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**PHARMACOKINETICS OF SELECTED ANTIRETROVIRAL AND
ANTIMALARIAL DRUGS IN UGANDAN ADULTS**

P. Byakika-Kibwika.

A thesis submitted for the degree of Doctor in Philosophy.

Trinity College, Dublin.

2011

**PHARMACOKINETICS OF SELECTED ANTIRETROVIRAL AND
ANTIMALARIAL DRUGS IN UGANDAN ADULTS**



Thesis 9592

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SUMMARY

Human immunodeficiency virus (HIV) and malaria are two major infectious diseases causing significant morbidity and mortality worldwide. The two have overlapping geographical distribution in sub-Saharan Africa, where over 90% of the world malaria burden and 68% of the global HIV burden occur. Infection with HIV increases risk of malaria infection. Severe malaria and death occur with higher frequency in HIV-infected individuals. There has been a roll-out of antiretroviral therapy (ART) for HIV treatment and artemisinin-based combination therapy (ACT) for malaria treatment. To facilitate ART scale-up, less expensive generic ART formulations are widely prescribed. While these facilitate rapid scale-up, their quality and bioequivalence need to be monitored to ensure long term success of ART regimens.

Highly active ART is a combination of at least three active antiretroviral drugs from at least two different pharmacological classes. Combination therapy has potential for pharmacokinetic drug-drug interactions which may result in high plasma drug concentrations causing excessive toxicity or sub-therapeutic concentrations leading to treatment failure with risk for development of resistance. Treatment of HIV-malaria co-infected patients receiving ART with ACT creates potential for drug interactions. This thesis presents a series of intensive pharmacokinetic studies evaluating the pharmacokinetic profiles and drug interactions of some ART and artemether-lumefantrine (AL) which is the first-line ACT in Uganda and a description of the pharmacokinetic profile and clinical response to intravenous (IV) artesunate.

The first study was a comparison of the pharmacokinetics of generic and branded ART in a cross-over study of 16 HIV-infected participants. Participants received both generic and branded formulations of stavudine, lamivudine and nevirapine. Intensive blood sampling was performed and drug concentrations measured. Pharmacokinetic profiles of generic and branded drugs were similar and patients tolerated both formulations very well. The second study was a two-arm parallel study of 16 HIV-infected ART naïve and 16 HIV-infected adults stable on LPV/r 400/100mg to compare the pharmacokinetics of AL when administered alone and in combination with LPV/r. Each participant received a single dose of AL 80/480mg. Intensive blood sampling was performed and plasma concentrations of

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DEDICATION

To the memory of Prof John Feely, who was head of the Department of Pharmacology and Therapeutics at Trinity College, Dublin, at the time I enrolled into the PhD program. He warmly welcomed me to the department and provided guidance and mentorship. He took keen interest in my work and visited my study site at Makerere University, Kampala, Uganda.

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List of abbreviations

ACT	-	Artemisinin based combination therapy
AIDS	-	Acquired Immuno-Deficiency Syndrome
AL	-	Artemether-Lumefantrine
ART	-	Antiretroviral Therapy
ATIC	-	AIDS Treatment Information Centre
CD	-	Cluster differentiation
CI	-	Confidence Interval
CL/F	-	Clearance
C_{max}	-	Maximum concentration
C_{min}	-	Minimum concentration
CRF	-	Case Record Form
C_{trough}	-	Trough concentration
CYP	-	Cytochrome
DNA	-	Deoxyribonucleic acid
ECG	-	Electrocardiograph
FDA	-	Food and Drug Administration
FDC	-	Fixed Dose Combination
GCP	-	Good Clinical Practice
GIT	-	Gastro Intestinal Tract
GMR	-	Geometric Mean Ratio
gp	-	Glycoprotein
hCAR	-	constitutive androstane receptor
HIV	-	Human Immunodeficiency Virus
HPLC	-	High Pressure Liquid Chromatography
hPXR	-	human nuclear pregnane X receptor
IDI	-	Infectious Diseases Institute
IQR	-	Inter Quartile Range
IV	-	Intravenous
LLOQ	-	Lower limit of quantification
LOD	-	Level of detection
LPV/r	-	Ritonavir boosted Lopinavir
MEC	-	Minimum Effective Concentration

ms	-	milliseconds
MU-JHU	-	Makerere University – Johns Hopkins University
NNRTIs	-	Non-Nucleoside Reverse Transcriptase Inhibitors
NRTIs	-	Nucleoside Reverse Transcriptase Inhibitors
NtRTI	-	Nucleotide Reverse Transcriptase Inhibitor
P-gp	-	P-glycoprotein
PIs	-	Protease Inhibitors
SD	-	Standard Deviation
SE	-	Standard Error
SOP	-	Standard operating procedure
STD	-	Standard
$T_{1/2}$	-	Half-life
T_{lag}	-	Absorption lag time
T_{max}	-	Maximum concentration
T_{min}	-	Minimum concentration
UGT	-	Dihposphoglucuronyltransferase
UK	-	United Kingdom
UV	-	Ultra Violet
V/F	-	Volume of distribution
WHO	-	World Health Organization

PREFACE

HIV and malaria are leading causes of morbidity and mortality worldwide. In the last decade, significant effort has been put into scaling up ART for HIV treatment and ACT for malaria treatment. As we scale up treatment, the great challenge is to ensure long-term success of the regimens. The increased risk for infections such as malaria in HIV-infected individuals requiring concomitant treatment creates several challenges such as increased pill burden, synergistic toxicity and pharmacokinetic drug interactions. Drug interactions resulting in sub-therapeutic concentrations are a risk for treatment failure and resistance. There is paucity of data on the drug-interactions of ART and antimalarial drugs yet they are frequently co-prescribed.

The over-arching objective of this thesis is to investigate and provide pharmacokinetic data essential to the safe rollout of life-saving ART and ACT by examining drug concentrations *in vivo* at steady state. Studies presented in this thesis are derived from questions that health care providers are faced with during routine clinical care of patients. Chapter 1 consists of relevant literature review on HIV and malaria with special emphasis on challenges of treatment of co-infections. Chapter 2 is a comparison of the steady-state pharmacokinetics of generic and branded formulations of stavudine, lamivudine and nevirapine in HIV-infected Ugandan adults. Chapter 3 compares artemether-lumefantrine pharmacokinetics when administered with and without lopinavir/ritonavir in HIV-infected patients. Chapter 4 is a presentation of the cardiac safety profile of co-administration of LPV/r with AL. Chapter 5 and 6 are comparisons of the pharmacokinetics of AL, efavirenz or nevirapine when administered alone and in combination. Chapter 7 provides a description of the pharmacokinetic profile and clinical response to intravenous artesunate during treatment of severe malaria in Ugandan adults. The final chapter is a discussion of the clinical relevance of the data generated from the six studies with recommendations plus research and policy implications.

CHAPTER 1

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1.1 Overview of the global HIV epidemiology

Infection with the human immune deficiency virus (HIV) has been a global pandemic and major cause of morbidity and mortality for over three decades. Despite significant progress made in preventing new HIV-infections; the number of people living with HIV worldwide is still very high; estimated at 32.8 million (30.9 million – 34.7 million) (1). In 2009 alone, an estimated 2.6 million (2.3 – 2.8 million) people became newly infected with HIV. The greatest burden of HIV is in sub-Saharan Africa where 68% of the global burden occurs with an estimated prevalence of 5.2% (4.9 – 5.4%), translating into 22.5 (20.9 – 24.2) million people infected (1).

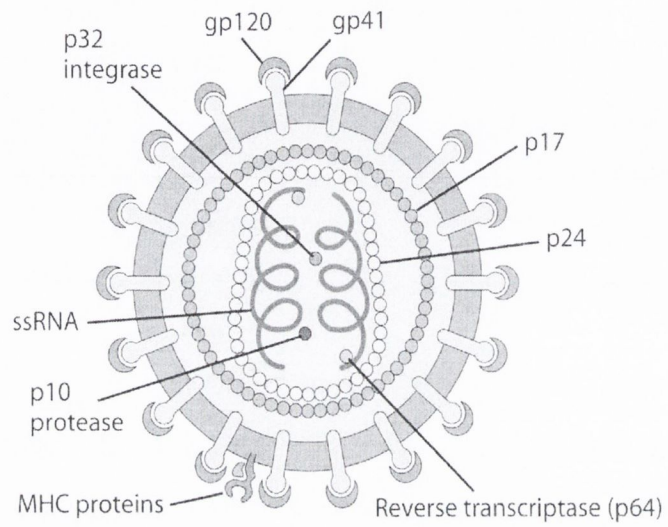
1.1.1 Classification, Structure, Transmission and Life-Cycle of HIV

Classification

The HIV belongs to the genus *Lentivirus*, part of the family of *Retroviridae*, a family that causes the acquired immunodeficiency syndrome (AIDS) (2). This virus has the ability to infect its host chronically, and progressively damages the host immune system (3). Two viral species have been characterised in humans: HIV type 1 (HIV-1) and HIV type 2 (HIV-2). The HIV-1 is more virulent and more infective and has spread globally (4) while HIV-2 has lower infectivity and is largely confined to West Africa (5). HIV-1 is classified into groups; M, O and N. The vast majority of HIV-1 strains belong to group M and have been classified into clades designated by letters A, B, C, D, F, G, H, J and K.

The virus is spherical in shape with a diameter of 80-100 nm (figure 1.0). It has a viral core or capsid made of viral protein 24. Inside the core is viral genetic material, consisting of two identical single strands of ribonucleic acid (RNA) and three enzymes (reverse transcriptase, integrase and protease) that are required for replication. A matrix composed of viral protein 17 surrounds the capsid (2) and all this is enclosed in an envelope. The envelope carries glycoprotein (gp) 120 which is non covalently linked to gp 41 which make up the spikes that project from HIV particles.

Figure 1.0. HIV structure

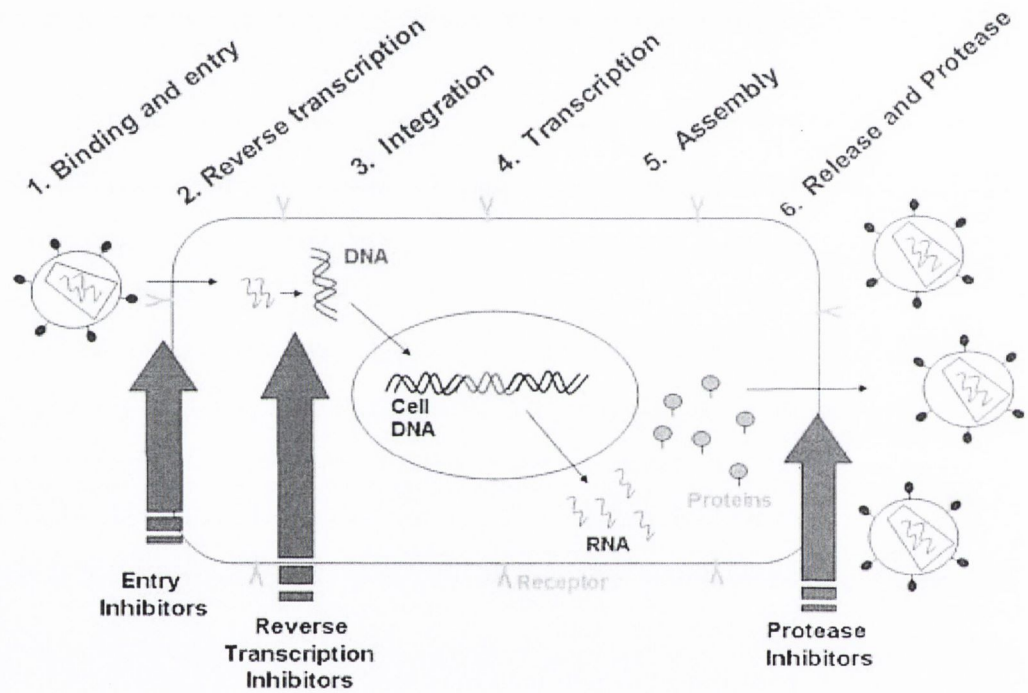


(Adopted from <http://biomems.co.za/HIV.html>)

HIV transmission and life-cycle

Transmission of HIV to humans predominantly occurs through sexual contact (heterosexual and homosexual). Other routes of transmission include; sharing drug injecting equipment, contamination of blood supply for transfusion and perinatally from mother to child. The HIV has affinity for CD4 T cells and monocytes and only replicates inside human cells. The HIV life-cycle begins when viral gp 120 interacts and binds to a CD4 receptor and a co-receptor on the surface of a CD4 T lymphocyte. The viral gp120 and gp 41 recognize the CD4 T cell ligand on host cells, bind to it and mediate entry into the cell. Upon binding a conformation change within gp120 is induced which exposes co-receptor binding sites in gp120. The co-receptors bind a host chemokine receptor either CCR5 or CXCR4 depending on whether the HIV particle is M tropic or T-tropic. M-tropic HIV recognize CCR5; infect macrophages and primary T-cells. These strains are called R5 viruses. The T-tropic viruses recognize CXCR4, which is highly expressed in CD4 T cells and induce fusion of cells to create one large cell with many nuclei (syncytia). These strains are called X4 viruses. Viruses that use only the CCR5 receptor are termed R5, those that use only the CXCR4 termed X4, and those that use both are X4R5 and described as having dual tropism. The presence of R5, X4 and/or dual tropic virus together in one host is called mixed tropism. Upon interaction with gp120 and the host chemokine receptor, gp41 binds to a host heparan sulphate, triggering fusion of host and viral membranes and permitting entry of the viral capsid into the cytoplasm with release of RNA into the host cell. Inside the host cell; the viral reverse transcriptase enzyme converts the single stranded HIV RNA into double stranded HIV deoxyribonucleic acid (DNA). The DNA is then integrated into the host cell's nucleus by the viral integrase enzyme; this is referred to as provirus and it may remain inactive for long periods of time. The provirus uses a host enzyme RNA polymerase to create copies of the HIV genomic material and copies of messenger RNA which is used to make long chains of HIV proteins. The long chains are cut into smaller proteins by HIV protease enzyme to form new virus particles which are then assembled and bud out of the host cell to infect new cells (6). The HIV life-cycle is presented in figure 1.1.

Figure 1.1. The HIV life cycle



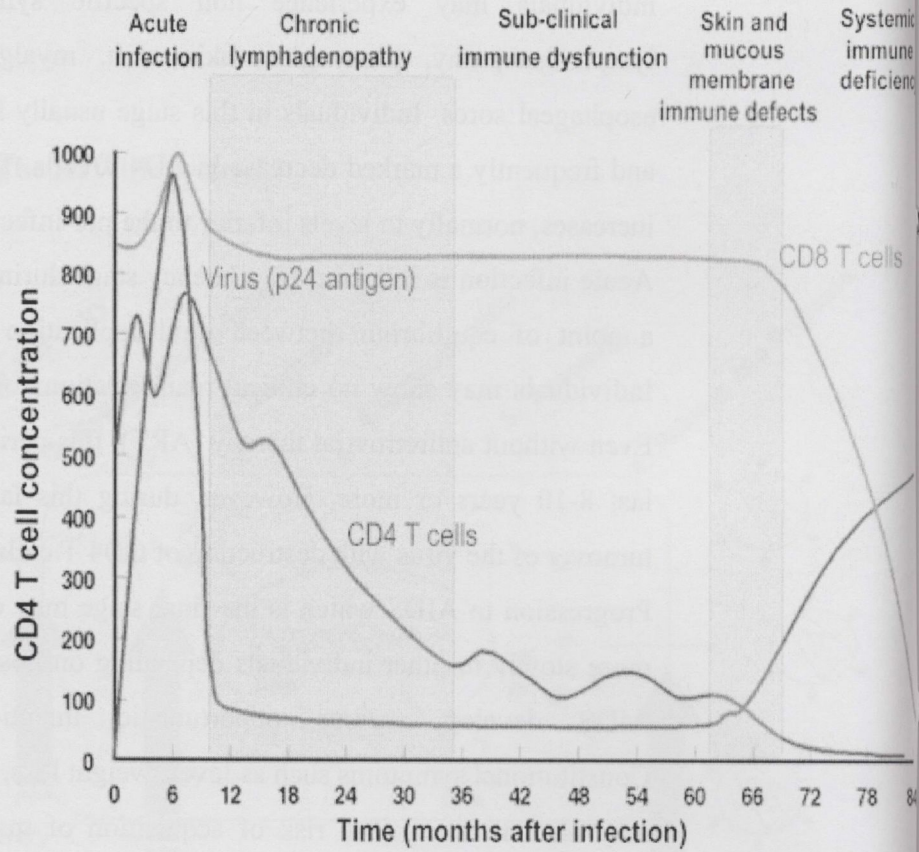
(Adopted from http://chemistry.ewu.edu/jcorkill/biochem/rama11_10.htm)

After infection with HIV, the course of disease varies with individuals. Patients may experience the acute viral syndrome of primary HIV infection. This acute stage is the time period from initial infection with HIV to the development of an antibody response. It lasts an average of 12 weeks and individuals may experience non specific symptoms such as fever, lymphadenopathy, pharyngitis, skin rash, myalgia, malaise, mouth and esophageal sores. Individuals in this stage usually have high plasma viremia and frequently a marked decrease in CD4 T cells. The CD4 T cell count later increases, normally to levels inferior to the pre-infection values.

Acute infection is followed by a latency stage during which individuals reach a point of equilibrium between viral replication and the host immunity. Individuals may show no clinical manifestations of HIV infection for years. Even without antiretroviral therapy (ART), this period of clinical latency may last 8-10 years or more. However, during this latency stage there is high turnover of the virus with destruction of CD4 T cells.

Progression to AIDS which is the final stage may occur rapidly in some and more slowly in other individuals depending on host factors. Individuals with AIDS develop various opportunistic infections and malignancies. Constitutional symptoms such as fever, weight loss, night sweats, and diarrhea may also manifest. The risk of acquisition of many of the AIDS-defining illnesses increases with CD4 T cell counts below 200 cells/ μ l (7). Figure 1.2 is a graphical presentation of the natural history of HIV/AIDS (7).

Figure 1.2. The Natural History of HIV infection



Adopted from (<http://pathmicro.med.sc.edu/lecture/hiv3.htm>)

1.1.2 HIV Immunopathogenesis

The HIV primarily infects vital cells of the human immune system such as CD4 T cells, macrophages, and dendritic cells. Following infection, chronic systemic immune activation, an almost pathognomonic feature of progressive HIV infection occurs. Such immune activation is manifest in many ways including polyclonal B-cell activation, increased T-cell turnover and increased serum levels of proinflammatory cytokines and chemokines (8-9). Immune activation results in detrimental effects to the immune system with high turnover of CD4 and CD8 T cells (8-9). Destruction of infected CD4 T cells by CD8 cytotoxic lymphocytes, increased rates of apoptosis in infected cells and diminished production of interferon gamma and tumor necrosis factor alpha result in depletion of CD4 T lymphocytes and diminished T helper 1 and T helper 2 responses. Depletion of T helper 2 response prevents B cell activation with resultant humoral immunodeficiency while T helper 1 deficiency causes impaired cellular immune response and impaired killing of intracellular pathogens.

Chronic immune activation results in inflammatory damage to lymphoid tissues with transforming growth factor-beta mediated fibrosis of lymph nodes and thymic dysfunction (10-11). These effects are associated with abnormal retention of effector type T cells and poor immune reconstitution with antiretroviral therapy (ART). Immune activation drives viral replication by generation of activated T cell targets which the virus can infect. Constant damage to the cellular sources and anatomical niches of CD4 T cell compartments caused by interplay between the virus and the immune system further exacerbates the progressive loss in CD4 T cell numbers and function and inevitably leads to AIDS. Without ART most people will progress to AIDS within 10 years of HIV infection. ART delays progression to AIDS and increases life expectancy of people infected with HIV. Death is mostly a result of opportunistic infections or malignancies associated with progressive immune system failure (9-10).

1.1.3 Antiretroviral therapy

The discovery of ART was a major milestone in the history of medicine that has significantly reduced HIV related morbidity and mortality. Highly active ART is a combination of at least three active drugs from at least two different pharmacological classes. Combination therapy offers potent suppression of viral replication while preventing emergence of drug resistance and progression to AIDS and death.

Combination of drugs from the same pharmacological class such as triple nucleoside regimens has previously been prescribed. Although triple nucleoside regimens play significant role in dose simplification and are associated with more favourable lipid profiles, they are associated with decreased antiviral efficacy (11).

There are over 20 drugs available for treatment of HIV (Table 1.0); broadly classified into 5 classes according to the mechanism of action and the phase of the retrovirus life-cycle that they inhibit. The 5 classes are nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs/NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors and entry inhibitors.

Table 1.0. Antiretroviral drugs approved for use

Class	NRTIs/NtRTIs	NNRTIs	PIs	Integrase inhibitors	Entry inhibitors	
					Fusion inhibitors	CCR5 antagonists
Mechanism of action	Competitive inhibition of HIV reverse transcriptase enzyme	Non-competitive inhibition of HIV reverse transcriptase enzyme	Inhibition of HIV protease enzyme	Inhibition of HIV integrase enzyme	Inhibition of viral membrane fusion	Inhibition of HIV-co receptor binding
Drugs	Zidovudine Stavudine Didanosine Lamivudine Abacavir Tenofovir disoproxil fumarate Emtricitabine	Nevirapine Efavirenz Etravirine Ralpivirine	Saquinavir Indinavir Ritonavir Nelfinavir Lopinavir Atazanavir Fosamprenavir Tipranavir Darunavir	Raltegravir	Enfuvirtide	Maraviroc

Nucleoside/nucleotide Reverse Transcriptase Inhibitors

The NRTIs and NtRTIs are analogues of naturally occurring deoxynucleotides needed to synthesize viral DNA. They inhibit the reverse transcriptase enzyme by acting as false building blocks for DNA synthesis. They differ from physiological nucleosides by a minor modification in the ribose molecule. They are administered in inactive form and converted to the active metabolite after endocytosis by phosphorylation catalyzed by cellular kinase enzymes (6). Addition of three phosphate groups to the deoxyribose moiety of NRTIs forms NRTI triphosphates. Tenofovir disoproxil fumarate is the only NtRTI available. It contains a phosphonate group attached to the adenine base, thus only two steps are required in its phosphorylation. The phosphorylated forms compete with the natural deoxynucleotides for incorporation into the viral DNA chain. Incorporation inhibits formation of phosphodiester bridges to stabilise the DNA strand thus preventing viral DNA synthesis and elongation (7). The NRTIs include; zidovudine, didanosine, stavudine, lamivudine, abacavir and emtricitabine.

Non-nucleoside reverse transcriptase inhibitors

The NNRTIs inhibit reverse transcriptase enzyme by binding directly and noncompetitively to the enzyme at a position in close proximity to the substrate binding site for nucleosides. The interaction of NNRTIs with reverse transcriptase induces conformational changes that impact the catalytic activities of the enzyme. The resulting complex blocks the catalyst activated binding site which in turn, can bind fewer nucleosides, slowing down polymerization significantly (7). Nevirapine, and efavirenz are the older NNRTIs. Tolerance and cross-resistance easily occurs with these older NNRTIs because of a single amino-acid substitution in HIV-1 reverse transcriptase which can result in pan-class cross-resistance. Newer NNRTIs are now available including; etravirine and rilpivirine with rilpivirine being the newest drug recently approved by FDA (6, 12).

