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Pharmacokinetics and safety of rifabutin in young HIV-infected children receiving rifabutin and lopinavir/ritonavir

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Objectives: Co-treatment of HIV and TB in young children is complicated by limited treatment options and complex drug–drug interactions. Rifabutin is an alternative to rifampicin for adults receiving a ritonavir-boosted PI. We aimed to evaluate the short-term safety and pharmacokinetics of rifabutin when given with lopinavir/ritonavir in children.

Patients and methods: We conducted an open-label study of rifabutin dosed at 5 mg/kg three times a week in HIV-infected children ≤5 years of age receiving lopinavir/ritonavir. Intensive steady-state pharmacokinetic sampling was conducted after six doses. The Division of AIDS 2004, clarification 2009, table for grading severity of adverse events was used to classify drug toxicities. The study was registered with ClinicalTrials.gov, number NCT01259219.

Results: Six children completed the study prior to closure by institutional review boards. The median (range) AUC_{0-48} of rifabutin was 6.91 (3.52–8.67) μg·h/mL, the median (range) C_{max} of rifabutin was 0.39 (0.19–0.46) μg/mL, the median (range) AUC_{0-48} of 25-*O*-desacetyl rifabutin was 5.73 (2.85–9.13) μg·h/mL and the median (range) C_{max} of 25-*O*-desacetyl rifabutin was 0.17 (0.08–0.32) μg/mL. The neutrophil count declined in all children; two children experienced grade 4 neutropenia, which resolved rapidly without complications. There was strong correlation between AUC_{0-48} measures and neutrophil counts.

Conclusions: Rifabutin dosed at 5 mg/kg three times per week resulted in lower AUC_{0-48}, AUC_{0-24} and C_{max} values for rifabutin and 25-*O*-desacetyl rifabutin compared with adults receiving 150 mg of rifabutin daily, the current recommended dose. We observed high rates of severe transient neutropenia, possibly due to immaturity of CYP3A4 in young children. It remains unclear whether a safe and effective rifabutin dose exists for treatment of TB in children receiving lopinavir/ritonavir.

Keywords: treatment, tuberculosis, TB

Introduction

TB is the commonest opportunistic infection in HIV-infected children, with almost one in three children in South Africa on TB treatment at the time of initiating combination ART (cART).[1] While cART should be initiated as soon as TB treatment is tolerated, initiation of cART in young children on TB treatment is complicated by clinically significant drug–drug interactions between rifampicin and antiretroviral drugs, as well as limited paediatric drug formulations for concomitant TB and HIV treatment. In resource-poor countries, most adults with HIV/TB coinfection receive a combination of efavirenz-based cART and rifampicin-based TB treatment. Efavirenz is not recommended in children <3 years of age despite recent FDA approval as there is considerable pharmacokinetic variability and data are limited.[2] For children <3 years with TB/HIV coinfection, the 2013 WHO guidelines recommend either a triple NRTI regimen or two NRTIs plus nevirapine in children co-treated with rifampicin-containing TB therapy.[2] While no randomized controlled trials (RCTs) of these regimens have been performed in young HIV-infected children with active TB, data from studies in children without active TB suggest that both of these regimens are suboptimal. Two RCTs have demonstrated lower virological suppression rates in young children without TB when treated with nevirapine- compared with

lopinavir/ritonavir-based cART.[3,4] Furthermore, there are concerns that co-treatment with rifampicin might result in a reduction in nevirapine concentrations in children.[5] The ARROW trial demonstrated significantly worse long-term viral load suppression rates in those children who were switched to a triple NRTI regimen 36 weeks after commencing a four-drug regimen consisting of nevirapine and three NRTIs.[6,7]

The induction of hepatic and intestinal cytochrome P450 3A4 (CYP3A4) and P-glycoprotein results in inadequate lopinavir exposure in children when lopinavir/ritonavir is administered at either standard or double dose together with rifampicin.[8] In South Africa, the recommended cART regimen for children <3 years who receive concomitant treatment for TB is to add additional ritonavir such that the lopinavir/ritonavir ratio is increased from 4:1 to 1:1 together with two NRTIs.[9] This regimen is also suboptimal as the short shelf life of ritonavir results in frequent stock outs and the palatability of ritonavir is poor.

