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Low antileishmanial drug exposure in HIV-positive visceral leishmaniasis patients on antiretrovirals: an Ethiopian cohort study

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Background: Despite high HIV co-infection prevalence in Ethiopian visceral leishmaniasis (VL) patients, the adequacy of antileishmanial drug exposure in this population and effect of HIV-VL co-morbidity on pharmacokinetics of antileishmanial and antiretroviral (ARV) drugs is still unknown.

Methods: HIV-VL co-infected patients received the recommended liposomal amphotericin B (LAmB) monotherapy (total dose 40 mg/kg over 24 days) or combination therapy of LAmB (total dose 30 mg/kg over 11 days) plus 28 days 100 mg/day miltefosine, with possibility to extend treatment for another cycle. Miltefosine, total amphotericin B and ARV concentrations were determined in dried blood spots or plasma using LC–MS/MS.

Results: Median (IQR) amphotericin B C_{max} on Day 1 was 24.6 μg/mL (17.0–34.9 μg/mL), which increased to 40.9 (25.4–53.1) and 33.2 (29.0–46.6) μg/mL on the last day of combination and monotherapy, respectively. Day 28 miltefosine concentration was 18.7 (15.4–22.5) μg/mL. Miltefosine exposure correlated with amphotericin B accumulation. ARV concentrations were generally stable during antileishmanial treatment, although efavirenz C_{min} was below the 1 μg/mL therapeutic target for many patients.

Conclusions: This study demonstrates that antileishmanial drug exposure was low in this cohort of HIV co-infected VL patients. Amphotericin B C_{max} was 2-fold lower than previously observed in non-VL patients. Miltefosine exposure in HIV-VL co-infected patients was 35% lower compared with adult VL patients in Eastern Africa, only partially explained by a 19% lower dose, possibly warranting a dose adjustment. Adequate drug exposure in these HIV-VL co-infected patients is especially important given the high proportion of relapses.

Introduction

HIV co-infection is reported in 2%–9% of all visceral leishmaniasis (VL) patients in endemic regions, with rates up to 20% in some regions of Ethiopia.[1] Treatment outcome in this patient population is of particular concern, with high rates of treatment failure and relapse.[2] Conventional antimony treatment leads to unacceptable rates of severe toxicity (pancreatitis, cardiotoxicity and severe vomiting) and a 10-fold higher mortality rate than in non-co-infected patients,[2,3] stressing the need for the development and evaluation of new, more efficacious and safer treatment regimens for HIV co-infected VL patients. A recent randomized open-label clinical trial in north Ethiopia strongly supports a change in first-line treatment of this vulnerable patient population from liposomal amphotericin B (LAmB) monotherapy to a LAmB/miltefosine combination therapy with a treatment duration dependent on reaching negative parasitology.[4]

Defining drug exposure–response relationships has been shown to be pivotal in clinical decision-making regarding dosing regimens against various infectious diseases.[5–8] In the case of antileishmanial treatment, lower miltefosine exposure has been associated with lower probability of cure,[9] and higher risk of relapse in VL.[10] Also for antiretroviral (ARV) drugs, exposure–response relationships have been established, such as lower treatment efficacy in patients with efavirenz steady-state trough levels below 1 μg/mL or nevirapine trough levels below 3.4 μg/mL.[11,12]

In VL patients co-infected with HIV, both diseases could potentially have effect on the pharmacokinetics (PK) of both antileishmanial and ARV drugs. NNRTIs are metabolized by a multitude of liver enzymes [cytochrome P450 (CYP) 3A4, CYP2B6, CYP2C9, CYP2D6, etc.[13]]. Alterations in liver physiology associated with VL, caused by the parasitic infection and increased macrophage recruitment, could potentially affect NNRTI metabolism and thus ARV exposure. For most neglected tropical diseases, adequate PK studies are lacking or absent.[14] Neither the PK of ARVs in VL patients nor the PK of miltefosine and LAmB in HIV co-infected VL patients has been evaluated previously. Moreover, the PK of LAmB has not been studied in VL patients, while altered liver physiology could, e.g. affect liposome clearance of LAmB.

Besides possible disease-specific effects on PK, additional drug–drug interactions could affect exposure and thereby the efficacy of the concomitantly administered drugs. Amphotericin B deoxycholate has been associated with the inhibition of CYP enzyme activity,[15] which could affect the metabolism of and thus exposure to NNRTIs. No information is available on this mechanism for the liposomal formulation, although it can be expected that the effect is less profound due to a lower free fraction.[16] Both LAmB (>96%)[17] and miltefosine (96%–98%)[18] are highly protein-bound, as is the ARV drug efavirenz (>95%).[19] VL patients have severe hypoproteinaemia, which could potentially result in competition in protein binding.[20–22]

The PK of miltefosine has been studied in combination with LAmB,[10] but the potential effect of miltefosine co-administration on LAmB PK has not been evaluated. *In vitro*, no PK interactions could be observed, except for the incorporation of the free fraction of amphotericin B in miltefosine micelles that form above a critical micelle concentration of 11 μM (4.5 μg/mL).[23]

As part of the aforementioned clinical trial investigating LAmB as monotherapy and in combination with miltefosine in HIV co-infected VL patients,[4] the PK of concomitantly administered antileishmanial and ARV drugs was assessed. Our objective was to provide the first known description of LAmB PK in VL patients. Furthermore, our aims were to describe the PK of both LAmB and miltefosine in this particularly vulnerable patient population and to monitor any potential drug–drug interactions. Finally, NNRTI ARV drug exposure was characterized and compared with established therapeutic windows.

Methods

Study population

PK samples were collected in a clinical trial in Ethiopia investigating the safety and efficacy of LAmB in monotherapy or in combination with miltefosine in the treatment of HIV co-infected VL patients (registered as NCT02011958).[4] Patients received one of the two treatments: (i) LAmB (AmBisome®, Gilead, Foster City, CA, USA) monotherapy at a total dose of 40 mg/kg (5 mg/kg on Days 1 to 5, 10, 17 and 24), or (ii) combination therapy of 30 mg/kg LAmB (5 mg/kg on Days 1, 3, 5, 7, 9, 11) combined with 28 days of 50 mg oral miltefosine bi-daily (100 mg/day; Impavido®, Paladin Labs Inc., Canada). Only a sub-set of the trial subjects were enrolled in the present PK study (site of Gondar).