The next section reviews the pharmacology of the NNRTIs (nevirapine and efavirenz) which are some of the ART that make up the subject of this thesis.

Nevirapine

Nevirapine was the first NNRTI to be licensed for use in the treatment of HIV-1 infection. It is used as part of combination therapy with 2 NRTIs. Its chemical formula is $C_{15}H_{14}N_4O$ with molecular weight of 266.302 (13). The chemical structure is shown in figure 1.3.

Nevirapine is available in tablet and suspension formulations for oral administration. Its absorption is not affected by food, acids or alkali and more than 90% of the administered dose is absorbed (13). It achieves bioavailability of more than 90%. About 60% is protein bound (14-15). The steady-state maximum and minimum concentration (C_{max} and C_{min}) after continuous administration of daily adult doses are 7.2 and 4mg/l respectively. The median time to maximum concentration (T_{max}) is 4 hours and the elimination half-life ($T_{1/2}$) is 25-30 hours with mean apparent oral clearance rate of 44-52 ml/kg/h. Nevirapine is distributed throughout the body with a volume of distribution (V/F) of 1.36 l/kg (13). It is biotransformed by cytochrome (CYP) 3A4 and 2B6 and excreted via the liver and kidneys in the form of glucuronide conjugates of hydroxylated metabolites (13). It is both a substrate and inducer of CYP 3A4, and 2B6 (14). A summary of nevirapine pharmacokinetics is presented in table 1.1.

Tolerability to nevirapine is relatively good for majority of patients (16). The adverse event most commonly observed is a hypersensitivity rash, occurring in about 16% of patients with about 7% experiencing grade 3 or 4 rash with the Steven Johnson syndrome. The rash is more common during the first 6 weeks of treatment (13). The second common adverse event is hepatotoxicity with elevated liver enzymes. Female sex and a high CD4 T cell count are associated with a higher incidence of hepatitis arising from nevirapine-induced hypersensitivity (13, 17-18). Nevirapine hypersensitivity occurs with more frequency in individuals with higher CD4 counts (women greater than 250 and men greater than 400 cells/ul). This association between CD4 count and hypersensitivity reactions is seen in treatment naïve patients. Patients already on treatment with efavirenz may not experience hypersensitivity reactions when switched to nevirapine.

A new tablet formulation containing 400mg of nevirapine is available for once-daily dosing. This is the nevirapine extended-release (XR) tablet. It was shown to be noninferior to the immediate-release tablet in treatment-naïve patients and its safety and tolerability is as good as that of the immediate-release nevirapine tablet. It is hoped that this formulation will enhance dosing convenience and adherence, however, it is not yet available in resource limited settings.

Table 1.1 Pharmacokinetics of nevirapine, efavirenz, lopinavir, ritonavir, artemether and lumefantrine (with permission from Khoo et al, The potential for interactions between antimalarial and antiretroviral drugs. AIDS 2005, 9(10):995-1005.)

Drug	Bioavailability (%)	Protein binding (%)	Half-life	Active metabolite	Metabolism	Excretion	Potential for interaction
ART							
Nevirapine	93	60	30 hours	-	Metabolized by CYP3A4 and 2B6; inducer of CYP3A4	Hepatic	High
Efavirenz		>99	35 hours	-	Metabolized by CYP3A4 and 2B6; inducer of CYP3A4	Hepatic	High
Lopinavir	Poor, given with ritonavir	98-99	5-6 hours	-	Extensive metabolism mainly via CYP3A4; potent inhibitor of CYP enzymes	Hepatic	High
Ritonavir		98-99	3-5 hours	-	Used to boost other PIs by enhancing bioavailability or reducing hepatic clearance through inhibition of CYP 3A4 in the gut or liver	Hepatic	High
ACT							
Artemether	Good	95.4	1.4 hours	Dihydroartemisinin	CYP 3A4; induces CYP 2C19/3A4	Hepatic	
Lumefantrine	Poor (<10%)	99.9	2-3 days	Desbutyl-lumefantrine	Via CYP3A4	Hepatic	

Efavirenz

The chemical formula of efavirenz is $C_{14}H_9ClF_3NO_2$ and molecular mass is 315.68. Its chemical structure is shown in figure 1.3. The drug is available as capsules, film-coated tablets and liquid formulation for oral administration. The recommended adult dose is 600mg once daily. Efavirenz has good oral bioavailability which increases when taken with food especially fatty food thus the recommendation to be taken on an empty stomach, preferably at bedtime to diminish possible neuropsychiatric side effects because administration with food enhances efavirenz absorption and increases the plasma exposure. (19-20). Efavirenz is highly protein bound (>99%), predominantly to albumin (20). The C_{max} is achieved within 3-5 hours post dose administration. It has a long serum $T_{1/2}$ of up to 45 hours and reaches steady-state plasma concentrations in 6 to 10 days. After a 600mg dose; mean \pm standard deviation (SD) steady state C_{max} , C_{min} and area under the concentration-time curve (AUC) were $12.9 \pm 3.7 \mu M$, $5.6 \pm 3.2 \mu M$ and $184 \pm 73 \mu M/h$ respectively (19).

Efavirenz is extensively metabolized by CYP2B6 with partial involvement of CYP3A4 and CYP2B6 to inactive hydroxylated metabolites that include 8-hydroxy and 7-hydroxyefavirenz (15, 20). Hydroxylated efavirenz metabolites undergo subsequent urinary and biliary excretion after glucuronidation. Efavirenz is an inhibitor and inducer of several CYP enzymes; 2B6, 3A4, 2A6, 2C9 and 2C19 and induces its own metabolism (20-21) plus that of co-administered drugs metabolized by the same enzymes (20). A summary of efavirenz pharmacokinetics is presented in table 1.1.

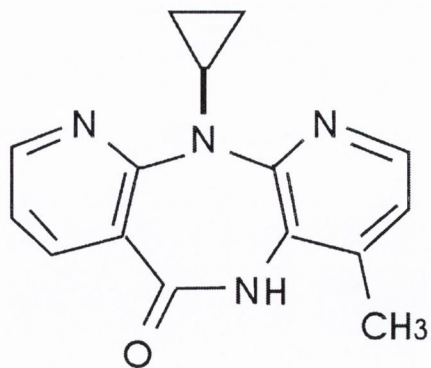
Efavirenz has good safety profile with minor side effects including; skin rash and neuropsychiatric events. These occur during the first few days to weeks in about 40 – 50% of patients. Most of them resolve spontaneously. They may be severe enough to warrant discontinuation in 3% of patients. The rash is maculopapular, often of mild to moderate intensity (grade 1 or 2), occurring between the first and third week of treatment; with incidence up to 34% (19). It resolves spontaneously within one month or with treatment interruption. The incidence of grade 3 or 4 rash with Stevens-Johnson syndrome is only 0.1% and once it occurs, treatment should be stopped immediately.

This formulation required storage under refrigeration which was not ideal for resource limited settings. A new tablet formulation, stable at room temperature is available (Aluvia[®]), each tablet containing 200mg lopinavir and 50mg ritonavir. The standard adult dose of LPV/r is 400mg/100mg twice daily in treatment experienced patients. A once-daily dosing regimen of 800mg/200mg may be used in therapy-naïve patients. At steady state, lopinavir is approximately 98%–99% bound to plasma proteins (15). A summary of lopinavir and ritonavir pharmacokinetics is presented in table 1.1.

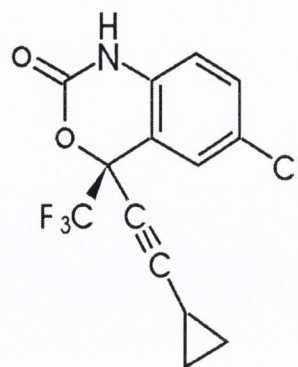
Both drugs are metabolized via CYP3A4 and primarily eliminated by the fecal route with urinary excretion accounting for <2% of the eliminated drug (22). LPV/r inhibits CYP3A4 and induces several CYP enzymes; 1A2, 2B6, 2C9 and 2C19 (21-24) resulting in significant drug interactions with CYP substrates. A major example of such interaction is with rifampicin, a potent inducer of CYP3A4 which causes significant reduction in lopinavir concentration. Co-administration of LPV/r at a dose of 400/100mg with rifampicin is thus contraindicated. Adjusted dosing of LPV/r as either 800/200mg or 400/400mg twice daily with rifampicin demonstrated less reduction in lopinavir AUC; however patients experienced more toxicity (25).

Combination of LPV/r with NRTIs is effective in suppressing viral replication (26) and regimens are generally well tolerated. The most frequent side effects are mild to moderate; mainly in the gastro-intestinal tract (GIT) with diarrhea, nausea, and vomiting. Other side effects include; hypertriglyceridemia, hypercholesterolemia, pancreatitis, transient elevations in transaminase levels, insulin resistance, new onset diabetes and worsening of pre-existing diabetes. Fat redistribution occurs including central obesity, dorsocervical fat enlargement, peripheral and facial wasting, breast enlargement, and cushingoid appearance. Less common adverse effects include allergic reaction, malaise, headache, myalgias, arthralgias, myocardial infarction and lactic acidosis (6, 27-28).

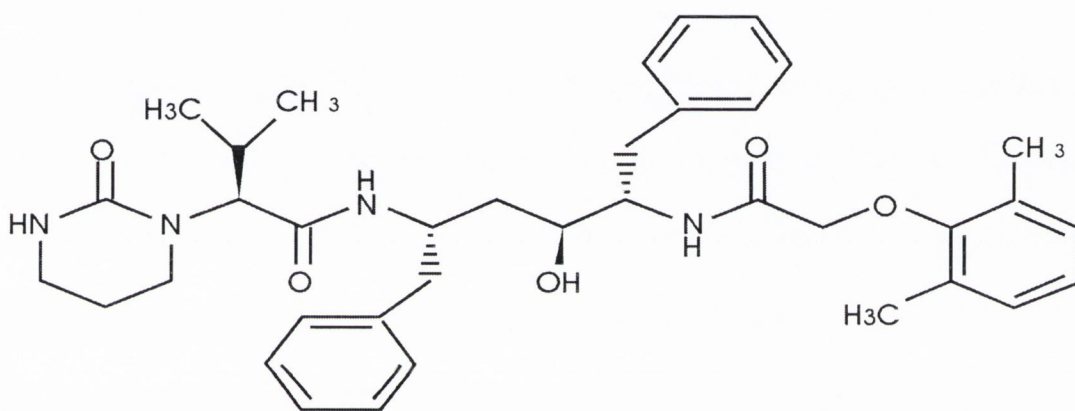
Figure 1.3. Chemical structures of nevirapine, efavirenz, lopinavir and ritonavir



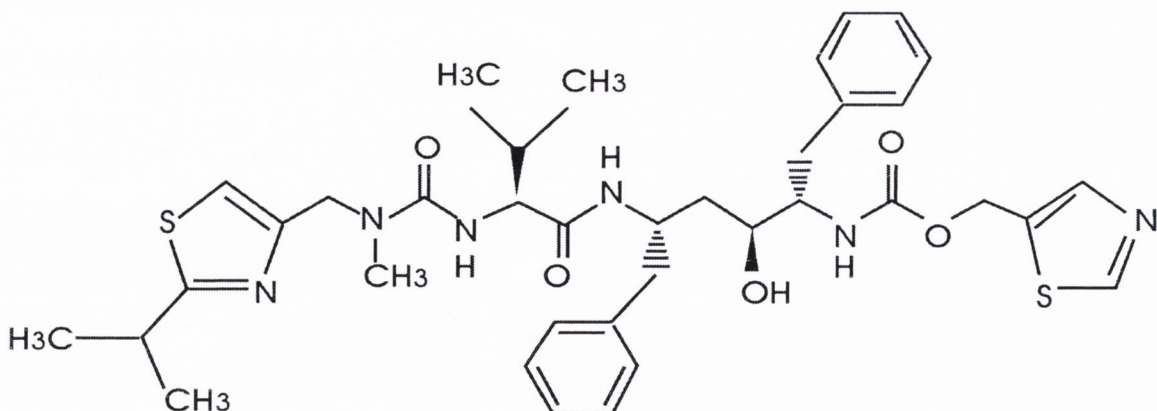
Nevirapine



Efavirenz



Lopinavir



Ritonavir

Integrase inhibitors

Integrase inhibitors interfere with activity of integrase enzyme thus inhibit ability of the HIV DNA to insert itself into the host DNA (6). Since integration is a vital step in retroviral replication, blocking it can halt further spread of the virus. Raltegravir was the first integrase inhibitor approved by the FDA, initially for treatment-experienced patients and subsequently for treatment-naïve patients (6).

Entry inhibitors

Entry inhibitors interfere with binding, fusion and entry of HIV into the host CD4 T cell by blocking one of several targets or receptors therefore preventing HIV entry. Entry inhibitors are classified into three groups depending on the actual step of viral entry that they inhibit namely; fusion inhibitors, co-receptor antagonists and CD4-receptor inhibitors (6). Drugs in this category include maraviroc; a chemokine receptor antagonist (29) and enfurvitide; the prototype of fusion inhibitors which prevents HIV from infecting the CD4 T cell by specifically inhibiting the function of the transmembrane gp41 of HIV-1 (30).

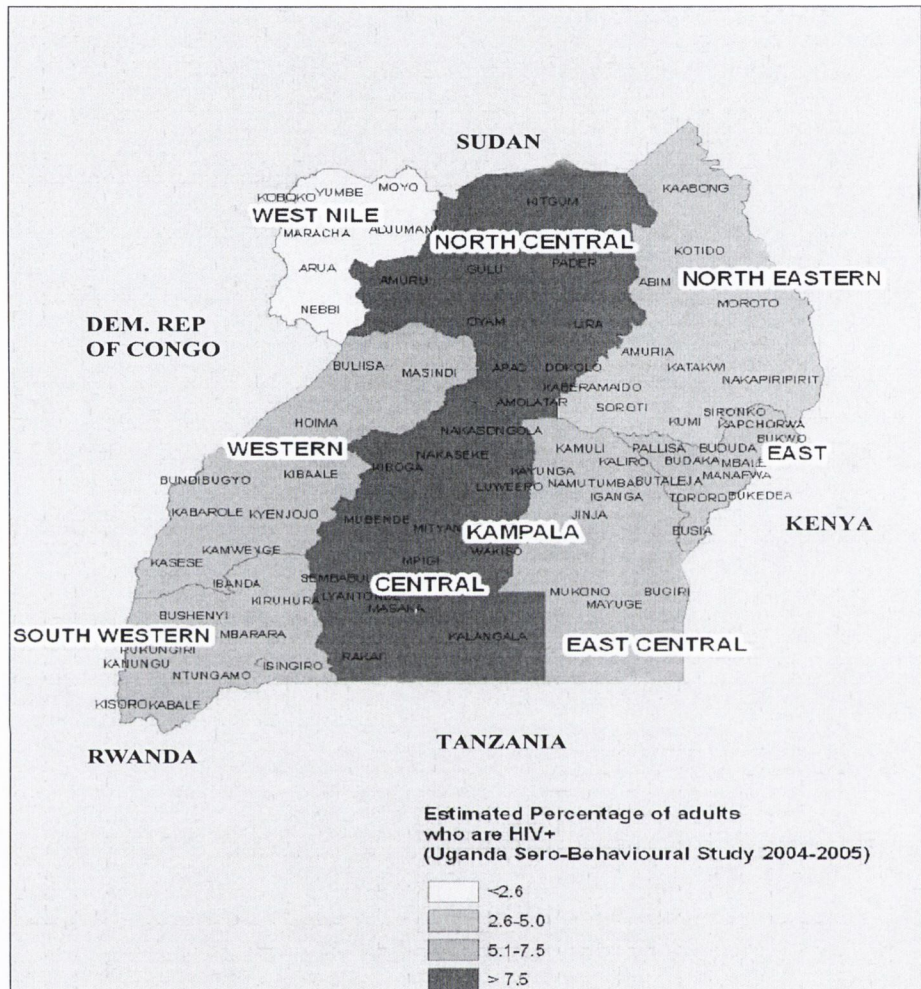
1.2 Uganda and HIV

Uganda is a landlocked country in East Africa with an area of 236,040 square kilometers (146,675 square miles) making it the 81st largest country in the world. Uganda has a fast growing population, currently estimated at 32 million. The high population growth rate is due to the high fertility rate of almost seven children per woman. This is the third highest birth rate in the world. The population is very young with a median age of 15 years. Uganda is a resource limited country where 31% of the population lives below one US dollar a day. Life expectancy in Uganda is 52 years, mostly due to HIV/AIDS (31).

HIV-infection was first described in Uganda in 1982, as ‘slim’ disease because of the significant wasting observed among patients (32-33). In 1991, data from rural south western Uganda reported prevalence rates among adults ranging from 38.5% in trading centers to 8.6% in agricultural villages. Having multiple sexual partners was the strongest factor favoring HIV transmission. Significant effort has

been made towards prevention of new infections; however, to date, Uganda continues to bear a heavy burden of HIV as one of the leading causes of morbidity and mortality. The national HIV prevalence reduced from over 18% during the early 1990s to the current 6-7% with approximately 1.1 million people infected (34-35). The HIV prevalence differs across the country as shown on the map of Uganda in figure 1.4. The reduction in HIV prevalence was achieved through national efforts to educate people on HIV transmission, provision of condoms plus testing and linkage to care to prevent the spread of HIV. HIV clades A, D and A/D recombinant strains are responsible for approximately 95% of HIV-1 infections in Uganda (36).

Figure 1.4. Map of Uganda showing the distribution of HIV prevalence



1.2.1 Antiretroviral therapy in Uganda

Until recently, the major constraint for widespread use of ART in Uganda, as in many other African countries, was the high cost of medications and associated monitoring tests. In 1997 the Uganda government embarked on efforts to provide access to ART, in collaboration with the Joint United Nations Program on HIV. A nationwide program was launched to provide free ART through the public sector and initiatives such as the Multicountry AIDS Program, the Presidents' Emergency Plan for AIDS Relief, the Global Fund to Fight AIDS, tuberculosis and malaria, the World Health Organisation's (WHO) 3 by 5 initiative and Medecins sans Frontiers among others were established to provide free care and treatment.

Uganda adopted the WHO guidelines based on public health principles, aiming for universal access to ART, and adapted them to national circumstances. Initial ART guidelines recommended initiation of therapy at CD4 count of 250/ul and below (37), however these guidelines have been updated and today, ART is recommended for HIV-infected patients with CD4 T cell count of 350 cells/ul and below, HIV-tuberculosis co-infected patients and those with WHO stage IV disease irrespective of CD4 counts. However, the Ugandan public health sector is continuously challenged with drug stock-outs consequently ART initiation is not uncommonly reserved for patients with CD4 counts of 50 cells/ul and below.

Currently, first-line ART regimens in Uganda consist of a NNRTI with 2 NRTIs and second-line regimens consist of a PI (LPV/r) with 2 NRTIs (37). Efavirenz and nevirapine are the NNRTIs available and LPV/r is the main PI in the Ugandan public sector (37).

In persons who have been accidentally exposed to HIV through needle-stick inoculation or through contamination of mucous membranes by secretions or non-medical exposure e.g. rape and defilement, immediate administration of antiretrovirals may prevent infection from occurring. In this situation ART needs to be provided for one month. In Uganda recommended regimens for post exposure prophylaxis include a 2-drug combination which may be either zidovudine or tenofovir plus lamivudine or emtricitabine for low risk exposure. For high risk exposure a 3-drug combination is recommended which is made up of the 2 drugs above plus efavirenz or a protease inhibitor

Due to the high cost of branded ART, 93% of ART prescribed in Uganda are generic fixed dose combinations (FDC), initially mainly from India, such as Triomune® (stavudine + lamivudine + nevirapine) manufactured by Cipla, Mumbai. These have made ART simpler, cheaper and more accessible. Infection with HIV is no longer a death sentence in Uganda as HIV-infected people receiving ART can survive longer and live more productive lives. However to ensure success of long term ART, several factors need to be considered. The next section is a discussion of some of these factors with a bias towards pharmacological considerations.

1.3 The role of clinical pharmacology in supporting the roll-out of ART

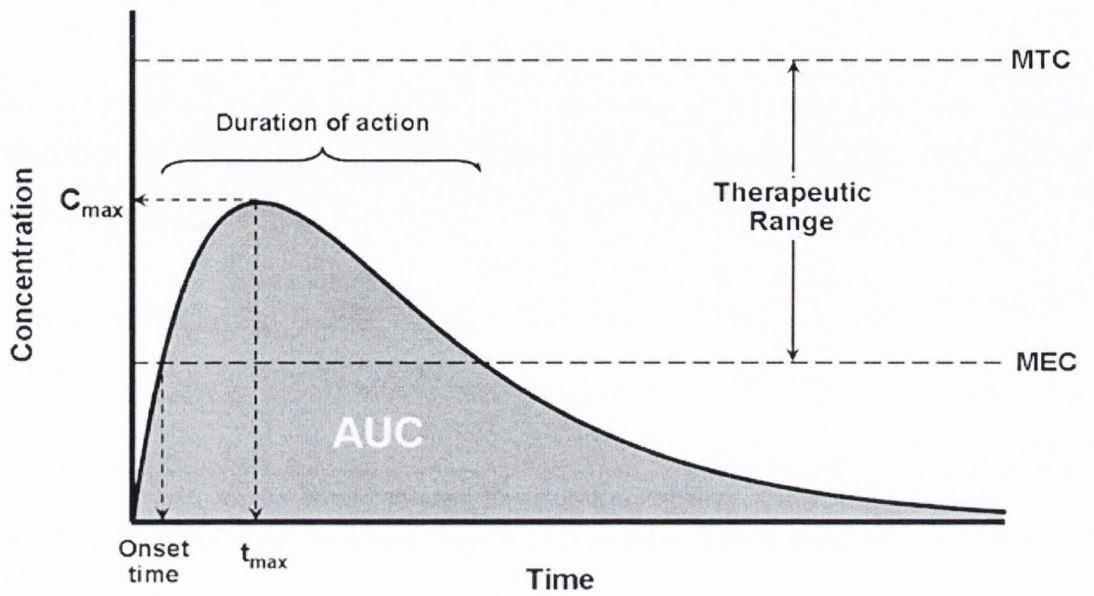
Successful treatment of infectious diseases such as HIV is the result of a complex interaction between the patient, the drug and the infectious agent. Sub-therapeutic drug concentrations can result in treatment failure with risk for emergence of resistant forms of the infectious agent. Development of resistance at an individual level compromises patients' response to future therapeutic interventions (38-39) and at a population level results in the transmission of resistant virus (40) which is more expensive and difficult to treat.

Optimal regimens provide adequate drug concentrations at the target sites with maximal efficacy and minimal toxicity. Optimal regimens may be supported by evidence from pharmacokinetic studies performed in the target population. Below, we provide a brief overview of the concepts of pharmacokinetics before discussing some factors that may influence ART pharmacokinetics.

1.3.1 Pharmacokinetics

Pharmacokinetics is the study of drug disposition in the body. It includes the four processes of absorption, distribution in tissues and cells, metabolism and elimination from the body. Pharmacokinetic parameters may be obtained from the exposure-time profile (figure 1.5) which is a function of the rate and extent of drug input, distribution, and elimination.

