite rifabutin continuation.

Pharmacokinetics of rifabutin and 25-hydroxy-desacetyl rifabutin

Based on data from 43 pharmacokinetic visits, the median (IQR) rifabutin AUC_{0-24} was 5.21 (4.38–6.60) μg·h/mL, above the study target of 3.8 μg·h/mL (Table 4). While the median rifabutin AUC_{0-24} increased across study weeks (Figure 2), the intra-participant mean changes (95% CI) in rifabutin AUC_{0-24} from weeks 2 to 4 and 2 to 8 were not statistically significant, with a change of 0.95 (−0.56–2.47) and 1.60 (−0.47–3.67) μg·h/mL, respectively. Four instances of low rifabutin AUC_{0-24} (≤3.2 μg·h/mL) occurred at week 2; in all cases exposures were >3.8 μg·h/mL at the 4 and 8 week visits. One additional participant had low AUC at both the 4 and 8 week visits, after an AUC of 3.67 μg·h/mL at week 2.

The median (IQR) des-rifabutin metabolite AUC_{0-24} was 3.12 (2.16–4.28) μg·h/mL (Table 4). Similar to rifabutin, the intra-participant mean changes (95% CI) in des-rifabutin AUC from

Table 3. Laboratory abnormalities at baseline and during rifabutin+lopinavir/ritonavir co-treatment

AEs/SAEs/laboratory parameters	Prior to rifabutin initiation	During rifabutin+lopinavir/ritonavir co-treatment		P^a
	AEs (subjects with AEs, $N=15$; laboratory AEs, $N=60$)	laboratory AEs ($N=506$)	subjects with AEs ($N=15$)	
AEs, *n* (%)				
subjects with AEs[b]	13 (87)	–	10 (67)	
laboratory AEs	18 (30)	56 (11)	–	
SAEs, *n* (%)				
subjects with SAEs[b]	3 (20)	–	9 (60)	
laboratory SAEs	3 (5)	18 (4)	–	
ANC				
median (IQR) ANC (cells/mm^3)	1918 (1332–3400)	1877 (1606–2652)		0.61
AEs by severity[c], *n* (%)	($N=15$)	($N=127$)	($N=15$)	
grade 1	2 (13)	9 (7)	6 (40)	
grade 2	1 (7)	11 (9)	4 (27)	
grade 3	0	9 (7)	4 (27)	
grade 4	0	1 (1)	1 (7)	
Haemoglobin (Hb)				
median (IQR) Hb (g/dL)	9.3 (8.9–10.5)	10.2 (9.1–11.1)		0.09
AEs by severity[c], *n* (%)	($N=15$)	($N=127$)	($N=15$)	
grade 1	4 (27)	4 (3)	1 (7)	
grade 2	5 (33)	4 (3)	3 (20)	
grade 3	2 (13)	7 (6)	5 (33)	
grade 4	1 (7)	1 (1)	1 (7)	
Platelet count (Plt)				
median (IQR) Plt ($\times 10^9$/L)	229 (195–351)	300 (194–355)		0.65
AEs by severity[c], *n* (%)	($N=15$)	($N=127$)	($N=15$)	
grade 1	1 (7)	3 (2)	2 (13)	
grade 2	0	0	0	
grade 3	0	0	0	
grade 4	0	0	0	
ALT				
median (IQR) ALT (IU/L)	20 (17–33)	22 (19–28)		0.57
AEs by severity[c], *n* (%)	($N=15$)	($N=125$)	($N=15$)	
grade 1	2 (13)	7 (6)	1 (7)	
grade 2	0	0	0	
grade 3	0	0	0	
grade 4	0	0	0	

AEs, any grade 1–4 events; SAEs, grade 3–4 events.
Median values during rifabutin represent population medians of intra-participant means.
[a]*P* value for difference between paired samples.
[b]Subjects who experience one or more AEs or SAEs are counted only once.
[c]Subjects are counted only once within a particular severity grade.

weeks 2 to 4 and 2 to 8 were not significant, with a change of 0.49 (−0.14–1.11) and 1.04 (−0.14–2.21) μg·h/mL, respectively.

Utilizing mixed-effect longitudinal models, only study week and BMI (kg/m^2) correlated with rifabutin AUC_{0-24} in unadjusted models. However, in the adjusted model, the effect estimates were slightly attenuated and thus did not achieve significance. Finally, at weeks 4 and 8 the Spearman rank based correlation coefficients were −0.4 ($P=0.11$) and −0.3 ($P=0.25$), respectively, suggesting a moderate, not statistically significant, association between higher rifabutin AUC and lower ANC levels.