Rifabutin is recommended as an alternative to rifampicin to treat TB in combination with PI-containing cART in adults and was added to the WHO essential medicine list in 2009 for treatment of TB in HIV-infected adults treated with ritonavir-boosted PI-containing cART.[10,11] In contrast to rifampicin, rifabutin induces the activity of CYP3A4 and P-glycoprotein to a limited extent and does not have clinically significant effects on the concentrations of concomitantly administered lopinavir/ritonavir.[10,12] However, ritonavir-mediated CYP3A4 inhibition inhibits the metabolism of both rifabutin and its active metabolite, 25-*O*-desacetyl rifabutin and results in a substantial increase in exposure, necessitating a reduction in the dose of rifabutin.[12] Because of concerns of inadequate response associated with rifabutin dosed at 150 mg three times a week, the recommended dose of rifabutin in adults receiving concomitant ritonavir-boosted PI-based cART has recently been increased to 150 mg daily or 300 mg three times a week.[13]

Safety data for concomitant administration of rifabutin and lopinavir/ritonavir in HIV-infected individuals in resource-limited settings are scarce. Haematological toxicities, most commonly neutropenia and thrombocytopenia, associated with rifabutin dose and/or coadministration of CYP3A4 inhibitors have been well described, though it remains unknown whether the occurrence of toxicities is associated with specific pharmacokinetic parameters.[14–17] While resolution is generally rapid after discontinuation of rifabutin, monitoring of haematological parameters is recommended. In addition, monitoring for uveitis is required.[10]

Currently, the major obstacle to the use of rifabutin to treat TB in young HIV/TB-coinfected children receiving concomitant lopinavir/ritonavir is the absence of pharmacokinetic and safety data and no suitable paediatric formulation of rifabutin. We aimed to determine the optimal dosing strategy, pharmacokinetic profile and short-term safety of rifabutin when given concomitantly with a lopinavir/ritonavir-containing cART regimen in HIV-infected children ≤5 years of age in Soweto, South Africa.

Patients and methods

Study design and treatment

We conducted an open-label, adaptive study to determine the short-term safety and optimal dose of rifabutin when coadministered with lopinavir/ritonavir in young (age ≤5 years) HIV-infected children residing in a high TB/HIV burden, resource-limited setting. The optimal dose was defined as the dose that resulted in a geometric mean AUC_{0-48} of 4.5–6 μg·h/mL, in keeping with the levels reported from studies in adults receiving 150 mg three times a week,[17–19] the recommended adult dose at the time of enrolment into the study.[20] The initial rifabutin dose was selected based on the 150 mg used thrice weekly in adults adjusted for children based on their weight using the established scientific framework of an allometric scaling (clearance and volume having allometric exponents of 0.75 and 1, respectively[21]). Accordingly, a 10 kg child would be expected to have approximately twice the clearance (per kilogram of body weight) of an adult, corresponding to approximately twice the per kilogram dose requirement in children aged 1–5 years. A rifabutin dose of 5 mg/kg three times per week thus was anticipated to correspond to the 150 mg three times a week rifabutin dose for adults when co-treated with lopinavir/ritonavir.[12,20]

Participating children received a total of six doses of 5 mg/kg rifabutin (three times a week on Mondays, Wednesdays and Fridays over a 2 week period). All children took the first dose of rifabutin on a Friday and the final dose on a Wednesday. The rifabutin suspension was compounded by research pharmacists using 150 mg Mycobutin® capsules, OraSure® and Orasweet®.[22] Since one anticipates minimal change in lopinavir exposures with the addition of rifabutin, lopinavir/ritonavir dosages were dosed twice daily according to the WHO weight bands and not altered during coadministration with rifabutin.[23] The first and sixth doses of rifabutin were directly observed. Parents were telephonically contacted after all other rifabutin doses to confirm administration according to schedule. In addition, adherence to rifabutin and antiretroviral agents was assessed using diary cards and weighing of medicine bottles at dispensing and on return. A full medical history and clinical examination were conducted at screening, enrolment, the three consecutive pharmacokinetic sampling days (days 13, 14 and 15) and at the close-out visit, which was conducted between 2 and 4 weeks after the last dose.

