Title: 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

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Summary: 28 days of allometric miltefosine dosing in Eastern African children with visceral leishmaniasis demonstrated increased and less variable exposure than linear miltefosine dosing, with improved efficacy and a satisfactory safety profile.
Abstract

**Background:** Convenient, safe, and effective treatments for visceral leishmaniasis in Eastern African children are lacking. Miltefosine, the only oral treatment, failed to achieve adequate cure rates in Eastern Africa, particularly in children, in whom linear dosing (2.5 mg/kg/day for 28 days) resulted in 59% cure rate, with lower systemic miltefosine exposure than in adults.

**Methods:** We conducted a phase-II trial in Kenya and Uganda in 30 children, aged 4-12 years, with visceral leishmaniasis, to test whether 28 days of allometric miltefosine dosing is able to safely achieve a higher and more effective systemic exposure of miltefosine than linear dosing (historical data).

**Results:** Miltefosine accumulated during treatment. Median AUC$_{0-210}$ and $C_{\text{max}}$ values were slightly higher than those reported previously for children on linear dosing but not dose-proportionally. Miltefosine exposure at start of treatment was increased, with higher median day-7 plasma concentrations (5.88 vs 2.67 µg/mL). Concentration-time curves were less variable, avoiding the low levels of exposure observed with linear dosing. The 210-day cure rate was 90% (95% confidence interval, 73%-98%), similar to that previously described in adults. Nineteen miltefosine-treatment-related adverse events (AEs) occurred but none caused treatment discontinuation. Two serious AEs occurred, both unrelated to treatment and fully recovering.

**Conclusions:** Allometric miltefosine dosing achieved increased and less variable exposure than linear dosing, though not reaching the expected exposure levels. The new dosing regimen safely increased the efficacy of miltefosine for Eastern African children with visceral leishmaniasis. Further development of miltefosine should adopt allometric dosing in paediatric patients.

**Key Words:** visceral leishmaniasis; Eastern African children; miltefosine; allometric regimen; drug pharmacokinetics.

Introduction
Visceral leishmaniasis (VL) (kala-azar) is the most lethal form of leishmaniasis, inevitably requiring treatment to prevent mortality [1]. Around 200,000-400,000 new cases of VL occur worldwide each year (data from 2004-2008; [2]). Cases of VL have sharply decreased in South Asia following an elimination campaign [3, 4], leaving Eastern Africa region with the highest burden worldwide, with 29,400 to 56,600 cases estimated annually [2].

A limited number of drugs are available for VL treatment [5], all of them have limitations related to either toxicity, parenteral administration, cost and/or requirement of cold chain. In Eastern Africa, the WHO recommended 17 days of combination treatment of sodium stibogluconate (20 mg/kg/day Sb⁵⁺) and paromomycin (11 mg base/kg/day) [5]. This combination has an efficacy of 91.4% [6], but requires 17 days of two injections, and antimonials is associated with infrequent but significant life-threatening toxicity (cardio-toxicity, acute pancreatitis) [5].

Miltefosine, an alkylphosphocholine analogue initially developed as an anti-cancer drug, has re-emerged as the only effective oral VL treatment [7]. In initial trials performed in India, miltefosine 2.5 mg/kg/day for 28 days had high efficacy rates (>90% at 6 months follow-up) in patients aged ≥12 years [8] and in children aged 2-11 years [9]. In Ethiopian adult VL non-HIV-infected male patients (aged ≥15 years), miltefosine (100 mg/day for 28 days), achieved a 6-month cure rate of 75.6% (99/131), although 19.1% were lost to follow-up. The efficacy for those who completed the 6-months follow-up was 93.4%, with 5.7% relapse rate and 1 case of death [10]. A phase-II trial (LEAP 0208) (NCT01067443) [11] in Sudan and Kenya comparing miltefosine alone (2.5 mg/kg/day for 28 days) with miltefosine (2.5 mg/kg/day for 10 days) in combination with liposomal amphotericin B (10 mg/kg single dose) achieved 6-month cure rates of 72% and 77%, respectively. Although this trial produced discouraging efficacy data, it provided important pharmacokinetic data for miltefosine monotherapy in Eastern African population, showing lower systemic miltefosine exposure in children (<12 years) than in adults with conventional linear mg/kg miltefosine dosing [11]. There was a corresponding lower cure rate in these children; cure rates at 6 months were 59% in children and 86% in adults (p=0.05). Lower efficacy of miltefosine monotherapy in paediatric VL was evident in previous studies in Nepal and India; children with VL had higher rates of failure (6.4% versus 3.4% in adults) [9] and higher relapse risk [12, 13]. Both for Eastern Africa [14] and Asia [13, 15], higher failure rates due to relapse were associated with lower
miltefosine exposure [15]. Model-based simulations of an allometric dosing regimen, based on non-linear scaling of the dose in children based on fat-free mass, predicted a level of miltefosine exposure in children equivalent to that achieved in adults receiving conventional dosing [15]. We tested this prediction in Eastern African children with VL aiming to increase drug exposure and correspondingly increase the cure rate. Because the required allometric dosing would involve oral doses exceeding conventional dosing, we also assessed its safety in children.

