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Population pharmacokinetics of a combination of miltefosine and paromomycin in Eastern African children and adults with visceral leishmaniasis

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Objectives: To improve visceral leishmaniasis (VL) treatment in Eastern Africa, 14- and 28-day combination regimens of paromomycin plus allometrically dosed miltefosine were evaluated. As the majority of patients affected by VL are children, adequate paediatric exposure to miltefosine and paromomycin is key to ensuring good treatment response.

Methods: Pharmacokinetic data were collected in a multicentre randomized controlled trial in VL patients from Kenya, Sudan, Ethiopia and Uganda. Patients received paromomycin (20 mg/kg/day for 14 days) plus miltefosine (allometric dose for 14 or 28 days). Population pharmacokinetic models were developed. Adequacy of exposure and target attainment of paromomycin and miltefosine were evaluated in children and adults.

Results: Data from 265 patients (59% ≤12 years) were available for this pharmacokinetic analysis. Paromomycin exposure was lower in paediatric patients compared with adults [median (IQR) end-of-treatment AUC_{0-24h} 187 (162–203) and 242 (217–328) μg·h/mL, respectively], but were both within the IQR of end-of-treatment exposure in Kenyan and Sudanese adult patients from a previous study. Cumulative miltefosine end-of-treatment exposure in paediatric patients and adults [AUC_{D0-28} 517 (464–552) and 524 (456–567) μg·day/mL, respectively] and target attainment [time above the *in vitro* susceptibility value EC_{90} 27 (25–28) and 30 (28–32) days, respectively] were comparable to previously observed values in adults.

Conclusions: Paromomycin and miltefosine exposure in this new combination regimen corresponded to the desirable levels of exposure, supporting the implementation of the shortened 14 day combination regimen. Moreover, the lack of a clear exposure–response and exposure–toxicity relationship indicated adequate exposure within the therapeutic range in the studied population, including paediatric patients.

Introduction

Visceral leishmaniasis (VL), a potentially fatal disease caused by the *Leishmania* parasite, is endemic in Eastern Africa,[1] where effective, safe and affordable treatments are still lacking. In Eastern Africa in particular, the majority of those affected by VL are children. This population is vulnerable and often malnourished, and has a higher risk of failure of VL treatment. Paromomycin and miltefosine are favourable treatment options because of their relatively good safety profile and suitability for

use in remote areas. However, earlier studies with paromomycin or miltefosine monotherapy, or a combination with other drugs, resulted in unsatisfactory efficacy in Africa, with high geographical variability.[2–5] A combination therapy of miltefosine and paromomycin has been shown to be highly effective in Asia (96.9%–98.7% cure rate).[6] In Eastern Africa, an adapted 14 day regimen of paromomycin (20 mg/kg/day) plus miltefosine (twice-daily allometric dose) was recently shown to exhibit a satisfactory cure rate of 91.2%.[7] This regimen is better than the current first-line therapy (a 17 day combination therapy of sodium stibogluconate plus paromomycin), as it reduces hospitalization by 3 days, and removes one painful daily injection. More importantly, this is the first combination therapy for VL in Eastern Africa without an antimonial component, reducing the risk of fatal toxicities, such as antimony-associated cardiotoxicity and pancreatitis.

Pharmacokinetic studies have been performed to evaluate whether the lower treatment response to paromomycin and miltefosine in Africa was caused by underexposure to the drugs, and whether dose adaptations would be warranted for Eastern African patients. A previous pharmacokinetic study of paromomycin in Eastern African and Indian VL patients revealed important geographical differences in paromomycin exposure, although drug efficacy could not be linked to differences in drug exposure.[8] Although paromomycin treatment at 15 mg/kg/day for 21 days is effective in India, an increased regimen of 20 mg/kg/day for 21 days is required for patients in Eastern Africa to achieve satisfactory efficacy, suggesting a different exposure–response relationship between the populations.[7,9] Miltefosine monotherapy treatment failure using the conventional mg/kg dosing regimen (2.5 mg/kg/day for 28 days) in Kenya and Sudan has been associated with low drug exposure in paediatric patients.[3,10] The time above the *in vitro* intracellular susceptibility of 90% maximal effective concentration of miltefosine (EC_{90}) has been identified as a predictor for VL relapse in Eastern African VL patients.[10] An adapted allometric miltefosine dose for 28 days in paediatric patients led to increased and less variable exposure, with adequate exposure, pharmacokinetic target attainment, and satisfactory efficacy of 90%, equivalent to adults.[11]

Whereas underexposure to the drugs can lead to poor treatment efficacy, overexposure could lead to drug-induced complications, such as liver toxicity (miltefosine), renal toxicity or ototoxicity (paromomycin). For miltefosine, an exposure–response relationship has clearly been demonstrated, indicating lower exposure with higher risk of relapse.[3,10] An exposure–response relationship has not been demonstrated for paromomycin; however, the initial lack of paromomycin efficacy appears to be exposure-driven, as a dose increase from 15 to 20 mg/kg led to improved efficacy.[5,12] Although paromomycin efficacy does not appear to differ between adults and children,[5] paromomycin exposure in these subpopulations has not been compared so far. Since most patients affected by VL are children, adequate and similar exposure to miltefosine and paromomycin in children and adults is key to ensuring a good treatment response in the whole VL population. Therefore, we aimed to evaluate target attainment and adequacy of exposure of two adapted miltefosine plus paromomycin combination treatment regimens in Eastern African children and adults with VL using a population pharmacokinetic approach, in addition to exploring exposure–efficacy/toxicity relationships.

Methods

Study design and patients

Pharmacokinetic data were collected in patients from an open-label, Phase III, randomized, controlled, multicentre non-inferiority trial in VL patients in Eastern Africa (NCT03129646).[7] Patients in the investigational arms received a combination of paromomycin for 14 days plus miltefosine for 14 days (PM+MF14D) or paromomycin for 14 days plus miltefosine for 28 days (PM+MF28D). The study was conducted at seven clinical trial sites, with pharmacokinetic data available from six sites: Kacheliba, Kenya; Amudat, Uganda; Doka and Um El Kher, Sudan; and Gondar and Abdurafi, Ethiopia. Patients aged 4 to ≤50 years with VL symptoms and parasitological diagnosis were included. Patients were excluded when they had relapse, severe malnutrition, severe VL, positive HIV diagnosis or concomitant severe infection, or were women with child-bearing potential unwilling to use contraception. Other inclusion and exclusion criteria have been described previously.[7] Ethical approval was obtained from independent ethics committees in Sudan, Kenya, Uganda and Ethiopia and from the MSF Ethics Review Board. Informed consent was obtained per regulatory requirements in each country.

Study medication

Paromomycin (Gland Pharma, India) and miltefosine (Paladin Labs and Knight Therapeutics Inc., Canada) were administered simultaneously, both starting on Day 1. Patients received a once-daily intramuscular injection of 20 mg/kg/day paromomycin sulphate (equivalent to 15 mg/kg/day paromomycin base) for 14 days and oral miltefosine twice daily for 14 days (PM+MF14D) or 28 days (PM+MF28D). Children <30 kg received an allometric dosing of miltefosine based on sex, weight and height [Figure S1 (available as Supplementary data at *JAC* Online)], patients ≥30 kg to <45 kg received 100 mg/day, and patients ≥45 kg received 150 mg/day. If patients vomited within 30 min of miltefosine administration, they received a miltefosine re-dose.

Sample collection and bioanalysis

Miltefosine plasma concentrations were measured in patients at Day 14 (PM+MF14D arm only), Day 28 and Day 56 (PM+MF14D and PM+MF28D). More intensive pharmacokinetic sampling for both paromomycin and miltefosine was performed in a subset of patients from Kenya and Sudan who consented to participate in the intensive sampling cohort, with EDTA blood plasma samples collected at 0, 1, 2, 4 and 24 h, or at 0, 1, 2, 8 and 24 h at Day 1 and Day 14. Paromomycin and miltefosine were analysed using FDA/EMA-validated LC-MS/MS bioanalytical assays.[13,14] The lower limit of quantification was 5 ng/mL for paromomycin and 4 ng/mL for miltefosine.

Population pharmacokinetic analysis

A population approach was used to model paromomycin and miltefosine plasma concentrations, using the non-linear mixed-effects modelling software NONMEM (version 7.5, ICON Development Solutions, USA). Data management, model evaluation and graphical analysis were performed using R (version 4.1.2), Perl-speaks-NONMEM (PsN, version 5.2.6) and the graphical interface Pirana (version 3.0.0).

