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Pharmacokinetics of Lumefantrine in Adults Co-Infected with Malaria and HIV-1: with and Without Efavirenz-Based Antiretroviral Therapy

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Abstract: A parallel study design investigated the pharmacokinetic parameters of lumefantrine in adults co-infected with malaria and HIV-1 in 2 groups enrolled prospectively: Antiretroviral Therapy (ART) naive patients and patients on Efavirenz (EFV)-based therapy. Both groups were on co-Trimoxazole (TS) prophylaxis. Both groups received Artemether-Lumefantrine (AL) combination twice daily for 3 days. Venous blood was collected just before last dose and subsequently at 2, 4, 8, 24 and 120 h post dose. Concentrations of lumefantrine were determined by high performance liquid chromatography. Mean area under the plasma concentration-time curve ($AUC_{0-\infty}$) was 264.8 (243.1-286.5) μg h mL^{-1}, mean maximum Concentration (C_{max}) was 4.05 (3.77-4.33) μg mL^{-1}, mean day 7 Concentration ($C_{day\ 7}$) was 0.26 (0.20-0.32) μg mL^{-1}, mean elimination rate (K_0) was 0.020933 (0.020219-0.021647) μg h^{-1} and mean half life was ($T_{1/2}$) 33.3 (30.9-35.7) h for subjects on EFV-based ART and for ART naive subjects were 375.2 (349.7-400.7) μg h mL^{-1}, 6.08 (5.57-6.59) μg mL^{-1}, 0.64 (0.54-0.74) μg mL^{-1}, 0.0195 (0.0185-0.0205) μg h^{-1} and 36.0 (34.1-37.9) h, respectively. Time to maximum concentration (T_{max}) was 4 h in both groups. EFV based ART significantly decreases the exposure of lumefantrine. AL is well tolerated in these subjects.

Key words: Lumefantrine, efavirenz, HIV, malaria, Uganda

INTRODUCTION

Malaria and Human Immunodeficiency Virus (HIV) are of public health concern in Uganda and sub-Saharan Africa (WHO/UNAIDS, 2000; Proietti *et al*., 2011). Clinical studies have reported an increased frequency of malaria in people with HIV (Whitworth *et al*., 2000; French *et al*., 2001; Cohen *et al*., 2005). Co-Trimoxazole (TS) prophylaxis and Antiretroviral Therapy (ART) have been reported to reduce the occurrence of malaria infection in patients living with HIV (Kamya *et al*., 2007; Flateau *et al*., 2011; Nakanjako *et al*., 2011). TS is therefore recommended for prophylaxis for all people living with HIV (WHO/UNAIDS, 2000). Due to the scaling up of availability of antiretroviral medicines (ARVs), many people living with HIV have been started on ART (WHO, UNAIDS, UNICEF, 2007).

The World Health Organisation (WHO) recommends artemisinin-based combination therapies for treatment of uncomplicated malaria (WHO, 2010) with Artemether-Lumefantrine (AL) being the combination of choice in Uganda. AL is therefore by necessity being prescribed alongside ARVs and TS in cases of malaria and HIV co-infection. The recommended treatment for HIV infection in Uganda is a triple therapy regimen composed of Zidovudine (ZDV) and lamivudine (3TC) with either nevirapine or Efavirenz (EFV) (Katabira and Kamya, 2003).

ZDV and 3TC are intracellularly phosphorylated and eliminated via the renal system and do not involve CYP450 in their metabolism (Dooley *et al*., 2008). Lumefantrine is predominantly metabolized by the CYP450 (CYP) isoenzyme CYP3A4 (White *et al*., 1999). EFV is mainly metabolised by CYP2B6 and to a smaller extent by CYP3A4 but it is also reported to be a potent inducer of CYP3A4 (Khoo *et al*., 2005). Therefore, theoretically, the concomitant administration of EFV with AL should increase the metabolism of lumefantrine.

Artemether and lumefantrine complement each other in their activity against malaria parasites. Whereas

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artemether is associated with decreased parasite clearance time, the role of lumefantrine in the AL combination is to eliminate residual malaria parasites hence avoiding recrudescence (Ezzet *et al*., 2000; White *et al*., 1999). It has been reported that day 7 concentrations of lumefantrine below 280 ng mL^{-1} result in an increased risk of treatment failure (Ezzet *et al*., 1998; Ashley *et al*., 2007; McGready *et al*., 2008).