Primary clinical outcome was evaluated after one treatment cycle at Day 29 for both arms. Patients who were clinically well but had persistent parasites by microscopy of tissue aspirate at Day 29 (spleen or bone marrow aspirate) received another cycle of the allocated treatment regimen ('extended treatment'). Patients that were parasite positive and clinically unwell received rescue treatment (antileishmanial treatment at discretion of the treating physician). After extended treatment, patients that were still parasite positive received rescue treatment. Relapse-free survival was evaluated at 12 months after end of treatment (nominally Day 390).

Patients already on antiretroviral therapy (ART) continued their regimen. Patients not yet on ART started with a once-daily regimen of tenofovir (300 mg), lamivudine (300 mg) and efavirenz (600 mg), during or at the end of antileishmanial treatment. ART regimen modification was made for patients who showed ART failure after VL was treated.

Ethics

The clinical trial was approved by the appropriate institutional, local and national ethical review and regulatory bodies:[4] the University of Gondar Institutional Review Board (R/C/S/V/P/05/376/2013), the Ethiopian National Research Ethics Review Committee (3.10\454\05), the Médecins Sans Frontières Ethics Review Board (no reference number), the London School of Hygiene and Tropical Medicine Research Ethics Committee (6185), the Antwerp University Hospital Ethics Committee (12/20/184), the Prince Leopold Institute of Tropical Medicine Institutional Review Board (IRB/AB/ac/010) and the Food, Medicine and Healthcare Administration and the Control Authority of Ethiopia (02/6/22/41). Before enrolment, written informed consent was obtained from each patient.

Sample collection, storage and transport

Miltefosine and ARV concentrations were determined in dried blood spots (DBS). Miltefosine samples were collected pre-treatment, pre-dose on Day 10, Day 28 (~12 h after final dose), Day 56 (~12 h after final extended treatment dose, if applicable), and 1 and 6 months after treatment. ARV samples were collected pre-dose (trough level, C_{min}) and 4–5 h post-dose (peak level, C_{max}) on the first day of VL treatment and subsequently at Day 24 (monotherapy) or Day 28 (combination therapy), at the end of the extended treatment cycle (if applicable), and during follow-up at Day 56, Day 210 and Day 390 after initiation of VL treatment. If patients were not yet on ART at the start of antileishmanial treatment, ARV PK samples were collected on the first day of ART.

DBS samples were air-dried for at least 3 h after collection. Samples were stored on-site at room temperature in zip lock bags with >3 desiccant packages. Under the same conditions, samples were transported to and subsequently stored at the bioanalytical laboratory in Amsterdam, the Netherlands.

K_2-EDTA plasma samples were collected for amphotericin B quantification on the first and last day of LAmB treatment, corresponding to Day 24 (monotherapy) or Day 11 (combination therapy). Samples were collected at 2, 6 and 24 h (trough level) after start of infusion. As LAmB was nominally administered by a 2 h IV infusion, the sample collected 2 h after start of infusion should represent the maximum observed concentration (C_{max}). Amphotericin B plasma samples were stored and transported at nominally −20°C.

Bioanalysis

Miltefosine concentrations were quantified as described previously.[24] The lower limit of quantitation (LLOQ) was 10 ng/mL.

Efavirenz and nevirapine drug concentrations were quantified as previously described, with slight alterations.[25] Calibration standards and quality control samples were prepared in whole blood adjusted to 30%±1% haematocrit (Hct) mimicking typical VL patients' Hct values. The Hct effect on method accuracy and precision was acceptable for both efavirenz and nevirapine (Hct 21%–40%). NNRTI plasma concentrations were calculated

from analysed DBS concentrations using analysed individual Hct values during treatment (median [range] 29.6% [11.4%–44.0%]), and 35% Hct for the follow-up samples.[26]

Total (free, protein-bound and liposomal encapsulated) amphotericin B plasma concentrations were analysed in a range from 0.5 to 100 μg/mL with LC–MS/MS. Sample pre-treatment involved protein precipitation by adding 1000 μL of methanol to 50 μL of plasma. Further details on the amphotericin B bioanalytical method, including its validation, can be found in the Supplementary Information (Supplementary Information is available as Supplementary data at *JAC* Online).

Data analysis

Data analysis was performed in R (version 3.3.1), and R package ggplot2 was used for the graphical presentation. Non-compartmental analysis (NCA) was performed with the R package 'ncappc'.

For AMB, the amphotericin B concentration at $t = 0$ is set to zero, to integrate the AUC during infusion. The AUC is integrated between $t = 0$ and $t = 24$ h (AUC_{0-24h}) on Day 1 ($AUC_{D1,0-24h}$) and the last day of treatment ($AUC_{D24,0-24h}$/$AUC_{D11,0-24h}$). Amphotericin B accumulation was expressed as the D24/D1 (monotherapy) or D11/D1 (combination therapy) AUC_{0-24h} ratio, calculated by dividing the individual AUC_{0-24h} on the last treatment day by the individual AUC_{0-24h} on Day 1.

For miltefosine, the AUC was calculated from Day 0 to 28 (AUC_{0-D28}) and from Day 0 to 210 ($AUC_{0-\infty}$). Day 210 concentrations below the LLOQ were set to zero for $AUC_{0-\infty}$ calculations.

To evaluate the effect of antileishmanial treatment on ARV drug exposure, the ARV drug concentration ratio, of the end compared with the start of the first antileishmanial treatment cycle, was calculated. Patients not yet on ART at start of antileishmanial treatment were excluded from this analysis.