Previously published population pharmacokinetic models of paromomycin and miltefosine in (paediatric) Eastern African VL patients were chosen as the starting point.[8,15] Model development was carried out in four consecutive steps: (i) re-evaluation of the initial structural model; (ii) re-evaluation of the initial stochastic model; (iii) selected covariate analysis; and (iv) model evaluation. In the paromomycin structural model, the presence of a second compartment was evaluated, as well as a decrease in paromomycin clearance after start of treatment, as described previously.[8] In the paromomycin structural model, relative bioavailability

was fixed to 1.17, for comparability with previously obtained parameter estimates from Sudanese and Kenyan patients.[8] In the miltefosine structural model, the presence of lower bioavailability at the start of treatment and stagnation in miltefosine accumulation associated with increased cumulative dose was evaluated, as described previously.[15] In the stochastic models, between-subject variability (BSV) on all parameters was evaluated and implemented assuming a log-normal distribution (Eq. 1). Residual variability was implemented using a proportional error model (Eq. 2).

$$P_i = P_{pop} * e^{\eta_i} \quad (1)$$

$$Y_{i,j} = C_{i,j} * (1 + \varepsilon) \quad (2)$$

where P_i is the individual parameter estimate for an individual i, P_{pop} is the population estimate for a parameter, and η_i is the BSV of the ith individual, assumed to be distributed N(0, ω^2). $Y_{i,j}$ is the observed concentration, $C_{i,j}$ is the predicted concentration for observation j of individual i, and ε is the residual error, distributed N(0, σ^2).

Covariate analysis

Body weight was maintained as a covariate in the paromomycin and miltefosine models, with fixed allometric exponents of 0.75 for clearance, and 1 for volume of distribution. Selection of other covariates was based on physiological plausibility and graphical inspection of covariate–parameter relationships, and were tested univariately in the model. Estimated glomerular filtration rate (eGFR), unadjusted to typical body surface area (BSA),[16] here referred to as absolute eGFR ($eGRF_{abs}$) (Eq. 3), was evaluated on paromomycin clearance, as the drug is mainly cleared renally.[17]

$$eGFR_{abs} = \frac{eGFR}{1.73} * BSA \quad (3)$$

Specific formulas to estimate GFR in African or other malnourished populations are lacking.[18] For adults, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula,[19] without the adjustment for ethnicity, has previously been suggested for this population and was used here for adults and adolescents >14 years old.[20] For children ≤14 years old, the Schwartz formula was used, as previously suggested.[21] Clearance was also evaluated as a fraction of $eGFR_{abs}$, where renal clearance is assumed to be the only route of elimination, or in combination with a nonrenal clearance route. Furthermore, neutropenia has been associated with an increased clearance and sometimes increased volume of distribution of several aminoglycosides[22,23] and, therefore, an inverse correlation between serum neutrophils and paromomycin clearance and volume of distribution was evaluated. Likewise, an inverse correlation between serum albumin and paromomycin volume of distribution was evaluated, as this has been observed in patients with haematological malignancies treated with other aminoglycosides.[24–27] After the inclusion of the above-mentioned covariates, a potential remaining population difference between countries was evaluated on paromomycin bioavailability, absorption rate, volume of distribution and clearance. Time was evaluated on paromomycin clearance to investigate a change in clearance over time, which was not explained by the covariates included.

A decrease in miltefosine bioavailability at the start of treatment might be related to disease severity and/or malnutrition, as patients are severely ill, often combined with anaemia, leucopenia and malnourishment, when they start VL treatment. Evaluated covariates that might represent disease severity included the number of *Leishmania* parasites in either spleen, bone marrow or lymph nodes (expressed by a microscopy score ranging from 0 to 6) at the start of treatment, serum albumin, neutrophil levels and total WBC levels. Covariates representing nutritional status included BMI for patients from 19 years old and height-for-age and BMI-for-age Z-scores for patients up to 19 years old. Malnutrition Z-scores were derived using the R package 'zscorer', based on the WHO Child Growth Standards.

Continuous covariates were normalized to the median value in the population and included using a linear relationship (Eq. 4).

$$P_{TV} = P_{pop} \times (1 + (Cov_{i,t} - Cov_{med}) \times l) \quad (4)$$

where P_{TV} is the typical parameter value at covariate value $Cov_{i,t}$, P_{pop} the population estimate of this parameter, $Cov_{i,t}$ the covariate value for individual i at time t, Cov_{med} the median covariate value in the population, and l the slope factor. Exponential and power functions were also evaluated. Categorical covariates were tested as proportional changes relative to the reference category.

Model selection and evaluation

Model selection was based on scientific plausibility, minimum objective function value (OFV), goodness-of-fit (GoF) plots, and precision of parameter estimates. A significance level of $P < 0.01$ in a likelihood ratio test was considered statistically significant. Predictiveness of the final models was evaluated by a visual predictive check (VPC), stratified per treatment arm. Precision in parameter estimates was obtained by sampling importance resampling (SIR).[28]

Exposure and target attainment

Paromomycin exposure in children and adults was derived using the final individual maximum *a priori* Bayesian pharmacokinetic model estimates (obtained by the POSTHOC option in NONMEM), expressed by the area under the plasma concentration–time curve (AUC) for 24 h determined on Day 1 ($AUC_{0-24,\ D1}$) and Day 14 ($AUC_{0-24,\ D14}$). Paromomycin exposure was compared to previously reported AUCs on Day 21 in VL patients from Kenya and Sudan, in which most patients were adults, receiving paromomycin monotherapy (20 mg/kg/day) for 21 days.[8] Miltefosine exposure was represented by the AUC from Day 1 until Day 7, Day 21 and Day 210 (AUC_{D0-7}, AUC_{D0-21} and AUC_{D0-210}, respectively) and the time that the miltefosine concentration was over the *in vitro* susceptibility value EC_{90} ($T_{>EC90}$), equivalent to 10.6 μg/mL.[10] Exposure parameters were derived in children and adults, and compared with previously reported adult exposure following a conventionally dosed monotherapy dosing regimen.[10] Furthermore, miltefosine exposure and target attainment were compared with clinical efficacy (final cure determined 6 months after the end of treatment versus relapse of disease between the end of treatment and 6 months follow-up), and the relationship between paromomycin exposure and occurrence of renal toxicity and ototoxicity was explored.

Results

Patients and data

Data from 265 patients (232 paromomycin observations and 927 miltefosine observations), of which 59% were paediatric patients ≤12 years old, were available for the pharmacokinetic analysis, with intensive paromomycin and miltefosine pharmacokinetic sampling in 26 patients (Figures 1 and 2, Tables 1 and 2). Longitudinal serum creatinine, albumin and neutrophil levels were available for all patients until the end of follow-up (Figures S2 and S3). Albumin and neutrophil levels were low at the start of treatment (IQR 23.4–32.4 g/L and 0.74–1.38 × 10^3 cells/μL, respectively), but levels increased during treatment, as expected. Three paromomycin observations were excluded from the analysis because the quantification was not reliable ($n = 1$), or because trough samples at Day 1 were taken after the next dose was administered ($n = 2$). There were no paromomycin observations below the limit of quantification (BLQ). Seventeen miltefosine observations were excluded from the analysis, including observations

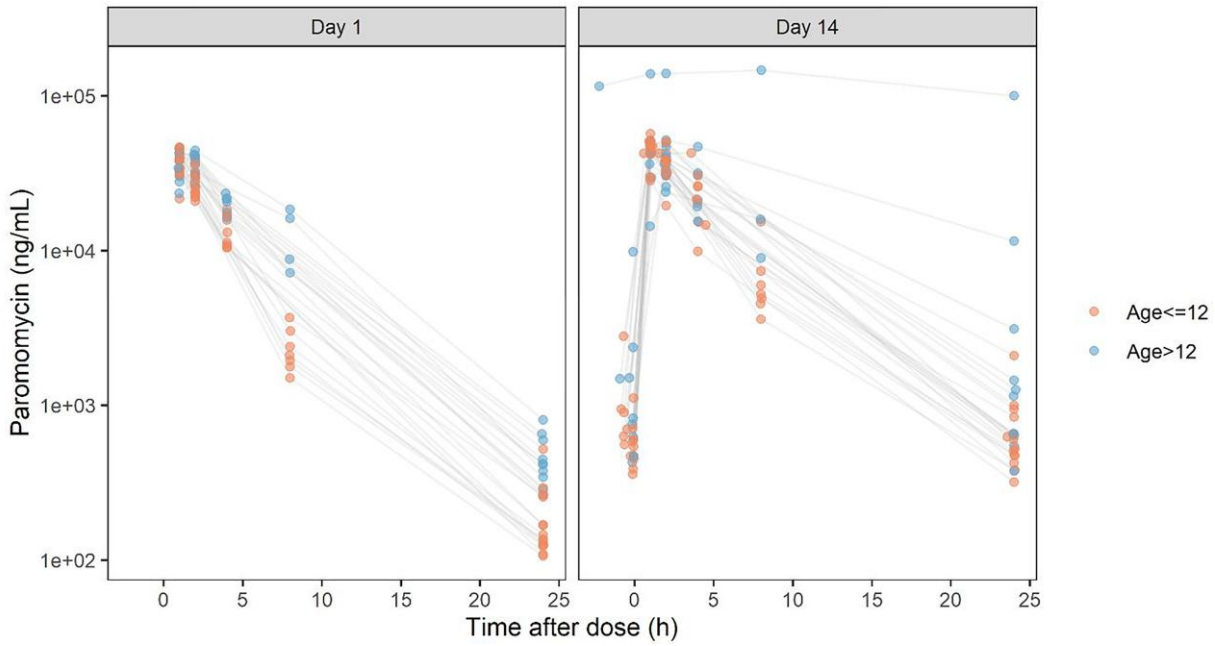

Figure 1. Paromomycin plasma concentrations included in the pharmacokinetic analysis, stratified by sampling day. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