Figure 1.5. A graph showing the drug concentration-time profile (41)



Adopted from

(<http://img.medscape.com/fullsize/migrated/556/234/ijir556234.fig1.gif>)

The exposure-time profile also called concentration-time profile is plotted using data obtained from pharmacokinetic studies. Following drug administration; biological samples are obtained and drug concentrations measured. The concentration-time profile is obtained by plotting drug concentrations on the y-axis against the corresponding time at which samples were obtained on the x-axis. The measure of total drug exposure (AUC) is obtained by calculating the area under the concentration-time curve. AUC_{0-last} is the area under the curve for concentration-time from time 0 to the last observation while $AUC_{0-\infty}$ is the area under the plasma concentration-time curve from time 0 extrapolated to infinity. The highest concentration attained post dosing is the C_{max} and can be obtained by direct inspection of the data. The time period taken to reach C_{max} is the time of maximum concentration (T_{max}). The lowest concentration of drug measured along a dosing interval is the C_{min} . C_{trough} is the measured concentration at the end of a dosing interval at steady state, taken immediately before the next dose administration. The therapeutic range of a drug is the range of plasma concentrations of drug associated with effective therapy without undue toxicity. Minimum effective concentration is the lowest concentration required for optimal therapeutic response. Maximum tolerated concentration is the highest concentration beyond which toxicity occurs. The $T_{1/2}$ is the time required for a given drug concentration to decrease by 50% while clearance (CL/F) is a measure of the efficiency of removal of drug from the blood or plasma. The apparent volume of body fluids into which a drug distributes at equilibrium is the V/F. Steady-state plasma concentration: is achieved when the total amount of drug delivered into the body is equal to the amount eliminated. The time to steady state depends on elimination $T_{1/2}$ and for over 95% of drugs, steady state is achieved after about 4-5 elimination $T_{1/2}$.

1.3.2 Factors affecting ART pharmacokinetics

Optimizing ART requires comprehensive characterization of the pharmacokinetics of ART in the target population. We have recently published a paper on pharmacologic considerations for ART in developing countries (42). Some of the factors that influence drug concentration in target populations are discussed below.

Individual variables; age, sex, weight and genetic background

Differences in covariates such as age, sex, weight and genetics may cause differences in drug disposition (43) either due to differences in expression of drug transporting proteins and metabolizing enzymes or rates of elimination. Some evidence to support differences in drug disposition arising from individual variables and polymorphisms of genes encoding metabolizing enzymes exists (44). Evidence suggests that differences in NNRTI metabolism are due to polymorphisms of genes encoding metabolising enzymes. The frequency of these polymorphisms varies with race and their effects may be clinically significant. Earlier work by Haas and colleagues demonstrated an association between central nervous system toxicity, higher efavirenz levels and CYP2B6 516 polymorphisms that occurred more commonly in blacks (38). Recent studies (45) also report associations between NNRTI levels and racially distributed genetic differences. Analogous to earlier reports with efavirenz, CYP2B6 516 variant alleles were associated with higher nevirapine concentrations. A 1.5 fold increase in 12 hour nevirapine concentrations was observed in TT versus GG individuals in one Ugandan study (46). Studies are needed to identify other pharmacogenetic factors that may affect the pharmacokinetics of ART in the target populations.

Diet, food intake and malnutrition

The oral bioavailability of lipophilic drugs varies widely and for some of them improves markedly when administered with a high-fat meal or drink. Some drugs require acidic pH for absorption while others are better absorbed in alkaline pH. Food enhances absorption and bioavailability of some drugs such as efavirenz (20), while absorption of other drugs may be delayed in presence of food (47). Given that the diet in resource limited settings markedly differs from that in the developed world, bioavailability of drugs may differ. Malnutrition reduces the amount of plasma protein so influences the fraction of drug bound by plasma protein, with more of the free drug available. Malnutrition also increases risk of suboptimal response to treatment and death when ART is initiated. Some types of food may interact with ART, while ART side effects such as nausea and vomiting may affect food intake and nutrition in general (48). Food may induce changes in the bioavailability of some drugs due to chelation by components in the food. The

physiological response to food intake such as gastric acid secretion may reduce bioavailability of other drugs (49).

Pregnancy

The physiological changes that occur during pregnancy such as alterations in plasma protein concentrations; increased volume of distribution; and changes in absorption rate may alter drug pharmacokinetics (50). The increase in progesterone levels during pregnancy reduces gastric emptying and small intestine motility thus affects absorption. The higher gastric pH may impair drug absorption (51). Drug distribution is modified by the elevated body water and fat, which increase the volume of distribution of both hydrophilic and lipophilic drugs. Plasma albumin and alpha₁-acid glycoprotein decrease during pregnancy thus affect drug protein binding (51). The high variability of expression of CYP450 enzymes during pregnancy affects drug metabolism while increased renal blood flow may enhance excretion of renally excreted drugs (51).

Previous studies demonstrated conflicting results regarding nevirapine pharmacokinetic exposure during pregnancy compared to the non pregnant state (52-54). A review by Roustit et al attributed this to the small sample sizes in the different studies and the high inter-individual variability (51). In Ugandan women, nevirapine exposure was reduced during the third trimester compared with post partum (55). Studies demonstrated a reduction in the steady-state pharmacokinetics of lopinavir during pregnancy (56-58) and higher dosage adjustments produced higher lopinavir exposure in the third trimester similar to the non-pregnant exposure (59-60). In contrast to these data, Lyons et al demonstrated trough plasma levels in pregnant mothers that were closer to those in the non-pregnant population (61). The authors suggested pharmacogenetic differences as possible explanations of the differences in handling of LPV/r in the different populations. Adequate ART pharmacokinetics is particularly important in pregnancy to achieve viral suppression and prevent mother to child transmission therefore characterization of ART pharmacokinetics in pregnancy is of utmost importance.

Plasma protein binding

Drug distribution in the body is influenced by plasma protein binding and drug transporting proteins. Albumin and alpha₁-acid glycoprotein are the major drug binding proteins in the blood. Albumin binds acidic drugs while basic drugs bind to the globulin fraction; alpha₁-acid glycoprotein. Binding of drugs to plasma proteins and tissue components is reversible and usually rapid, reaching a state of equilibrium between the bound and unbound drug fractions within seconds. For example following oral administration, 60% of nevirapine is protein bound (14) while efavirenz is highly protein bound (>99%), predominantly to albumin (20). The unbound drug is available for action at the target sites. Only the free drug crosses membranes and is pharmacologically active.

Drug distribution may involve transport of drugs by carriers such as P-glycoprotein, expressed in many tissues such as the intestine, liver, kidney, testes and brain. These facilitate cellular uptake and efflux of drug molecules across membranes. P-glycoprotein is a transmembrane efflux protein that may affect drug disposition by inhibiting drug absorption from the GIT and facilitating drug excretion into the bile and urine. Some drugs inhibit and/or induce drug transporters influencing concentrations of co-administered drugs (62).

Drug quality

Qualified generic drugs offer great promise in the treatment of HIV/AIDS; however, proven bioavailability and bioequivalence are key to successfully implementing their use. Substandard and counterfeit ART formulations produce sub-therapeutic plasma concentrations with risk for treatment failure and emergence of drug resistance. In addition, they may contain toxic substances harmful to patients (63). Although the magnitude of the problem of counterfeit drugs in Uganda is not well established, reports are often made in the public media such as newspapers (64-65).

Bioequivalence testing assesses the expected *in vivo* biological equivalence of two preparations of a drug. According to the US Food and Drug Administration (FDA), if two products are bioequivalent, then their bioavailability should not differ significantly when administered at the same dosage under similar conditions. The two products should not have significant difference in the rate and

extent to which the active ingredient becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study. Both AUC and C_{\max} can be analyzed as bioequivalence markers. The FDA definition for bioequivalence is a 90% confidence interval of the geometric mean ratio that lies between 0.8 and 1.25 when comparing the test to the reference drug (66).

Adherence

Sub-optimal adherence leads to sub therapeutic drug concentrations with incomplete viral suppression, treatment failure and risk for drug resistance (48-49). At least 95% adherence to ART is necessary for successful HIV treatment (67) especially with NNRTIs. Significant effort has been put into enforcing adherence to ART in Uganda. Interventions such as regular ART counseling and use of fixed-dose combination (FDC) ART regimens have been adopted. Such interventions have improved ART adherence to levels greater than 90% for majority of patients in Uganda and other areas in sub-Saharan Africa (68-70). However, ART toxicity as well as the immune reconstitution inflammatory syndrome may impact adherence (71). Data from a large multicentre trial demonstrated a high rate of life-threatening adverse events exceeding AIDS defining events (72). The occurrence of such events may cause poor adherence necessitating a switch in ART regimens. About 25% of patients stop therapy within the first year on ART because of side effects. About the same number do not take the recommended dosages due to concerns of side effects. Patients who report significant side effects are more often non-adherent to therapy (7).

Drug pharmacokinetics

Drugs are metabolized into more hydrophilic forms that are easily excreted from the body; some are biotransformed into more active metabolites. Metabolism reactions are broadly classified into phase 1 and phase 2 reactions catalyzed by a number of enzymes. Phase 1 reactions are oxidation, reduction and hydrolysis reactions; which provide a chemical group to a drug increasing its polarity and water solubility while phase 2 reactions involve conjugation or synthetic reactions in which hydrophilic moieties such as glucuronic acid, sulphate, glutathione and

acetate are attached to a metabolite resulting from a phase 1 reaction (73). The metabolites may have less or more pharmacologic and toxic properties than the parent drug.

Phase 1 reactions mostly involve oxidation catalyzed by the super family of heme protein enzyme isoforms of CYP450 enzymes. These enzymes are electron transporting proteins containing a heme prosthetic group, found in the liver endoplasmic reticulum, adrenal mitochondria and GIT. Their nomenclature is derived from cyto for the cellular location, chrome for spectrophotometric characteristics and P for pigment. It includes an Arabic numeral indicating the isoform family, a capital letter for the subfamily and an Arabic numeral for the individual gene product in the subfamily. In the presence of carbon monoxide, they have an absorption maximum at wavelengths near 450 nanometers. The CYP enzymes play a major role in the activation of chemical carcinogens, detoxification of numerous xenobiotics as well as oxidative metabolism of endogenous compounds such as steroids, fatty acids and prostaglandins. The liver is the major site for CYP-mediated oxidative metabolism. Other sites include the intestine, lungs and kidneys (74).

Although 14 human families of CYP enzymes have been identified, approximately 95% of all drug oxidation occurs through the action of 6 CYP enzymes: CYP1A2, CYP2C8/9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4/5. The CYP3A subfamily is the major form in the human liver and other tissues accounting for 30 per cent of the cytochrome P450 enzymes and responsible for metabolism of majority of drugs in clinical use (21). Regarding ART, both PIs and NNRTIs are extensively metabolized by CYP3A4. Nevirapine and efavirenz are inducers while most PIs are inhibitors of CYP3A4, ritonavir being the most potent. Considering the multiple pharmacotherapy that HIV-infected patients often receive and that CYP3A4 is involved in metabolism of majority of drugs, the risk for drug interactions is considerable.

Pharmacokinetic drug-interactions

Pharmacokinetic interactions may occur when drugs are co-administered. These interactions may result from induction or inhibition of CYP enzymes or drug transporters by drugs causing synergistic or antagonistic therapeutic effects and/or side effects. Enzyme induction occurs when there is enhanced activity of existing enzymes due to increased enzyme synthesis or decreased degradation. Increased enzyme synthesis occurs when there is increase in transcriptional activation of messenger RNA causing enhanced protein synthesis. The molecular mechanisms of CYP gene regulation involve interaction between transcription factors such as nuclear receptors with promoters of the CYP genes (74). An inducer binds to nuclear receptors and the inducer-nuclear complex binds to a DNA response element, enhancing DNA transcription thus increasing protein synthesis and enzyme production. The liver-enriched transcription factors and nuclear receptors include the human nuclear pregnane X receptor (hPXR) and the constitutive androstane receptor (hCAR) which regulate expression of the CYP enzyme genes, and transactivation of these receptors leads to upregulation of CYP enzyme activity (74-77).

Inducers such as efavirenz and nevirapine activate the hPXR and hCAR, markedly increasing CYP3A4 and 2B6 functional activity (75-76). Induction of CYP enzyme activity causes increased drug metabolism with decreased plasma concentration of the drug metabolized by that enzyme and reduced availability to sites of action. Known inducers include; rifamycins (78), anticonvulsants (79), efavirenz (21), nevirapine (14) and herbal medications like St John's wort (80).

Enzyme inhibition on the other hand, leads to reduced clearance, prolonged $T_{1/2}$ and accumulation of drug. It may be reversible or irreversible with the former being more common. Reversible inhibition may be competitive or non-competitive. In competitive inhibition; the inhibitor competes with the substrate for the same binding site within a CYP enzyme. In non-competitive inhibition, the inhibitor binds to the same enzyme as the substrate, but the binding site differs. Known reversible inhibitors of CYP enzymes include; antifungals and antibiotics like ciprofloxacin and clarithromycin (81). With irreversible inhibition; the inhibitor forms inhibitory intermediate metabolites which form stable inactive complexes with the prosthetic heme of CYP. The enzyme involved is unavailable

for drug metabolism and synthesis of new enzymes is required to overcome the inhibition.

Drug-drug interactions arising from concomitant medication use are an important widely recognized cause of differences in drug disposition (82). Antiretroviral drugs are among the drugs with high potential for drug-drug interactions due to the potent inhibition and induction of CYP enzymes as well as transport proteins. The combination of at least three drugs for highly active ART increases the risk for drug-drug interactions. The potential for drug interactions is complicated when additional drugs for treatment of co-morbidities and infections are administered (14-15, 82-83).

Over the counter drugs and herbal products although poorly studied are commonly used by HIV-infected individuals and may be a source of drug interactions. Herbal medicines may have significant interactions with ART. By inducing CYP3A4 and P-glycoprotein, St John's wort reduces PI and NNRTI concentrations (84), additional data suggests that garlic and vitamin C also reduce ART concentrations (85). A survey in Uganda documented 103 species of medicinal plants prescribed by traditional medicine practitioners for treatment of HIV/AIDS (86). It is important to identify the active ingredients and drug interactions of herbal remedies with ART.

The risk of clinically significant interactions is considerable and may result in high concentrations with excessive toxicity or reduced concentrations with reduced efficacy and risk for development of resistance. Clinically significant CYP mediated drug-drug interactions are more likely to occur with NNRTIs and PI because they are inducers and/or inhibitors of CYP enzymes (83). NRTIs do not undergo CYP mediated metabolism and their effect depends on the rate and extent of intracellular phosphorylation (83). They are less likely to cause CYP mediated drug interactions. They are excreted mainly via the liver and kidneys. They may cause competition with other drugs for renal tubular secretion. A comprehensive review of drug interactions is available at <http://www.hiv-druginteractions.org/> and <http://idi.mak.ac.ug/docs/druginteractionchart.pdf> and health care providers are advised to review this information before prescribing co medication to patients receiving ART.

Disease and co-morbidity

The immune suppression caused by HIV-infection predisposes patients to various infections and co-morbidities requiring co-treatment. Disease and co-morbidity may influence drug pharmacokinetics in various ways; plasma proteins increase during acute infections and may alter the bound and unbound drug fractions. Drug excretion from the body through various routes, including the kidneys, GIT, lungs, breast milk and sweat may be affected by disease states causing accumulation of drug and necessitating dose reduction. Disease interactions, increased pill burden, drug interactions, and synergistic drug toxicity arising from concomitant treatment may influence adherence and drug pharmacokinetics with influence on treatment outcome.

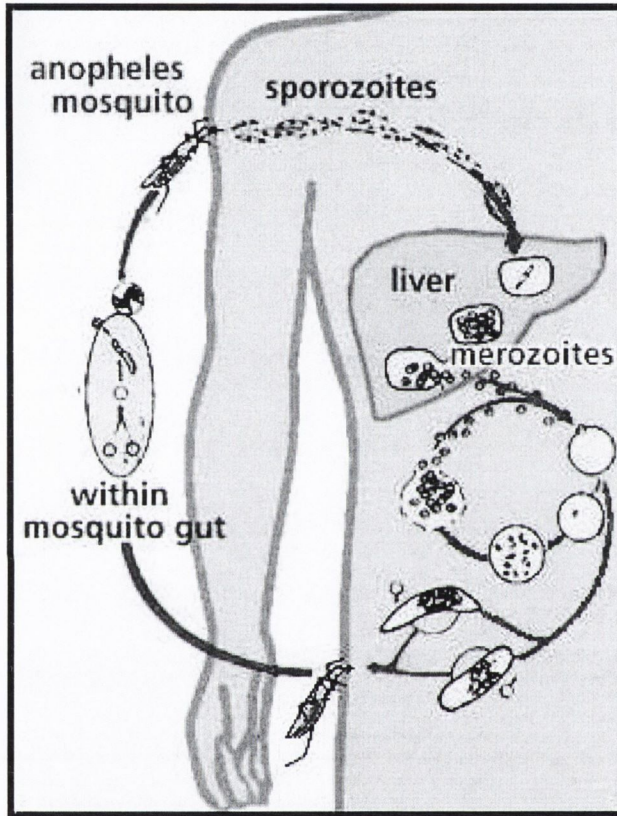
In sub-Saharan Africa, malaria is a major cause of morbidity and mortality in HIV-infected patients. Malaria and HIV together account for more than 4 million deaths annually worldwide (87). The geographical overlap in epidemiological distribution of HIV and malaria creates significant epidemiological, clinical and socio-economic interactions with profound consequences (87-92). Treatment of the two diseases is currently being scaled-up in sub-Saharan Africa; however, optimizing treatment of HIV-malaria co-infected patients faces several challenges. In the next chapter we present literature review on malaria and HIV interactions with focus on potential drug-drug interactions between antimalarial drugs and ART.

1.4 HIV and Malaria interactions

Malaria is a febrile illness caused by intracellular protozoa of the *genus Plasmodium*, transmitted by the bite of an infected female *anopheles* mosquito. *Plasmodium* species that cause disease in humans include *P.falciparum*, *P.vivax*, *P.malariae*, and *P. ovale* (93). *P. falciparum* is the most prevalent in Uganda and the most virulent causing the most severe form of disease (94). Worldwide, malaria ranks as one of the most important causes of morbidity and mortality with 300-500 million clinical episodes and 1.5 – 2.7 deaths annually. Over 90% of these occur in sub-Saharan Africa, the region with 68% of the global HIV burden (1, 95).

The malaria parasite has part of its life-cycle in humans and part in the mosquito (figure 1.7). The mosquito injects sporozoites into the human host. Sporozoites travel in the blood to infect hepatocytes where they mature into schizonts. Schizonts rupture releasing merozoites which infect red blood cells. At the end of a 48 to 72 hour cycle red blood cells rupture releasing merozoites responsible for the clinical manifestations of disease including; fever, chills and rigors, joint pains, abdominal pain, vomiting and headache. In the absence of adequate treatment, individuals may develop severe malaria with organ dysfunction and death.

Figure 1.7. The life-cycle of the malaria parasite (96)



(Adopted from http://sickle.bwh.harvard.edu/malaria_sickle.html)

Development of malaria infection may be prevented by destruction of sporozoites by cytotoxic T cells. Both humoral antibody responses and adequate T cell immunity are critical to development of an effective immune response against malaria. CD4 T cells modulate production of antimalarial antibodies and production of cytokines directed against malaria parasites. Activated CD4 and CD8 cells facilitate antibody dependent cytotoxicity of infected red blood cells which results in death of intraerythrocytic parasites (97).

By depleting CD4 T cells HIV-infection causes diminished antimalarial immune responses due to deficiency in humoral and cell mediated immunity. Studies have established increased malaria incidence and severity in HIV-1 infected individuals (87, 91, 98). The HIV-infected individuals are over twice as likely to get infected with malaria as HIV-uninfected individuals (98-101). The risk worsens with advanced immune suppression (98-99). On the other hand, the immunological consequences of malaria infection stimulate HIV replication with increase in viral load (90, 102). Transient but repeated elevations in HIV viral load during recurrent malaria attacks could have an impact on HIV disease progression and transmission (92).

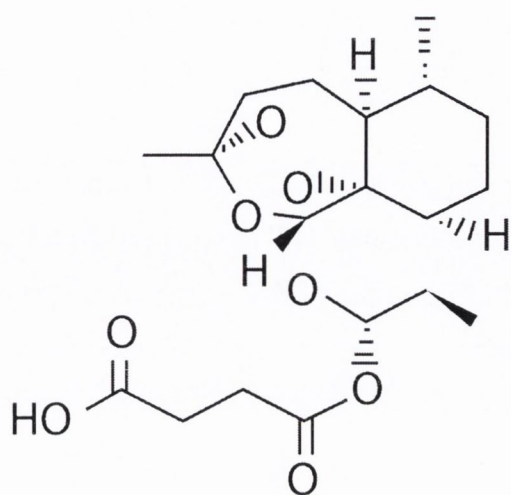
Uncomplicated malaria if not promptly and adequately treated, progresses to severe malaria with high risk for death. The risk for severe malaria is higher among HIV-infected individuals (103-104). Severe malaria is a spectrum of clinical syndromes unified by the single causative organism *P. falciparum*. Operationally it is defined as any malaria syndrome that is associated with a high mortality (>5%), even after appropriate treatment in hospital (105). Severe falciparum malaria is defined according to the following criteria; presence of coma, severe anaemia, respiratory distress, hypoglycemia, circulatory collapse, spontaneous bleeding, haemoglobinaemia, acidosis and repeated convulsions. The supporting criteria include: impaired consciousness, jaundice, prostration, hyperpyrexia and hyperparasitaemia (106). Severe malaria is the commonest cause of death, particularly in rural areas that are not serviced by the formal health system. However, even under ideal conditions in specialized hospitals, the case fatality rate still remains unacceptably high. The increasing seriousness of this problem calls for the need to evaluate and embrace new interventions for the management of this disease.

The drug of choice for treatment of severe malaria in most parts of Africa has been IV quinine; however, recent evidence suggests IV artesunate to replace quinine (107-108). A large multi-center randomized placebo-control trial carried out in South-east Asia reported a 35% reduction in mortality in adults treated with IV artesunate compared to IV quinine. Artesunate use was also associated with very few adverse events (107). Data from a very recent multi-centre randomised trial demonstrated superiority of artesunate over quinine with a 22.5% reduction in mortality, and significantly less incidence of coma, convulsions and deterioration of coma score in patients treated with artesunate (108).

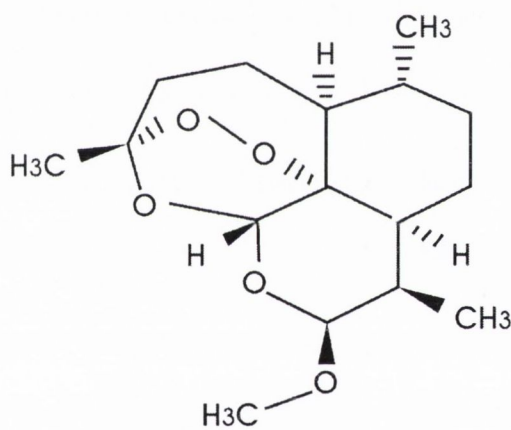
Artesunate

Artesunate is a water soluble hemisuccinate artemisinin derivative of the Chinese herb; *Artemisia annua* (*sweet wormwood*); manufactured to Good Manufacturing Practice standards as artesunic acid for injection (60mg/ampule) by Guilin Pharmaceutical Factory, Guangxi, and People's Republic of China. The chemical structure of artesunate is shown in figure 1.8. Like other artemisinin derivatives, the artesunate structure includes an endoperoxide bridge (C-O-O-C), which in the presence of intraparasitic iron, is converted into free radicals and other electrophilic intermediates which alkylate specific malaria target proteins.