Data are represented as median (IQR), unless indicated otherwise. For normally distributed variables, the two-sample *t*-test was used when comparing groups with equal variances, and the Welsh two-sample *t*-test when comparing groups with unequal variances. In case of non-normal distribution, the Mann–Whitney *U*-test was applied. In evaluating correlations, a linear regression was performed in R.

Results

Demographics and treatment

A total of 30 male HIV co-infected VL patients were included in this PK study: 10 patients on LAmB monotherapy and 20 patients on LAmB + miltefosine combination therapy (Table 1). At the start of antileishmanial treatment, 8 patients in the monotherapy and 15 patients in the combination therapy arm were already on ART for

Table 1. Demographics and treatment information of study population

Parameter	Total	Monotherapy LAmB	Combination therapy LAmB + MIL
Total no. of patients	30	10	20
Male patients, *n* (%)	30 (100)	10 (100)	20 (100)
Age (years)	33 (27–45)	36 (27–45)	33 (28–44)
Body weight Day 0 (kg)	47.0 (36.0–73.0)	48.5 (41.5–67.0)	46.5 (36.0–73.0)
Body weight Day 28 (kg)	50.0 (35.0–75.0)	52.5 (37.0–70.5)	49.5 (35.0–75.0)
Height (cm)	170 (158–180)	170 (158–180)	170 (159–180)
Treatment outcome after one treatment cycle			
parasite negative, *n* (%)	13 (43)	3 (30)	10 (50)
parasite positive, rescue treatment, *n* (%)	3 (10)	2 (20)	1 (5)
parasite positive, extended treatment, *n* (%)	14 (47)	5 (50)	9 (45)
Treatment outcome after extended treatment			
parasite negative, *n* (%)	9 (64)	1 (20)	8 (89)
parasite positive, rescue treatment, *n* (%)	5 (36)	4 (80)	1 (11)
Primary infection, *n* (%)	14 (47)	5 (50)	9 (45)
Secondary infection, relapse, *n* (%)	16 (53)	5 (50)	11 (55)
ART at start antileishmanial treatment, *n* (%)			
TDF/3TC/EFV (300/300/600 mg)	15 (50)	7 (70)	8 (40)
other treatments including EFV	3 (10)		3 (15)
other treatments including NVP	4 (13)	1 (10)	3 (15)
other treatments including LPV/r	1 (3)		1 (5)
no treatment	7 (23)	2 (20)	5 (25)
ART at end antileishmanial treatment, *n* (%)			
TDF/3TC/EFV (300/300/600 mg)	23 (77)	9 (90)	14 (70)
other treatments including EFV	2 (7)		2 (10)
other treatments including NVP	4 (13)	1 (10)	3 (15)
other treatments including LPV/r	1 (3)		1 (5)

LAmB, liposomal amphotericin B; MIL, miltefosine; EFV, efavirenz; NVP, nevirapine; LPV, lopinavir; /r, ritonavir; TDF/3TC/EFV, tenofovir/lamivudine/efavirenz.
All values are given as median (range), unless stated otherwise.

2–1937 days (median 244 and 346 days for mono- and combination therapy, respectively). At the end of antileishmanial treatment, all patients were on ART, most commonly tenofovir/lamivudine/efavirenz (23/30 patients, Table S1). At the end of the first treatment cycle, 3/10 patients in the monotherapy arm and 10/20 in the combination therapy arm were clinically well and had no detectable parasites by microscopy. Three patients (3/30, 10%) had a concomitant TB infection at baseline, all of which received at least rifampicin and isoniazid during VL treatment.

Amphotericin B pharmacokinetics

Amphotericin B concentrations on the first and last day of treatment were available for all 30 patients. For three patients, Day 1 samples were excluded from analysis as they were not collected according to protocol (4, 8, 26 h instead of 2, 6, 24 h after start infusion).

Exposure variables are described in Figure 1 and Table 2. No statistically significant difference was found between the treatment arms for any of these variables. Amphotericin B accumulation was observed upon repeated dosing (Table 2). The amphotericin B D24/D1 AUC_{0-24h} ratio was 1.3 (1.1–1.6) for the monotherapy and the D11/D1 AUC_{0-24h} ratio was 2.4 (1.5–3.8) for the combination therapy, which cannot be directly compared due to different intermittent dosing time spans. There was no significant effect of body weight on the accumulation (monotherapy $P = 0.48$, combination therapy $P = 0.28$).

There was no significant difference in observed amphotericin B C_{max} on the first treatment day between patients already on and not yet on ART [24.1 (17.1–34.4) μg/mL versus 28.3 (16.5–50.9) μg/mL, respectively]. In addition, there were no significant differences in the amphotericin B C_{max} or AUC_{0-24h} on the last VL treatment day between different ART regimens. No correlation between C_{max} or AUC_{0-24h} and body weight could be observed.

Miltefosine pharmacokinetics

The average daily miltefosine dose received was 2.1 mg/kg/day (range 1.4–2.8 mg/kg/day). All pre-treatment miltefosine concentrations were below the LLOQ. Three PK samples with