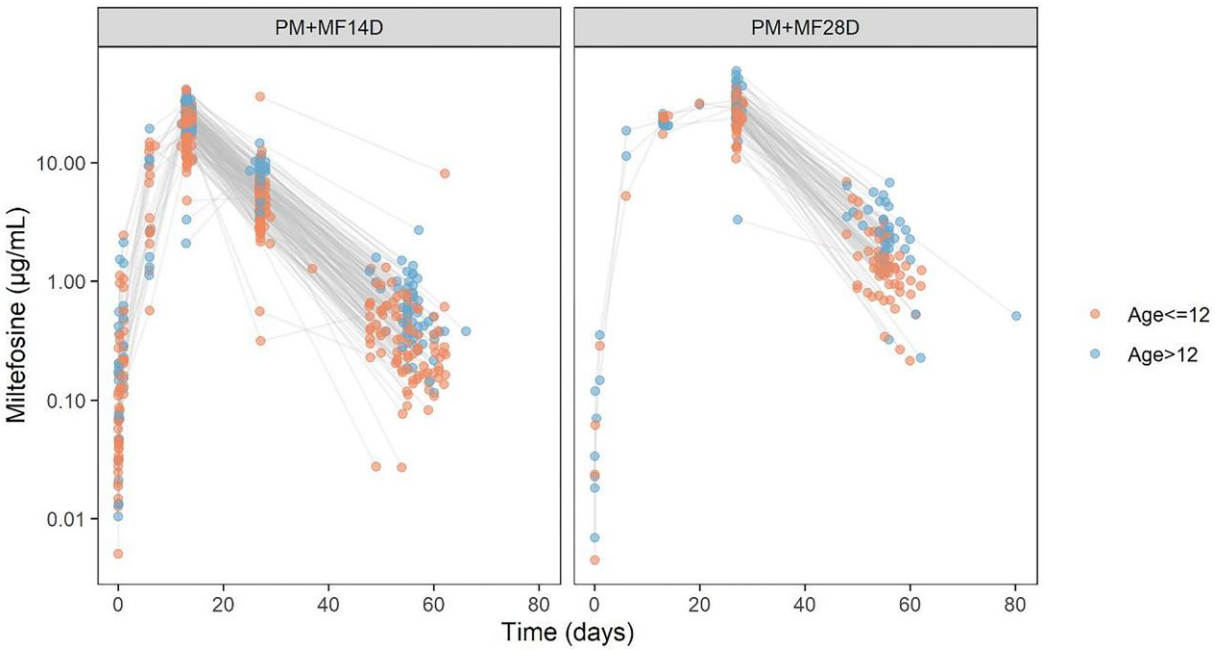

Figure 2. Miltefosine plasma concentrations included in the pharmacokinetic analysis, stratified by treatment arm. PM+MF14D, paromomycin 14 days+miltefosine 14 days; PM+MF28D, paromomycin 14 days+miltefosine 28 days. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

that were physiologically implausible and, therefore, unreliable ($n=3$), and BLQ observations ($n=14$, 2.8% of miltefosine observations). In the case of vomiting after miltefosine dosing, the dose before vomiting was excluded and the re-dose was included in the dataset ($n=20$).

Population pharmacokinetic models

A two-compartment model with first-order absorption best described paromomycin pharmacokinetics (Table 3, Figure 3). A decrease in clearance over time was observed, which was significantly associated with the increase in plasma neutrophils

Table 1. Patient characteristics

	Intensive PK sampling			Sparse PK sampling				
	Kenya	Sudan	Total	Kenya	Sudan	Ethiopia	Uganda	Total
Subjects, *n*	18	8	26	68	96	59	16	239
Male, *n* (%)	14 (78)	7 (88)	21 (81)	51 (75)	73 (76)	58 (98)	11 (69)	193 (80)
Age (years)[a]	14.7 (7–35)	11.8 (9–15)	13.8 (7–35)	9.7 (4–30)	10.5 (4–45)	23.4 (14–40)	12.6 (5–35)	13.6 (4–45)
Paediatrics ≤12 years, %	61	63	62	75	81	0	69	59
Body weight (kg)[a]	34.7 (20.5–54.0)	31.1 (25.0–52.0)	33.6 (20.5–54.0)	26.4 (13.5–53.5)	27.3 (11.0–70.0)	49.1 (28.5–61.0)	34.3 (14.5–71.0)	32.9 (11.0–71.0)
BMI[a,b]	17.4 (16.7–18.9)	NA	17.4 (16.7–18.9)	18.3 (17.5–19.4)	19.0 (17.2–21.3)	17.4 (14.6–20.2)	18.9 (17.0–20.8)	17.7 (14.6–21.3)
HAZ[a,c]	−0.07 (−2.3 to 2.7)	−0.9 (−3.1 to 1.7)	−0.4 (−3.1 to 2.7)	−0.3 (−4.2 to 3.3)	−0.8 (−4.1 to 2.8)	−1.7 (−4.2 to 0.3)	0.2 (−3.5 to 3.3)	−0.6 (−4.2 to 3.3)
BAZ[a,c]	−1.6 (−2.6 to 0.9)	−1.5 (−2.7 to −0.8)	−1.6 (−2.7 to 0.9)	−1.6 (−2.9 to 0.8)	−1.3 (−3.1 to 2.3)	−2.0 (−2.8 to −1.2)	−1.6 (−2.9 to 2.0)	−1.5 (−3.1 to 2.3)
Creatinine[a] (mg/dL)	1.0 (0.3–1.4)	0.5 (0.3–0.8)	0.8 (0.3–1.4)	1.0 (0.3–1.3)	0.6 (0.1–1.3)	0.8 (0.5–1.1)	0.9 (0.4–1.5)	0.8 (0.1–1.5)
Absolute eGFR[a] (mL/min)	55.8 (28.8–110.3)	83.7 (41.7–135.0)	64.4 (28.8–135.0)	40.1 (14.9–122.2)	68.8 (18.4–288.1)	107.0 (61.9–151.1)	51.5 (17.9–100.4)	68.9 (14.9–288.1)
Albumin[a] (g/L)	29.9 (20.8–49.7)	26.7 (22.5–32.4)	28.9 (20.8–49.7)	31.4 (12.9–54.6)	28.0 (16.0–54.0)	27.0 (1.5–50.0)	22.2 (14.6–45.8)	28.3 (1.5–54.6)
Neutrophils[a] (cells/μL)	0.92 (0.54–1.64)	1.51 (0.92–3.33)	1.10 (0.54–3.33)	0.92 (0.22–2.9)	1.4 (0.22–2.9)	0.87 (0.17–4.3)	1.2 (0.39–2.1)	1.11 (0.17–4.3)

BAZ, BMI-for-age Z-score; HAZ, height-for-age Z-score; NA, not available.
[a]Mean (range) at baseline.
[b]For patients >19 years old ($n=67$).
[c]For patients ≤19 years old ($n=198$).