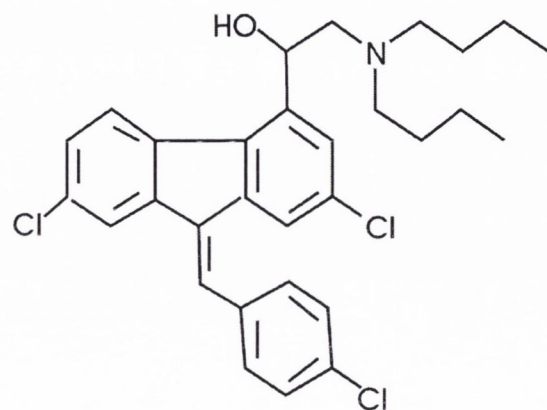
Figure 1.8. Chemical structures of artesunate, artemether and lumefantrine



Artesunate



Artemether



Lumefantrine

The mechanism of action of artesunate and the other artemisinin derivatives is not well understood but is thought to be linked to the peroxide bridge (109-110). Artesunate exerts oxidant stress on the intraerythrocytic malaria parasites (111). When the malaria parasite enters the red blood cell, it consumes hemoglobin and liberates heme in its digestive vacuole. The iron of heme reduces the artesunate peroxide bond generating high oxygen radicals which kill the parasite.

Artesunate is highly effective with very fast schizonticidal action. It may be administered orally, intramuscularly, rectally or IV. Following IV administration, artesunate is very rapidly hydrolysed to dihydroartemisinin (112) by blood esterases and the hepatic CYP3A4 (113). Artesunate's excellent antimalarial properties demonstrated by rapid parasite and fever clearance are attributed to its rapid hydrolysis to dihydroartemisinin (114-115), a metabolite with potent antimalarial properties (112, 114, 116-119). The excellent efficacy and safety plus the ease of administration make artesunate a very attractive option for use in remote peripheral areas, although widespread use is hampered by the cost of drug acquisition. To prevent emergence of drug resistance, artesunate monotherapy is not recommended and therapy should be completed with a full course of an oral ACT.

1.4.1 Treatment of uncomplicated malaria

Reduction of malaria-associated morbidity and mortality in sub-Saharan Africa largely depends on provision of prompt, safe, affordable, and effective antimalarial therapy. Response to antimalarial therapy is dependent on the abilities of both antimalarial drugs and host immune responses to inhibit infecting parasites. Malaria-specific immunity is acquired with repeated exposure to malaria parasites and improves with age. Similarly response to therapy improves with acquired immunity. The HIV depletes acquired immunity predisposing patients to poor treatment outcomes. The HIV-malaria co-infected patients present with higher parasite counts (91), an independent predictor of treatment failure (120), underscoring the need for prompt, effective and safe antimalarial treatment. Data on the effect of HIV-infection on antimalarial treatment response is accumulating but not conclusive (121-125); one study reported no effect of HIV-infection on treatment failure among children treated with quinine (125). A few

studies have suggested decreased antimalarial treatment response in HIV-infected patients (121, 123, 126), and higher rates of malaria re-infection in adults after malaria treatment (122). Cotrimoxazole prophylaxis and ART greatly reduce the risk for malaria in HIV-infected individuals (127-128), however, once infected with malaria; individuals should receive effective and safe antimalarial treatment. Due to the wide-spread resistance to older antimalarial drugs such as chloroquine and sulphadoxine-pyrimethamine, WHO recommends use of artemisinin based combination therapy (ACT) as first-line treatment for uncomplicated malaria (93). The ACTs combine fast-acting artemisinins which clear parasites rapidly, with structurally unrelated and more slowly eliminated compounds which permit elimination of residual parasites.

The impact of HIV induced immunosuppression on antimalarial treatment outcomes progressively increases as antimalarial drug efficacy decreases. ACTs are highly effective in management of malaria in HIV infected individuals though there is an increased risk of new malaria infections after treatment and recrudescence malaria occurs more commonly in adults with advanced immunosuppression (122, 129). Co-administration of ART and ACT to HIV-malaria co-infected patients has potential for drug interactions due to the central role played by CYP enzymes in biotransformation of the NNRTIs, PIs and the ACTs.

1.4.2 The potential for pharmacokinetic interactions between ACT and ART

The potential for interactions between ACTs and ART was demonstrated when a combination of amodiaquine plus artesunate was co-administered with efavirenz to healthy volunteers (130). The amodiaquine AUC increased by 100-300% and the individuals manifested with hepatotoxicity. Co-administration of amodiaquine and efavirenz is therefore contraindicated (130). In a recent study in Uganda, amodiaquine plus artesunate was associated with remarkably high risk of neutropenia in HIV infected children, worse with concurrent ART especially zidovudine (131).

Artemether-lumefantrine

Artemether-lumefantrine (AL) is an ACT manufactured to Good Manufacturing Practice standards as a FDC tablet containing 20mg artemether and 120mg lumefantrine (Coartem®) by Novartis Pharma AG, Basel, Switzerland) (132). A six-dose regimen of AL has excellent efficacy against sensitive and multidrug resistant falciparum malaria (133). Artemether-lumefantrine is the first-line ACT for treatment of uncomplicated malaria in Uganda. We have published an update on efficacy, effectiveness and safety of AL for treatment of uncomplicated malaria (134). Artemether and lumefantrine have different modes of action and act at different points in the parasite life cycle. Artemether interferes with parasite transport proteins, disrupts parasite mitochondrial function, inhibits angiogenesis and modulates host immune function. Lumefantrine is an aryl-amino alcohol that prevents detoxification of heme, such that toxic heme and free radicals induce parasite death (135-136).

Artemether is an artemisinin derivative of the Chinese herb *artemisua annua*. Its chemical formula is $C_{16}H_{26}O_5$ with a molecular weight of 298.4. The chemical structure of artemether is shown in figure 1.8. Artemether is absorbed very rapidly after oral administration reaching peak plasma concentrations within 2 hours (136-137). It acts rapidly to clear malaria parasites with $T_{1/2}$ of 1-3 hours and is metabolized quickly via CYP2B6, 3A4 and possibly 2A6 to dihydroartemisinin (15, 132, 138). Dihydroartemisinin is converted to inactive metabolites by glucuronidation via uridine diphosphoglucuronyltransferases (UGT), UGT1A1, 1A8/9 and 2B7 (132, 138). Artemether induces CYP2C19 and 3A4 (15). A summary of artemether pharmacokinetics is presented in table 1.1. Both artemether and dihydroartemisinin offer potent anti-malarial properties causing significant reduction in asexual parasite mass of approximately 10,000 fold per reproductive cycle, with prompt resolution of symptoms (139).

Lumefantrine is an aryl-amino alcohol (138) with chemical formula $C_{30}H_{32}Cl_3NO$ and molecular weight of 528.9. The chemical structure of lumefantrine is shown in figure 1.8. Lumefantrine absorption occurs 2 hours after oral intake reaching peak concentration after 3-4 hours (139). It has a $T_{1/2}$ of 3-6 days and is responsible for preventing recurrent malaria parasitemia (15, 139). It is absorbed and cleared more slowly than artemether and dihydroartemisinin and eliminates

residual parasites thus prevents recrudescence (135-136). Lumefantrine inhibits CYP2D6 (15) and is metabolized by N-debutylation mainly via CYP3A4 to desbutyl-lumefantrine (15, 138). Food enhances bioavailability of both artemether and lumefantrine (132, 139). A summary of lumefantrine pharmacokinetics is presented in table 1.1.

Co-administration of CYP inducers such as nevirapine and efavirenz with AL could potentially result in clinically significant drug interactions (15) with reduction in plasma concentrations of artemether and lumefantrine with increase in the concentrations of dihydroartemisinin and desbutyl-lumefantrine. On the other hand, the inhibitory effect of LPV/r could theoretically result in elevated artemether and lumefantrine concentrations with reduced dihydroartemisinin and desbutyl-lumefantrine concentrations when co-administered.

We have published a paper on the potential for complex interactions between AL and ART (140). There are very scanty data on these interactions and their effects yet AL and ART continue to be co-prescribed in malaria endemic regions. A study that investigated the pharmacokinetics of the standard six-dose AL as 80/480mg twice daily when administered with LPV/r 400/100mg twice daily in 13 healthy HIV-seronegative volunteers demonstrated 2 to 3-fold increases in lumefantrine AUC and trends towards decreases in artemether C_{max} and AUC with decrease in dihydroartemisinin AUC. The authors concluded that co-administration of AL and LPV/r can be carried out but highlighted the need for formal safety analysis of concomitant therapy (141). Data from another pharmacokinetics study of HIV-infected participants without malaria, unexpectedly demonstrated significantly increased lumefantrine exposure when co-administered with nevirapine although toxicity was not increased (142). Since lumefantrine has structural similarity to halofantrine, a drug known to cause cardiac arrhythmias and sudden death (143) and elevated concentrations may be associated with toxicity (144), more data are needed on the safety of elevated lumefantrine concentrations.

It is not known what plasma levels of ART will result if AL is co-administered; however, since malaria infection occurs as an acute illness requiring a short course of therapy, the effect of AL on ART may only be transient with clinically insignificant results. However, in malaria endemic regions; if individuals are

exposed to repeated malaria infections requiring recurrent treatment, the effect of drug interactions combined with the transient increase in viral replication and viral load during malaria infection may be similar to effects of sub-optimal adherence to ART or treatment interruptions which predispose to ART failure and development of resistance (145). The effects of drug interactions may be more relevant in patients with borderline therapeutic concentrations due to CYP variability and in presence of physiological factors such as pregnancy that influence drug pharmacokinetics.

1.5 Problem statement, rationale, research questions and objectives

Problem statement and rationale

There has been a rapid roll-out of ART in sub-Saharan Africa in the last decade with significant improvement in survival of HIV-infected individuals. However, long term success of any ART regimen requires ensuring continuous therapeutic concentrations of drugs at the target sites. Achieving adequate concentrations is dependent on both drug and host characteristics such as quality of drugs, physiological and nutritional status, diet, presence or absence of disease and adherence. In addition health system factors such as adequate human resource, laboratory infrastructure and continuous supply of drugs play a major role.

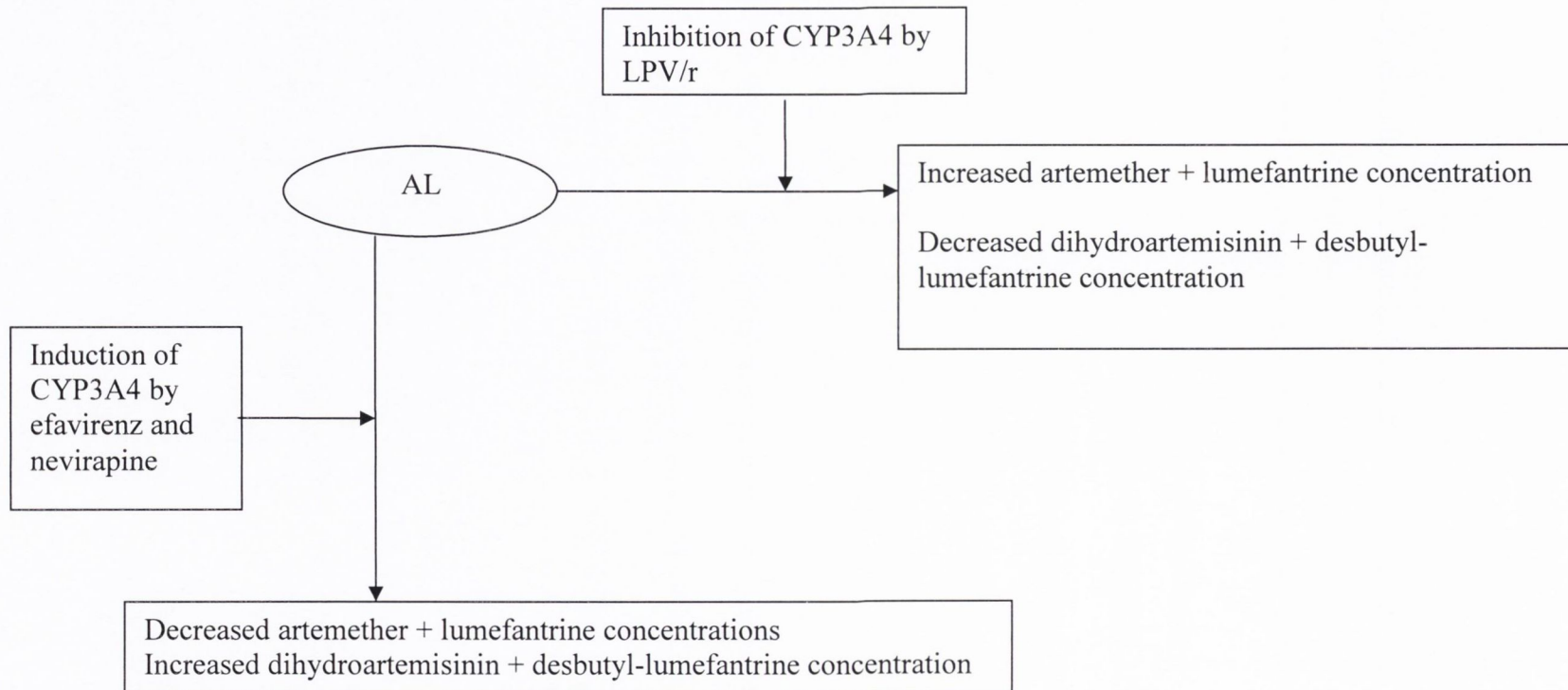
Sub-therapeutic drug concentrations predispose to treatment failure with risk for development of drug resistance while very high concentrations predispose to increased toxicity which may affect adherence. Many of the treatment programs in sub-Saharan Africa rely on the less expensive generic ART formulations to attain treatment goals. This creates the need to test and ensure that the generic products are bio-equivalent to the innovator ones. In addition, although pharmacokinetic studies are performed during drug development, drug pharmacokinetics may differ with differing genetics making it important that additional pharmacokinetic studies are performed in the target population.

The geographical overlap in the distribution of HIV and malaria in sub-Saharan Africa, causes significant clinical, epidemiological and social-economic interactions with profound public health consequences. Impaired host immunity resulting from HIV infection causes increased incidence and severity of malaria attacks with poor treatment response. On the other hand, infection with malaria

parasites accelerates HIV replication with increase in HIV viral load. Optimization of treatment for HIV–malaria co-infected patients is of utmost importance and requires characterisation of the pharmacokinetics and drug-drug interactions between ART and ACT.

Highly active ART is a combination of a NNRTI or PI with 2 NRTIs. The NNRTIs and PIs are substrates and/or inducers or inhibitors of CYP enzymes; therefore have potential for drug interactions when co-administered with CYP substrates. Current WHO malaria treatment guidelines recommend ACTs such as AL which is a CYP substrate. Co-administration of ART with AL could generate significant drug-drug interactions with influence on plasma drug concentrations and treatment outcomes of HIV-malaria co-infected patients. The conceptual framework for the effect of these ART on AL is shown in figure 1.9.

Figure 1.9. Conceptual framework for potential pharmacokinetic interactions between AL and ART



There are very scanty data on the interactions between AL and ART although these are commonly co-prescribed. There are no recommendations to guide treatment of HIV-malaria co-infected patients and there are no study data available to guide policy. The studies presented in this dissertation include evaluation of the quality of generic FDC ART and pharmacokinetic drug-drug interactions of LPV/r, efavirenz and nevirapine with AL. The last study describes the pharmacokinetic profile and clinical response to IV artesunate during treatment of severe malaria. Data generated from these studies will help address these important knowledge gaps and will contribute to evidence for policy guidelines.

Research questions and objectives

General objective

To evaluate the pharmacokinetics and drug interactions of selected antiretroviral and antimalarial drugs in Ugandan adults when administered alone and in combination.

Specific objectives

Chapter 2

Research question: Does the generic FDC of stavudine, lamivudine and nevirapine (Triomune40® from Cipla, Mumbai, India) provide equivalent pharmacokinetic exposure to the branded products; Zerit® (Bristol Myers Squibb, Princeton, NJ, USA), Epivir® (GlaxoSmithKline, Research Triangle Park, NC, USA) and Viramune® (Boehringer Ingelheim, Columbus, OH, USA).

Study objective: To compare the steady-state pharmacokinetics, safety and tolerability of generic and branded formulations of stavudine, lamivudine and nevirapine in HIV-infected Ugandan adults

Hypothesis: The pharmacokinetic parameters of the generic FDC of stavudine, lamivudine and nevirapine (Triomune40®) are equivalent to those of the branded formulations Zerit®, Epivir® and Viramune® in HIV-infected Ugandan adults.

Chapter 3

Research question: Does co-administration of AL with LPV/r result in clinically significant drug interactions?

Study objective: To compare the pharmacokinetics of AL when administered as FDC to HIV-infected patients receiving LPV/r and HIV-infected ART naïve patients.

Hypothesis: Co-administration of LPV/r with AL leads to increased pharmacokinetic exposure of artemether and lumefantrine.

Chapter 4

Research question: What is the cardiac safety of co-administration of AL with LPV/r?

Study objective: To compare the cardiac function during AL administration to HIV-infected patients receiving LPV/r and HIV-infected ART naïve patients.

Hypothesis: Co-administration of LPV/r with AL leads to increased pharmacokinetic exposure of lumefantrine with adverse effects on cardiac function.

Chapter 5

Research question: Does co-administration of AL with efavirenz result in clinically significant drug interactions?

Study objective: To compare the pharmacokinetics of artemether, dihydroartemisinin, lumefantrine and efavirenz when administered alone or in combination to HIV-infected patients.

Hypothesis: Co-administration of efavirenz with AL leads to decreased pharmacokinetic exposure of artemether and lumefantrine.

Chapter 6

Research question: Does co-administration of AL with nevirapine result in clinically significant drug interactions?

Study objective: To compare the pharmacokinetics of artemether, dihydroartemisinin, lumefantrine and nevirapine when administered alone or in combination to HIV-infected patients.

Hypothesis: Co-administration of nevirapine with AL leads to decreased pharmacokinetic exposure of artemether and lumefantrine.

Chapter 7

Research question: What is the pharmacokinetic profile and clinical response to IV artesunate during treatment of severe malaria in Ugandan adults?

Study objectives: To describe the pharmacokinetic profile and clinical response to IV artesunate in Ugandan adults with severe malaria.

1.6 Materials and Methods

Study setting

The studies were conducted at the Infectious Diseases Institute (IDI) and Mulago Hospital, Kampala, Uganda (Figure 1.10). The IDI was established in 2004 to support the rapid scale up of ART in Uganda. It is situated within the Makerere University College of Health Sciences campus next to Mulago hospital. It is a centre of excellence whose pragmatic areas include; prevention, care and treatment, training, research, laboratory services and community outreach. About 20,000 HIV-infected patients are registered, with 10,000 actively in care; about 5,500 are on first-line (nevirapine or efavirenz plus 2NRTIs) and 520 on second line ART (LPV/r plus 2NRTIs) regimens. Patients receive HIV and ART counseling, safe water vessels, mosquito nets, reproductive health services, cotrimoxazole prophylaxis and treatment of opportunistic infections.

Mulago Hospital is the national referral hospital of Uganda and teaching hospital of Makerere University College of Health Sciences, with a bed capacity of 1500. All patients recruited in the pharmacology studies presented in this thesis were admitted to Mulago Hospital private ward where pharmacokinetic sampling and clinical monitoring was performed.

Figure 1.10. Mulago Hospital and the Infectious Diseases Institute, Kampala, Uganda



Picture taken by Charles Steinberg.

Supporting HIV care using medicines information at the AIDS Treatment Information Centre

Within IDI is the AIDS Treatment Information Centre (ATIC) a toll free call-in centre established to support the roll-out of ART. It is run by pharmacists and physicians with significant expertise in HIV/AIDS and infectious disease management. The centre provides medicines information by telephone, email, drug information charts and newsletters. The ATIC publishes reference charts such as drug identification and drug interaction charts that are freely and widely available for use even in the most remote areas. Our antimalarial-antiretroviral charts can be found at <http://idi.mak.ac.ug/docs/antimalarial.pdf>.

Health care providers submit questions using either the toll free telephone service or email and responses are submitted back after thorough literature search by ATIC staff. Quality assurance is provided by pharmacists from the National Medicine Information Centre at Saint James' Hospital, Dublin and North-Western Memorial Hospital in Chicago, USA. Some of the questions received at this centre form part of the research agenda at the IDI and are included in this thesis.

Capacity building for clinical pharmacology research in Uganda

The pharmacology research unit was established to support the roll-out of ART and treatment of co-morbidities and infections through research and training. The staffs received training in principles of pharmacology research, regulatory procedures, filing and documentation, standard operating procedures (SOPs), high pressure liquid chromatography (HPLC) techniques, data review, statistical analysis and Good Clinical Practice (GCP).

With a grant from the Irish government, HPLC equipment was purchased and installed in the College of American Pathologists' certified Makerere University-Johns Hopkins University (MU-JHU) research collaboration laboratory at the IDI in October 2006. The laboratory technologist was trained at the University of Cape Town and has since gained significant expertise in conducting drug assays. Collaboration with senior pharmacologists and technicians from the Departments of Pharmacology and Therapeutics of Trinity College Dublin, Ireland, University of Liverpool, United Kingdom (UK), University of Cape Town, South Africa, Northwestern University, Chicago, IL, Mahidol-Oxford Tropical Medicine

Research Unit, Mahidol University, Thailand and Pfizer has enabled the IDI pharmacology research team to gain significant expertise. In June 2007, a workshop was hosted by the IDI clinical pharmacology research team to consolidate, co-ordinate and expand their research, training and capacity building activities. The workshop brought together a panel of Clinical Pharmacology experts from Zambia, Uganda, South Africa, Nigeria, UK, United States and Ireland. The clinical pharmacology research team was trained by these experts and has significantly benefited from collaboration with them.

The Clinical Pharmacology unit had the opportunity to host a team of experts from Trinity College Dublin in 2009. The memorandum of understanding signed at this visit between Makerere University College of Health Sciences and Trinity College, Dublin in 2009 has enhanced collaboration between the two institutions.

Ethical considerations

All studies received scientific and ethics approval from the institutional review committees at the IDI, Uganda National HIV/AIDS Research Committee or the Makerere University Faculty of Medicine Research and Ethics committee and were registered with Uganda National Council of Science and Technology and ClinicalTrials.gov. All studies were conducted in accordance with GCP principles. All study procedures were explained to participants in their local languages and information leaflets were provided. All participants provided informed written consent prior to study entry. Data were kept in secured cabinets and study staff maintained confidentiality of study participant information. Study participation was voluntary. Any individual who declined to participate received standard treatment and care from the clinic with no prejudice.