Methods

Study Patients

Thirty children, aged 4-12 years, with primary VL were recruited at two clinical sites: Kacheliba, West Pokot County, Kenya, and Amudat, Karamoja sub-region, Uganda. All patients fulfilled the trial inclusion and exclusion criteria (supplemental material). They showed VL clinical signs and symptoms and had a confirmatory parasitological microscopic diagnosis. Their age varied between ≥4 and ≤12 years and they weighed <30 kg. All had primary, non-severe VL (based on clinical and haematological parameters, as per exclusion criteria) and had not received anti-leishmanial drugs within the previous 6 months. None suffered severe malnutrition or any serious underlying disease or concomitant severe infection.

Study drug

Miltefosine medication was Impavido® 10 mg and 50 mg capsules (Paladin Labs Inc., Montreal, Canada), in aluminium-aluminium blister foil packs.

Treatment and Procedures

Patients were hospitalized for screening, baseline procedures and for the 28-day treatment duration, and assessed during out-patient follow-up on days 56 and 210 after treatment start. All patients received 28-day allometric miltefosine dosing, twice daily after a meal, under observation by the nurse until 30 minutes after each administration to record possible vomiting. The allometric dose was determined using patient’s sex, baseline height and weight, according to tables adapted from Dorlo and colleagues [13] (supplementary
material). Plasma samples were collected at screening, during treatment at days 1 (8 hours after first dose), 7, 14, 21, 28 (before miltefosine administration), and at days 56 and 210 during the follow-up visits.

**Trial design**

This was a phase-II, open-label clinical trial, registered at the U.S. clinical trial registry (under NCT02431143) and conducted in accordance with the trial protocol, the International Conference on Harmonization guideline for Good Clinical Practice, local regulations and the Declaration of Helsinki. Ethical approvals were obtained from institutional ethics committees at the Kenya Medical Research Institute and at Makerere University, Uganda. Individual informed consent was obtained from parents/guardian and assent was obtained from participating patients, when applicable, as per country regulation.

The study objectives were to characterize the pharmacokinetics, safety and efficacy of miltefosine allometric regimen given for 28 days in Eastern African children with primary VL. The primary pharmacokinetic endpoints were total drug plasma exposure (area-under-the-concentration-time-curve [AUC] from 0 to day 210) and plasma maximum concentration ($C_{\text{max}}$).

Primary safety endpoints were frequency and severity of adverse events (AEs), serious adverse events (SAEs) and AEs necessitating treatment discontinuation. Secondary efficacy endpoints were cure rates at days 28 and 210 after start of treatment (cure rate = proportion of patients recovering from clinical signs and symptoms of infection, having a negative microscopic reading for parasitaemia at day 28 and not requiring any rescue treatment up to day 210 follow-up).

**Plasma miltefosine bioanalysis**

Samples storage and transportation conditions were monitored and maintained at maximally -20°C; no deviations were noted. Plasma miltefosine concentrations were quantified using liquid chromatography coupled to tandem mass spectrometry as previously published [16]. The performance of the bioanalysis is described in detail in the online supplementary information.

**Pharmacokinetic analyses**
The plasma miltefosine data were managed using R (version 3.1.2). Standard two-stage non-compartmental pharmacokinetic analysis was performed with the R-package “ncappc” [17]. Unless indicated otherwise, data are represented as median (range) and statistical tests were performed using a Mann-Whitney U-test.

Safety assessment

Treatment safety was assessed at each visit by routine recording of adverse events (AEs) that occurred since the previous visit, by blood sampling for measurement of haematological and clinical chemistry parameters and by assessment of vital signs and physical condition. The laboratory parameters were graded according to CTCAE v4.0 and clinically relevant values recorded.