Discussion

Novel co-treatment strategies are urgently needed to expand options for TB/HIV-coinfected children. To the best of our knowledge, this study provides the first pharmacokinetic data

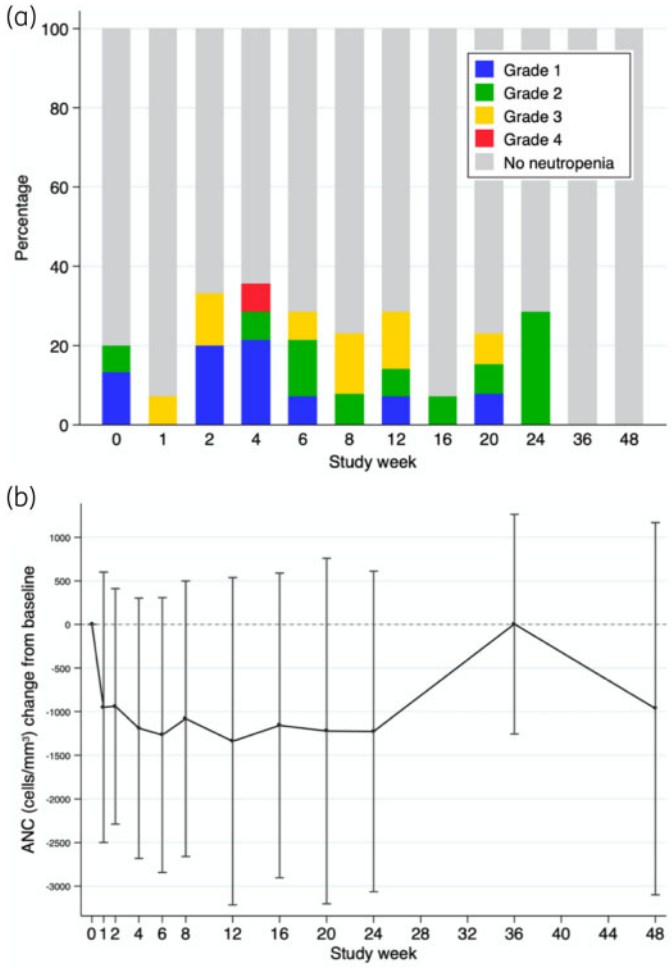

Figure 1. (a) Percentage of children with neutropenia by severity grade across study weeks. (b) Mean (95% CI) intra-participant change in ANC from baseline value.

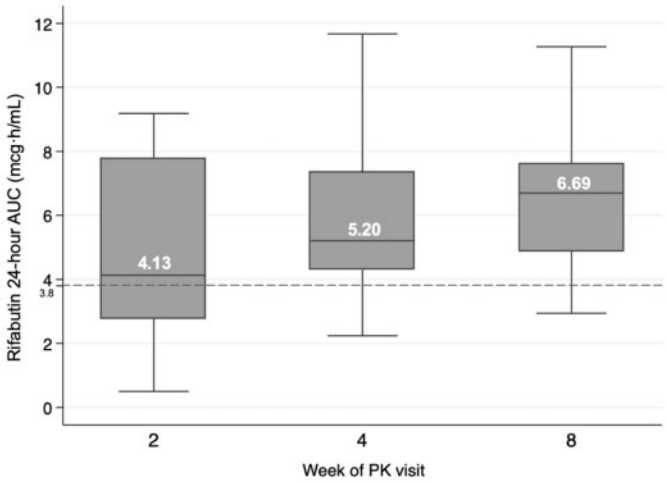

Figure 2. Rifabutin AUC_{0-24} by study visit week. Median AUC_{0-24} with IQR (box) and range (whiskers) by study week, with reference line at rifabutin AUC_{0-24} of 3.8 μg·h/mL, the target population average among adults receiving rifabutin 300 mg daily without lopinavir/ritonavir.

examining a novel rifabutin dosing strategy among coinfected children receiving lopinavir/ritonavir. We found that rifabutin dosed at 2.5 mg/kg daily among children aged 10–15 years and receiving lopinavir/ritonavir-based ART achieved concentrations comparable to adults receiving standard dosing of rifabutin without a drug–drug interaction.[12–14] Severe neutropenia was infrequent and resolved despite rifabutin continuation, which supports our findings in clinical practice, but contrasts with the only other pharmacokinetic study of this combination in children.[16,17] Our findings support rifabutin use among children who require concurrent lopinavir/ritonavir-based ART, a crucial addition to the co-treatment armamentarium for this vulnerable population.