The primary objective of this study was to determine the pharmacokinetics of lumefantrine in patients co-infected with malaria parasites and HIV with and without EFV based antiretroviral therapy. The secondary objective was to assess the safety of the AL combination in these patients.

MATERIALS AND METHODS

Study site: This study was conducted at the ART clinic of Gulu Regional Referral and Teaching Hospital, Uganda during the period between December 2010 and November 2011. Malaria in this region is holoendemic and with a high transmission rate.

Study design and sample size: This was a three-arm, parallel design, open-label, pharmacokinetic study of lumefantrine at steady state in adult co-infected with malaria and HIV. One arm was ART naive while the second arm was for patients experienced on ART regimen composed of ZDV 300 mg and 3TC 150 mg twice daily and EFV 600 mg once daily. All patients in both arms were taking TS for prophylaxis. Sample size calculation was based on difference in area under the plasma concentration-time curves (AUC) of lumefantrine between the ART naive group and the group receiving EFV based antiretroviral therapy. It was to detect a difference of 30% in lumefantrine AUC at a 5% significance level, given a 60% between subject co-efficient variations with a power of 80%. Twenty two subjects were required in each arm. These subjects were recruited consecutively until the numbers reported were achieved.

Inclusion and exclusion criteria: All adult patients whose blood smears (thin and thick films) tested positive for malaria parasites and had a confirmed diagnosis of HIV-1 were eligible for recruitment onto the study. Those initiated on EFV based ART must have been on treatment for at least 4 weeks.

Individuals with complicated malaria, (i.e., impaired consciousness, multiple/recurrent convulsion, deep breathing or respiratory distress, difficulty in breathing or demonstrable pulmonary oedema, circulatory collapse or shock, jaundice, generalized weakness and severe anaemia), abnormal cardiac, liver or renal function, pregnant woherbal medication or any other medication that may interfere with cytochrome P450 System were excluded from the study.

Drug administration and compliance: The subjects received 6 doses of AL (each dose was 80 mg artemether and 480 mg lumefantrine) (Artefan® Ajanta pharma limited, India) administered at 0, 8, 24, 36, 48 and 60 h. Each subject swallowed the medicines with 200 mL of milk containing 3.5% fat to ensure optimal absorption (Borrmann *et al*., 2010). The drug administration was directly supervised and observed by the research assistants. The study nurses and clinicians ensured that the patients had strictly adhered to TS and ARVs using pill counts and self-reports at all their clinical visits.

Pharmacokinetic sampling: Venous blood was sampled just before the last dose and at 2, 4, 8, 24 and 120 h post dose. About 2-3 mL of whole blood were collected into Ethylenediaminetetraacetic Acid (EDTA) containing tubes at each time point. This was then centrifuged at 2000 g for 10 min at room temperature. The plasma was then stored at -80°C until analysis.

Lumefantrine assay: Plasma concentrations of lumefantrine were determined by a validated method using high performance liquid chromatography with UV detection (Zeng *et al*., 1996). The absolute recovery of lumefantrine in spiked plasma samples was 90% over the concentration range 5-4000 ng mL^{-1}. The internal standard used was halofantrine whose recovery was 88% at a concentration of 300 ng mL^{-1} in spiked plasma. The detection limit of lumefantrine was 14.7 ng mL^{-1}. The total assay coefficients were <6% for inter and intraday precisions.

Safety and tolerability assessment: Patients were continuously assessed by the clinicians for any adverse events using a checklist developed from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult adverse events. These were to be graded as mild, moderate, severe or life threatening.

The laboratory measurements were assessed on day 0 and then repeated on days 7 and 14. The hematology parameters assessed were red blood cell count, haemoglobin concentration, differential white blood cell count and platelet count. The biochemistries assessed were total bilirubin, direct bilirubin and creatinine, serum glutamic pyruvic transaminase and serum glutamic oxaloacetic transaminase. Electrocardiographs (ECGs) were performed at enrolment and on day 4.

Pharmacokinetic parameters: The pharmacokinetic parameters were obtained by STATA® Version 11.2 (StataCorp, College Station, Texas, USA). These were area under the plasma concentration-time curve ($AUC_{0-\infty}$), half life ($T_{1/2}$), maximum concentration (C_{max}), time to maximum concentration (T_{max}) and elimination rate (K_0). The area under the curve was calculated using the trapezoidal rule.