Table 2. Data summary

	Intensive PK sampling			Sparse PK sampling		
	PM+MF14D[b]	PM+MF28D[b]	Total	PM+MF14D[b]	PM+MF28D[b]	Total
Subjects, *n*[a]	23	3	26	145	94	239
Paromomycin observations, *n*[a]	202	27	229	0	0	0
Paromomycin observations BLQ, *n*	0	0	0	0	0	0
Miltefosine observations, *n*[a]	271	38	309	413	188	601
Miltefosine observations BLQ, *n*	14	0	14	0	0	0

[a]Subjects and observations after exclusion of unreliable data.
[b]PM+MF14D, paromomycin 14 days+miltefosine 14 days; PM+MF28D, paromomycin 14 days+miltefosine 28 days.

over time (dOFV −19.9), corresponding to a 13% decrease in clearance for each increase in neutrophils of 1×10^3 cells/μL. A typical VL patient with a neutrophil level of 1.0×10^3 cells/μL at the start of treatment and 2.5×10^3 cells/μL at the end of treatment would have a corresponding decrease in paromomycin clearance from 2.61 to 2.10 L/h. Age or country of origin could not explain remaining variability in any of the pharmacokinetic parameters on top of the identified covariates, and $eGFR_{abs}$ could not explain variability in clearance. The final paromomycin model including covariates adequately described the change in pharmacokinetics over time, shown by the VPC stratified for the first and last day of paromomycin treatment (Figure 4).

Miltefosine was best described by a two-compartment model with first-order absorption (Table 4, Figures 5 and 6), including two non-linearities influencing bioavailability (Figure S4). As in the previous study, bioavailability was 65% (95% CI 57%–73%) lower during the first week of treatment (dOFV −196), and this decrease was highly variable between patients [BSV 74.8% (95%CI 62.0%–90.3%)]. As previously described, miltefosine accumulates over time due to its slow absorption and long elimination half-life, reaching higher exposure at the end of therapy (Figure 2). A decrease in miltefosine bioavailability was also related to increased miltefosine exposure over time, represented by the total miltefosine dose administered, resulting in stagnation of miltefosine accumulation in plasma in the third week of treatment. For example, when a typical patient of 35 kg received 100 mg/day miltefosine, bioavailability was 21% lower on Day 28. No other covariates could be identified to explain the non-linear effects on bioavailability.

Paromomycin and miltefosine exposure and exposure–response relationships

At the end of treatment, paromomycin exposure ($AUC_{0-24,\ D14}$) was lower in children than adults (Table 5). However, paromomycin exposure of all individuals, including paediatrics, was within the IQR of end-of-treatment exposure in Kenyan and Sudanese patients in the previous study,[8] in which the majority of patients

Table 3. Parameter estimates of the final paromomycin pharmacokinetic model

	Estimate	95% CI[a]	Shrinkage (%)
Population parameters[b]			
CL (L/h)	2.62	2.08–3.25	
V_c (L)	9.17	8.42–9.92	
Q	0.26	0.22–0.31	
V_p (L)	6.55	4.58–9.58	
k_a (h^{-1})	2.05	1.55–2.92	
F1	1.17 (fixed)		
$COV_{CL,neutr}$[c] (fractional change/10^3 cells/μL)	−0.13	−0.16 to −0.10	
Between-subject variability			
CL (CV%)	56.1	44.1–76.6	0.0
Residual variability			
Proportional error (CV%)	53.4	50.9–56.5	4.9

$$CL_{TV} = CL_{pop} \times \left(\frac{WT_{i,t}}{WT_{med}}\right)^{0.75} \times (1 + (NEUTR_i - NEUTR_{med}) \times COV_{CL,neutr})$$

$$V_{d,TV} = V_{d,pop} \times \left(\frac{WT_{i,t}}{WT_{med}}\right)^{1.00}$$

CL, apparent oral clearance; COV, covariate factor; F1, bioavailability; k_a, absorption rate constant, NEUTR, neutrophils (10^3 cells/μL); $NEUTR_{med}$, median population neutrophils (0.98×10^3 cells/μL); Q, intercompartmental clearance; V_c, central volume of distribution; V_p, peripheral volume of distribution; WT, body weight (kg); WT_{med}, median population body weight (27.5 kg).

[a]Obtained by SIR.

[b]Parameter values relative to a bioavailability of 1.17.

[c]CL decreases by 13% per 1×10^3 cells/μL increase of neutrophils.

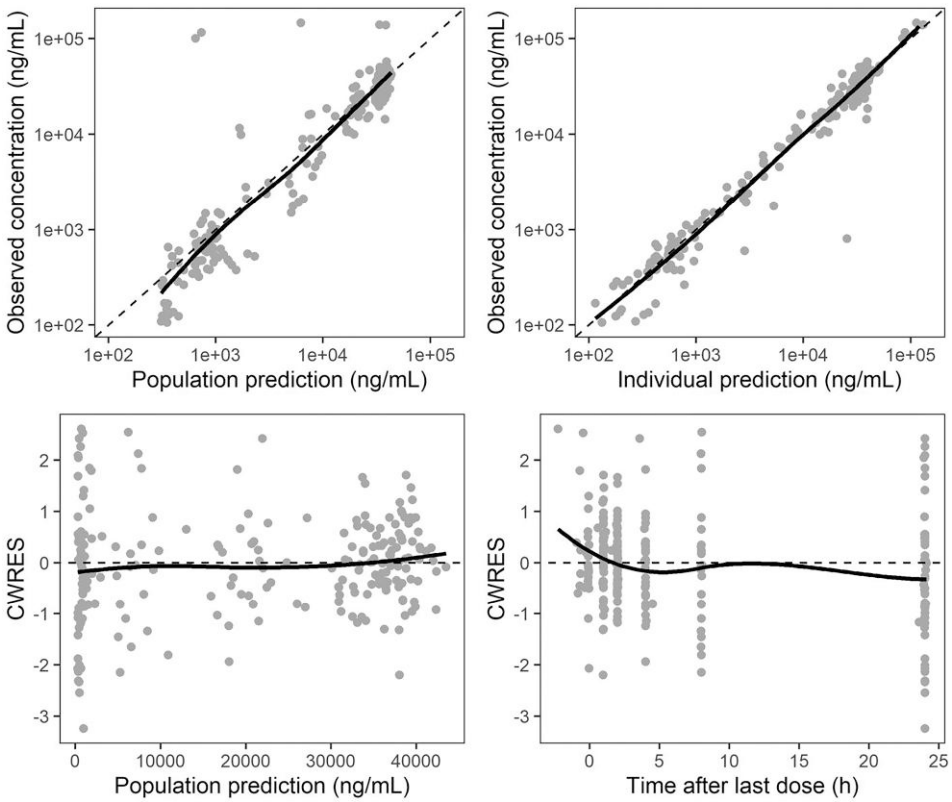

Figure 3. GoF plots for the final paromomycin pharmacokinetic model. Observed versus population-predicted paromomycin concentrations; observed versus individually predicted paromomycin concentrations; conditional weighted residuals (CWRES) versus population predicted concentrations; and CWRES versus time after last dose.

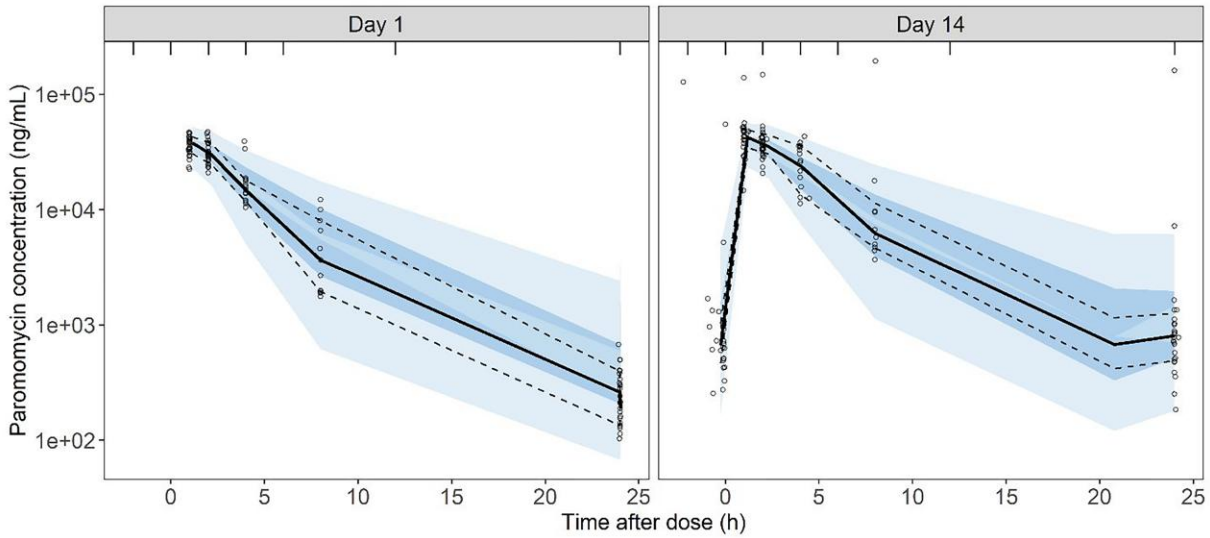

Figure 4. Prediction-corrected VPC of the final paromomycin pharmacokinetic model. The solid lines represent the median of the observed values, and the dashed lines the 20th and 80th percentiles of the observed values. The dark and light areas indicate the 90% CIs of the simulated median and percentiles, respectively, based on 1000 simulations. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 4. Parameter estimates of the final miltefosine pharmacokinetic model