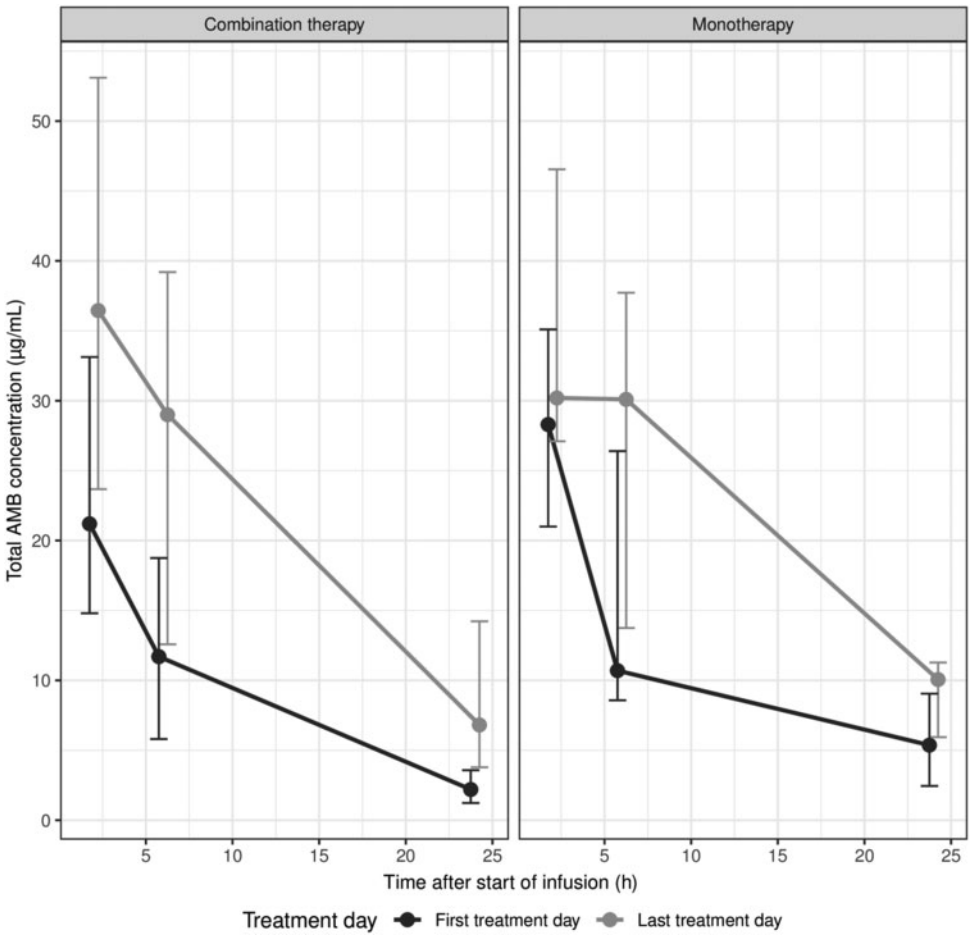

Figure 1. Median total amphotericin B (AMB) plasma concentration on the first treatment day (black lines) for monotherapy ($n = 9$) and combination therapy ($n = 18$) and the last treatment day (light grey lines) for monotherapy (Day 24, $n = 10$) and combination therapy (Day 11, $n = 20$). Error bars indicate the IQR.

Table 2. Amphotericin B plasma exposure

	First treatment day		Last treatment day	
	monotherapy, $N=9$	combination therapy, $N=18$	monotherapy, $N=10$	combination therapy, $N=20$
C_{min} (μg/mL)	5.37 (2.45–9.05)	2.20 (1.23–3.58)	10.1 (5.94–11.3)	6.82 (3.79–14.2)
C_{max} (μg/mL)	28.3 (21.0–40.8)	21.2 (14.8–33.1)	33.2 (29.0–46.6)	40.9 (25.4–53.1)
AUC_{0-24h}(μg·h/mL)	209 (173–570)	195 (114–305)	492 (271–587)	436 (240–703)

Last treatment day was Day 24 for the monotherapy arm and Day 11 for the combination therapy arm.
Values are presented as median (IQR).

Table 3. Miltefosine exposure

	All patients	Parasite negative (Day 29)	Parasite positive (Day 29)
Received daily dose (mg/kg)	2.2 (2.0–2.4)	2.2 (2.1–2.3)	2.1 (2.0–2.4)
C_{day28} (μg/mL)	18.7 (15.4–22.5)	17.5 (15.3–22.8)	19.2 (17.9–22.0)
C_{day56} (μg/mL)	N/A	N/A	20.1 (17.3–24.6)
AUC_{0-D28} (μg·day/mL)	314 (275–377)	330 (285–395)	314 (263–364)
$AUC_{0-\infty}$ (μg·day/mL)	N/A	524 (428–685)	1066 (1016–1317)

N/A, not applicable.
Values are presented as median (IQR).

physiologically improbable values were excluded from the results (all collected on Day 210).

Miltefosine exposure is described in Table 3. Figure 2 depicts the miltefosine concentration–time curves per patient, stratified by outcome at the end of the first treatment cycle (Day 29). There was no further accumulation of miltefosine between Day 29 and Day 56 for patients receiving extended treatment.

Median Day 28 miltefosine concentrations (C_{day28}) were significantly higher for patients treated with nevirapine (25 100 ng/mL, IQR 23 700–26 300 ng/mL) compared with patients treated with efavirenz (18 000 ng/mL, IQR 15 020–20 300 ng/mL, $P=0.04$, two-sample *t*-test), but only three patients received nevirapine in the combination therapy arm. There was no difference in miltefosine C_{day28} for the five patients who were not yet on ART at start of antileishmanial treatment compared with patients who were receiving ART.

There was a significant, but highly variable, correlation between the D11/D1 amphotericin B AUC_{0-24h} ratio, a measure of amphotericin B drug accumulation, and the miltefosine AUC_{0-D28}, a measure of total miltefosine accumulation ($P=0.0313$, $R^2=0.26$, Figure 3).

Miltefosine exposure (C_{day28}) for the two patients co-infected with TB enrolled in the combination treatment arm was relatively low: 7330 (parasite positive) and 13 400 (parasite negative) ng/mL compared with the 18 700 ng/mL median.

Antileishmanial exposure in relation to treatment outcome

There was no significant difference in miltefosine exposure, either C_{day28} or AUC_{0-D28}, between patients with negative and patients with positive parasitology at Day 29. Two patients showed particularly low miltefosine exposure with a C_{day28} of 8420 ng/mL and 7330 ng/mL and both were still parasite positive at the end of the first treatment cycle. One of these patients received extended treatment and showed increasing miltefosine levels. No significant difference was present for any of the amphotericin B exposure parameters between cured patients and patients requiring rescue or extended treatment. No correlation was detected between combined miltefosine and amphotericin B exposure and treatment outcome (Figure 3 and Figure S1).

ARV pharmacokinetics

C_{min} and C_{max} of efavirenz on the first and last antileishmanial treatment day of the first treatment cycle are described in Table 4.