Quality control

The PhD student received hands on training and exposure to clinical pharmacology research at the Chelsea Westminster Hospital, London, UK and at the department of Pharmacology and Therapeutics, University of Liverpool, UK. I attended a postgraduate course in clinical pharmacology, drug development, and regulation by Tufts Centre for Study of Drug Development, Tufts University, Boston, USA, pharmacokinetic analysis at the University of Amsterdam,

Netherlands and training in Good Clinical Practice (GCP) and advanced human subject protection, investigator responsibilities, documentation and essential documents, simple and advanced statistics at Makerere University in Kampala, Uganda.

SOPs were developed for all study procedures prior to study start. All members of the study team were trained on each study protocol prior to the onset of the study. Study group meetings were conducted regularly to review study progress, address any difficulties and provide performance feedback to the members of the team.

Data was collected using pre-tested Case Report Forms (CRFs). All CRFs were reviewed by the clinical trial investigator. The study clinical monitors reviewed all CRFs before start of the studies for adequacy and again before data entry for completeness and accuracy.

Pre-analytical steps such as food and drug intake, sampling methods, sample handling, storage and transport to the laboratory were performed according to recommended methods (132, 146). On the morning of sampling all participants received standard breakfast before administering study drugs. Intake of breakfast and study drugs was directly observed by study staff. Blood was collected in polypropylene tubes with anticoagulant (lithium-heparin for stavudine, lamivudine, efavirenz, nevirapine plus AL, and fluoride-oxalate for artesunate). Fluoride-oxalate prevents degradation of artesunate by plasma esterases. Sampling tubes for artesunate measurement were chilled before samples were drawn and samples were transported on ice to the laboratory for processing. All tubes were properly labelled and cross-checked for accuracy. All drug concentrations were measured from venous plasma obtained by high speed centrifugation of anticoagulated blood. Blood was centrifuged within 60 minutes of sampling at 1000-3000 times for 7-15 minutes and the separated plasma was stored at -80 degrees centigrade until estimation of drug concentrations. Plasma samples were shipped on dry ice to the respective laboratories for drug concentration measurement. All drug measurements were performed using validated methods in the respective laboratories. Study screening tests and sample processing were performed by experienced laboratory technologists in the MU-JHU research laboratory at IDI. To optimise the quality of thick malaria blood slide readings, each slide was read by two expert microscopists. Any

discrepancies in slide readings were reviewed and resolved by a third microscopist.

The initial nevirapine drug assays were performed by standard HPLC with UV detection at the Department of Infectious Diseases, HIV Pharmacology laboratory, University of Turin, Italy. Subsequently we set up nevirapine assays at the MU-JHU core laboratory at the IDI where all subsequent nevirapine assays were performed. For purposes of quality control, we collected blood samples in duplicate. One set was shipped to Turin for assays while the second set was retained at the IDI for assays. The data generated from the two laboratories was compared and found to be in agreement.

Adherence assessment

In all the studies presented study participants were given information on the importance of taking their medication. This information was repeated on each study visit. Adherence to medication was assessed using self report as well as pill count on each study visit.

Data management

The core components of the data management system were housed on a Server running a Linux operating system with OpenClinica installed. OpenClinica is open source clinical trials software for electronic data capture and clinical data management. Data from the CRFs were manually entered into Openclinica. Study events were defined in OpenClinica to which each CRF was attached. Double data entry was configured hence resulting into entry of each CRF by two different data entrants. Openclinica's discrepancy function enabled correction of any differences between the first and second entry. After the discrepancies were corrected, data was extracted into a tab-delimited file and then transferred to the STATA format using STATA transfer.

The Openclinica CRFs that contained strong validation rules and regular expressions to limit the number of digits or strings to be entered by the data entrants was uploaded to OpenClinica. Openclinica CRFs were designed and developed in excel which later acted as a codebook during analysis. It provided a description of the variable names, option values and variable data types. During

the process of second entry, the data entrant entered a value and if that value did not exactly correspond to the value entered during first entry, then an error message popped-up notifying the second data entrant that the value entered did not match with the value entered by the first entrant. The second entrant then corrected the value or provided a discrepancy reason for the second value if there was any difference. The data manager revised all the discrepancies posted in the system to double check the corrections made in comparison with the paper CRF. Access and authentication to the OpenClinica system was at two levels; the first one being a system log-in authentication by the users with their role definitions. The system users always logged on with strong passwords and their specific roles such as data entry or manager specified. Openclinica also had an audit trail that enabled tracking of which data entrant entered specific data.

Pharmacokinetic analysis

Non-compartmental analysis was performed using WinNonlin Professional™ software, version 5.2 (Pharsight Corp., Mountain View, CA, USA). Calculated pharmacokinetic parameters included; the C_{max} , T_{max} , C_{min} or C_{trough} , AUC, CL/F, V/F, $T_{1/2}$ and absorption lag time (T_{lag}). All parameters were calculated using actual blood sampling times. Drug concentrations below the lower limit of quantification (LLOQ) of the bioanalytical assays were treated as missing data.

The trapezoidal rule (linear-up/log-down) was used to estimate AUC; where the area of a trapezoid is equal to one half the product of the sum of the heights times the width as in the equation below;

$$\text{Area} = (\frac{1}{2}) (C_1 + C_2) (t_2 - t_1) + (\frac{1}{2}) (C_2 + C_3)(t_3 - t_2) + (\frac{1}{2})(C_{n-1} + C_n)(t_n - t_{n-1})$$

Where C denotes drug concentration, t denotes time and the subscript n refers to the sample number.

Statistical analysis

Data were analyzed using STATA® version 10.0 (StataCorp, College Station, TX). The different study design required application of various statistical tests. Baseline study participant characteristics were summarized into means with standard error (SE) or standard deviation and medians with interquartile range.

Where comparisons were needed the Independent T-test was performed to test for a difference in means for two unrelated groups.

In the bioequivalence study, the pharmacokinetic parameters (AUC and C_{max}) were assumed to follow the log normal distribution. A log-normal distribution is a continuous probability distribution of a random variable whose logarithm is normally distributed. The geometric means were calculated after log transformation of the original data following which anti-log transformation was performed.

In subsequent chapters, pharmacokinetic parameters were summarized into medians with range and compared using non-parametric tests such as Wilcoxon rank-sum test and Wilcoxon matched pairs test depending on the study design as described in the various chapters. In all cases a p value < 0.05 was considered statistically significant. This analysis was performed because it was considered the most suitable for clinicians to better understand and interpret the data.

For artemether, dihydroartemisinin and lumefantrine; presentation of C_{min} was considered inappropriate and therefore C_{max} and AUC were calculated. This was based on the fact the artemether C_{max} has been demonstrated to correlate with malaria parasite clearance and total lumefantrine exposure is the main determinant of cure.

Statistical significance may differ from clinical significance. Statistical significance measures the likelihood of occurrence of an event that is not due to chance. In determining statistical significance, p values and confidence intervals are used. P values give the probability that the outcome would have been obtained by chance while the confidence intervals estimate the range within which the real results of the outcome would be if the trial or study was performed many times. The 95% CI gives the confidence that if the trial was performed 100 times one would be 95% confident that the true value lies with that interval. Clinical significance on the other hand, measures how large the difference in effect is in clinical practice. Measures such as relative risk, absolute risk reduction, numbers needed to treat may be used. The magnitude of the effect considered clinically significant depends on the severity of the disease and side effects of the treatment.

My role as the PhD student

As the PhD student, I received training in research methods, statistical analysis, GCP among others. I wrote the study proposals and submitted them to the ethics committees for review and approval following which I registered the studies with ClinicalTrials.gov. I drafted the case record forms for all studies and with the help of a clinical research team, I screened and enrolled research participants in all the studies, performed pharmacokinetic sampling and ensured timely and efficient transportation to the laboratory for processing and storage. We provided medical care to study participants till the end of follow-up as necessary. I drafted the material transfer agreements for all the studies and submitted them for ethical approval following which I shipped samples to the respective laboratories for drug assays. Some assays were performed at the MU-JHU core laboratory at the IDI and for these I participated in reviewing all the HPLC data generated. I performed all the statistical analysis for all the studies and wrote up the thesis as well as the manuscripts for submission to peer-reviewed journals.

Chapter 2

Comparison of the pharmacokinetics of generic and branded formulations of stavudine, lamivudine and nevirapine in HIV-infected Ugandan adults

2.1	Introduction.....
2.2	Study objective
2.3	Materials and Methods.....
2.4	Results.....
2.5	Discussion.....

Introduction

The introduction of the less expensive generic ART formulations has enabled the scale-up of ART in Uganda and other resource limited settings. Until recently, the three-drugs-in-one FDC tablet of generically manufactured Triomune[®] (stavudine 40mg, lamivudine 150mg, nevirapine 200mg) was one of the first-line regimens recommended in the Uganda National ART policy (147). Triomune[®] existed as two formulations; Triomune 40[®] and Triomune 30[®], both manufactured by Cipla Mumbai, India. Both contained 200mg of nevirapine and 150mg of lamivudine, however Triomune 40[®] contained 40mg of stavudine for patients weighing more than 60kg and Triomune 30[®] contained 30mg of stavudine for those weighing less than 60kg.

The available pharmacokinetic data for Triomune[®] at the time was limited to a single dose study in healthy Indian volunteers which was performed by the manufacturer of Triomune[®], Cipla, Mumbai (148) and only one independent bioequivalence study on the steady state pharmacokinetic parameters of Triomune[®] in HIV-infected patients in Malawi. Of concern in this study Triomune[®] was found not to be bioequivalent to the originator products with significantly higher stavudine levels in the patients on Triomune[®] when compared to the originator product. In this study the patients also reported more side-effects, principally peripheral neuropathy, when taking Triomune[®] and the authors postulated that this may have been a result of the higher stavudine levels. It was also noted that nevirapine levels were markedly higher in Malawians compared to western subjects of same weight, possibly due to genetic metabolic differences. The authors concluded that similar evaluation of drug exposure should be performed as these medications are introduced to new populations (149).

In Uganda, *in vitro* drug dissolution studies are conducted by the national drug regulatory authority, however, chemical studies conducted *in vitro* do not guarantee optimal drug dissolution and absorption in humans. Although the amount of drug in the generic tablet may be similar to brand formulations, drug pharmacokinetics may differ *in vivo*. Documentation of bioequivalence in the target population is therefore important. Bioequivalence is a term in pharmacokinetics used to assess the expected *in vivo* biological equivalence of

two different preparations of a drug. The United States Food and Drug Administration (US FDA) has defined bioequivalence as ‘the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study (66). Bioequivalence is used to compare two formulations of a single dose of drug given to healthy volunteers. Intensive pharmacokinetic studies may be performed to compare pharmacokinetics of two formulations in the target population.

2.2 Study objective

To compare the steady-state pharmacokinetic parameters of stavudine, lamivudine and nevirapine in Triomune 40[®] with the branded products in HIV-infected Ugandan adults.

2.3 Materials and Methods

Study design

We performed an open label, randomized, cross-over intensive pharmacokinetic study. Participants were electronically randomized to either Triomune 40[®] (Cipla, Mumbai, India.) or the patented brand version of the drugs: Zerit[®] (stavudine, Bristol Myers Squibb, Princeton, New Jersey, USA), Epivir[®] (lamivudine, GlaxoSmithKline, Research Triangle Park, North Carolina, USA) and Viramune[®] (nevirapine, Boehringer Ingelheim, Columbus, Ohio, USA). A regimen of stavudine 40mg, lamivudine 150mg and nevirapine 200mg was taken twice daily. Participants took one tablet twice daily while on the generic formulation (Triomune 40[®]) and one Zerit[®] capsule, one Epivir[®] tablet and one Viramune[®] capsule, twice daily while on the branded formulation. All participants took drugs for a month prior to pharmacokinetic sampling. Intensive blood sampling was performed during administration of both the generic and branded formulations and stavudine, lamivudine and nevirapine plasma concentrations were measured. Before discharge from the unit, patients were switched to brand formulations if on generic and vice versa and were given the drugs to administer at home. After 28

days, study subjects were readmitted and blood sampling repeated. All participants resumed their pre-study treatment at the end of the second sampling day.

Study participants

A sample size of 12 was calculated to have 80% power to detect a difference in means of drug C_{\max} and AUC between branded and generic formulations based on the definition of bioequivalence using the paired t-test with a significance level of 0.05. We anticipated a drop-out rate of 30%; therefore, planned to enroll 16 subjects for 12 to complete all pharmacokinetic assessments.

Participants were screened and enrolled consecutively from the cohort of patients attending the IDI clinic. Individuals were eligible to participate if they were HIV-infected and older than 18 years of age. Patients with liver and renal function test parameters greater than 3 and 1.5 times the upper limit of normal respectively, hemoglobin less than 8mg/dl and taking known inhibitors or inducers of CYP enzymes or any herbal medications and pregnant mothers were excluded. All participants received cotrimoxazole daily for prophylaxis against opportunistic infections.

Study procedures

Participants had detailed explanation of study procedures at enrolment. They were reminded to take their study drugs by telephone. Adherence to study drugs was assessed using self report and pill count and information on adverse and serious adverse effects was collected. On the evening prior to pharmacokinetic sampling, participants were reminded of their study day appointment and given detailed instructions to eat food, administer their medication by 8.00pm and arrive at the hospital by 7.00am the next morning in fasting state.

On the study day, patients were admitted in fasting state, an indwelling IV cannula was inserted following aseptic technique and blood samples were drawn for determination of pre-dose concentrations of study drugs. The intake of a standardized breakfast and morning doses of study drugs was directly observed by study staff. Blood sampling was performed at scheduled time points. Four mLs of blood was collected per sampling time in lithium-heparin tubes. Samples were

centrifuged immediately at 5000 revolutions per minute for 10 minutes and the separated plasma was stored at -80 degrees centigrade until estimation of drug concentrations.

Stavudine, lamivudine and nevirapine plasma concentration measurement

Stavudine, lamivudine and nevirapine plasma concentration measurement was performed at the Department of Infectious Diseases, HIV Pharmacology Laboratory, University of Turin, Italy and also at the MU-JHU laboratory at IDI in Kampala. Concentrations were measured using solid phase extraction and HPLC with photo diode array (HPLC-PDA) (Waters 2695 HPLC system with a 2998 photo diode array detector). Stock solutions of stavudine, lamivudine and nevirapine were prepared to a final concentration of 1mg/ml in HPLC grade water and refrigerated at 4 degrees centigrade until use. The highest calibration standard (STD 8) and 3 quality control samples were prepared by adding a determined volume of each of the stock solutions to blank plasma from healthy donors obtained from the blood bank. All other standards were prepared by serial dilution from STD 8 to STD 1 using blank plasma to obtain eight different spiked concentrations plus a blank sample (STD 0). These known solutions were used to make the standard curve. Internal standards were made with quinoxaline (7.5ug/mL), for nevirapine, and thymidine (10ug/mL), for lamivudine and stavudine, in methanol and HPLC grade water (50:50 vol/vol) and refrigerated at 4 degrees centigrade until use. The mobile phase was composed of mobile phase A (KH₂PO₄ 50 mM with orthophosphoric acid, final pH = 3.23) and mobile phase B (acetonitrile). All patient samples, STDs, and quality controls underwent a heat inactivation procedure for HIV (35 minutes at 58°C) and were stored at -20 degrees centigrade until analysis.

Patient plasma samples for analysis were thawed and 500ul of plasma was diluted with 500ul of HPLC mobile phase A, 50ul of IS was added to each tube and samples were vortexed for 10 seconds. The SPE C-18 cartridges were placed on a vacuum elution manifold WAT 200677 (Waters) and activated with 1mL of methanol, followed by 1 mL of HPLC mobile phase A. Loading of samples was carried out under gravity. The cartridges were washed with 500 mL of HPLC mobile phase A, followed by 250 mL of HPLC grade water, and elution was

carried out using 500 mL of methanol and acetonitrile solution (90:10, vol/vol). Eluted solutions were collected into glass tubes and treated by vortex vacuum evaporation to dryness at 60 degrees centigrade. Each extract was reconstituted with 150 mL of HPLC grade water and acetonitrile solution (60:40, vol/vol), and 30 μ L was injected into the column. Chromatographic separation was performed by a Luna 5 μ C18 column (150*4.6 mm ID; Phenomenex, CA) in 30 minutes with a gradient, and the run was performed at 1mL/min.

Absorbance was monitored at 284nm for nevirapine and quinoxaline, 260 nm for lamivudine, stavudine and for thymidine by the PDA detector. Peaks of nevirapine (retention time= 23.5 \pm 0.1minutes) lamivudine (retention time = 7.0 minutes) and stavudine (retention time = 12.1 minutes) were compared with calibration curves built on peaks obtained from the STDs and checked with QCs. Accuracy and precision were assessed using 15 percent relative standard deviation of all data for each run. The lower limit of quantification (LLOQ) was 25, 25 and 50 ng/mL for stavudine, lamivudine and nevirapine and the limit of detection was 5, 5 and 10 ng/mL respectively (101).

Statistical analysis

Descriptive statistics, including mean and SD, were calculated for stavudine, lamivudine and nevirapine pharmacokinetic parameters. Within-subject changes in drug pharmacokinetic parameters were evaluated by calculating geometric mean ratios (GMRs) and 90% confidence intervals (CIs). The concentrations measured during branded formulation administration were used as reference. The CIs were determined using logarithms of the individual geometric mean values; the calculated values were then expressed as linear values. Steady-state pharmacokinetic parameters were compared using the US FDA standards for bioequivalence. Parameters were considered similar if the 90% CI for the C_{max} and the AUC fell within the range of 0.8–1.25 (66).

2.4 Results

A total of 27 HIV-infected subjects (16 females) were screened between January and March 2007; 11 did not meet study eligibility criteria. A total of 16 (10 females) completed all sampling phases; their clinical and demographic characteristics are illustrated in table 2.1.

Table 2.1. Baseline characteristics of study participants according to the randomization arm

<i>Parameter</i>	<i>Generic→Brand</i> (N=7)	<i>Brand→Generic</i> (N=9)
Age (years)	36.8 (34-40)	37.4 (33-40)
Weight (kg)	64.5 (63.5-66)	64.7 (63-67)
CD4 count (cells/mm ³)	305.3 (220-349)	324.4 (255-343)
Females n (%)	5 (71)	5 (56)

Median (IQR) unless differently specified

Adherence, safety and tolerability

All participants reported 100% adherence during both study phases. Both the branded and generic formulations were well tolerated and no serious adverse events were reported while on either arm. All participants preferred the generic FDC formulation because of dosing convenience.

Bioequivalence evaluation

Table 2.2 shows the comparison of the pharmacokinetic parameters of generic and branded formulations of stavudine, lamivudine and nevirapine

Table 2.2. Pharmacokinetic parameters of branded and generic formulations of stavudine, lamivudine and nevirapine

Parameter	Stavudine	Lamivudine	Nevirapine
C_{max} (ng/mL)*			
Branded	203.5 (±127.7)	855.2 (±276.6)	8594.3 (±3699.0)
Generic	210.3 (±208.4)	966.8 (±279.6)	7017.3 (±2757.8)
GMR (90% CI)	0.92 (0.78-1.08)	1.11 (0.95-1.30)	0.84 (0.64-1.11)
AUC₀₋₁₂ (h*ng/ml)*			
Branded	685.6 (±219.6)	5522.7 (±2009.8)	75192.7 (±29294.9)
Generic	579.8 (±231.2)	6039.0 (±2370.8)	64338.3 (±19944.6)
GMR (90% CI)	0.83 (0.70-0.97)	1.06 (0.94-1.2)	0.88 (0.71-1.10)
C_{trough} (ng/mL)*			
Branded	3.1 (±3.8)	190.1 (±98.59)	6394 (±4144)
Generic	3.4 (±6.1)	211.3 (143.9)	4626 (±1462)
T_{1/2} (hours)⁺			
Branded	1.92 (1.54-3.45),	4.02 (2.49-10.10)	15.36 (2.46-81.90)
Generic	1.91 (1.21-3.57)	3.83 (2.04-7.61)	17.02 (6.07-255.29)

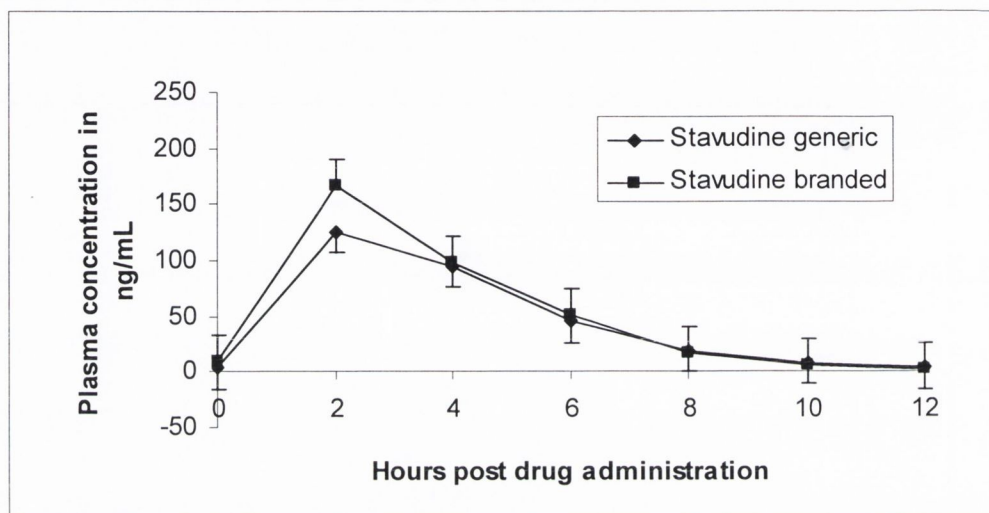
*mean (±SD); ⁺median (range); GMR, geometric mean ratio; CI, confidence interval

Stavudine

The GMR for stavudine C_{max} and AUC were significantly lower for the generic compared to the branded formulation. The FDA criteria of bioequivalence of 90% CIs of the GMR between 0.85 and 1.25 was not met for both C_{max} and AUC for stavudine; 0.92 (90% CI, 0.78-1.08) and 0.83 (90% CI, 0.70-0.97) respectively. There was a 17% reduction in stavudine exposure with the generic formulation.

The C_{trough} and $T_{1/2}$ for stavudine were not different between the generic and branded formulation. Mean (\pm SD) stavudine C_{trough} concentration was 3.1 (\pm 3.8)ng/mL on the branded and 3.4 (\pm 6.1) ng/mL on the generic formulation. Median (range) plasma $T_{1/2}$ was 1.91 (1.21-3.57) hours while on the generic and 1.92 (1.54-3.45) hours while on the branded formulation. Pharmacokinetic parameters and profiles of generic and branded formulations of stavudine are shown in table 2.2 and figure 2.1.

Figure 2.1. Mean plasma concentration-time profile of stavudine over 12 hours post oral administration of generic (diamond symbol) and brand (square symbol) formulations.