In this study, the median rifabutin AUC_{0-24} was 37% above the mean rifabutin AUC_{0-24} observed in studies of adults receiving rifabutin 300 mg daily without ART, the current standard of practice.[12–14] While the optimal rifabutin exposure is unknown, rifabutin AUC_{0-24} values less than 3.2 μg·h/mL are associated with acquired rifamycin resistance in one study.[22] The median rifabutin C_{max} value was also comparable to that among adults receiving rifabutin 300 mg daily (0.30, 0.79 and 0.29 μg/mL, respectively).[12–14] By comparison, among adults in two studies conducted in Vietnam and South Africa (rifabutin 150 mg daily versus 150 mg thrice weekly with lopinavir/ritonavir), the target rifabutin concentration was achieved with the daily strategy (AUC_{0-24} of 7.29 and 4.77 μg·h/mL, respectively), but not the thrice-weekly strategy (AUC_{0-24} estimated as 3.67 and 2.31 μg·h/mL, respectively).[13,14]

Moultrie *et al.*[16] examined concurrent rifabutin+lopinavir/ritonavir in six children utilizing a thrice-weekly dosing strategy at 5 mg/kg, resulting in a median AUC_{0-24} of 5.36 μg·h/mL (AUC_{0-48} 6.91 μg·h/mL). Their total weekly rifabutin dosage was thus 15 mg/kg, compared with 17.5 mg/kg in our study, yet we observed a total weekly AUC over 1.5 times that observed in their study (weekly AUC 24.2 versus 36.5 μg·h/mL, respectively), which is consistent with adult studies in which increasing from thrice-weekly to daily dosing of rifabutin 150 mg resulted in a > 2-fold increase in AUC_{0-24}.[14]

Overall, the median rifabutin AUC_{0-24} increased with time (Figure 2), though this change was not statistically significant given large interparticipant variability. A similar finding was observed among adults receiving combination lopinavir/ritonavir+rifabutin 300 mg thrice weekly.[12] A rifabutin half-life of 45 h results in an expectation of steady-state rifabutin concentrations by approximately 10 days, so the cause of this finding remains unclear, but the potent inhibitory effect of ritonavir on CYP3A4 activity may be more progressive than previously recognized. Alternately, improved adherence and/or absorption over time could cause a similar finding, though all participants reported perfect adherence through study week 8. Finally, the moderate, but statistically insignificant, association we observed between increasing AUC_{0-24} and decreasing ANC, also reported in the Moultrie *et al.*[16] study, needs to be evaluated in larger studies.

The primary rifabutin metabolite, des-rifabutin, has antimycobacterial activity equal to rifabutin.[26] In two adult studies, receipt of ritonavir-boosted PIs increased metabolite AUC_{0-24} by approximately 5–10-fold compared with rifabutin 300 mg daily without ART (4.13 versus 0.70 and 4.77 versus 0.27 μg·h/mL,

Table 4. Rifabutin and 25-hydroxy-desacetyl rifabutin pharmacokinetic parameters by pharmacokinetic study week

	Rifabutin			25-Hydroxy-desacetyl rifabutin		
	AUC_{0-24} (μg·h/mL)	C_{max} (μg/mL)	T_{max} (h)	AUC_{0-24} (μg·h/mL)	C_{max} (μg/mL)	T_{max} (h)
Median (IQR)[a]	5.21 (4.38–6.60)	0.45 (0.34–0.54)	3.3 (2–3.3)	3.12 (2.16–4.28)	0.17 (0.11–0.22)	5.3 (4–8)
By visit week						
2 weeks	4.13 (3.12–7.73)	0.38 (0.22–0.58)	4 (2–4)	2.34 (1.77–4.10)	0.12 (0.10–0.20)	8 (3–10)
4 weeks	5.20 (4.36–7.23)	0.47 (0.39–0.53)	2 (2–2)	2.84 (2.48–4.71)	0.18 (0.13–0.24)	4 (2–7)
8 weeks	6.69 (5.10–7.56)	0.48 (0.30–0.60)	4 (2–4)	4.01 (3.35–4.55)	0.19 (0.17–0.20)	4 (4–8)

[a]Population median of intra-participant mean of results from weeks 2, 4 and 8.

respectively),[13,14] which may contribute to both efficacy and toxicity. In this study, the median des-rifabutin AUC_{0-24} was similar to that observed in the study by Moultrie *et al.*[16] (3.12 versus 3.34 μg·h/mL, respectively).