	Estimate	95% CI[a]	Shrinkage (%)
Population parameters			
CL (L/day)	1.85	1.75–1.94	
V_c (L)	13.6	12.8–14.4	
Q (L/day)	0.17	0.13–0.21	
V_p (L)	2.22	1.96–2.59	
k_a (day^{-1})	0.037	0.036–0.038	
F1	1 (fixed)		
$COV_{F,W1}$[b] (fractional change)	−0.65	−0.57 to −0.73	
$COV_{F,CD}$[c] (exponent of power relationship)	−2.40	−3.79 to −1.21	
Between-subject variability			
CL (CV%)	16.3	14.3–18.5	14
$COV_{F,W1}$ (CV%)	74.8	62.0–90.3	48
Residual variability			
Proportional error (CV%)	31.5	29.7–33.6	14

$$CL_{TV} = CL_{pop} \times \left(\frac{FFM_{i,t}}{FFM_{med}}\right)^{0.75}$$

$$V_{TV} = V_{pop} \times \left(\frac{FFM_{i,t}}{FFM_{med}}\right)^{1.00}$$

$$F_{TV} = F_{pop} \times (1 - COV_{F,W1}) \times \left(\frac{CD_{i,t}}{CD_{med}}\right)^{COVF,CD}$$

CL, apparent oral clearance; CD, cumulative miltefosine dose (mg/kg); COV, covariate factor; F1, bioavailability; FFM, fat-free mass; k_a, absorption rate constant; Q, intercompartmental clearance; RSE, relative standard error; V_c, central volume of distribution; V_p, peripheral volume of distribution.
[a]Obtained by SIR.
[b]Fractional change in F. Applied during the first week.
[c]Exponent of power relationship between cumulative dose and F. Applied after a cumulative dose of 60 mg/kg is reached.

were adults (Figure 7). Miltefosine exposure during treatment was comparable between children and adults, although AUC_{D0-210} and $T_{>EC90}$ were slightly higher in adults (Table 6). Miltefosine exposure (AUC_{D0-28}) in both adults and paediatric patients was within the IQR of adult patients in the previous study,[10] when receiving 28 days of miltefosine (Figure 8). Miltefosine target attainment ($T_{>EC90}$) in adults (IQR 28–32 days) was within the previously observed IQR in adults (IQR 27–34 days), and numerically lower in paediatric patients (IQR 25–28) (Figure 9).

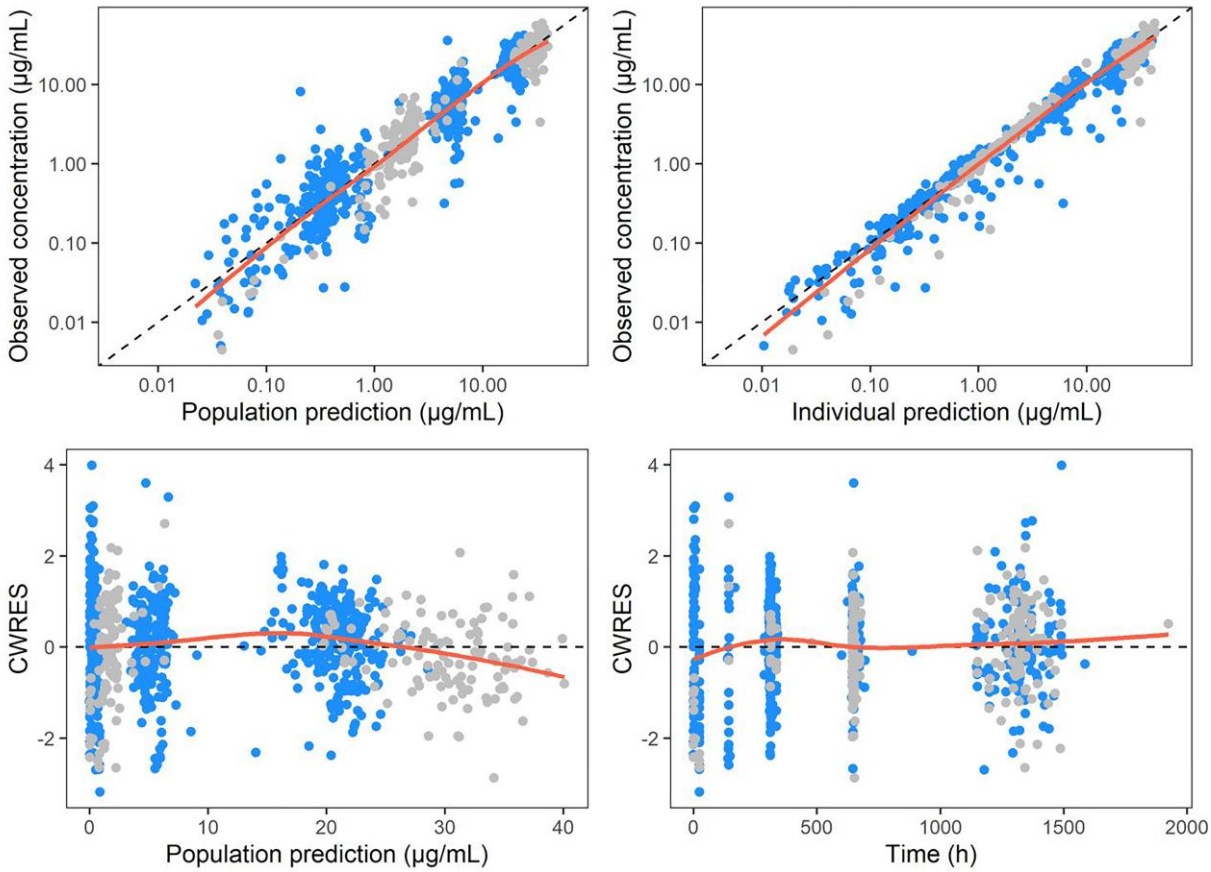

Figure 5. GoF plots for the final miltefosine pharmacokinetic model, coloured by treatment arm. Observed versus population-predicted paromomycin concentrations; observed versus individually predicted paromomycin concentrations; conditional weighted residuals (CWRES) versus population-predicted concentrations; and CWRES versus time after last dose. Blue dots, treatment arm 1 (miltefosine dosing for 14 days); grey dots, treatment arm 2 (miltefosine dosing for 28 days). This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

There were only 12 patients in the investigational regimen arms (PM+MF14D and PM+MF28D) with relapses, which happened between Day 68 and Day 228. Only pharmacokinetic data for miltefosine were available for these patients. There was no obvious correlation between miltefosine exposure at Day 7, Day 28 or Day 210 or target attainment ($T_{>EC90}$) and clinical outcome (Figure S5).

Twenty patients treated with paromomycin experienced ototoxicity. Paromomycin pharmacokinetic data were available for only two of these patients. One of these patients had extremely high paromomycin exposure on Day 14 due to renal failure ($AUC_{0-24,\ D14}$ 2388 μg·h/mL compared with a median $AUC_{0-24,\ D14}$ of 202 μg·h/mL), which explains the occurrence of severe ototoxicity. The other patient, who had a regular $AUC_{0-24,\ D14}$ of 297 μg·h/mL, presented mild hypoacusis (left and right ear) at the Day 28 visit, which resolved completely by Day 56.

Discussion

This pharmacokinetic study characterized the pharmacokinetics of paromomycin and miltefosine in both paediatric and adult Eastern African VL patients receiving a new, shortened combination regimen. The new treatment provides the desired levels of exposure, i.e. the exposure seen in adults that is associated with cure. Paromomycin exposure in adults was comparable to previously reported exposures in Kenyan and Sudanese VL patients. Children had a lower exposure to paromomycin than adults; however, the exposure seems to be adequate in combination with allometric miltefosine, given that there was similar efficacy for adults and paediatric patients, i.e. in the PM+MF14D regimen, final cure was 94.1% in patients aged ≤12 years and 86.8% in patients aged >12 years.[7] Total miltefosine exposure was also greater in adults than children, but comparable to a previous study in Eastern African VL patients. Since the exposures of paromomycin and miltefosine in this study are comparable to those found in previous monotherapy studies, this suggests the absence of any obvious drug–drug interaction between paromomycin and miltefosine. Moreover, based on graphical analysis (Figure S5), no clear exposure–response and exposure–toxicity relationships were observed for both paromomycin and miltefosine, suggesting that the currently used doses in this new combination regimen led to adequate and safe ranges of exposure for these drugs.