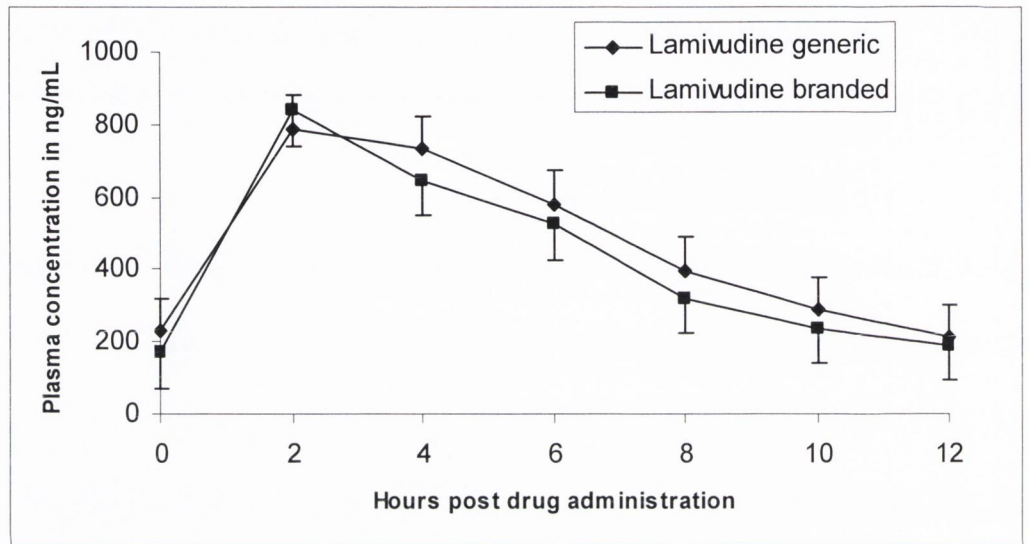


Vertical lines represent standard error

Lamivudine

The GMR for lamivudine C_{\max} and AUC were close to unity; 1.11 (0.95-1.30) and 1.06 (0.94-1.20) respectively, suggesting similar exposure for the two formulations. However, only the 90% CI for lamivudine AUC GMR was within 0.80 and 1.25 and met the FDA criteria for average bioequivalence (Table 2.2). There were no significant differences between the mean (\pm SD) lamivudine C_{trough} concentrations on the two formulations; 190.1 (\pm 98.59) ng/mL on the branded and 211.3 (143.9) ng/mL on the generic formulation. Similarly lamivudine median (range) plasma $T_{1/2}$ was not significantly different on the two formulations; 3.83 (2.04-7.61) hours for the generic and 4.02 (2.49-10.10) hours for the branded formulation. Pharmacokinetic profiles of generic and branded formulations of lamivudine are shown in figure 2.2.

Figure 2.2. Mean plasma concentration-time profiles of lamivudine over 12 hours post oral administration of generic (diamond symbol) and brand (square symbol) formulations.



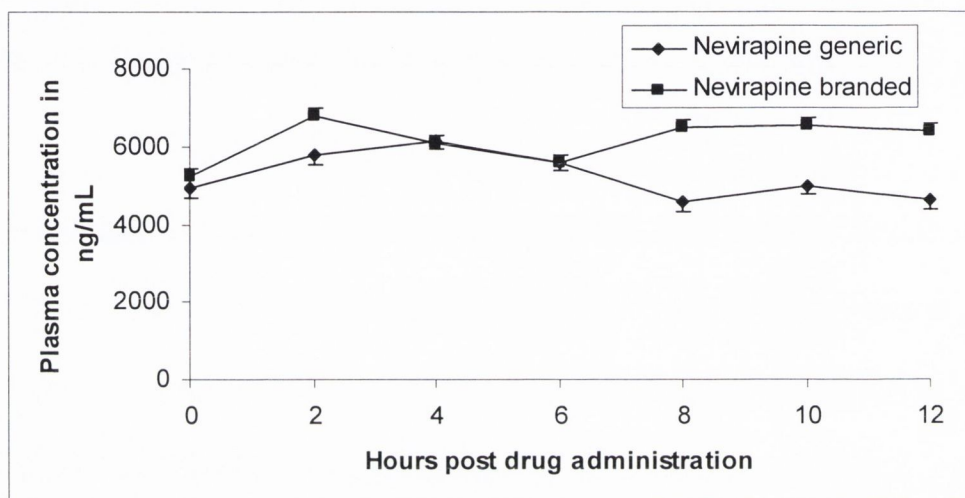
Vertical lines represent standard error

Nevirapine

Nevirapine plasma concentrations were not significantly different; GMR (90% CI) 0.84 (0.64-1.11) for C_{max} and 0.88 (0.71-1.10) for AUC. The generic formulation however, had lower concentrations; (16% lower for C_{max} and 12% lower for AUC). The 90% CI for GMR of both C_{max} and AUC were outside the predefined limits and did not meet the strict FDA criteria for average bioequivalence (Table 2.2).

Mean (\pm SD) nevirapine C_{trough} concentrations were 6394 (\pm 4144)ng/mL while on the branded and 4626 (\pm 1462)ng/mL while on the generic formulation. Six subjects (38%) had nevirapine C_{trough} concentrations below the MEC of 3,400 ng/mL on both formulations (2 on generic and 4 on branded nevirapine). The nevirapine median (range) plasma $T_{1/2}$ was 17.02 (6.07-255.29) hours for the generic and 15.36 (2.46-81.90) hours for the branded formulation. Pharmacokinetic profiles of generic and branded formulations of nevirapine are shown in figure 2.3.

Figure 2.3. Mean plasma concentration-time profiles of nevirapine over 12 hours post oral administration of generic (diamond symbol) and brand (square symbol) formulations.



Vertical lines represent standard error

2.5 Discussion

The pharmacokinetic profiles of stavudine, lamivudine and nevirapine in generic Triomune 40[®] were similar to those of the branded products; Zerit[®] (stavudine, Bristol Myers Squibb, Princeton, New Jersey, USA), Epivir[®] (lamivudine, GlaxoSmithKline, Research Triangle Park, North Carolina, USA) and Viramune[®] (nevirapine, Boehringer Ingelheim, Columbus, Ohio, USA). The strict US FDA standards for bioequivalence testing (66) were not met for nevirapine and stavudine parameters, however the differences were minimal and unlikely to be of clinical relevance. Bioequivalence studies may not be clinically relevant; but C_{trough} is clinically relevant especially when dealing with drugs that have low genetic barrier to resistance such as nevirapine.

There was lower nevirapine exposure in this study compared to that in a study performed in Malawi (149). The difference is likely due to genetic differences in rates of biotransformation of nevirapine. The functional single nucleotide polymorphism (516G>T) and CYP2B6 516 variant alleles are associated with higher nevirapine concentrations with TT versus GG individuals having higher nevirapine exposure. A 17% prevalence of this mutation was reported in a small Ugandan study (40). A higher prevalence of this and/or other polymorphisms in Malawians could account for the differences. Studies are needed to study the distribution of these polymorphisms within the African populations.

The need for optimal plasma concentrations of nevirapine above the MEC is crucial because of nevirapine's low genetic barrier to resistance. Nevirapine pharmacokinetics in this study were similar for the two formulations and steady state concentrations were adequate and above MEC for the majority of patients (11 out of 16 for the generic and 10 out of 16 for the branded). A few patients had nevirapine C_{max} values above the MTC of 6690ng/ml however; no serious adverse events were reported.

Triomune 40[®] produced lower stavudine concentrations compared to Zerit[®]. Stavudine is a pro-drug that requires intracellular phosphorylation to the active form. Intracellular concentrations rather than plasma concentrations correlate with virological suppression. The 17% reductions in the AUC of stavudine may not be clinically significant in patients with uncomplicated disease or those without

comorbidities; but become significant if patients have existing co-factors that further reduce concentrations. Our findings contrast to those of the Malawi study that reported a 12% increase in stavudine with Triomune 40[®]. Reasons for this are unclear and not related to changes in manufacturing practice arising from the reports of the Malawi study because the Triomune 40[®] tablets used in our study were produced before those data were known.

Given that Triomune 40[®] was one of commonest generic formulations prescribed at the time of study design, our data were very encouraging and supported its use to facilitate the roll-out of ART. However Triomune 40[®] is no longer recommended for HIV treatment in view of accumulated evidence on the significant toxicity arising from stavudine (150-152). In addition to providing evidence on pharmacokinetics of generic ART, this study confirmed the feasibility of performing pharmacokinetic studies in Kampala. Experience gained was applied to the subsequent studies in which we investigated pharmacokinetic drug interactions of LPV/r, efavirenz and nevirapine with AL. These are presented in the subsequent chapters.

Chapter 3

Pharmacokinetics of artemether-lumefantrine with and without lopinavir/ritonavir in HIV-infected Ugandan adults

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3.1 Introduction

Malaria and HIV contribute to significant morbidity in sub-Saharan Africa. Treatment of HIV-malaria co-infected patients has several challenges such as the potential for drug-interactions. Drug bioavailability may be influenced by CYP mediated drug-drug interactions. Ritonavir and lopinavir inhibit CYP3A4 and induce CYP1A2, 2B6, 2C9 and 2C19 (21-22, 153). Artemether and lumefantrine which offer excellent efficacy against sensitive and multi-drug resistant *Plasmodium falciparum* are both metabolized by CYP enzymes including CYP3A4/5 for artemether and lumefantrine and CYP2B6, 2C9, 2C19 and possibly 2A6 for artemether (132, 139). Elimination of dihydroartemisinin occurs after conversion to inactive metabolites via UGT1A1, 1A8/9 and 2B7 (138). The principal pharmacokinetic correlate for antimalarial cure is the total exposure to lumefantrine (139), although artemether and dihydroartemisinin play a significant role of rapidly clearing parasites from circulation.

Co-administration of LPV/r with AL to malaria-HIV co-infected patients may result in drug interactions with enhanced artemether and lumefantrine plasma concentrations. Whereas elevated plasma concentrations may be beneficial for malaria cure, they may cause adverse effects. In a previous study of healthy volunteers co-administration of LPV/r with AL resulted in significantly increased exposure to lumefantrine, decreased dihydroartemisinin and a trend towards decreased artemether exposure (141). Because drug pharmacokinetics may differ in healthy volunteers compared to diseased patients we investigated the pharmacokinetics of artemether, dihydroartemisinin and lumefantrine after administration of a single dose of AL 80/480mg to HIV-infected adults taken with and without LPV/r. To avoid unknown adverse effects, we administered a single dose of AL to HIV-infected patients without malaria.

3.2 Study objective

To compare the pharmacokinetics of AL when administered as FDC to HIV-infected patients receiving LPV/r and HIV-infected ART naive patients.

3.3 Materials and Methods

Study design

We performed a two-arm parallel intensive pharmacokinetic study. Arm 1 consisted of ART naïve HIV-infected participants who were not yet eligible for ART according to national guidelines and the second arm consisted of participants stable on 400/100mg of LPV/r plus 2 NRTIs for at least one month. All participants in the second arm took the non refrigerated formulation of LPV/r (Aluvia®)

Study participants

Participants were screened and enrolled consecutively from the cohort of patients attending the IDI clinic. Individuals were eligible to participate if they were HIV-infected and older than 18 years of age. Patients with liver and renal function test parameters greater than 3 and 1.5 times the upper limit of normal respectively, abnormal cardiac function, positive blood smears for malaria, hemoglobin less than 8mg/dl and those taking known inhibitors or inducers of CYP enzymes or any herbal medications and pregnant mothers were excluded. All participants received cotrimoxazole daily for prophylaxis against opportunistic infections.

Study procedures

Participants had detailed explanation of study procedures at enrolment. They were reminded to take their study drugs by telephone. Adherence to study drugs was assessed using self report and pill count and information on adverse and serious adverse effects was collected. On the evening prior to pharmacokinetic sampling, participants were reminded of their study day appointment and given detailed instructions to eat food, administer their medication by 8.00 pm and arrive at the hospital by 7.00 am the next morning in fasting state.

On the study day, patients were admitted in fasting state, an indwelling IV cannula was inserted following aseptic technique and blood samples were drawn for determination of pre-dose concentrations of study drugs. Because food especially fatty food enhances absorption of both artemether and lumefantrine (132) all participants received standard breakfast consisting of tea with milk, unleavened flat bread (chapatti), stuffed pastry (samosa) and bread with

margarine plus a single AL dose of 4 tablets equivalent to 80/480mg of AL (Coartem®, Novartis Pharma AG, Basel, Switzerland). Patients in the LPV/r arm took 400/100mg of LPV/r (Aluvia®, Abbott laboratories, US) plus 2 NRTIs (zidovudine plus lamivudine or stavudine plus lamivudine) with their AL dose. The intake of a standardized breakfast and morning doses of study drugs was directly observed by study staff. Intensive sampling was performed and artemether, dihydroartemisinin plus lumefantrine plasma concentrations measured.

Blood sampling was performed at scheduled time points. Four mLs of blood was collected per sampling time in lithium-heparin tubes. Samples were centrifuged immediately at 3000 revolutions per minute for 10 minutes and the separated plasma was stored at -80 degrees centigrade until estimation of drug concentrations.

Artemether, dihydroartemisinin and lumefantrine concentration measurement

Artemether, dihydroartemisinin and lumefantrine concentrations were measured at the Clinical Pharmacology Laboratory, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand. Artemether and dihydroartemisinin concentrations were measured using solid phase extraction and liquid chromatography-mass spectrometry (154). Internal standards were stable isotope-labeled artemether and dihydroartemisinin. Artemether and dihydroartemisinin were quantified using an API 5000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, USA) with a TurboV™ ionization source interface operated in the positive ion mode. Quantification was performed using selected reaction monitoring for the transitions m/z 302-163 and 307-166 for dihydroartemisinin and stable isotope-labeled dihydroartemisinin, respectively, and 316-163 and 320-163 for artemether and stable isotope-labeled artemether, respectively. Total-assay coefficients of variation (CV) for dihydroartemisinin and artemether during analysis were less than 5% at all quality control levels. The LLOQ for both drugs was 1.4 ng/mL.

Lumefantrine concentrations were determined using a solid phase extraction liquid chromatographic assay with ultra-violet (UV) detection (155). The internal

standard was a hexyl analogue of desbutyl-lumefantrine obtained from Novartis Pharma AG (Basel Switzerland). The coefficient of variation during the analysis was less than 6 at all quality control levels. The LLOQ was 25 ng/mL (155).

Statistical analysis

Data were analyzed using STATA[®] version 10.0 (StataCorp, College Station, TX). Baseline characteristics were summarized into means with standard error (SE) and compared using the independent T-test. Pharmacokinetic parameters were calculated using WinNonlin software and summarized into medians with range and compared using the Wilcoxon rank-sum test. A p value < 0.05 was considered statistically significant.

3.4 Results

Twenty nine participants completed the 72 hour pharmacokinetic sampling. Analyses were performed on data from all the 29 participants; 16 (9, 56% female) in the AL plus LPV/r arm and 13 (9, 69% female) in the AL arm who completed pharmacokinetic sampling. All participants taking LPV/r arm had viral load suppressed below the level of detection of 400 copies/ml while median (IQR) viral load was 49,786 (6668 -195321) copies/ml in the AL arm. Participants in the two study arms were comparable on other baseline characteristics measured (Table 3.1); except the hemoglobin which was significantly higher among participants taking LPV/r (mean (SE) 14.4 (0.2) vs 12.6 (0.5) mg/dl p = 0.003).

Table 3.1. Baseline characteristics of study participants

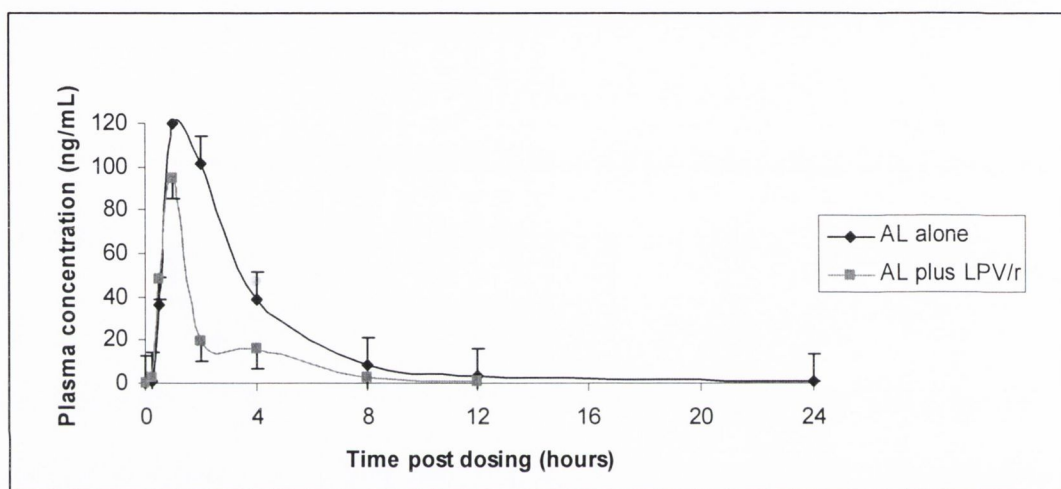
Variable	AL + LPV/r arm	AL arm	P value
	Mean (SE)	Mean (SE)	
Age (yrs)	38.2 (1.4)	34.5 (2.0)	0.1
Weight (kgs)	64.8 (3.1)	63.8 (2.6)	0.8
Height (cms)	166.3 (2.0)	160.1 (2.2)	0.05
BMI	23.6 (1.1)	25.1 (1.3)	0.3
Hb (mg/dl)	14.4 (0.2)	12.6 (0.5)	0.003
AST (ul)	25.7 (1.9)	30.3 (3.5)	0.2
ALT (ul)	16.5 (1.2)	35.6 (14.9)	0.1
QTc (ms)	418.2 (5.1)	411.5 (5.1)	0.3

CD4 count not included though advised

Effect of LPV/r on artemether and dihydroartemisinin

With the exception of T_{max} , all parameters for artemether were significantly different between the two study arms (Table 3.2). Artemether C_{max} and AUC were significantly reduced by 50% and 42% respectively, during co-administration with LPV/r. Pharmacokinetic profiles of artemether with and without LPV/r are shown in figure 3.1.

Figure 3.1. Mean (\pm SE) plasma concentration versus time of artemether with and without LPV/r



Vertical lines represent standard error

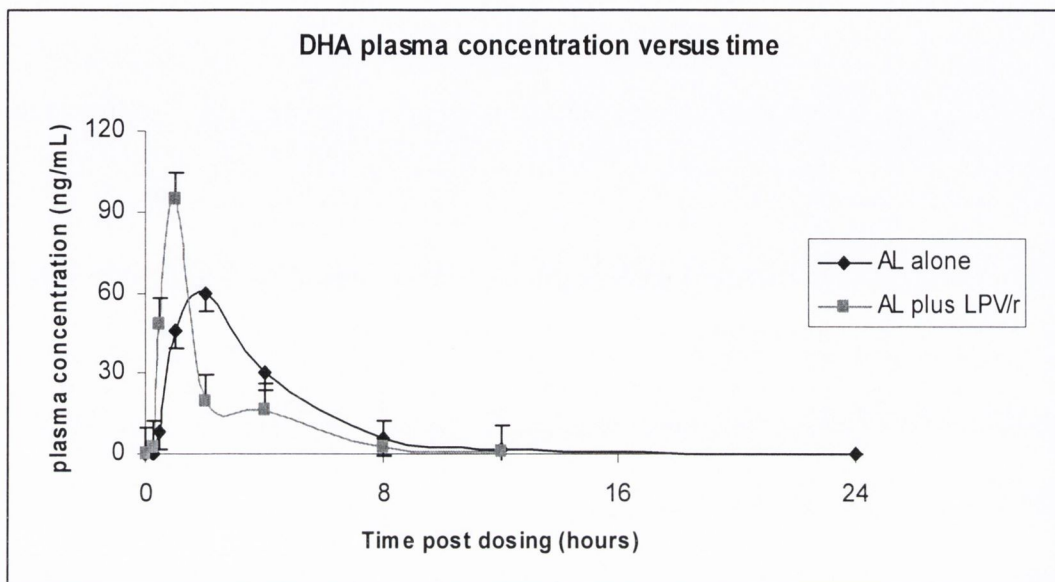
Artemether clearance and volume of distribution were significantly increased by 66% and 38% respectively during AL co-administration with LPV/r.

Parameters for dihydroartemisinin were not influenced by LPV/r co-administration (Table 3.2). Pharmacokinetic profiles of dihydroartemisinin with and without LPV/r are shown in figure 3.2.

Table 3.2. Artemether, dihydroartemisinin and lumefantrine pharmacokinetics with and without LPV/r

Parameter	AL (N= 13)	AL plus LPV/r (N=16)	p value
	Median (range)	Median (range)	
Artemether			
C _{max} (ng/mL)	112 (20 - 362)	56.7 (17 - 236)	0.03
T _{max} (hr)	1 (1 - 4)	2 (0.75 - 4)	0.38
CL/F (L/hr)	295 (69 - 817)	492 (129 -1805)	<0.01
V/F (L)	1072 (593 - 2651)	1487 (762 - 3485)	0.02
T _{1/2} (hr)	2.5 (1.2 - 5.9)	1.6 (0.9 - 6.6)	0.04
AUC _{0-last} (hr×ng/mL)	264 (92 - 1129)	151 (38 - 606)	<0.01
Dihydroartemisinin			
C _{max} (ng/mL)	66.9 (10 - 111)	73 (31 - 224)	0.55
T _{max} (hr)	2 (1 - 4)	2 (0.75 - 4.1)	0.89
CL/F (L/hr)	350 (210.27- 942.07)	424 (280 - 626)	0.23
V/F (L)	922.34 (498.77- 4779.16)	876 (734 - 1315)	1
T _{1/2} (hr)	1.8 (1.3 - 3.5)	1.51 (1.01- 2.69)	0.06
AUC _{0-last} (hr×ng/mL)	213 (68 - 343)	175 (118 - 262)	0.27
Lumefantrine			
T _{lag} (hr)	1 (0-4.03)	1 (0-1.02)	0.16
C _{max} (ng/mL)	2532 (1071 - 5957)	7097 (2396 - 9462)	<0.01
T _{max} (hr)	8 (3.9 - 12)	8 (4 - 12.03)	0.26
T _{1/2} (hr)	23.6 (6.25 - 51.6)	31.4 (24.2 - 43.7)	<0.01
AUC _{0-last} (hr×ng/mL)	41119 (12850 - 125200)	199678 (71205 - 251015)	<0.01

Figure 3.2. Mean (\pm SE) plasma concentration versus time of dihydroartemisinin with and without LPV/r

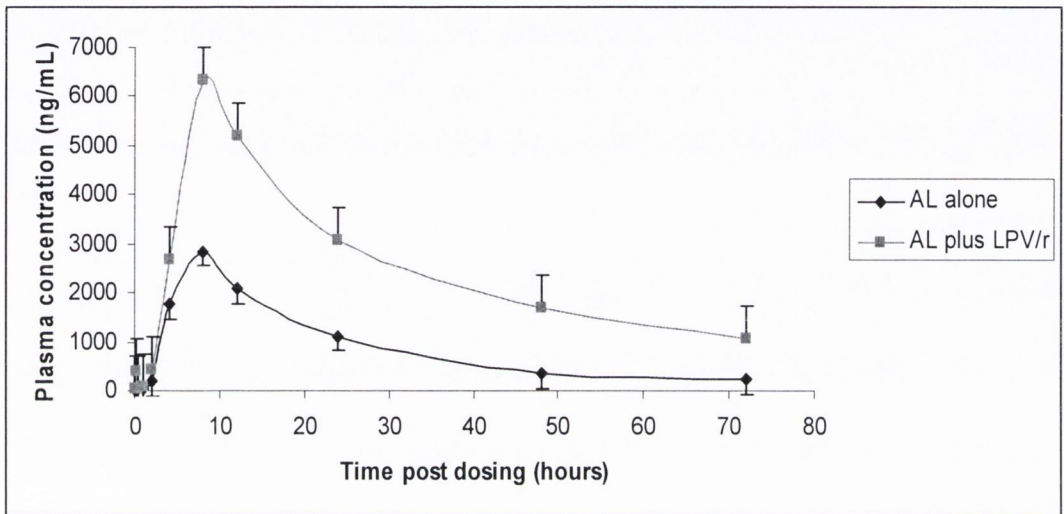


Vertical lines represent standard error

Effect of LPV/r on lumefantrine pharmacokinetics

Lumefantrine C_{\max} , AUC and $T_{1/2}$ were significantly increased while clearance and volume of distribution were significantly decreased during co-administration of AL with LPV/r (Table 3.2). Clearance was decreased by 82% and volume of distribution decreased by 54% during AL administration with LPV/r, correspondingly; lumefantrine C_{\max} was increased more than 2-fold while the AUC increased more than 4-fold during co-administration with LPV/r. Pharmacokinetic profiles of lumefantrine with and without LPV/r are shown in figure 3.3.