Observed efavirenz trough concentrations during and after treatment are depicted in Figure 4. The efavirenz concentration change during antileishmanial treatment was 0.81 (0.49–1.26) for C_{max} and 1.10 (0.71–1.67) for C_{min}, without significant differences between treatment arms. During follow-up efavirenz concentrations generally remained steady with no difference in the number of patients within the therapeutic window during antileishmanial treatment versus follow-up.

In general, nevirapine C_{max} and C_{min} (Figure 5) remained relatively stable. No obvious differences during antileishmanial treatment versus follow-up were detected.

Discussion

To our knowledge, this is the first PK study for LAmB in VL patients and the first describing the PK of concomitantly administered

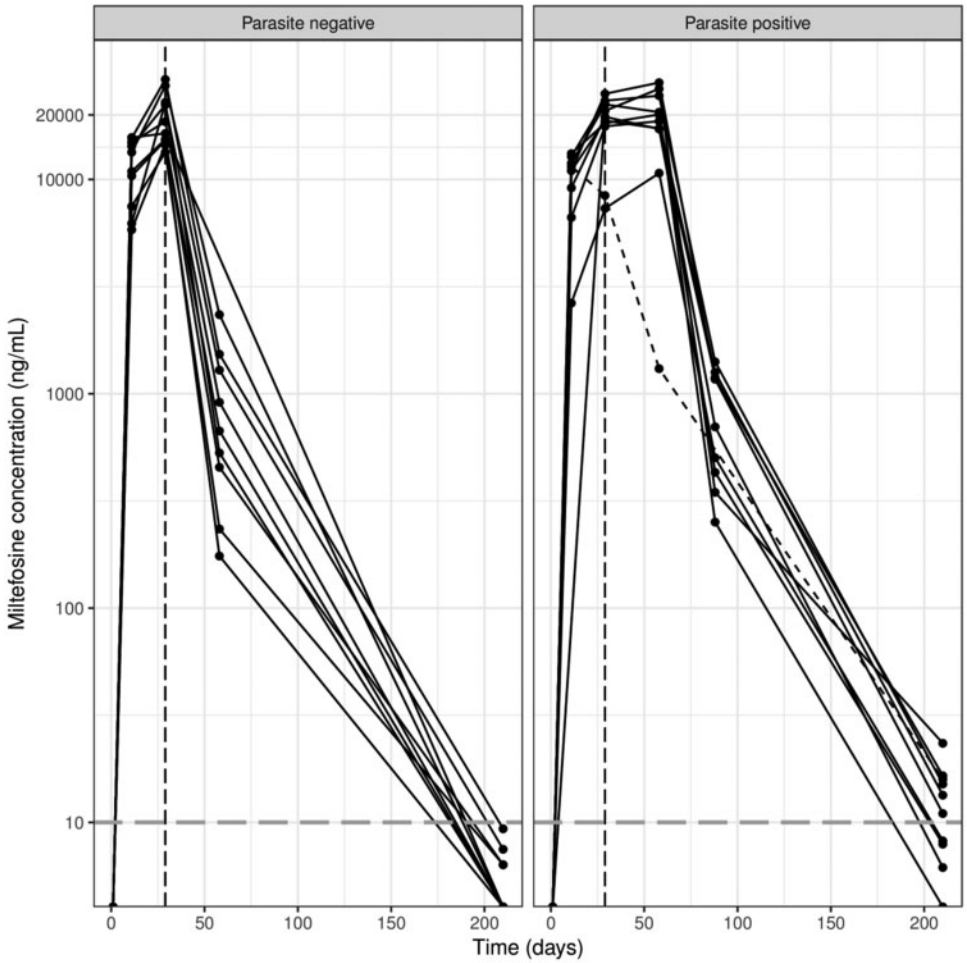

Figure 2. Miltefosine concentration–time curves for patients who were parasite negative at Day 29 (left, $n = 10$) and patients that were still parasite positive (right, $n = 10$) at the end of the first treatment cycle (Day 29, indicated with dashed vertical black line). The horizontal grey dashed line indicates the LLOQ of 10 ng/mL. For the 10 patients with positive parasitology at Day 29, one patient received rescue treatment (dashed line) and the others received an additional treatment cycle (until Day 56).

miltefosine, LAmB and ARV drugs in HIV-co-infected VL patients. Previous PK studies on LAmB were performed either in healthy volunteers or patients with invasive fungal infections. Total amphotericin B exposure was around 2-fold lower than previously described. In the AmBisome® manufacturer's product monograph a C_{max} of 57.6 ± 21.0 μg/mL (mean±SD) was reported after a single 5 mg/kg dose in 12 patients,[27] compared with 24.6 (17.0–34.9) μg/mL in this trial. Assuming dose proportionality, the value in the product monograph is in line with the reported values of 18.0–22.9 μg/mL after 2–3 mg/kg dose administration[16,28,29] and 75.9–95.5 μg/mL after 7.5 mg/kg dose administration.[30,31] The lower observed total amphotericin B exposure might be related to VL disease pathogenesis. Liposomes are cleared from the circulation by macrophages of the reticuloendothelial system mainly in the liver and spleen.[32] Clearance of LAmB could be affected by the increased liver macrophageal load leading to changes in drug distribution and possibly also an increased drug elimination. An additional effect of HIV on LAmB exposure cannot be excluded. As there was no difference in Day 1 amphotericin B exposure between patients already on ART versus patients that were not, no drug–drug interaction between ARV drugs administered in this trial and amphotericin B is to be expected.

No significant relationship could be identified between amphotericin B exposure and treatment outcome. However, it remains unknown what the best approximation of amphotericin B intracellular target site exposure is, e.g. whether free or encapsulated fraction in plasma relates best to the active moiety. Due to technical challenges, separation of the free amphotericin B fraction from the encapsulated fraction could not be performed. Increased clearance of the liposomes by the liver and spleen could actually indicate increased amphotericin B uptake at its target site of action.