A data and safety monitoring board (DSMB) consisting of two independent South African infectious diseases paediatricians and an independent US-based pharmacologist was constituted and reviewed all serious adverse events and any grade 3 or 4 adverse events according to the NIAID Division of AIDS (DAIDS) 2004, clarification 2009, grading tables.[24] The study protocol mandated that enrolment would be halted if the DSMB determined that any serious adverse event, grade 3 or 4 adverse event or uveitis was related to rifabutin exposure. In that case, all pharmacokinetic samples already collected would be analysed and a DSMB review of all data would be conducted. In addition, an interim analysis of rifabutin pharmacokinetic parameters was planned after the first 10 children completed pharmacokinetic assessments in order to determine the final sample size.

The study was approved by the Human Research Ethics Committees of the University of the Witwatersrand and University of Cape Town and the Institutional Review Board of the University of North Carolina Chapel Hill. The study was registered with ClinicalTrials.gov, number NCT01259219. Written informed consent was obtained from the parents of all participating children.

Study population

HIV-infected children ≤5 years of age who had successfully completed a course of therapy for active TB within the past 2–6 weeks and who were receiving a cART regimen containing lopinavir/ritonavir at the Harriet Shezi Children's Clinic in Soweto were invited to participate. The requirement to have recently successfully completed a course of therapy, defined as completed a course with resolution of symptoms and signs, was instituted to reduce the risk that an HIV-infected child with untreated or undiagnosed active TB would be exposed to rifabutin monotherapy. To reduce potential risk associated with administration of rifabutin in the absence of direct benefit, children were excluded if they had a history of symptomatic clinical hepatitis during TB treatment, ALT >2.5× the upper limit of normal (ULN), bilirubin >1.5× ULN, creatinine >1.1× ULN, haemoglobin <8 g/dL,

neutrophil <1000 cells/mm^3, platelets <125 000 cells/mm^3, any pre-existing eye condition at screening or required treatment with another drug with potential for interaction with rifabutin.

Pharmacokinetic and safety sampling

Blood for safety parameters was drawn for full blood count, white blood cell differential count, liver function tests, bilirubin, urea, creatinine and electrolytes at screening and full blood count, white blood cell differential count and liver function tests at first scheduled pharmacokinetic visit. Children were screened for symptoms and signs of uveitis at every visit and were referred for slit lamp examination by an ophthalmologist if required. Serial blood samples for pharmacokinetic analysis were collected pre-dose (0 h) and at 2, 4, 9, 24 and 48 h after the observed administration of the sixth rifabutin dose. Samples were centrifuged at 2600 rpm for 10 min and plasma was extracted and stored at −70°C.

Rifabutin and 25-*O*-desacetyl rifabutin were analysed with a validated LC/MS/MS assay. The samples were processed with a protein precipitation extraction method using 50 μL of plasma and 300 μL of acetonitrile. Rifaximin was used as an internal standard and was spiked into the precipitation solvent at a concentration of 100 ng/mL. Gradient chromatography was performed on a Phenomenex, Luna 5 μm PFP(2), 100 A, 50 mm×2 mm analytical column, using acetonitrile and 0.1% formic acid as mobile phase, and was delivered at a flow rate of 500 μL/min. An AB Sciex API 3200 mass spectrometer was operated at unit resolution in the multiple reaction monitoring mode, monitoring the transition of the protonated molecular ions at *m/z* 847.4 to the product ions at *m/z* 95.1 for rifabutin, the protonated molecular ions at *m/z* 805.4 to the product ions at *m/z* 95.1 for 25-*O*-desacetyl rifabutin and the protonated molecular ions at *m/z* 786.3 to the product ions *m/z* 151.1 for the internal standard. The accuracies (%Nom) for rifabutin and 25-*O*-desacetyl rifabutin were between 99.1% and 109.0% at low, medium and high quality control (QC) levels during interbatch validation. The percentage coefficient of variation (%CV) for rifabutin and 25-*O*-desacetyl rifabutin during interbatch validation was <9.2% at low, medium and high QC levels. The calibration range for rifabutin was between 3.91 ng/mL and 1000 ng/mL and for 25-*O*-desacetyl rifabutin the calibration range was between 0.780 ng/mL and 200 ng/mL.

Statistical analysis

Age- and sex-adjusted *Z*-scores for weight and height were calculated using WHO 2006 growth references.[25] Pharmacokinetic data were analysed in Stata 12.2 (StataCorp) using non-compartmental methods and the trapezoid rule to obtain the area under the curve from 0 to 24 h (AUC_{0-24}) and from 0 to 48 h (AUC_{0-48}), C_{max}, T_{max} and elimination half-life. Pearson correlation coefficients were calculated to assess the strength of correlation between the absolute neutrophil count (ANC) and pharmacokinetic parameters.