Clinical assessment of efficacy

The clinical assessment of VL was performed at screening, on days 3, 7, 14, 21 and 28 of treatment, and on follow-up days 56 and 210. This involved measurement of axillary temperature, size of spleen and liver and body weight. VL symptoms were also recorded. Parasitological assessments were performed at baseline and day 28, using microscopic examination of spleen aspirate or, under specific circumstances (see Study Protocol in online supplementary methods), bone marrow aspirates. Cure at the end of treatment (day 28) was defined as recovery of clinical signs and symptoms (patient afebrile, spleen size reduced, improvement of symptoms and haematological parameters) and microscopic absence of parasites from spleen or bone marrow aspirate. Definitive cure at day 210 (6 months) was defined as absence of signs and symptoms of VL (no fever, reduced spleen, haematological parameters recovered, weight gained) and having not required any rescue treatment during the trial.

Statistical analyses

The minimal sample size was based on pharmacokinetic clinical trial simulations for the primary pharmacokinetic endpoint using the method of Dorlo and colleagues [18]. Including potential non-compliance, this provided a trial sample of 30 patients. For the primary pharmacokinetic endpoint, plasma miltefosine concentrations were measured in all patients receiving at least one miltefosine dose. All patients who were administered the first dose of miltefosine constituted the safety population. The primary population for
efficacy analysis at days 28 and 210 was the intention-to-treat population (ITT). The per-protocol (PP) population included patients with no pre-specified major protocol deviations relating to treatment compliance and baseline exclusion criteria.

Descriptive analyses were used for patient characteristics and biological data. Categorical variables were summarized using proportions. Continuous variables are presented as means (standard deviation) and medians (interquartile range). Box plots are used to present laboratory parameters and spleen size. Time to relief of fever was analyzed using Kaplan-Meier curve. For efficacy analyses, the primary analysis population at day 28 & day 210 was intention to treat population (ITT). Furthermore, per protocol (PP) analysis was also performed. The STATA version 13.1 program [19] was used for data analysis.

Results

Patients

Thirty subjects out of 158 suspected VL cases were enrolled and their disposition during the trial is shown in Figure 1. Patients were excluded for various reasons but mostly (87/158) because of age. Of those enrolled, three required rescue treatment, one during miltefosine treatment and two during the follow-up. The trial was completed without any loss to follow-up. The intention-to-treat and per-protocol populations were of identical size (n=30).

The age distribution of the children was evenly spread between four and 12 years (Table 1). All children had fever and most had abdominal swelling (96.7%), mucosal pallor (96.7%) and splenomegaly (93%). Most were of normal weight, with 36.7% underweight and none overweight, but 63.3% had lost weight since disease onset, with 53.3% having muscle wasting and 26.7% appetite loss.

Pharmacokinetics

The patients received a median daily allometric miltefosine dose of 3.2 mg/kg/day (range, 2.7-3.9 mg/kg/day). Quantitative analysis of plasma miltefosine met standard Food and Drug Administration criteria, with accuracies and precisions within ±15% and ≤15%, respectively. Excluding pre-treatment samples, which were
all below the lowest level of quantification (LLOQ) as expected, a total of 206 samples were collected from 30 patients, as per protocol. Three samples were excluded from data analysis, since a steep (>70%) decrease in miltefosine concentration was observed during treatment, physiologically improbable due to the long elimination half-life of miltefosine [18].

**Observed miltefosine exposure after allometric dosing**

Miltefosine plasma concentration-time profiles after allometric dosing are depicted in Figure 2. Of the three patients who experienced treatment failure or relapse, two had substantially lower miltefosine accumulation than the cured patients (Figure 2). Unexpectedly, for 37% of patients, the miltefosine concentrations plateaued or even decreased between day 14 and day 21 (change in concentration between -19% and +10%), after which concentrations increased >18% (range 18-58%) at day 28.

**Descriptive comparison of allometric miltefosine dosing with historical conventional-dosing data**

Miltefosine exposure in the current allometric study was compared with paediatric data for conventional linear dosing (from the LEAP 0208 trial [11]). Due to differences in age limits for inclusion, the LEAP 0208 cohort (n=21) had a higher median age (10 years, range 7-12 years) and a higher median body weight (24 kg, range 16-34 kg) [11]. As there was no difference in exposure between the 4-6 years and 7-12 years age groups in the present study, all patients with available PK data were included in this comparison with the LEAP 0208 trial.