While exposure was lower than previously described, the wide inter-individual variability in observed concentrations is in line with previous LAmB PK studies, and has been previously explained by inter-individual variability in liposomal uptake into tissue compartments or differences in amphotericin B release from the liposome

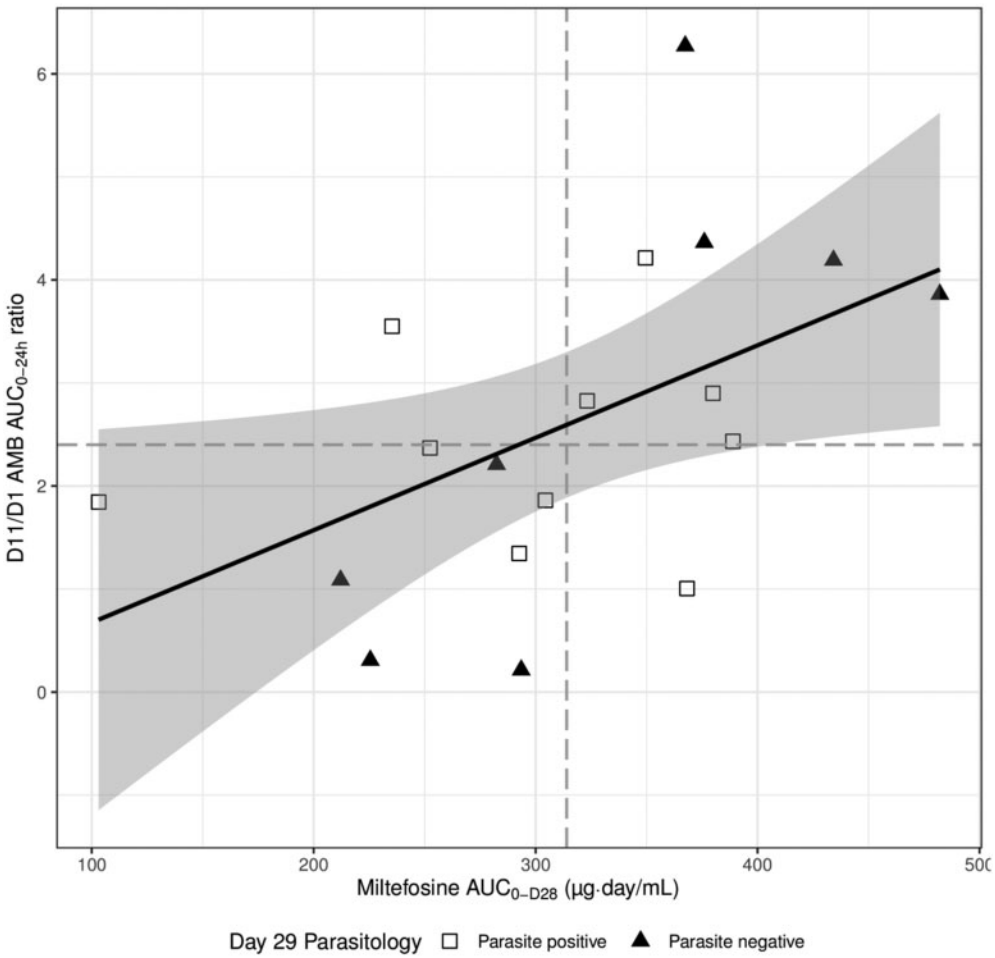

Figure 3. Correlation between amphotericin B (AMB) accumulation and miltefosine exposure in patients receiving combination therapy. AMB accumulation is expressed in terms of D11/D1 AMB AUC_{0-24h} ratio, while miltefosine exposure is expressed in cumulative area under the contration–time curve until the end of the first treatment cycle (miltefosine AUC_{0-D28}). The black line indicates the linear regression line ($P = 0.0313$, $R^2 = 0.26$), and the grey shaded area the 95% CI. Individual observations are indicated in solid triangles for patients who were parasite negative and open squares for patients still parasitologically positive after one treatment cycle (Day 29). The horizontal dashed line indicates the median D11/D1 AMB AUC_{0-24h} ratio of 2.4, while the vertical dashed line depicts the median miltefosine exposure AUC_{0-D28} at 314 µg·day/mL. Two patients had aberrant sampling schedules and were excluded.

Table 4. Efavirenz C_{max} and C_{min} in combination therapy and monotherapy, stratified by ART status on the day of VL treatment initiation

	ART on first antileishmanial treatment day?	Day	Total patients (*n*)	Efavirenz C_{min} (µg/mL), [median (IQR)]	Efavirenz C_{max} (µg/mL), [median (IQR)]	C_{min} <1 µg/mL, [*n* (%)]	C_{min} >4 µg/mL, [*n* (%)]
Combination therapy[a]	yes	1	11	1.28 (0.65–2.66)	4.91 (2.97–5.32)	5 (45)	1 (9.1)
		28	10[c]	1.32 (0.98–1.97)	3.24 (2.50–4.56)	3 (30)	0 (0)
	no[a]	28	5	1.06 (0.58–1.76)	4.00 (3.16–4.62)	2 (40)	1 (20)
Monotherapy[b]	yes	1	7	1.35 (1.08–1.86)	3.85 (2.73–4.22)	2 (29)	0 (0)
		24	7	1.83 (1.22–1.97)	4.60 (2.23–4.76)	2 (29)	1 (14)

[a]Five patients in the combination therapy were not on ART, but started ART during antileishmanial treatment on Day 11, 14, 16, 27 and 28, respectively. Only Day 28 concentrations are depicted for these patients in this table.
[b]Two patients in the monotherapy were not on ARVs, but started ART on the last day of antileishmanial treatment. These data were therefore excluded from this table.
[c]One patient excluded since both C_{max} and C_{min} on Day 28 were below LLOQ, due to a switch in ART.