Results

Participant characteristics

Between December 2010 and July 2011, informed consent was obtained for seven children; six were enrolled and one died of acute pneumonia prior to the screening visit. The median (range) age and weight of the children were 27 (10–41) months and 10.6 (8.8–12.2) kg, respectively (Table 1). Three children had a history of WHO stage IV-defining conditions: two extrapulmonary TB and one confirmed cytomegalovirus infection without ocular involvement. Children had been on lopinavir/ritonavir-based cART for a median of 7 months (range 6–28) and had completed TB treatment a median of 4 weeks (range 3.9–5.7) prior to the first dose of rifabutin. The median CD4% at enrolment was 24.9% (range 15.3%–45.8%). HIV-1 RNA viral load on ART was available for four children; all had ≤400 copies/mL. All six children received daily co-trimoxazole prophylaxis according to South African guidelines.[26] Adherence to rifabutin as assessed by weighing of medicine

Table 1. Baseline demographic and clinical characteristics of children

	Child					
	1	2	3	4	5	6
Age (months)	12	21	29	10	41	33
Weight (kg)	10.1	9.5	11.8	8.8	12.2	11.1
Weight-for-age *Z*-score	0.9	−1.2	−1.0	−0.8	−1.8	−1.9
Height-for-age *Z*-score	−0.1	−2.3	−3.1	−2.4	−2.6	−1.3
WHO stage	3	3	4	4	4	3
Prior WHO AIDS condition	PTB	PTB	EPTB	CMV	EPTB	PTB
CD4%	15.3	24.0	24.9	NA	29.5	45.8
CD4 count (cells/mm^3)	1004	1084	1695	NA	1108	982
Viral load (copies/mL)	400	149	NA	NA	<400	190
Haemoglobin (g/dL)	12.6	11.9	10.3	12.0	10.9	11.8
White cell count (10^9/L)	13.2	11.6	10.3	10.5	16.9	5.3
ANC (10^9/L)	1.8	1.8	2.7	2.0	8.6	1.5
Platelets (10^{12}/L)	451	333	627	385	330	401
ALT (U/L)	19	26	18	20	43	22
ART drugs in addition to LPV/r	d4T+3TC	3TC+ABC	3TC+ABC	3TC+ABC	d4T+3TC	d4T+3TC
Months on LPV/r-based ART	7	6	6	7	28	19
Time since end of TB treatment (weeks)	5.7	3.9	4.3	4.4	4.4	4.1

PTB, pulmonary TB; EPTB, extrapulmonary TB; CMV, cytomegalovirus; NA, not available; d4T, stavudine; 3TC, lamivudine; ABC, abacavir; LPV/r, lopinavir/ritonavir.

containers was excellent with adherence ranging between 98% and 110% in the five children for whom this could be assessed.

Pharmacokinetics of rifabutin

All children had measurable rifabutin concentrations at all timepoints, but there was substantial interindividual variation despite uniform dosing (Table 2). The median AUC_{0-48} of rifabutin was 6.91 μg·h/mL, ranging from 3.52 to 8.67 μg·h/mL. Only one child had a rifabutin AUC_{0-48} below the target range of 4.5–6.0 μg·h/mL. The median rifabutin C_{max} was 0.385 μg/mL, ranging from 0.19 to 0.46 μg/mL. One of the six children had a C_{max} >0.45 μg/mL. The rifabutin half-life was 16.0 h (range 10.9–38.6). Time of C_{max} indicated delayed absorption in child 1.

Pharmacokinetics of 25-O-desacetyl rifabutin

All children had measurable 25-*O*-desacetyl rifabutin concentrations at all timepoints (Table 2). The median AUC_{0-48} of 25-*O*-desacetyl rifabutin was 5.73 μg·h/mL (range 2.9–9.1). The median 25-*O*-desacetyl rifabutin C_{max} was 0.17 μg/mL. The half-life of 25-*O*-desacetyl rifabutin was 36.2 h (range 11.9–55.0).