Although not statistically significant, children receiving allometric dosing showed a trend (p=0.07) for much more rapid miltefosine accumulation than children on conventional dosing, with median day-7 plasma concentrations of 5.88 µg/mL (range 0.66-14.3 µg/mL) versus 2.67 µg/mL (range 0.70-12.8), respectively. However, the median total exposure was only slightly increased with allometric dosing, resulting in a 6% and 8% higher $C_{\text{max}}$ and $\text{AUC}_{0-210}$, respectively (Table 2). For comparison, the observed total exposure after allometric dosing was still lower than that in adults receiving the conventional linear dosing (median non-compartmental analysis $\text{AUC}_{0-210}$ 582 versus 836 µg·day/mL, respectively [data not published]). The variability (CV%) of miltefosine $C_{\text{max}}$ values was two-fold lower with allometric dosing (15.7%) than with conventional
dosing (30.5%) when comparing the paediatric populations. Similarly, AUC$_{0-210}$ values were less variable with allometric dosing than with conventional dosing (Table 2). Furthermore, the proportion of patients with $C_{\text{max}}$ lower than the target of 17.9 µg/mL was 14.8% in patients treated with allometric dosing as compared to 28.6% in conventional dosing (Table 2).

Safety

Table 3 summarises the frequency of AEs recorded during the trial and Table 4 lists the AEs according to the MedDRA categorisation system (System Organ Class / Preferred Term), relationship to study drug and severity (as per CTCAE v4.0). A total of 110 treatment emergent adverse events (TEAEs) were reported in 30 subjects. All patients had at least one TEAE, the most frequent being anaemia, neutropenia, malaria and upper respiratory infection. Thirteen patients (43%) presented a total of 19 TEAEs related to the study drug, which are referred to as treatment-emerging adverse drug reactions (TEADRs). Among these, the most common were neutropenia and vomiting, which were reported in 20% and 17% of patients, respectively. No patient discontinued treatment due to an AE and cases of vomiting were not associated with treatment compliance failure. Five (4.5%) Grade-4 TEAEs (1 anaemia, 4 neutropenia) were reported. The Grade-4 neutropenia cases (<500/mm$^3$) occurred in subjects with Grade-3 low neutrophil counts at baseline that worsened in severity after treatment initiation. The majority of neutropenia cases occurred during the first days of treatment, were temporary and asymptomatic, resolved spontaneously and did not require any intervention. Only mild (Grade-1 CTCAE) increases in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were observed. Creatinine levels remained stable for all subjects during the treatment period and only one Grade-2 increase was observed. These were not considered clinically significant and not reported as AE by the investigator.

Two SAEs occurred in 2 patients: one case of “transfusion reaction” (PT) was considered an important medical event by the investigator, occurring on day 203 after treatment-start. The other case was life-threatening anaemia (PT), occurring on day 19. This patient was later assessed as initial failure. Both SAEs were reported as unrelated to miltefosine treatment by the investigator and the patients fully recovered.
Efficacy

Each enrolled patient belonged to both the PP and ITT populations. The 28-day and 210-day cure rates were 96.7% (95% CI, 83%-100%) (29/30 patients) and 90% (95% CI, 74%-98%) (27/30 patients), respectively. Three patients received Ambisome® rescue treatment: one at day 25 (initial failure), one at day 168 (relapse), one at day 206 (relapse).

Clinical response was observed in several parameters, including spleen and liver sizes, which decreased progressively during the treatment and follow-up period (up to day 210) (Figure 3). Haemoglobin and haematocrit increased towards normal levels by the end of treatment and remained normal during follow-up (Figure 3). Fever was cleared in nearly all patients by day 14 of treatment and in all patients by the end of treatment (see Figure S1 in supplementary material). Weight gain was observed for all patients, with an 8% average increase at the end of the study compared to baseline.

Discussion

The present study is the first to assess the pharmacokinetics, safety and efficacy profile of miltefosine in paediatric VL patients treated with an allometric dosing regimen, which provided a 28% higher median daily dose than conventional linear dosing. Our pharmacokinetic data indicate more rapid accumulation after allometric dosing in the first weeks of treatment, with $C_{\text{max}}$ and $\text{AUC}_{0-210}$ being only slightly increased (6% and 8%, respectively) probably due to a plateau in accumulation in the third week of treatment, implying non-linear dose proportionality of miltefosine pharmacokinetics (see online supplementary material).