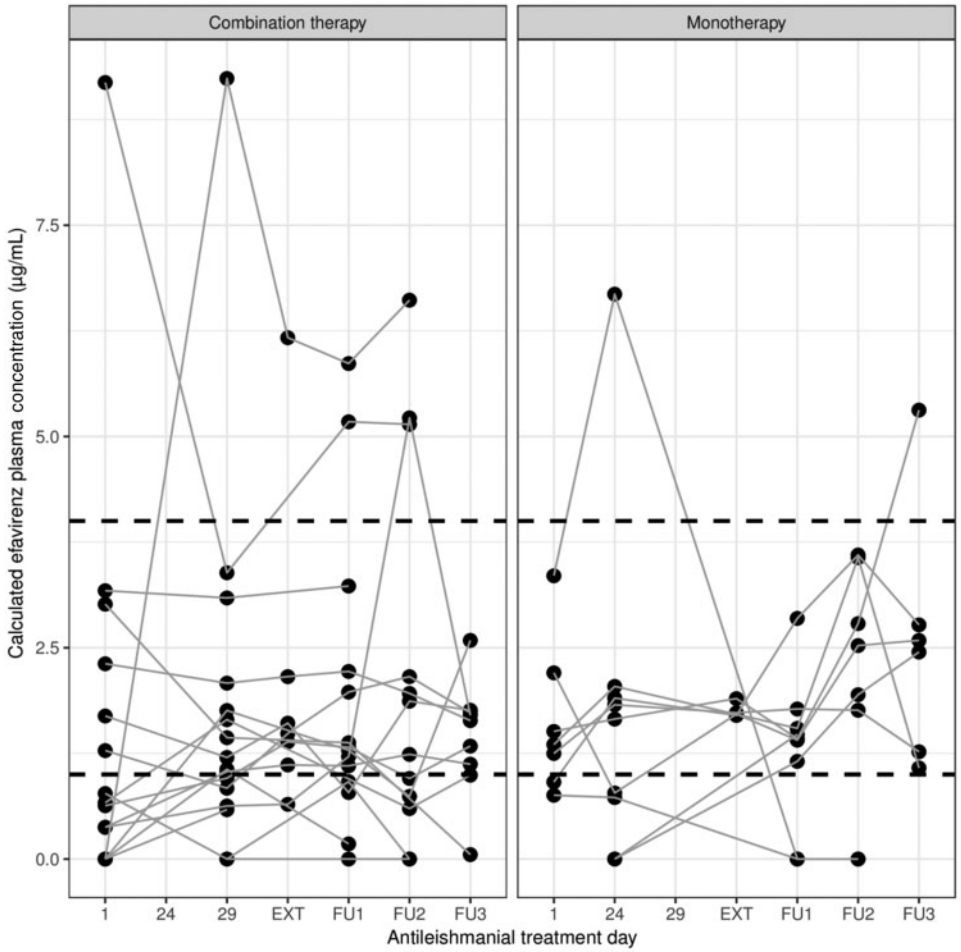

Figure 4. Efavirenz C_{min} over time per patient during the treatment follow-up for combination therapy ($n = 16$) and monotherapy ($n = 9$). This figure also includes patients not yet on ART on the first antileishmanial treatment day and patients that had a treatment switch or otherwise showed undetectable ARV levels. The horizontal dashed black lines depict the 1–4 μg/mL therapeutic window previously described for efavirenz. FU, follow-up timepoint; EXT, additional extended treatment timepoint.

carrier.[28,29,33] As has been documented previously, accumulation was observed upon multiple dosing.[28]

The median miltefosine C_{day28} of 18 700 (15 400–22 500) ng/mL was approximately 35% lower than the previously reported C_{day28} of around 30 000 ng/mL,[34,35] and the miltefosine AUC_{0-D28} of 314 (275–377) μg·day/mL was 37% lower compared with the previously observed 497 (191–767) μg·day/mL in adult Eastern African non-HIV-infected VL patients.[10] Extended treatment did not result in higher miltefosine concentrations. The low miltefosine exposure in this patient cohort can partially be attributed to the flat dosing of 50 mg miltefosine twice daily, which corresponded to a 19% lower daily dose compared with that previous study (2.1 versus 2.6 mg/kg/day, respectively).[10,36] In adults, miltefosine dosing should be adjusted by body weight, with patients ≥45 kg receiving 150 mg of miltefosine daily.[37] High compliance is expected, as miltefosine administration was directly observed, while gastrointestinal side effects were not more pronounced in this patient cohort compared with non-HIV-infected VL patients.[4]

Patients treated with efavirenz had a significantly lower miltefosine C_{day28}, which could imply a potential effect of efavirenz on miltefosine accumulation, although the sample size is small ($n = 16$). It is possible that the highly protein-bound (99.5%) efavirenz competes with miltefosine for binding albumin, which is extensively decreased in VL patients, while this competition could be less marked for nevirapine (60% protein-bound). Stronger up-regulation of P-glycoprotein expression by efavirenz compared with nevirapine (observed *in vitro*)[38] might have influenced miltefosine intracellular accumulation.[39]

Two patients co-infected with TB showed a relatively low miltefosine C_{day28}. Co-medication of these patients with rifampicin could potentially have contributed to the lower exposure, as rifampicin is known to induce the P-glycoprotein transporter.[40]

Although we did not find a significant relationship between the miltefosine C_{day28} and initial treatment outcome at Day 29, the patient receiving rescue treatment (i.e. clinically unwell and with positive parasitology) showed a 27% decline in miltefosine concentrations between Day 11 and Day 28 (Figure 2). This decrease