Table 2. Rifabutin and 25-*O*-desacetyl rifabutin pharmacokinetic parameters from non-compartmental analyses in six HIV-infected children

	AUC_{0-24} (μg·h/mL)	AUC_{0-48} (μg·h/mL)	Half-life (h)	C_{max} (μg/mL)	T_{max} (h)
Rifabutin					
Child 1	6.11	7.31	10.9	0.45	9
Child 2	5.77	8.03	14.3	0.35	4
Child 3	2.33	3.52	37.1	0.19	2
Child 4	4.96	6.49	17.4	0.46	4
Child 5	3.46	5.67	38.6	0.21	2
Child 6	6.29	8.67	14.6	0.42	2
median	5.36	6.91	16.0	0.39	3
25-*O*-desacetyl rifabutin					
Child 1	5.95	9.13	21.0	0.32	9
Child 2	3.40	6.01	39.8	0.17	9
Child 3	1.62	2.85	55.0	0.08	4
Child 4	3.29	5.46	32.6	0.17	9
Child 5	2.19	3.95	54.2	0.10	4
Child 6	4.61	6.80	11.9	0.23	4
median	3.34	5.73	36.2	0.17	7

Combined rifabutin and 25-O-desacetyl rifabutin pharmacokinetic parameters

The median AUC_{0-48} and C_{max} ratios of 25-*O*-desacetyl rifabutin to rifabutin were 0.80 (range 0.70–1.24) and 0.49 (range 0.38–0.72), respectively. The median combined AUC_{0-48} for rifabutin plus 25-*O*-desacetyl rifabutin was 12.99 μg·h/mL (range 6.36–16.55).

Adverse events

The ANC decreased in all six children. Two children developed grade 4 neutropenia, one grade 2 neutropenia and one grade 1 neutropenia when graded according to the DAIDS 2004, clarification August 2009, table (Table 3).[24] Both of the grade 4 neutropenias resolved to grade 1 without sequelae, 2 and 6 days after cessation of drug exposure. Apart from one child who had an intercurrent infection, no other potential causes for decline in ANC were identified. When neutrophil counts were graded according to the pre-2004 grading table, there were no grade 4 events, one grade 3, one grade 2 and two grade 1 neutropenias (Table 3).[27]

The median platelet count decreased from 393 000 cells/mm^3 (range 330 000–627 000) to 260 000 cells/mm^3 (range 130 000–493 000). None of the six children developed elevated liver enzymes, uveitis, myalgia, arthralgia or gastrointestinal symptoms.

Correlation between ANC and pharmacokinetic parameters

When excluding the one outlier (child with an elevated baseline ANC of 8600 cells/mm^3 and who had had an intercurrent illness and whose ANC therefore could be expected to change substantially in the time period of interest independent of rifabutin exposure), there was a strong correlation between pharmacokinetic parameters and ANC measured during rifabutin administration (Figure 1a–c): $r=-0.85$ ($P=0.07$) for correlation between rifabutin AUC_{0-48} and ANC; $r=-0.94$ ($P=0.02$) for correlation between AUC_{0-48} of the metabolite 25-*O*-desacetyl rifabutin and ANC; and $r=-0.96$ ($P=0.01$) for correlation between the combined AUC_{0-48} of rifabutin and its metabolite 25-*O*-desacetyl rifabutin and ANC. The correlation between C_{max} and ANC was $r=-0.67$ ($P=0.21$) for rifabutin C_{max} and $r=-0.90$ ($P=0.04$) for 25-*O*-desacetyl rifabutin C_{max}. There was no correlation between any of these pharmacokinetic parameters and ANC when the outlier was not excluded.

Table 3. Decrease in ANC and DAIDS adverse event gradings

Child	Rifabutin dose (mg)	Rifabutin dose (mg/kg)	Baseline ANC (cells/mm^3)	Post-rifabutin ANC[a] (cells/mm^3)	DAIDS 2004 grading[24]	DAIDS 1994 grading[27]
6	56	5.00	1540	450	4	2
1	50	4.95	1810	330	4	3
2	48	5.05	1800	770	2	1
4	44	5.00	1960	1160	1	1
3	60	5.08	2690	1490	—	—
5	62	5.05	8600	3270	—	—

[a]Taken on first pharmacokinetic day (day 13).