Figure 3.3. Mean (\pm SE) plasma concentration versus time of lumefantrine with and without LPV/r



Vertical lines represent standard error

Discussion

Co-administration of AL with LPV/r significantly increased artemether clearance with consequent significant reduction in artemether exposure. Dihydroartemisinin pharmacokinetic parameters were not affected by LPV/r. Lumefantrine clearance significantly decreased with consequent significant increase in exposure.

With the exception of dihydroartemisinin, data from our HIV-infected participants are similar to those from the only published study by German et al conducted among 13 healthy volunteers (141). The previous study demonstrated a trend towards decreased artemether exposure, significant reduction in dihydroartemisinin and significant increase in lumefantrine exposure following standard 6-dose AL administration with LPV/r (141). Inhibition of CYP3A4 is the most likely explanation for the increased lumefantrine exposure, given that lumefantrine metabolism is mainly by CYP3A4 which is inhibited by LPV/r. The reduction in artemether exposure is unexpected since CYP3A4 is said to be the predominant CYP enzyme in the metabolism of artemether (132). However other CYP enzymes including CYP2B6, 2C9, 2C19 and possibly 2A6 are involved in artemether metabolism (132) and LPV/r was shown to induce them (22). The observed decreased artemether exposure is likely due to induction of these enzymes by LPV/r.

We found non-significantly lower total dihydroartemisinin exposure with LPV/r co-administration. This contrasts with data by German et al and reasons for this are unclear. It may be due to the small numbers and large inter-individual variability. Dihydroartemisinin is converted to inactive metabolites via UGT1A1, 1A8/9 and 2B7. Induction and inhibition of UGTs by xenobiotics have been described previously and LPV/r was demonstrated to inhibit UGTs; 1A1, 1A3, 1A4, 1A6, 1A9 and 2B7 (76-77, 156). This however, does not provide an explanation for our findings.

Artemether and dihydroartemisinin are very potent antimalarial agents with very short half lives. Lumefantrine has a much longer $T_{1/2}$ and mainly clears residual parasites, preventing recrudescence (135). Higher artemether and dihydroartemisinin exposure decreases parasite clearance time, (137) but the major determinant of radical cure is lumefantrine exposure (157). Given that HIV-malaria co-infected patients present with higher parasite counts (91, 98) which is

an independent predictor of poor treatment response (120), reduction in artemether exposure may predispose patients to severe malaria due to slower parasite clearance. Our findings should be extrapolated to clinical relevance with caution given that we administered a single AL dose whereas a six-dose AL regimen is administered for malaria treatment. The reduction in artemether exposure by LPV/r after the single AL dose may be offset by the increase in lumefantrine exposure. However, rapid clearance of artemether and reduced clearance of lumefantrine may create pharmacokinetic mismatch possibly exposing parasites to lumefantrine monotherapy with risk for development of resistance.

The parallel study design was adequate for the objectives of this study but may be a limitation since it did not take the great inter-individual variability of artemether into account. However, comparison of pharmacokinetic exposures in the same individuals using the sequential design was not possible given that LPV/r is used for second-line HIV treatment in this study setting. Due to inadequate capacity we were unable to evaluate the effect of AL on LPV/r pharmacokinetics, however, in a previous study AL did not affect LPV/r pharmacokinetics (141).

In conclusion, co-administration of AL with LPV/r significantly reduces artemether exposure with increase in lumefantrine exposure. Reduction in artemether exposure may cause slower parasite clearance. High lumefantrine exposure may offer additional benefit, however, the reduced artemether exposure causes pharmacokinetic mismatch with exposure to lumefantrine monotherapy and risk for drug resistance. High lumefantrine concentrations may be associated with enhanced toxicity. In the next chapter we present data on the cardiac safety of co-administration of AL with LPV/r.

Chapter 4

Cardiac conduction safety during co-administration of artemether-lumefantrine and lopinavir/ritonavir in HIV-infected Ugandan adults

4.1	Introduction.....
4.2	Study objective
4.3	Materials and Methods.....
4.4	Results.....
4.5	Discussion.....

4.1 Introduction

Concerns over safety of LPV/r became more crucial following the FDA alert on LPV/r cardiotoxicity (158). Safety information on LPV/r includes warnings and precautions regarding QT/QTC interval and PR interval prolongation. According to the revised safety label, LPV/r prolongs the PR interval, and cases of second- or third-degree atrioventricular block have been reported in some patients. Indeed LPV/r should be used with caution in patients at increased risk of developing cardiac conduction abnormalities, such as those with underlying structural heart disease, preexisting conduction system abnormalities, ischemic heart disease, or cardiomyopathies (158).

The effect of co-administration of LPV/r with drugs that prolong the QTc and PR interval has not been determined and should be undertaken with caution. Clinical monitoring is recommended during co-administration of LPV/r with drugs metabolized by CYP3A (158) such as lumefantrine. Lumefantrine has some structural similarity to halofantrine which is cardiotoxic mainly causing QTc prolongation. Since lumefantrine has structural similarity with halofantrine, a drug associated with cardiac arrhythmias and sudden death (159-161) the effects of enhanced lumefantrine concentration resulting from drug interactions need to be investigated. Vigilant monitoring and evaluation of the effects of increased lumefantrine exposure on cardiac function is warranted. We co-administered a single dose of AL with LPV/r and monitored cardiac function for adverse effects. This study was part of the study presented in chapter 3.

.2 Study Objective

To assess the cardiac safety of co-administration of a single dose of AL (80/480mg) with LPV/r in HIV-positive Ugandan adults.

4.3 Materials and Methods

Study site, design and population

This study was performed as part of the study described in chapter 3, using the same participants with the same study drugs.

Study Procedures

Medical history, physical examination, vital signs, renal and liver function tests, blood smears for malaria parasites, ECGs and urine screens for pregnancy were performed at screening. On the study day, medical history, physical examination, vital signs examination and a blood smear for malaria parasites were repeated. We collected information on adverse drug events and serious adverse drug events. Standard 12-lead electrocardiograms (ECG) were recorded at screening, immediately prior to dosing (T=0hour), and continuously for 12 hours post dose of AL. Patients were discharged after 12 hours and returned for the following three mornings (T= 24, 48, 72 hour) for a single ECG tracing. QTc-intervals were calculated using the Bazett formula ($QTc = QT \div \sqrt{RR}$) to correct for the influence of heart rate. A senior cardiologist evaluated the PR, QRS and QT intervals visually on the ECG.

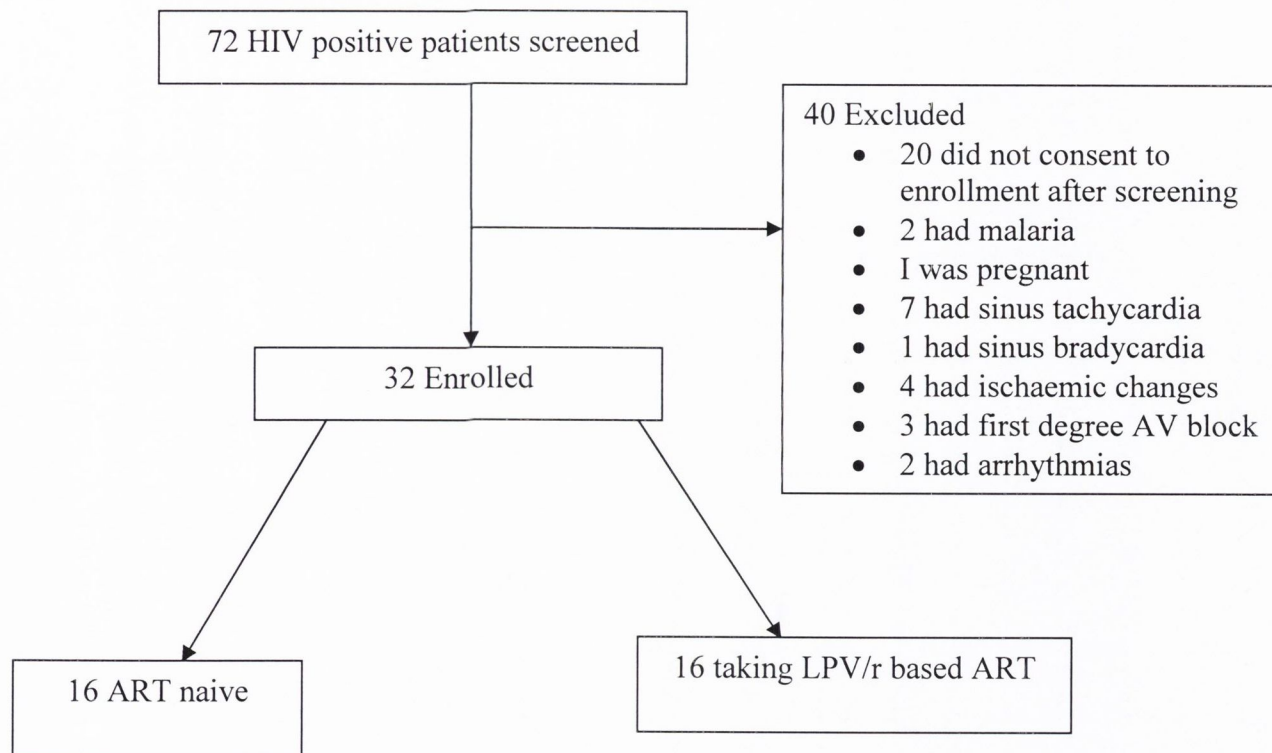
Statistical analysis

Data were analyzed using SPSS version 12.0 and STATA version 10.0. Continuous variables were summarized into means and medians. Means were compared using the Independent T-test. A p-value <0.05 was considered statistically significant.

4.4 Results

A total of 72 HIV-positive patients (41, 65% females) were screened between January and June 2009; 32 were enrolled with 16 in each arm. 40 (56%) were excluded as follows; 20 (28%) did not consent to enrollment after screening, 2 (3%) had malaria with a positive blood smear and received treatment with a complete dose of AL, 1 (1%) was pregnant, 7 (10%) had sinus tachycardia, 1 (1%) had sinus bradycardia, 4 (6%) had ischaemic changes on ECG, 3 (4%) had first degree AV block and 2 (3%) had arrhythmias (Figure 4.1).

Figure 4.1 Flow chart to show participants screened, excluded and enrolled



Baseline characteristics of study participants are presented in Table 3.1 in chapter 3. The QTc interval measured in milliseconds (ms) at baseline was higher for participants taking LPV/r although the difference was not statistically significant. There were no serious adverse events during the study period. ECG parameters (heart rate, PR-interval, QRS-complex and QTc) remained well within normal limits in both study arms. The mean QRS-complex and QTc interval post AL administration were higher in the LPV/r arm compared to the ART naïve arm (87.4 vs 82.8 ms, $p=0.06$ and 421 vs 404ms, $p= 0.03$, respectively) but the mean PR-interval was significantly higher in the ART naïve arm (154 vs 169ms, $p=0.02$) (Table 4.1).

Table 4.1. Mean ECG parameters in milliseconds post AL dosing

Variable	LPV/r arm	ART naive arm
	Mean (SD)	Mean (SD)
Heart rate	69 (8.1)	71 (5.9)
PR	154 (18.4)	169 (15.9)
QRS	87.4 (6.6)	82.8 (6.6)
QTc	421 (20.0)	404 (20.7)

Mean (SD) change in QTc interval values from the pre-AL QTc interval values was greater for the ART naïve arm compared to the LPV/r arm; 6.7 (15.4) vs -0.8 (13), $p= 0.17$. The QTc interval measurements for participants in both study arms remained within normal ranges over the 72 hour period (Table 4.2). The values for QTc at 24 hours and 72 hours were higher for participants taking LPV/r, however none were greater than the upper limit of normal (450ms for males and 470ms for females). These data were published (162).

Table 4.2. Median QTc interval measurements post AL dosing

Time	QTc (milliseconds)		p value
	Median (IQR)		
	LPV/r arm	ART naïve arm	
Screening	415 (403-439)	395 (388-425)	0.14
12hour	415 (404-439)	419 (403-427)	0.7
24 hour	424 (401-434)	406 (393-411)	0.02
48 hour	411 (396-432)	409 (401-419)	0.7
72 hour	424 (416-441)	408 (392-417)	0.004

4.5 Discussion

In this study, we found that HIV-positive patients taking LPV/r had a higher QTc interval prior to administration of AL compared to HIV-positive ART naïve patients, nevertheless, the difference was not statistically significant. It is possible that this could have been a result of the effects of LPV/r on the heart; however, we can not establish a causal relationship since we did not have QTc measurements for these patients prior to initiation of LPV/r. This however, raises concern especially in view of the recent FDA alert over the effects of LPV/r on the heart. Indeed the label for LPV/r includes warnings and precautions regarding QT/QTc interval and PR interval prolongation (158).

Although lumefantrine exposure was markedly elevated with LPV/r (chapter 3), all patients tolerated study drugs very well with no evidence of cardiac conduction abnormalities (162). Although the QTc interval for participants who took LPV/r with AL was significantly higher than that for participants who took AL without LPV/r at 72 hours post dosing, the difference could not be attributed to LPV/r because participants in the LPV/r arm had higher baseline QTc interval. The QTc interval values remained well within normal limits for participants in both study arms. It is possible that the increment in the QTc intervals could have been higher if patients had received the standard six-dose AL regimen. Previous studies found no changes in the QTc interval after a single dose of AL in healthy volunteers (163-164), however, these were conducted in patients with malaria without LPV/r. Since we do not know what levels and effects of lumefantrine would result if the standard six-dose AL regimen is co-administered with LPV/r in HIV-malaria co-infected patients, we suggest close clinical monitoring of patients during concomitant administration of LPV/r with AL until more data become available.

Chapter 5

Pharmacokinetics of artemether-lumefantrine and efavirenz when administered alone and in combination to HIV-infected Ugandan adults

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5.5	Discussion.....	129

5.1 Introduction

Efavirenz is a NNRTI, used as part of first-line ART regimens in Uganda. It is administered in an adult dose of 600mg daily and metabolized mainly by CYP2B6 with some involvement of 3A4, into hydroxylated inactive metabolites which undergo subsequent glucuronidation before elimination (15, 20, 165). In combination with 2 NRTIs efavirenz offers good clinical efficacy. It is the preferred NNRTI in combination with 2 NRTIs for ART naive patients in sub-Saharan Africa.

Efavirenz is also used for post exposure prophylaxis in combination with 2 NRTIs (20). For low risk exposure, a 2-drug combination which may be either zidovudine or tenofovir plus lamivudine or emtricitabine is used while a 3-drug combination made up of the 2 drugs above plus efavirenz or a protease inhibitor is recommended for high risk exposure. Due to potent induction of hepatic CYP3A4 by efavirenz (21, 166-167) clinically relevant CYP mediated drug-drug interactions occur when it is co-administered with CYP substrates such as rifampicin. Co-administration with rifampicin decreases efavirenz plasma concentration (114). Efavirenz decreases rifabutin plasma concentration (168). Clarithromycin's AUC and C_{max} decrease by 39% and 26% respectively, when co-administered with efavirenz (169). Efavirenz significantly decreased methadone concentrations resulting in manifestations of opiate withdrawal warranting an increase in the maintenance dose of methadone (170-171). Anticonvulsant agents such as carbamazepine, phenobarbitol and phenytoin, oral contraceptives, St. John's wort (*Hypericum perforatum*) and midazolam interact with efavirenz (20, 172). Efavirenz significantly decreases plasma concentrations of atazanavir, fosamprenavir, indinavir, lopinavir nelfinavir, and saquinavir such that dose adjustment, boosting with ritonavir and close monitoring are required when co-administered (20, 173-174).

Both artemether and lumefantrine are metabolized via cytochrome (CYP) enzymes, predominantly CYP3A4 (132, 135). The key pharmacokinetic determinant of cure is the area under the concentration time curve (AUC) of lumefantrine (137). In multidrug-resistant areas, day-seven lumefantrine concentration is a surrogate marker for AUC and a threshold venous plasma

concentration of 280ng/mL predicts treatment failure (137, 139). More recently this threshold has been reduced to 175ng/mL (157).

Since efavirenz is a potent inducer of CYP3A4 and 2B6, co-administration with AL has potential for drug interactions (83, 175-176). We hypothesized that co-administration of AL with efavirenz reduces artemether and lumefantrine exposure. We compared pharmacokinetics of AL and efavirenz when administered alone and in combination to HIV-infected adults.

5.2 Study objective

To compare the pharmacokinetic parameters of artemether, dihydroartemisinin, lumefantrine and efavirenz when AL and efavirenz are administered alone and in combination to HIV-infected patients.

5.3 Materials and Methods

Study participants

Twenty-seven participants provided 80% power to reject the null hypothesis that lumefantrine AUC estimated during administration of AL without efavirenz is equivalent to lumefantrine AUC during administration of AL with efavirenz. We anticipated a 10% drop out rate so enrolled 30 participants.

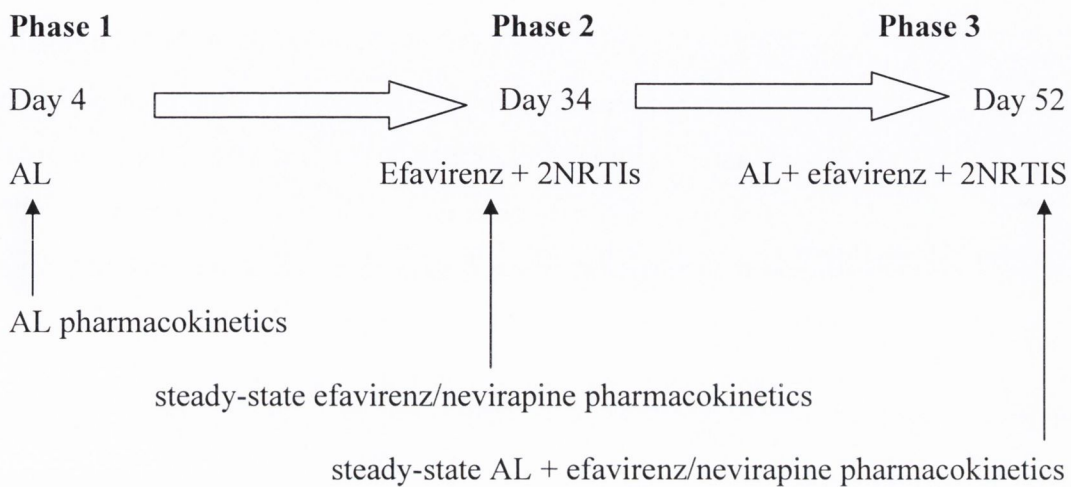
Participants were screened and enrolled consecutively from the cohort of patients attending the IDI clinic. Individuals were eligible to participate if they were HIV-infected and older than 18 years of age. Patients with liver and renal function test parameters greater than 3 and 1.5 times the upper limit of normal respectively, hemoglobin less than 8mg/dl, abnormal cardiac function and positive blood smears for malaria, pregnant mothers and those taking known inhibitors or inducers of CYP enzymes or any herbal medications were excluded. All participants received cotrimoxazole daily for prophylaxis against opportunistic infections.

Study design and procedures

We performed a one-sequence cross-over intensive pharmacokinetic study. Participants had detailed explanation of study procedures at enrolment. All participants received a standard 6-dose AL regimen (Coartem®, Novartis Pharma

AG, Basel, Switzerland) prior to and at efavirenz steady-state. Each AL dose consisted of 4 tablets, each tablet containing 20mg artemether and 120mg lumefantrine (total 80mg artemether/480mg lumefantrine). Study procedures were carefully explained to participants with clear instructions not to take any medication not prescribed by the study physicians. Participants were encouraged to come back to the clinic on their appointment days as well as any other time they felt unwell. Pharmacokinetic sampling was performed in three phases as shown in the study scheme in figure 5.1.

Figure 5.1. Study 3 scheme



In phase 1, participants received AL (Coartem®, Novartis Pharma AG, Basel, Switzerland) and sampling was performed for artemether, dihydroartemisinin and lumefantrine plasma concentration measurement. In phase 2, participants were initiated on efavirenz at a dose of 600mg daily in combination with 2NRTIs; zidovudine plus lamivudine or tenofovir disoproxil fumarate plus emtricitabine. When participants had achieved ART steady-state (after 1 month) blood sampling was performed for efavirenz plasma concentration measurement.

In phase 3, participants received both six-dose AL and efavirenz based ART and samples were drawn for artemether, dihydroartemisinin, lumefantrine and efavirenz concentration measurement.

Participants were reminded to take their study drugs by telephone. Adherence to study drugs was assessed using self report and pill count and information on adverse and serious adverse effects was collected. On the evening prior to pharmacokinetic sampling, participants were reminded of their study day appointment and given detailed instructions to eat food, administer their medication by 8.00 pm and arrive at the hospital by 7.00am the next morning in fasting state.

On the study day, patients were admitted in fasting state, an indwelling IV cannula was inserted following aseptic technique and blood samples were drawn for determination of pre-dose concentrations of study drugs. The intake of a standardized breakfast and morning doses of study drugs was directly observed by study staff. Breakfast consisted of unleavened flat bread (chapatti), stuffed pastry (samosa), bread with margarine and tea with milk. Blood sampling was performed at scheduled time points. Four mLs of blood was collected per sampling time in lithium-heparin tubes. Samples were centrifuged immediately at 3000 revolutions per minute for 10 minutes and the separated plasma was stored at -80 degrees centigrade until estimation of drug concentrations.

Efavirenz concentration measurement

Efavirenz plasma concentration measurement was performed at the Department of Pharmacology and Therapeutics, Makerere University College of Health Sciences. Concentration was determined by reverse-phase HPLC with UV detection using a previously validated method. The mobile phase consisted of

30% acetonitrile, 30% methanol, 4 mmol l⁻¹ potassium hydroxide and 10 mmol l⁻¹ acetic acid (pH 4.3).

Plasma proteins were precipitated with acetonitrile before centrifuging. Supernatant (6 µl) was injected and eluted at 0.80 ml min⁻¹ for 3.5 min. The retention time for efavirenz was 2.42 min as detected at UV-VIS 1, 210 nm, UV-VIS 2, 220 nm. This method was linear, with a within-day coefficient of variation of 3.2, 3.3 and 5.1% at concentrations of 2.0 µM (*n*= 17), 8.0 µM (*n*= 17), and 20 µM (*n*= 16), respectively, and a between-day coefficient of variation of 4.1% (*n*= 50). The LLOQ for the method was set at 0.35 µM.