Pharmacokinetic and statistical data analysis: The baseline demographic characteristics were summarised as mean with 95% Confidence Interval (CI) and compared using the independent t-test. The pharmacokinetic parameters in the two groups were summarised as mean 95% CI and compared using the Wilcoxon rank-sum test. The $p<0.05$ was considered statistically significant.

The changes in the biochemistry and hematological measurements on days 3, 7 and 14 were each compared to the measures on day 0, respectively using the Wilcoxon signed rank test. The $p<0.05$ was considered statistically significant.

Ethical review: Written informed consent was obtained from all literate subjects who participated in this study. For the patients who were unable to read and write, an impartial witness was present during the process of obtaining the consent. It was made abundantly clear to all the subjects that they were free to withdraw from the study at any time without any consequences. This study was carried out in accordance with the Helsinki declaration and adhered to good clinical practice. It was approved by the Gulu University Research and Ethics Committee. Permission to carry out this study was granted by the Uganda National Council of Science and Technology (HS 877).

RESULTS

Patient demographics and baseline characteristics: A total of 36 adults co-infected with malaria and HIV were recruited and completed the study. Total 21 were on TS only and 15 were on TS and EFV-based ART. There was no significant difference in sex, age, weight and height in the two arms but the CD4 count was significantly different (Table 1).

Pharmacokinetic parameters of lumefantrine: Subjects on EFV based ART had significantly lower area under the plasma concentration-time curve, maximum concentration and day 7 concentration by 31% ($p<0.05$), 33% ($p<0.05$) and 59% ($p<0.05$), respectively. Half life was 33.3 h and elimination rate was 0.020933 μg h^{-1} in subjects on EFV-based ART and that for ART naive subjects was 36 h and 0.0195 μg h^{-1}, respectively. Time to maximum concentration was 4.0 h in both groups (Table 2).

Table 1: Summary of baseline patient demographics and clinical characteristics of participants

Prescription	TS	EFV based ART+TS	p-value[a]
No. (%) of females	17 (81.0)	20 (90.9)	0.11[b]
Age (years)	35.0 (30.6-39.3)	38.5 (34.6-42.4)	0.24
Weight (kg)	56.8 (53.2-60.4)	59.2 (55.3-63.1)	0.40
Height (cm)	165.5 (161.8-169.2)	165.6 (161.1-170.1)	0.97
CD 4 (cell mL^{-1})	301 (256-346)	382 (310-454)	0.05

Age, weight, height and CD4 count are expressed as mean (95% confidence interval); [a]p was calculated using the independent t-test unless otherwise indicated; [b]p value was calculated using χ^2-test for categorical variables

Table 2: Pharmacokinetic parameters of lumefantrine in adults co-infected with malaria and HIV-1 on TS prophylaxis and on TS+EFV-based ART

Parameters	AL with TS	AL with EFV/ 3TC/NVP+TS	p-value
$AUC_{0-\infty}$ (μg h mL^{-1})	375.2 (349.7-400.7)	264.8 (243.1-286.5)	0.0000
C_{max} (μg mL^{-1})	6.08 (5.57-6.59)	4.05 (3.77-4.33)	0.0000
C_{trough} (μg mL^{-1})	5.73 (4.68-6.78)	3.88 (3.33-4.43)	0.0000
$C_{day\,7}$ (μg mL^{-1})	0.64 (0.54-0.74)	0.26 (0.20-0.32)	0.0000
K_0 (μg h^{-1})	0.0195 (0.0185-0.0205)	0.020933 (0.020219-0.021647)	0.0369
$t_{1/2}$ (h)	36.0 (34.1-37.9)	33.3 (30.9-35.7)	0.0355
T_{max} (h)	4.0	4.0	-

Data are expressed as mean (95% CI; n = 22 for ART naive and 22 for ART experienced); AUC: Area Under the plasma Concentration-time curve at steady state; C_{max}: maximum Concentration; $C_{day\,7}$: Concentration at day 7; CI: Confidence Interval; $t_{1/2}$: half-life; K_0: elimination rate, T_{max}: Time to maximum concentration; p-value was calculated using the Wilcoxon rank-sum test

Safety and tolerability: The adverse events in the ART naive subjects were headache (n = 3), vomiting (n = 1), nausea (n = 1), chills (n = 2) and pruritis (n = 1). While those in the subjects on EFV based ART were: headache (n = 1), chills (n = 1) and nausea (n = 4). All the events were graded as mild in both groups. There were no changes in electrocardiogram parameters on admission compared to the 2 h after the last dose in both groups.