Variability in exposure decreased almost twofold with the allometric dosing compared to the conventional dosing (as shown by the spread of $C_{\text{max}}$ and of $\text{AUC}_{0-210}$ Table 2). The low variability observed in miltefosine concentrations between patients can also be seen in Figure 2. These data indicate that allometric dosing of miltefosine allows a more consistent systemic exposure to the drug treatment than linear dosing. The individual patients’ $C_{\text{max}}$ values from the non-compartmental pharmacokinetic analyses (Table 2) also indicate that fewer children (15%) on allometric dosing than children (29%) on linear dosing had plasma miltefosine levels below the threshold of 17.9 μg/mL, which has been related previously to a higher probability of disease
relapse [21]. Additionally, miltefosine concentrations in the first week of treatment were highly increased after allometric dosing compared to linear dosing when comparing the paediatric populations (data not shown). This may be pivotal both in terms of efficacy as well as driving emergence of drug resistance, given that the parasite biomass is highest at this stage of treatment.

The improved miltefosine pharmacokinetics could at least partially explain the much improved 6-month cure rate (90%), compared to the rate we previously reported in Eastern African children (59%, LEAP 0208) [11]. In fact, the efficacy observed in children treated with allometric dosing was similar to that observed in adults (86%) treated with 28-days conventional treatment regimen [11], despite the lower average drug exposure compared to adults.

The present non-compartmental analysis is limited by the sparse sampling and the observed pharmacokinetic non-linearity, which may, for example, cause underestimation of exposure during treatment and overestimation during follow-up. We are currently developing a pooled model-based analysis of pharmacokinetic data pooled from several trials to characterize the observed non-linearities in paediatric VL patients in Eastern Africa.

Our findings are an important basis for further developing miltefosine as an effective oral medicine for use in combination treatment of VL in Eastern African children. We show that allometric miltefosine dosing in children is safe and more effective than conventional dosing. Further development of miltefosine should adopt allometric dosing in patients weighing <30 kg. A phase III trial is envisaged to assess combining an allometric regimen of miltefosine with paromomycin in VL patients in Eastern Africa as compared to SSG-PM. Positive results would provide a basis for provision of an alternative treatment that is more patient-friendly and requires shorter hospitalization than the current standard treatment, by replacing the much more toxic SSG with oral miltefosine. Miltefosine allometric dosing would be of potential benefit beyond Eastern Africa, as there is also an urgent need in South America and Asia for more tolerable and more convenient oral treatments for children affected by cutaneous leishmaniasis and post-kala-azar dermal leishmaniasis.
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References


Figure Legends

Figure 1: Disposition of patients

Figure 2: Individual plasma miltefosine concentration-time profiles: the grey lines indicate the patients who were cured of visceral leishmaniasis, the full black lines indicate the two patients who required rescue treatment during follow-up and the dashed black line indicates the patient who initially failed on treatment. The vertical dotted line shows the end of treatment (day 28).

Figure 3: Box plots of selected efficacy clinical parameters during treatment (day 0 to day 28) and follow-up. The box plots represent the interquartile ranges, whiskers represent minimum and maximum values, and dots outside the whiskers are outlier values. Red lines in the Haemoglobin figure represent the lower and upper limits of normal.
Figure 1

Screened, n = 158

Enrolled, n = 30

Completed treatment, n = 29

Completed trial, n = 28

Screen failures:
parasite detection –ive, n = 3
abnormal clinical lab. values, n = 9
age, out of range, n = 87
relapse, n = 8
severely undernourished, n = 1
pregnant / lactating, n = 1
body weight >30 kg, n = 2
others, n = 17

received rescue treatment, n = 1

received rescue treatment, n = 1

received rescue treatment, n = 1

Analysed:
Intention-to-treat, n = 30
Per-protocol, n = 30

Completed trial, n = 28

Received rescue treatment, n = 1

Completed treatment, n = 29

Received rescue treatment, n = 1

Enrolled, n = 30

Received rescue treatment, n = 1

Screened, n = 158
Figure 2: 

![Graph showing Melflufen concentration over time for different treatment outcomes: Cure, Relapse, and Initial treatment failure.](https://academic.oup.com/cid/advance-article-abstract/doi/10.1093/cid/ciy747/5090844)
Figure 3:

Haemoglobin (g/dl) vs Spleen size (cm)