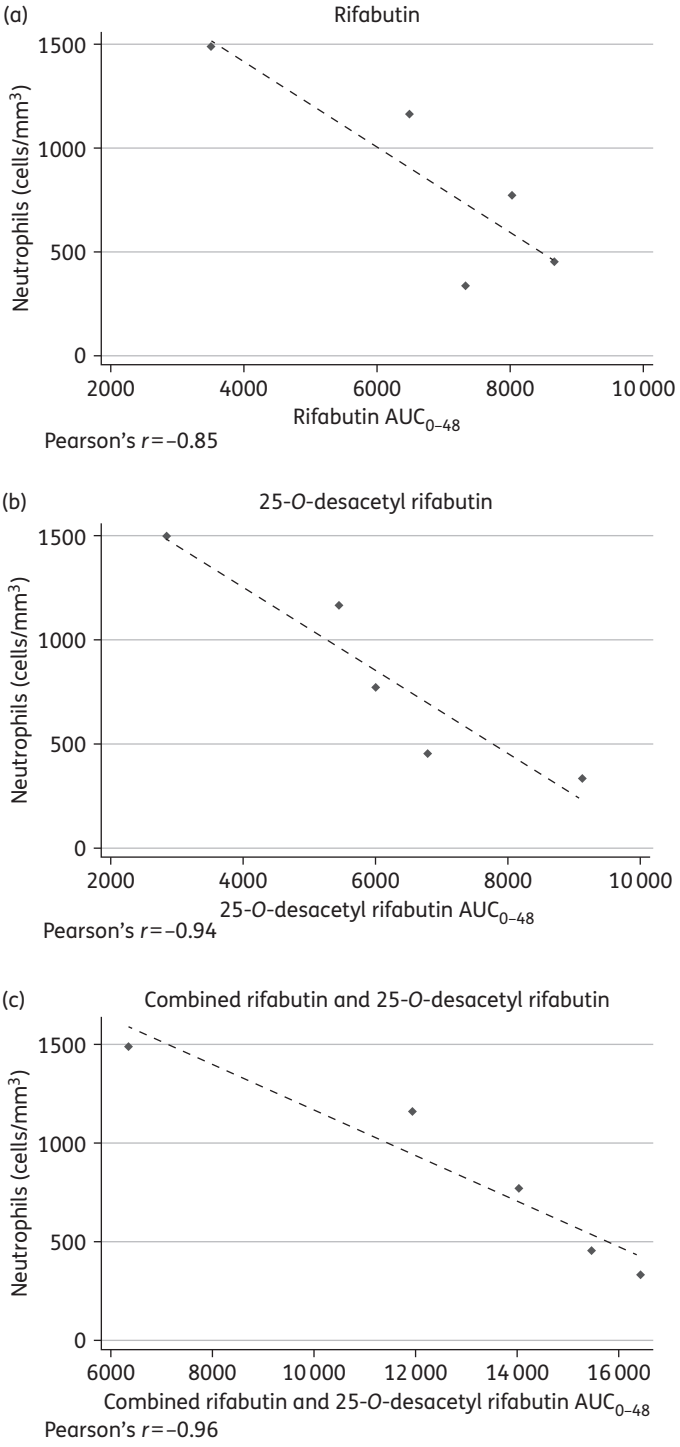

Figure 1. Correlation between ANC on day 13 of rifabutin exposure and (a) rifabutin AUC_{0-48}, (b) 25-*O*-desacetyl rifabutin AUC_{0-48} and (c) combined rifabutin and 25-*O*-desacetyl rifabutin AUC_{0-48}.

Review of study by DSMB, ethics committees and funding agency

Per protocol DSMB reviews were conducted after the first and sixth children had grade 4 neutropenia. The DSMB classified the first case as unrelated to rifabutin as the child had an intercurrent infection at the time and the second case as potentially related to rifabutin. The DSMB and Human Research Ethics Committees of the University of the Witwatersrand and Cape Town approved an amended protocol with a reduced rifabutin dose (from 5 to 3 mg/kg three times a week), an additional safety visit after the third dose of rifabutin and a switch to the DAIDS 1994 grading tables to address the issue of ethnic neutropenia. The Institutional Review Board of the University of North Carolina determined that the study required approval under 45 CFR 46.407 because of greater than minimal risk without direct benefit. The study was stopped when the funding agency decided not to pursue the 407 process.