For the ART naive patients, there was a statistically significant change in total bilirubin ($p<0.05$) on day 3 as compared to day 0. This was graded as mild and was found to have normalised by day 7 and 14. For the ART experienced group, there was a statistically significant change in haemoglobin concentration ($p<0.05$) on day 3 as compared to day 0. This was graded as mild and transient. It was found to have normalised by day 7 and 14.

DISCUSSION

Researchers investigated the pharmacokinetics of lumefantrine when administered concomitantly with TS only in ART naive subjects and with TS and EFV-based ART in subjects co-infected with malaria and HIV-1. The safety and tolerability of administering AL to these subjects was also assessed. This study demonstrates that the exposure to lumefantrine decreases significantly in subjects on EFV-based ART (Fig. 1). Gender and age did

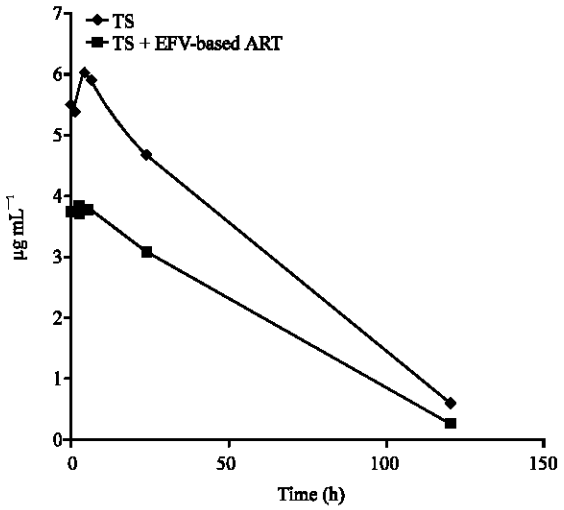

Fig. 1: Mean plasma concentration of lumefantrine (µg/mL) versus time (h) in adults co-infected with malaria and HIV-1 after the last dose of AL in subjects on TS prophylaxis and on TS+EFV-based ART

not have significant effect on the pharmacokinetic parameters. The AL combination was well tolerated with no clinically significant adverse events.

This study confirms the decreased exposure of lumefantrine when administered concomitantly with EFV that has earlier been reported in healthy volunteers and HIV infected adults free of malaria (Byakika-Kibwika *et al*., 2010; Huang *et al*., 2012). This is because EFV is an inducer of CYP3A4 which in turn is responsible for the increased metabolism of lumefantrine. There is probably an increased risk of recrudescence because the mean day 7 concentration of lumefantrine obtained in this study was <280 ng mL^{-1}. This concentration is regarded as the threshold for successful treatment (Ezzet *et al*., 2000; Ashley *et al*., 2007).

The ART naive subjects on TS prophylaxis had a higher exposure of lumefantrine when compared to historical data obtained in Ugandan patients from a earlier study (Piola *et al*., 2005). This may be attributed to the components of co-trimoxazole (trimethoprim + sulfamethoxazole) which have been shown to significantly influence the metabolism of several drugs frequently used concomitantly with the antibiotic (Masters *et al*., 2003). However, much higher concentrations of lumefantrine have been reported in subjects in Asia and Europe due to the highly lipophilic nature of lumefantrine and the higher fat content of the diets in these regions (Djimde and Lefevre, 2009).

Artemether-Lumefantrine (AL) combination is well tolerated in ART naive subject on TS prophylaxis. It's also safe and well tolerated in ART experienced subjects on EFV based ART with TS prophylaxis. The mild increases of total bilirubin observed in the ART naive subjects and the mild decrease in haemoglobin in the ART experienced subjects are transient and normalise by day 7. This good safety and tolerability profile of AL is consistent with that reported in previous studies (Tshefu *et al*., 2010; Rasheed *et al*., 2011).

Only 6 (14%) men were enrolled onto this study and this is a reflection of the gender distribution of the patients that attend this clinic. All the women participants on this study were not pregnant therefore gender could not have had an effect on the pharmacokinetic parameters of lumefantrine as this has been earlier established (Ezzet *et al*., 2000). The CD 4 counts in the patients receiving EFV based ART was significantly higher because of the benefits of antiretroviral medicines to the immune system of these individuals (Idigbe *et al*., 2005).

CONCLUSION

The concomitant administration of artemether-lumefantrine to subjects on Efavirenz based antiretroviral therapy is well tolerated but leads to decreased exposure of lumefantrine.

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