One exceptional patient developed renal failure (serum creatinine increased from 0.4 mg/dL on Day 1 to 10.8 mg/dL on

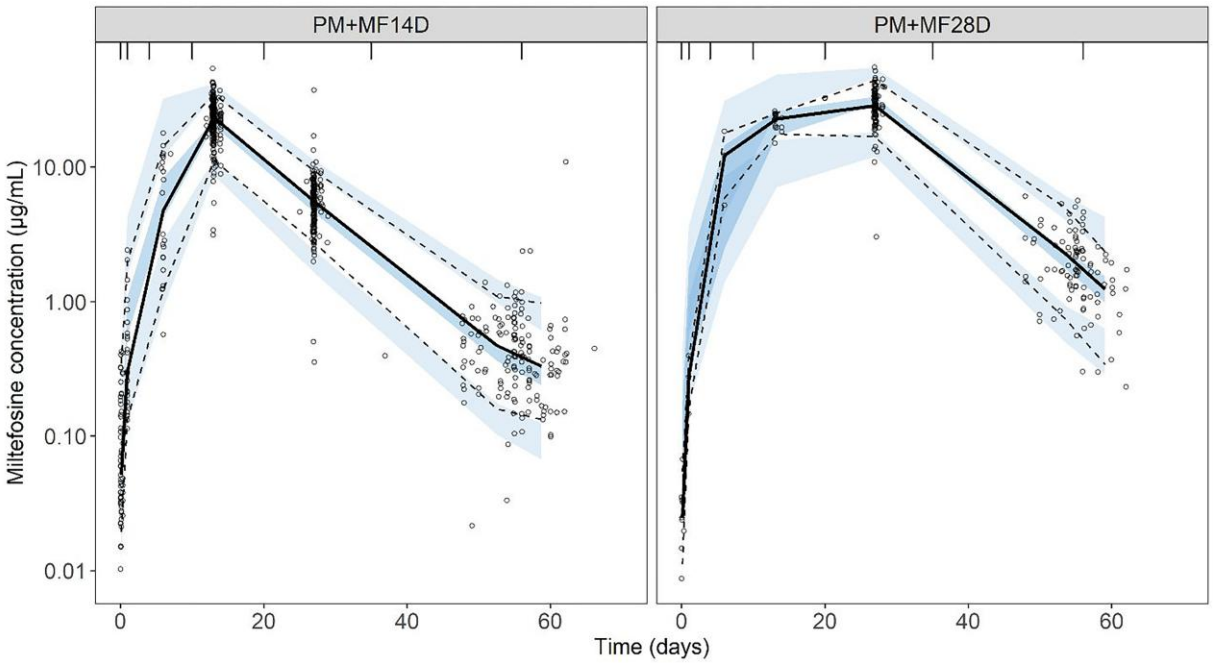

Figure 6. Prediction-corrected VPC of the final miltefosine pharmacokinetic model. The solid lines represent the median of the observed values, the dashed lines the 5th and 95th percentiles of the observed values. The dark and light areas indicate the 90% CIs of the simulated median and percentiles, respectively, based on 500 simulations. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Table 5. Paromomycin exposure at the first and last day of treatment

	Paromomycin exposure (AUC_{0-24}[a])		
Treatment day	Children[b] ($n=16$)	Adults[b] ($n=10$)	Total ($n=26$)
1	145 (136–167)	219 (199–252)	171 (144–199)
14	187 (162–203)	242 (217–328)	202 (185–240)

[a]AUC_{0-24} (µg·h/mL), median (IQR)is the area under the plasma concentration–time curve for 0 to 24 h after dosing.
[b]Children: ≤12 years; adults: >12 years.

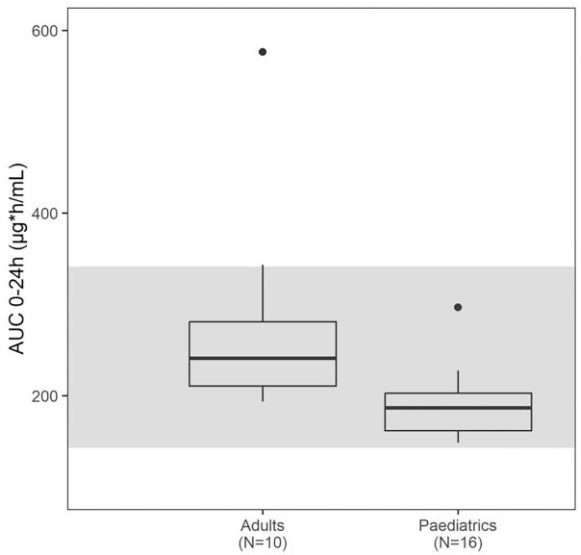

Figure 7. Paromomycin exposure (AUC_{0-24}) at last day of treatment (Day 14) in paediatrics (≤12 years) and adults (>12 years), compared with previously observed paromomycin exposure (Day 21) in adult Sudanese and Kenyan patients receiving 20 mg/kg/day paromomycin for 21 days (the marked area represents the IQR).[8] One outlier patient with an extremely high AUC_{0-24} of 2388 µg×h/mL is not shown in this figure. This patient developed ototoxicity due to renal failure.

Day 14) leading to extremely high paromomycin exposure at the end of treatment, causing bilateral deafness.[7] Renal impairment can lead to prolonged exposure to aminoglycosides, which is a risk factor for ototoxicity. Although the occurrence of renal failure is rare in this study (2/268 patients developed acute kidney injury),[7] this observation highlights the need to monitor renal function during treatment with this combination regimen or, preferably, therapeutic drug monitoring if logistically feasible within the clinical setting and context.

The developed paromomycin and miltefosine population pharmacokinetic models adequately described the pharmacokinetic data, including changes in paromomycin clearance and miltefosine bioavailability over time. Previously, the increase in paromomycin exposure has been described by an empirical decrease in clearance over time,[8] while in this study, we linked this decrease in clearance to recovery of disease, more specifically recovery of depleted neutrophil levels over time. Increased clearance of other aminoglycosides in neutropenic patients has been described before,[22,23] potentially due to augmented renal clearance.[29,30] In this study, patients were neutropenic at the start of treatment and, therefore, paromomycin clearance

Table 6. Miltefosine exposure and target attainment

	Miltefosine exposure, median (IQR)			
	PM + MF14D[a]		PM + MF28D[a]	
	Children[b] (n = 102)	Adults[b] (n = 66)	Children[b] (n = 54)	Adults[b] (n = 43)
AUC_{D0-7} (μg·day/mL)	20 (17–25)	22 (15–27)	20 (18–22)	18 (16–20)
AUC_{D0-EOT}[c] (μg·day/mL)	114 (98–130)	111 (94–136)	517 (464–552)	524 (456–567)
AUC_{D0-210} (μg·day/mL)	336 (293–384)	379 (329–440)	790 (687–824)	898 (784–961)
$T_{>EC90}$[d] (days)	12 (11–14)	14 (12–16)	27 (25–28)	30 (28–32)

EOT, end of treatment.
[a]PM + MF14D, paromomycin 14 days + miltefosine 14 days; PM + MF28D, paromomycin 14 days + miltefosine 28 days.
[b]Children: ≤12 years; adults: >12 years.
[c]AUC from Day 1 until the end of treatment (Day 14 for PM + MF14D, Day 28 for PM + MF28D).
[d]Time that the miltefosine concentration was over the *in vitro* susceptibility value EC_{90}, equivalent to 10.6 μg/mL.