Discussion

In this study, we found that rifabutin dosed at 5 mg/kg three times a week together with a lopinavir/ritonavir-containing cART regimen in young (≤5 years) children resulted in generally higher AUC_{0-48}, AUC_{0-24} and C_{max} values for rifabutin and 25-*O*-desacetyl rifabutin compared with adults receiving 150 mg of rifabutin three times a week,[18,19] though lower than the values associated with the more recent recommendations of 150 mg of rifabutin daily.[19,28] High rates of severe neutropenia were observed and values for rifabutin and 25-*O*-desacetyl rifabutin AUC were strongly correlated with low neutrophil counts during rifabutin administration.

Despite the dose adjustments made to account for expected differences in clearance between children and adults,[29] the median rifabutin AUC_{0-24}, AUC_{0-48} and C_{max} parameters observed in these young children were higher than those reported in a study of 10 adults in the USA receiving 150 mg of rifabutin three times a week in combination with lopinavir/ritonavir.[18] In these adults, Boulanger *et al.*[18] observed a median rifabutin C_{max} of 0.23 (0.04–0.32) μg/mL and AUC_{0-48} of 4.42 (0.96–7.48) μg·h/mL, lower than the median C_{max} of 0.39 (0.19–0.46) μg/mL and AUC_{0-48} of 6.91 (3.52–8.67) μg·h/mL observed in our paediatric study. In contrast, a study of 13 Vietnamese adults receiving 150 mg of rifabutin thrice weekly in combination with lopinavir/ritonavir observed higher median values for C_{max} [0.54 (0.06–0.96) μg/mL] and AUC_{0-48} [7.34 (1.43–10.90) μg·h/mL] than those observed in our study.[28]

The rifabutin AUC_{0-48} in our study participants receiving rifabutin three times per week were not surprisingly substantially lower than those in both Vietnamese (14.58 μg·h/mL) and South African (9.53 μg·h/mL) adults when dosed at 150 mg of rifabutin daily, the current recommended dose in combination with lopinavir/ritonavir.[19,28]

We also found high AUC_{0-48} values for the active metabolite 25-*O*-desacetyl rifabutin [5.73 (2.85–9.13) μg·h/mL], higher than those observed in US adults [2.70 (1.39–4.23) μg·h/mL] and Vietnamese adults [3.81 (0.87–7.63) μg·h/mL] when dosed at 150 mg three times a week. This is consistent with potent inhibition of CYP3A4 by ritonavir and the immaturity of the CYP3A4 enzyme in young infants.[29]

The decline in ANC in all children soon after administration of rifabutin, with two of six children experiencing severe neutropenia after six doses of rifabutin, is cause for concern, even if the neutropenia resolved rapidly and without sequelae after withdrawal of rifabutin. While neutropenia has been described in adults receiving rifabutin, the rate observed in this group of young children was

higher than expected. In the study of Vietnamese adults, only 1 in 12 adults developed a grade 3 neutropenia and no grade 4 neutropenia was observed. Similarly, in the study of US adults, two patients developed grade 2 neutropenia and no grade 3 or 4 neutropenia was observed. The strong correlation between the ANC under rifabutin and high plasma concentration of both rifabutin and its metabolite in our study suggest that the neutropenia observed may be the consequence of immaturity of CYP3A4 in these young children. It is important to note however that when 1994 DAIDS grading tables were applied the grades of neutropenia experienced in our study were less severe, supporting the need for population-specific grading tables for neutrophil counts to accommodate relative ethnic neutropenia.[30–32]

In conclusion, the 5 mg/kg dose of rifabutin resulted in lower rifabutin and 25-*O*-desacetyl rifabutin exposures in young children than those obtained in adults dosed at 150 mg of rifabutin daily, the current recommended dose for co-treatment with a ritonavir-boosted PI. The high interindividual variability in plasma concentrations, the high rates of neutropenia and the strong association between rifabutin and 25-*O*-desacetyl rifabutin plasma concentrations and neutrophil count suggest that it will be difficult to identify a safe and effective dose of rifabutin for young children in need of concomitant treatment with lopinavir/ritonavir.

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Transparency declarations

None to declare.

Author contributions

H. Moultrie and A. V. R. contributed to the conceptualization, design, acquisition, analysis and interpretation of the data and drafting and revision of the manuscript. H. McIlleron contributed to the design, acquisition, analysis and interpretation of the data and drafting and revision of the manuscript. S. S., G. K. and H. G. contributed to the conduct and safety of the study, acquisition of data and drafting and revision of the manuscript. T. K. and L. W. contributed to the pharmacokinetic data acquisition and analysis and drafting and revision of the manuscript. All authors approved the final manuscript.

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