Among children, use of rifabutin has been limited by lack of data to support a safe and effective dosing strategy. In the present study, rifabutin at a dose of 2.5 mg/kg daily as part of TB treatment together with lopinavir/ritonavir was well tolerated. No severe clinical AEs occurred during rifabutin+lopinavir/ritonavir co-treatment and severe laboratory abnormalities were uncommon. Treatment-limiting neutropenia in one-third of participants in the Moultrie *et al.*[16] study resulted in early discontinuation. Four of our participants (27%) experienced grade 3 or 4 neutropenia, but this was transient in three (i.e. next available ANC within 2–4 weeks was grade 2 or less). While no instances were associated with clinical events, and neutropenia improved despite ongoing rifabutin therapy, close clinical and laboratory monitoring during co-treatment is indicated. Further, while safety data from this cohort of 15 adolescents are reassuring, larger studies evaluating the safety of this approach are needed.

Overall, we observed favourable clinical outcomes in this population of adolescents for whom long-term adherence may be challenging.[27,28] Symptoms attributed to TB infection resolved by 6 months in all evaluable participants with no subsequent relapse through 12 months of follow-up. Most children (92%) achieved virological suppression at 6 months, though ongoing suppression was limited by suboptimal adherence as indicated by no protease resistance among the five children who failed to maintain suppression at 12 months.

These data are limited by the age (range = 10.2–15.0 years) and nutritional status of enrolled children; all participants were underweight and nearly half met criteria for moderate to severe malnutrition. While it is reassuring that dosing remained adequate among underweight children in this study, an observed weak association between AUC and BMI (data not shown) indicates additional evaluation among malnourished children is needed.[29] Currently, lopinavir/ritonavir-based ART remains the most widely used second-line ART regimen in children, as well as first-line regimen among children less than 3 years of age. We are currently studying rifabutin pharmacokinetics and safety among coinfected children 12–36 months of age, which will add further data to this practice. Strengths of this study include intensive clinical and laboratory follow-up with few missing data points. Specifically, subjects had near perfect compliance with 11 clinical and laboratory evaluations during 12 months of follow-up with few missing values and three pharmacokinetic visits during treatment, providing robust data for the patient population. Finally, children in this study received a rifabutin suspension that was prepared by pharmacists, which may limit broader implementation, but highlights the importance of developing paediatric formulations.

In conclusion, these data support the use of rifabutin 2.5 mg/kg daily among coinfected children who require lopinavir/ritonavir. This co-treatment strategy achieved AUC_{0-24} comparable to adults, with favourable HIV and TB outcomes observed and relatively uncommon SAEs. Among adults who require PI-based ART, rifabutin is the rifamycin of choice and is recommended by the WHO.[5] To the best of our knowledge, this study provides the first data that support paediatric rifabutin use, a novel addition to current limited co-treatment options for children.

Acknowledgements

Results from a subset of participants included in this analysis were presented at the International Workshop on Clinical Pharmacology of Antiviral Therapy, Baltimore, MD, USA, 2018 (Abstract 13).

We would like to thank all the children and their parents/caregivers who participated and acknowledge the hard work and dedication of the clinical, data and laboratory staff at the APIN paediatric ART sites. We would also like to thank the data safety and monitoring board for their expert review.

Funding

This research was supported by a Harvard University Center for AIDS Research grant (P30 AI060354) and the National Institute of Allergy and Infectious Diseases (K23AI125122 to H.E.R.). Clinical care was supported by the President's Emergency Plan for AIDS Relief through the US Centers for Disease Control and Prevention (CDC) under the terms of Cooperative Agreement (U51HA02522). H.M.M. is funded by the Wellcome Trust (206379/Z/17/Z). The University of Cape Town Clinical Pharmacokinetics Laboratory is supported in part by the AIDS Clinical Trials Group (ACTG), by NIAID (UM1AI068634, UM1AI068636 and UM1AI106701), as well as the Infant Maternal Pediatric Adolescent AIDS Clinical Trials Group (IMPAACT; U01 AI068632).

Transparency declarations

None to declare.

Disclaimer

The contents are solely the responsibility of the authors and do not necessarily represent the official views of the funding institutions.

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