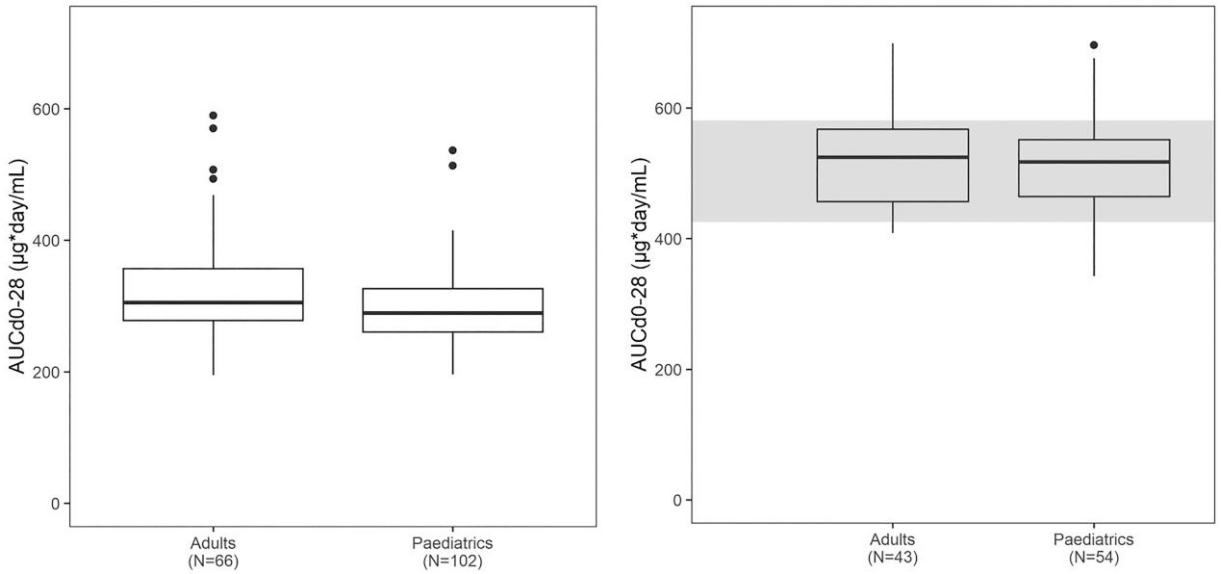

Figure 8. Cumulative miltefosine exposure until Day 28 (AUC_{D0-28}) in paediatrics (≤12 years) and adults (>12 years), receiving 14 days of miltefosine (PM + MF14D, left panel) and 28 days of miltefosine (PM + MF28D, right panel), compared with previously observed Day 28 miltefosine exposure in adult Eastern African patients receiving miltefosine allometric dosing for 28 days (the marked area represents the IQR).[10] One outlier patient with an AUC_{D0-28} of 2106 μg × day/mL (PM + MF14D, 10 years old) is not shown in this figure.

might be increased at the beginning. However, $eGFR_{abs}$ as calculated in this study did not indicate glomerular hyperfiltration, which is present when the eGFR exceeds 160 mL/min/1.73 m^2 in men and 150 mL/min/1.73 m^2 in women.[29] The decrease in paromomycin clearance over the treatment period might also be explained by the nephrotoxic effect of paromomycin.[31,32] It was expected that $eGFR_{abs}$ would decrease during paromomycin treatment due to drug-induced nephrotoxicity resulting in increased creatinine levels, but this was not observed in this population. Additionally, $eGFR_{abs}$ was not associated with paromomycin clearance, despite the fact that this is the main route of excretion of paromomycin.[17] This may indicate that serum creatinine and $eGFR_{abs}$ do not adequately reflect renal function in this malnourished African VL population, which is in line with earlier studies demonstrating that eGFR based on serum creatinine is overestimated in malnourished patients with low muscle mass and low creatinine production.[33,34] In the final paromomycin model, no significant pharmacokinetic differences between countries were identified, indicating that there are no geographical differences that are not already explained by demographic differences between populations or other covariates.

The effects on miltefosine bioavailability were the same as previously described: decreased bioavailability during the first week of treatment, and decreased bioavailability with increased cumulative dose (Figure S4). The lower bioavailability might be caused by initial malnourishment and malabsorption, although variables associated with malnutrition, such as height-for-age or BMI-for-age, were not identified as explanatory covariates

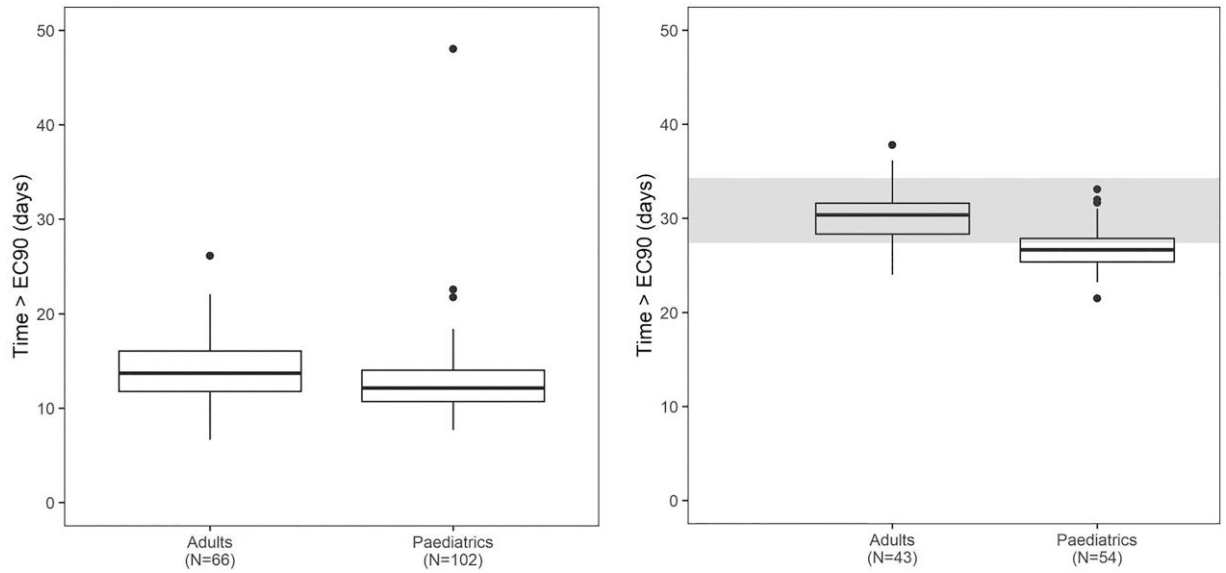

Figure 9. Miltefosine $T_{>EC90}$ in paediatrics (≤12 years) and adults (>12 years), receiving 14 days of miltefosine (PM + MF14D, left panel) and 28 days of miltefosine (PM + MF28D, right panel), compared with previously observed $T_{>EC90}$ in adult Eastern African patients receiving miltefosine conventional dosing for 28 days (the marked area represents the IQR).[10]

on the initially decreased bioavailability. The lower than dose proportional increase in miltefosine exposure was related to a decrease in bioavailability with increasing cumulative dose. This could be due to the slow and saturable transport of miltefosine over the gastrointestinal membrane, resulting in a saturated absorption route after extended exposure to miltefosine.[35]

Using the population pharmacokinetic models developed, we identified different disease-associated factors, such as neutropenia and renal failure, that influence the pharmacokinetics of antileishmanial drugs. It should be noted that this study predominantly involved male patients, potentially confounding any sex-related effects, if any. Additionally, assessing the impact of malnourishment on pharmacokinetics was challenging due to potential confounding by disease severity. Nevertheless, the high efficacy rates in this study, the few cases of toxicity, and the lack of clear exposure–response and exposure–toxicity relationships indicate adequate exposure within the therapeutic range in the population studied, including paediatric patients. This is supported by the satisfactory cure rates observed in the trial, in both children and adults.[7]

The results of this pharmacokinetic analysis are supportive of the implementation of the shorter 14 day paromomycin plus allometric miltefosine combination regimen for VL in Eastern Africa. This study reaffirms the fact that an increased allometric miltefosine dosing regimen is more suitable for East African paediatric patients with VL than the conventional mg/kg regimen, also in a 14 day combination regimen with paromomycin. The paromomycin and miltefosine exposure levels achieved could serve as pharmacokinetic targets when monitoring treatment efficacy over time in endemic regions. The development of this combination therapy illustrates the importance of adapting treatment regimens for children, who in this case comprise approximately 50% of cases globally.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 to S5 are available as Supplementary data at *JAC* Online.

References

1 WHO. Global leishmaniasis surveillance: 2019–2020, a baseline for the 2030 roadmap. Weekly epidemiological record. 2021. https://www.who.int/publications/i/item/who-wer9635-401-419.