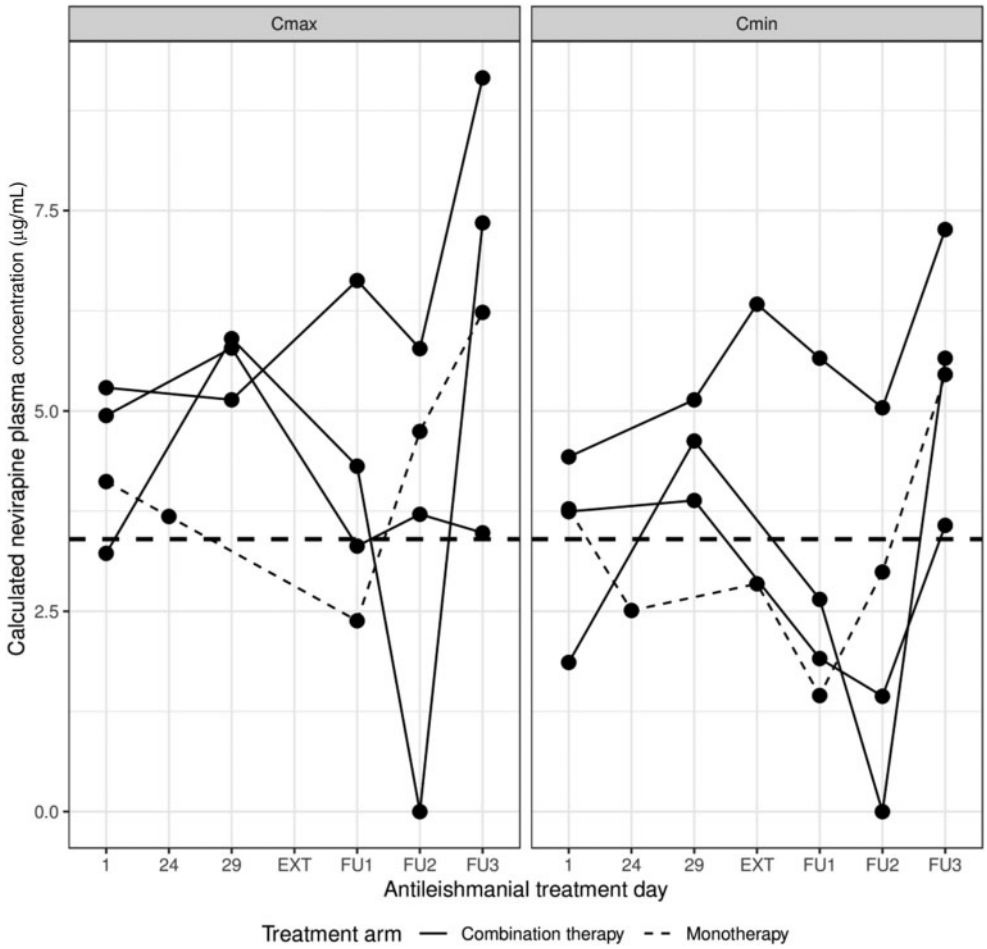

Figure 5. Nevirapine C_{max} (left) and C_{min} (right) during the antileishmanial treatment period, per patient. Indicated with the black dashed line is the lower limit of the therapeutic window at 3.4 μg/mL. One patient had undetectable levels at FU2, probably due to non-adherence. FU, follow-up timepoint; EXT, additional extended treatment timepoint.

in exposure coincided with a decrease in body weight (48 to 39 kg), indicating a worsened clinical condition, possibly resulting in lower absorption and bioavailability.

Nevertheless, overall efficacy was better for the combination therapy,[4] which could potentially be due to the immunomodulatory activity of miltefosine, driving activation of Th1 response and reversing Th2 activation.[41,42] A relationship between host immunity and treatment response was suggested by a transcriptomics study in this patient cohort.[43]

Interestingly, a significant correlation between amphotericin B and miltefosine accumulation was observed, which has not been described previously to our knowledge. This correlation might be caused by similar distribution patterns and mechanisms for both LAmB and miltefosine. Furthermore, it could be hypothesized that free amphotericin B accumulates within miltefosine micelles when concentrations are above the critical micelle concentration of 11.1 μM (4.5 μg/mL), as reported previously *in vitro*.[23] However, as both the free fraction of miltefosine and amphotericin B are small,[17,18] this effect is probably negligible. Additionally, miltefosine micelles and amphotericin B liposomal carriers could theoretically fuse, altering their composition and possibly clearance. Liposome clearance in the liver has been found to be largely dependent on liposome composition, such as size, charge and headgroup composition.[44]

Efavirenz C_{min} on the first day of antileishmanial treatment were similar to the previously reported C_{min} of 1.21 (0.83–1.86) in a large Ethiopian population ($n = 215$).[45] The therapeutic window of efavirenz (1–4 μg/mL) is well defined, with higher risk of treatment failure when efavirenz trough concentrations are below 1 μg/mL and increased risk of neuropsychiatric adverse reactions with peak concentrations above 4 μg/mL.[11,12] A large proportion of patients had efavirenz C_{min} below 1 μg/mL, which was observed previously as well for non-VL patients in Ethiopia,[45] but this proportion did not change upon antileishmanial treatment. In general, no profound effect of antileishmanial treatment could be observed on efavirenz or nevirapine concentrations, with individual exceptions.

New WHO ART guidelines propose lowering the efavirenz dose to 400 mg.[46] While this might lower the observed efavirenz effect on miltefosine pharmacokinetics, it will most probably also lead to an even larger proportion of Ethiopian HIV-co-infected VL patients at risk of ART failure (C_{min} <1 μg/mL).

Conclusions

Both amphotericin B and miltefosine exposure were lower than previously observed in non-VL and non-HIV-VL patients, respectively. The decreased amphotericin B exposure could potentially be caused by a change in clearance due to altered liver physiology in VL. The lower miltefosine exposure can partially, but not exclusively, be attributed to the 19% lower dosing. This indicates that miltefosine dosing in this primarily adult population should be adjusted by weight as per recommendations to achieve equivalent exposure to non-HIV-infected East African adult VL patients, given the established exposure–response relationship for miltefosine in VL. The lower than expected antileishmanial drug exposure to both LAmB and miltefosine emphasizes the importance of dose finding studies and investigating the PK of co-administered antileishmanial and ARV drugs in these specifically vulnerable patients. Adequate drug exposure in these HIV co-infected patients is of utmost importance to optimize treatment efficacy, as relapse incidence is especially high in this population and treatment options are highly limited.

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Transparency declarations

None to declare.

Supplementary data

Table S1, Figure S1 and Supplementary Information are available as Supplementary data at *JAC* Online.

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