2 Omollo R, Alexander N, Edwards T *et al*. Safety and efficacy of miltefosine alone and in combination with sodium stibogluconate and liposomal amphotericin B for the treatment of primary visceral leishmaniasis in East Africa: study protocol for a randomized controlled trial. *Trials* 2011; **12**: 166. https://doi.org/10.1186/1745-6215-12-166

3 Wasunna M, Njenga S, Balasegaram M *et al*. Efficacy and safety of AmBisome in combination with sodium stibogluconate or miltefosine and miltefosine monotherapy for African visceral leishmaniasis: phase II randomized trial. *PLoS Negl Trop Dis* 2016; **10**: e0004880. https://doi.org/10.1371/journal.pntd.0004880

4 Hailu A, Musa A, Wasunna M *et al*. Geographical variation in the response of visceral leishmaniasis to paromomycin in East Africa: a multicentre, open-label, randomized trial. *PLoS Negl Trop Dis* 2010; **4**: e709. https://doi.org/10.1371/journal.pntd.0000709

5 Musa A, Khalil E, Hailu A *et al*. Sodium stibogluconate (SSG) & paromomycin combination compared to SSG for visceral leishmaniasis in East Africa: a randomised controlled trial. *PLoS Negl Trop Dis* 2012; **6**: e1674. https://doi.org/10.1371/journal.pntd.0001674

6 Sundar S, Sinha PK, Rai M *et al*. Comparison of short-course multidrug treatment with standard therapy for visceral leishmaniasis in India: an open-label, non-inferiority, randomised controlled trial. *Lancet* 2011; **377**: 477–86. https://doi.org/10.1016/S0140-6736(10)62050-8

7 Musa AM, Mbui J, Mohammed R *et al*. Paromomycin and miltefosine combination as an alternative to treat patients with visceral leishmaniasis in Eastern Africa: a randomized, controlled, multicountry trial. *Clin Infect Dis* 2023; **76**: E1177–85. https://doi.org/10.1093/cid/ciac643

8 Verrest L, Wasunna M, Kokwaro G *et al*. Geographical variability in paromomycin pharmacokinetics does not explain efficacy differences between Eastern African and Indian visceral leishmaniasis patients. *Clin Pharmacokinet* 2021; **60**: 1463–73. https://doi.org/10.1007/s40262-021-01036-8

9 Sundar S, Jha TK, Thakur CP *et al*. Injectable paromomycin for visceral leishmaniasis in India. *N Engl J Med* 2007; **356**: 2571–81. https://doi.org/10.1056/NEJMoa066536

10 Dorlo TPC, Kip AE, Younis BM *et al*. Visceral leishmaniasis relapse hazard is linked to reduced miltefosine exposure in patients from Eastern Africa: a population pharmacokinetic/pharmacodynamic study. *J Antimicrob Chemother* 2017; **72**: 3131–40. https://doi.org/10.1093/jac/dkx283

11 Mbui J, Olobo J, Omollo R *et al*. Pharmacokinetics, safety, and efficacy of an allometric miltefosine regimen for the treatment of visceral leishmaniasis in Eastern African children: an open-label, phase II clinical trial. *Clin Infect Dis* 2019; **68**: 1530–8. https://doi.org/10.1093/cid/ciy747

12 Musa AM, Younis B, Fadlalla A *et al*. Paromomycin for the treatment of visceral leishmaniasis in Sudan: a randomized, open-label, dose-finding study. *PLoS Negl Trop Dis* 2010; **4**: e855. https://doi.org/10.1371/journal.pntd.0000855

13 Dorlo TPC, Hillebrand MJX, Rosing H *et al*. Development and validation of a quantitative assay for the measurement of miltefosine in human plasma by liquid chromatography-tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2008; **865**: 55–62. https://doi.org/10.1016/j.jchromb.2008.02.005

14 Roseboom IC, Thijssen B, Rosing H *et al*. Highly sensitive UPLC-MS/MS method for the quantification of paromomycin in human plasma. *J Pharm Biomed Anal* 2020; **185**: 113245. https://doi.org/10.1016/j.jpba.2020.113245

15 Palić S, Kip AE, Beijnen JH *et al*. Characterizing the non-linear pharmacokinetics of miltefosine in paediatric visceral leishmaniasis patients from Eastern Africa. *J Antimicrob Chemother* 2020; **75**: 3260–8. https://doi.org/10.1093/jac/dkaa314

16 Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* 1989; **5**: 303–11. http://doi.org/10.1001/archinte.1916.00080130010002

17 Seyffart G. *Drug Dosage in Renal Insufficiency*. Springer, 1991.

18 Eastwood JB, Kerry SM, Plange-Rhule J *et al*. Assessment of GFR by four methods in adults in Ashanti, Ghana: the need for an eGFR equation for lean African populations. *Nephrol Dial Transplant* 2010; **25**: 2178–-87. https://doi.org/10.1093/ndt/gfp765

19 Levey AS, Stevens LA, Schmid CH *et al*. A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; **150**: 604. https://doi.org/10.7326/0003-4819-150-9-200905050-00006

20 Azzi A, Cachat F, Faouzi M *et al*. Is there an age cutoff to apply adult formulas for GFR estimation in children? *J Nephrol* 2015; **28**: 59–66. https://doi.org/10.1007/s40620-014-0148-y

21 Schwartz GJ, Muñoz A, Schneider MF *et al*. New equations to estimate GFR in children with CKD. *J Am Soc Nephrol* 2009; **20**: 629–37. https://doi.org/10.1681/ASN.2008030287

22 Bury D, Ter Heine R, van de Garde EMW *et al*. The effect of neutropenia on the clinical pharmacokinetics of vancomycin in adults. *Eur J Clin Pharmacol* 2019; **75**: 921–8. https://doi.org/10.1007/s00228-019-02657-6

23 Lortholary O, Lefort A, Tod M *et al*. Pharmacodynamics and pharmacokinetics of antibacterial drugs in the management of febrile neutropenia. *Lancet Infect Dis* 2008; **8**: 612–20. https://doi.org/10.1016/S1473-3099(08)70228-7

24 Hodiamont CJ, Juffermans NP, Bouman CSC *et al*. Determinants of gentamicin concentrations in critically ill patients: a population pharmacokinetic analysis. *Int J Antimicrob Agents* 2017; **49**: 204–11. https://doi.org/10.1016/j.ijantimicag.2016.10.022

25 Romano S, de Gatta MMF, Calvo MV *et al*. Population pharmacokinetics of amikacin in patients with haematological malignancies. *J Antimicrob Chemother* 1999; **44**: 235–42. https://doi.org/10.1093/jac/44.2.235

26 Etzel JV, Nafziger AN, Bertino JS Jr. Variation in the pharmacokinetics of gentamicin and tobramycin in patients with pleural effusions and hypoalbuminemia. *Antimicrob Agents Chemother* 1992; **36**: 679–81. https://doi.org/10.1128/AAC.36.3.679

27 Davis RL, Lehmann D, Stidley CA *et al*. Amikacin pharmacokinetics in patients receiving high-dose cancer chemotherapy. *Antimicrob Agents Chemother* 1991; **35**: 944–7. https://doi.org/10.1128/AAC.35.5.944

28 Dosne AG, Bergstrand M, Karlsson MO. An automated sampling importance resampling procedure for estimating parameter uncertainty. *J Pharmacokinet Pharmacodyn* 2017; **44**: 509–20. https://doi.org/10.1007/s10928-017-9542-0

29 Fuster-Lluch O, Gerónimo-Pardo M, Peyró-García R *et al*. Glomerular hyperfiltration and albuminuria in critically ill patients. *Anaesth Intensive Care* 2008; **36**: 674–80. https://doi.org/10.1177/0310057X0803600507

30 Campassi ML, Gonzalez MC, Masevicius FD *et al*. Augmented renal clearance in critically ill patients: incidence, associated factors and effects on vancomycin treatment. *Rev Bras Ter Intensiva* 2014; **26**: 13–20. https://doi.org/10.5935/0103-507X.20140003

31 Lopez-Novoa JM, Quiros Y, Vicente L *et al*. New insights into the mechanism of aminoglycoside nephrotoxicity: an integrative point of view. *Kidney Int* 2011; **79**: 33–45. https://doi.org/10.1038/ki.2010.337

32 Rougier F, Claude D, Maurin M *et al*. Aminoglycoside nephrotoxicity. *Curr Drug Targets Infect Disord* 2004; **4**: 153–62. https://doi.org/10.2174/1568005043340858

33 Beddhu S, Samore MH, Roberts MS *et al*. Creatinine production, nutrition, and glomerular filtration rate estimation. *J Am Soc Nephrol* 2003; **14**: 1000–5. https://doi.org/10.1097/01.ASN.0000057856.88335.DD

34 Hari P, Bagga A, Mahajan P *et al*. Effect of malnutrition on serum creatinine and cystatin C levels. *Pediatr Nephrol* 2007; **22**: 1757–61. https://doi.org/10.1007/s00467-007-0535-x

35 Ménez C, Buyse M, Farinotti R *et al*. Inward translocation of the phospholipid analogue miltefosine across Caco-2 cell membranes exhibits characteristics of a carrier-mediated process. *Lipids* 2007; **42**: 229–40. https://doi.org/10.1007/s11745-007-3026-8