Artemether, dihydroartemisinin and lumefantrine concentration measurement

Artemether, dihydroartemisinin and lumefantrine concentrations were measured at the Clinical Pharmacology Laboratory, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand as described in chapter 3.

Statistical analysis

Baseline characteristics were summarized into medians with interquartile range (IQR) and pharmacokinetic parameters into medians with range. Comparison of pharmacokinetic parameters was made with the Wilcoxon matched-pairs signed-rank test. A *p*-value <0.05 was considered statistically significant. Comparisons between treatments were made using individual ratios of parameters calculated for each participant by dividing AL parameters obtained during co-administration of AL with efavirenz to parameters obtained during AL administration as reference. Similarly for efavirenz, individual ratios were calculated by dividing efavirenz parameters obtained during co-administration of efavirenz with AL to parameters obtained during efavirenz administration as reference. Individual ratios were calculated for patients with parameters from both phases and summarized into medians with range.

5.4 Results

Pharmacokinetic data were available for all the 30 participants; (20, 66% female). Participants' characteristics are shown in table 5.1.

Table 5.1. Baseline characteristics of study participants

Parameter	(N=30)
Females n (%)	20 (66)
Age (years)*	38 (33.7 – 43.0)
Weight (kgs)*	62.5 (55.0 – 68.2)
Height (cm)*	160.4 (154.6 – 168.5)
BMI*	23.0 (20.6 – 25.8)
CD4 (cells/uL)	158 (76 – 256)

**Presented as median (IQR)*

In phase 1; artemether pharmacokinetic parameters were calculated for 29 of 30 participants and dihydroartemisinin parameters for all 30 participants. One participant whose artemether pharmacokinetic parameters were not calculated had artemether plasma concentration quantified 4 hours post dosing with the next concentrations less than the LLOQ while dihydroartemisinin was measured all through the sampling time.

In phase 3; artemether parameters were calculated for 22 of 30 and dihydroartemisinin parameters for 25 of 30 participants. Of the participants whose artemether and dihydroartemisinin parameters were not calculated; two had artemether below LLOQ and one of these had dihydroartemisinin below LLOQ throughout the sampling period. Six participants had very low artemether concentrations at 2 and 4 hours post dosing after which levels fell less than LOD. Four of these had very low dihydroartemisinin quantified once post dosing. Lumefantrine parameters were calculated for all 30 participants in both phases.

Effect of efavirenz arm on artemether, dihydroartemisinin and lumefantrine pharmacokinetics

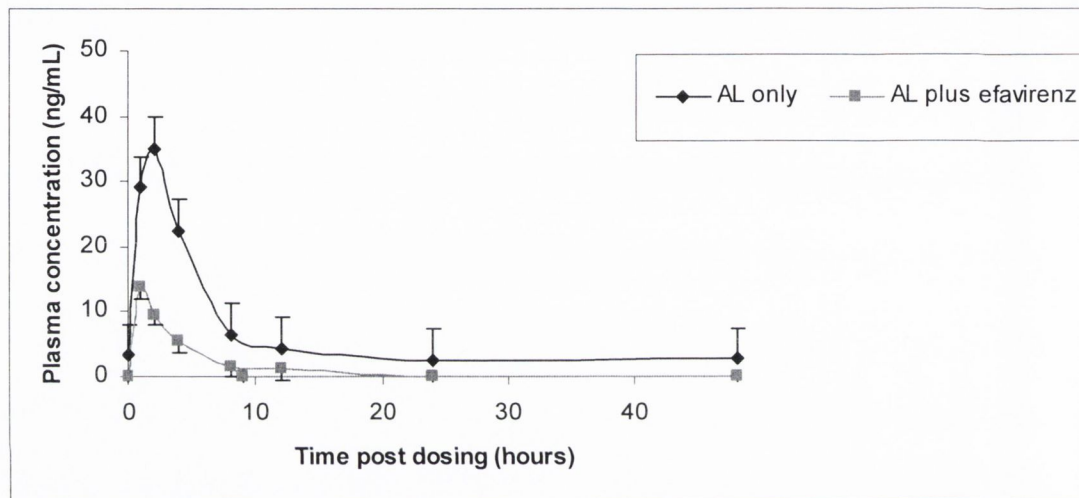
Pharmacokinetic exposure of artemether, dihydroartemisinin and lumefantrine was significantly reduced during co-administration of AL with efavirenz (Table 5.2)

Table 5.2. Comparison of pharmacokinetics of artemether, dihydroartemisinin and lumefantrine with and without efavirenz

Parameter	Median (range)	Median (range)	Median (range) of Individual Ratio	p value
	AL	AL plus efavirenz		
Artemether	(N = 22)	(N = 22)		
C _{max} (ng/mL)	29 (10 - 247)	12 (2 - 88)	0.2 (0.03 - 2.6)	<0.01
T _{max} (hr)	2 (1 - 4)	1 (1 - 4)	0.9 (0.2 - 2)	0.05
CL/F (L/hr)	591 (80 - 2273)	2558 (414 - 9960)	3.1 (0.4 -35)	<0.01
V/F (L)	4523 (374 - 10402)	4715 (1078 - 28925)	1.6 (0.2 -20)	0.02
T _{1/2} (hr)	4 (1 - 24)	1.8 (0.6 - 4)	0.5 (0.07 - 3.1)	<0.01
AUC _{0-last} (hr×ng/mL)	119 (26 - 917)	25 (5 - 185)	0.17 (0.03 - 2.3)	<0.01
AUC _{0-∞} (hr×ng/mL)	135 (35 - 997)	31 (8 - 192)	0.32 (0.03 - 2.1)	<0.01
Dihydroartemisinin	(N = 22)	(N = 22)		
C _{max} (ng/mL)	120 (39 - 230)	26 (4 - 114)	0.27 (0.05 - 0.9)	<0.01
T _{max} (hr)	2 (1 - 4)	2 (1 - 4)	1 (0.25 - 3.9)	0.78
CL/F (L/hr)	216 (82 - 382)	844 (234 - 5704)	3.6 (1.2 - 19.2)	<0.01
V/F (L)	754 (212 - 1494)	2082 (608 - 14013)	2.6 (1.2 - 18.6)	<0.01
T _{1/2} (hr)	2 (1 - 5)	1 (0.8 - 3)	0.6 (0.3 - 1.1)	<0.01
AUC _{0-last} (hr×ng/mL)	341 (187 - 908)	84 (8 - 321)	0.2 (0.03 - 0.8)	<0.01
AUC _{0-∞} (hr×ng/mL)	352 (199 - 921)	90 (13 - 325)	0.2 (0.05 - 0.8)	<0.01
Lumefantrine	(N = 30)	(N = 30)		
C _{max} (ng/mL)	8737 (4073 - 20470)	6331 (2996 - 16576)	0.7 (0.2 - 1.7)	0.03
	280370 (105127 - 774338)	124381 (26992 - 309305)		<0.01
AUC _{0-last} (hr×ug/mL)			0.4 (0.07 - 1.3)	

Artemether CL/F and V/F significantly increased (591 vs 2558L/hr, $p<0.01$ and 4523 vs 4715L, $p=0.02$, respectively) with consequent significant reduction in the C_{max} and AUC by 58% (29 vs 12ng/mL, $p<0.01$) and 77% (119 vs 25hr \times ng/mL, $p<0.01$) respectively when co-administered with efavirenz. Pharmacokinetic profiles of artemether with and without efavirenz are shown in figure 5.2.

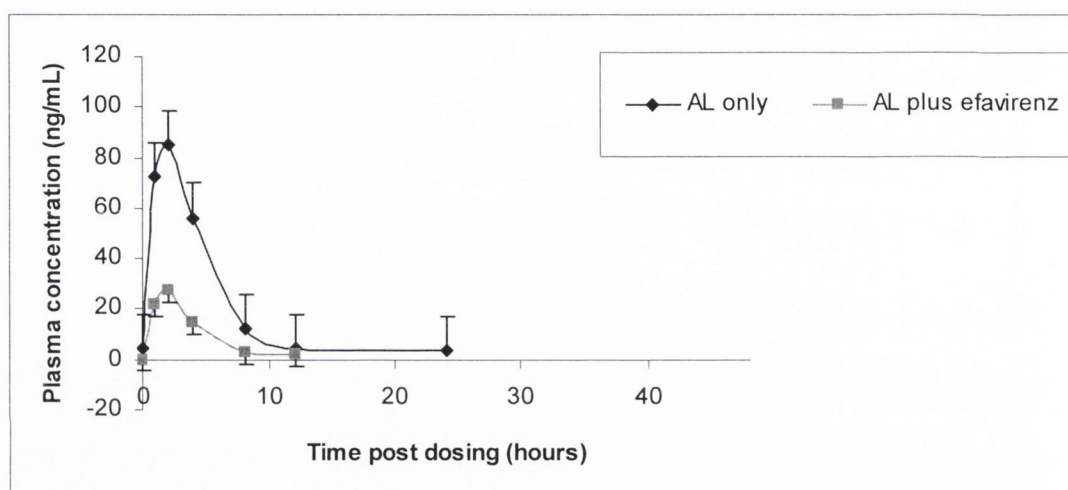
Figure 5.2. Mean (\pm SE) plasma concentration versus time of artemether with and without efavirenz



Vertical lines represent standard error

Dihydroartemisinin CL/F and V/F significantly increased (216 vs 844L/hr, $p<0.01$) and (754 vs 2082L, $p<0.01$, respectively) while the C_{\max} and AUC significantly reduced by 94% (120 vs 26ng/mL, $p<0.01$) and 75% (341 vs 84hr \times ng/mL, $p<0.01$) respectively during AL co-administration with efavirenz. Pharmacokinetic profiles of dihydroartemisinin with and without efavirenz are shown in figure 5.3.

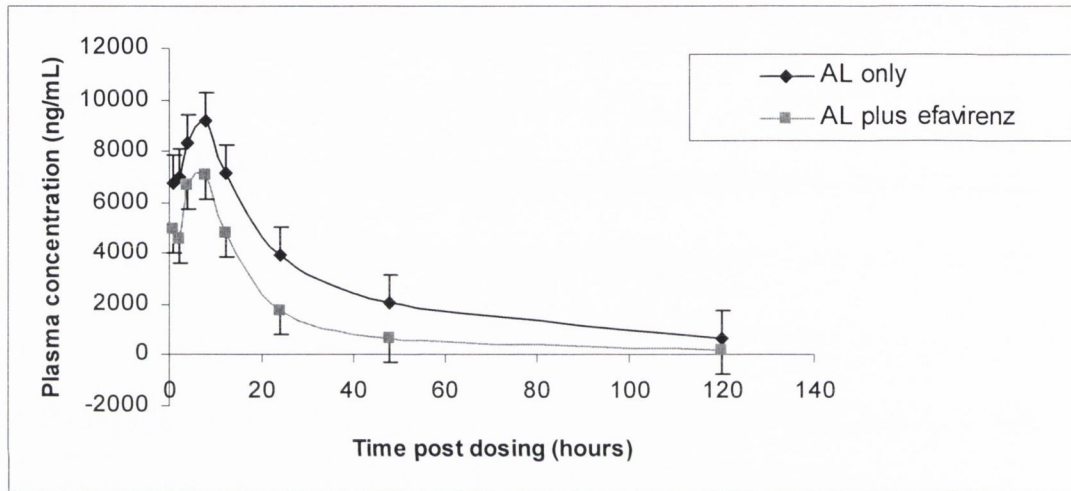
Figure 5.3. Mean (\pm SE) plasma concentration versus time of dihydroartemisinin with and without efavirenz



Vertical lines represent standard error

Lumefantrine C_{\max} and AUC were significantly reduced by 25% (8737 vs 6331 ng/mL, $p=0.03$) and 55% (280370 vs 124381 hr \times ng/mL $p<0.01$) respectively with efavirenz co-administration. Pharmacokinetic profiles of lumefantrine with and without efavirenz are shown in figure 5.4.

Figure 5.4. Mean (\pm SE) plasma concentration versus time of lumefantrine with and without efavirenz



Vertical lines represent standard error

Day 7 concentrations were significantly lower during co-administration with efavirenz, during which mean (range) was 204 (35 – 686) compared to 684 (249 – 1969) ng/mL without efavirenz, $p < 0.01$. Two of 30 (7%) participants had day 7 lumefantrine concentration less than 280ng/mL without efavirenz compared to 21 out of 30 (70%) with efavirenz. Using the 175ng/mL day 7 threshold; no participant had less than this threshold when AL was taken without efavirenz compared to 17 out of 30 (57%) when AL was taken with efavirenz.

Efavirenz pharmacokinetic parameters

Efavirenz C_{max} and AUC were not affected by AL co-administration (3.77 vs 4.02 ug/mL, $p=0.7$ and 1.99 vs 2.07hr \times ug/mL, $p=0.7$) respectively. Median (IQR) efavirenz C_{trough} was not affected by co-administration with AL (4.2 (1.3 – 4.2) vs 3.8 (2.1 – 4.6) ug/mL, $p = 0.7$).

5.5 Discussion

In this study we investigated interactions between AL and efavirenz in HIV-infected Ugandan adults. Co-administration of AL with efavirenz significantly reduced artemether, dihydroartemisinin and lumefantrine exposure. The reduction in artemether, dihydroartemisinin and lumefantrine exposure is most likely due to induction of CYP3A4 and 2B6 by efavirenz (14, 21, 176). Mechanisms of induction involve both decreased enzyme degradation and enhanced protein synthesis by increasing transcriptional activation of messenger ribonucleic acid. The hPXR and hCAR regulate expression of CYP3A4 and 2B6 genes. Transactivation of these receptors leads to upregulation of CYP3A4 and 2B6 activity. Efavirenz activates the hPXR and hCAR, markedly increasing CYP3A4 and 2B6 functional activity (76, 167). Efavirenz has mixed effects on CYP3A4, with inhibition during acute and induction during chronic exposure (176). Induction is more likely in this study given that we measured concentrations at steady-state. Activation of the hPXR and hCAR can induce specific UGT1A isoforms,(77) which likely explains the reduction in dihydroartemisinin exposure. Our data implies that reduced AL exposure due to these drug interactions may predispose to decreased parasite clearance, slower symptom resolution and treatment failure with risk for development of drug resistance. Extrapolation of

data from our study to clinical relevance should be performed in consideration of changes in drug bioavailability during acute disease states. Previous studies demonstrated low bioavailability of AL with acute malaria and higher concentrations during recovery from malaria (136, 177), due to improvement in food intake and changes in volume of distribution. Population pharmacokinetic studies would have yielded information from the target HIV-malaria co-infected population, however, the cross-over study design which was most suitable for our research question and for minimizing inter-individual variability was not feasible with the population pharmacokinetic studies in HIV-malaria co-infected patients. Although drug metabolism may be influenced by presence or absence of disease, the effect is unlikely to differ in HIV-malaria co-infected patients. More so, HIV-malaria co-infected patients are more likely to present with more severe disease and less likely to return to normal food intake early. In conclusion efavirenz significantly reduced AL exposure which is likely to result in antimalarial treatment failure.

An important question may arise on the ethical considerations made in the design of this study. Using the sequential study design meant that participants had a period of time during which they did not receive ART. These participants were ART naïve at the start of the study although were eligible to receive ART. As occurs in many health facilities in Uganda, not all HIV-infected individuals who need ART are receiving it due to several reasons such as drug stock outs, poor health seeking behavior etc. All study participants were eligible to start ART; however had not initiated due to inadequate ART stock at the time. Enrollment in the study was voluntary and the study provided participants with an opportunity to access ART. The study team provided adequate pre-ART counseling, and consenting participants were initiated on ART. Therapy continued as recommended post study completion. In the next chapter we present data on AL interactions with nevirapine, a study performed in the same study setting with the same study design.

Chapter 6

Pharmacokinetics of artemether-lumefantrine and nevirapine when administered alone and in combination to HIV-infected Ugandan adults

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6.1 Introduction

Nevirapine is a NNRTI prescribed widely for treatment of HIV-infected ART naïve patients. In combination with 2 NRTIs, nevirapine offers good efficacy in suppression of HIV. In addition nevirapine plays a significant role in prevention of mother to child HIV transmission. Given that efavirenz is not prescribed to pregnant mothers in the first trimester as well as women with pregnancy intentions, nevirapine is preferentially prescribed for these populations.

Due to the potent induction of CYP3A4 and 2B6, nevirapine demonstrates pharmacokinetic interactions with CYP substrates (14). Studies demonstrated clinically significant drug interactions when nevirapine was co-administered with methadone (170, 178). Women using oral contraceptives may require alternate methods of birth control when receiving nevirapine due to reduced concentrations of the contraceptives (179). Initiation of nevirapine in HIV-tuberculosis co-infected patients receiving rifampicin led to sub-therapeutic nevirapine concentrations on day 21 after either the nevirapine dose escalation regimen or the 200mg twice daily regimen (180) and co-administration with rifampicin led to marked reduction in nevirapine trough concentrations (181).

The populations in whom nevirapine will most likely be prescribed are also the same populations at higher risk for malaria infections such as very young children and pregnant mothers. Given the widespread availability of ACTs, co-administration of nevirapine with AL is highly likely. However there is very scanty data on the pharmacokinetic interactions that may occur when nevirapine is co-administered with AL. Data is limited to one study among HIV-infected participants without malaria, that demonstrated significantly increased lumefantrine exposure when AL was co-administered with nevirapine (142). We investigated the pharmacokinetics of artemether, dihydroartemisinin, lumefantrine and nevirapine when AL and nevirapine were administered alone or in combination.

6.2 Study objective

To compare the pharmacokinetics of artemether, dihydroartemisinin, lumefantrine and nevirapine administered alone or in combination to HIV-infected adults.

6.3 Materials and Methods

Study design

The study design, participant selection and procedures were performed as described in chapter 5. Participants received nevirapine instead of efavirenz at a dose of 200mg once daily for the first 2 weeks then 200mg twice daily thereafter in combination with 2NRTIs. Pharmacokinetic sampling was performed as in chapter 5. Artemether, dihydroartemisinin and lumefantrine concentrations were measured at the Clinical Pharmacology Laboratory, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand as described in chapter 3.

Nevirapine plasma concentration measurement

Nevirapine concentrations were measured using reversed-phase HPLC with UV detection at MU-JHU Research Laboratory, at IDI, using a validated method developed at the University of Liverpool (182). The LLOQ was 450ng/mL. Inter-assay and intra-assay CV were 8.2% and 6.1%, respectively.

Statistical analyses were performed as described in chapter 5.

6.4 Results

A total of 30 participants were enrolled; one was discontinued due to non compliance to study procedures and one developed severe immune reconstitution inflammatory syndrome with tuberculosis following ART initiation and died. Participants' characteristics are shown in table 6.1.

Table 6.1. Baseline characteristics of study participants

Parameter	(N=28)
Females n (%)	27 (96)
Age (years)*	33.5 (28.0 – 36.0)
Weight (kgs)*	54.5 (48.0 – 62.0)
Height (cm)*	156.9 (151.0 – 159.0)
BMI*	21.9 (19.6 – 26.3).
CD4 (cells/uL)	195 (41 – 269)

**Presented as median (IQR)*

Pharmacokinetic data were available for 28 participants (27, 96% female). In phase 1; artemether and dihydroartemisinin parameters were calculated for all the 28 participants. In phase 3; artemether parameters were calculated for 21 of 28 participants and dihydroartemisinin parameters for all 28 participants. Lumefantrine parameters were calculated for all 28 participants in both phases. Among the participants whose parameters were not calculated, five participants had artemether quantified for 4 hours post dosing after which concentrations were less than LLOQ. Two participants had artemether less than LLOQ throughout the sampling period.

Effect of nevirapine on artemether, dihydroartemisinin and lumefantrine pharmacokinetics

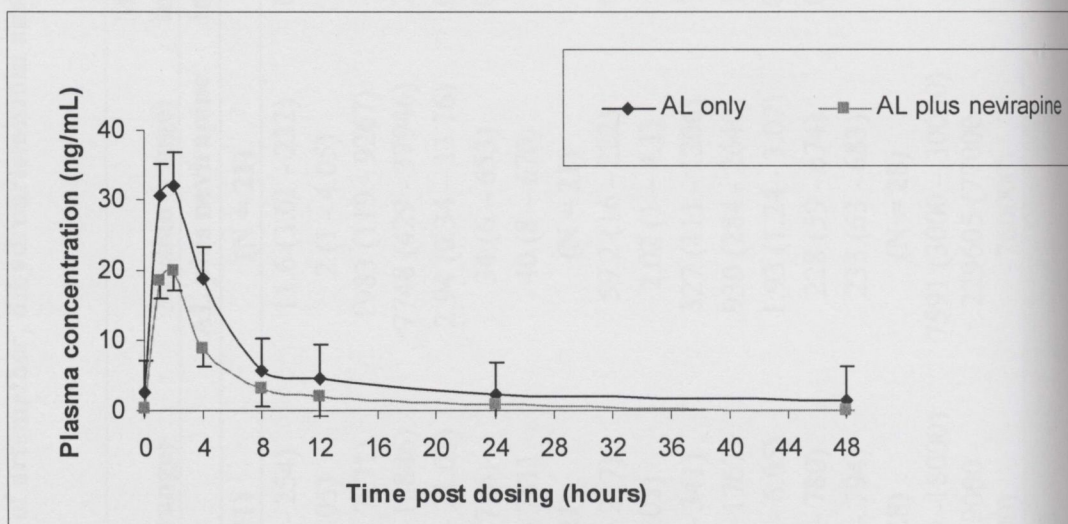
The pharmacokinetic parameters of artemether and dihydroartemisinin were significantly affected by co-administration of AL with nevirapine while lumefantrine parameters remained unaffected (Table 6.2).

Table 6.2. Pharmacokinetic parameters of artemether, dihydroartemisinin and lumefantrine with and without nevirapine

Parameter	Median (range)	Median (range)	Median (range) of	p value
	AL	AL plus nevirapine	Individual Ratio	
	AL	AL plus nevirapine	Individual ratio	
Artemether	(N = 21)	(N = 21)		
C _{max} (ng/mL)	28.5 (3.4 - 254)	11.6 (3.01 - 232)	0.32 (0.04 - 2.71)	<0.01
T _{max} (hr)	2 (1 - 4.05)	2 (1 - 4.05)	1 (0.25 - 4.05)	0.2
CL/F (L/hr)	601 (102 - 7271)	1983 (119 - 9267)	3.57 (0.64 - 20)	<0.01
V/F (L)	4095 (866 - 18886)	7748 (429 - 37946)	2.07 (0.24 - 9.66)	<0.01
T _{1/2} (hr)	4.08 (1.26 - 21.03)	2.94 (0.34 - 13.76)	0.58 (0.13 - 2.02)	0.04
AUC _{0-last} (hr×ng/mL)	123 (7 - 756)	34 (6 - 653)	0.24 (0.04 - 1.58)	<0.01
AUC _{0-∞} (hr×ng/mL)	133 (11 - 781)	40 (8 - 670)	0.28 (0.05-1.56)	<0.01
Dihydroartemisinin	(N = 21)	(N = 21)		
C _{max} (ng/mL)	107 (55 - 217)	59.2 (16 - 222)	0.57 (0.21 - 1.57)	<0.01
T _{max} (hr)	2 (1 - 4.05)	2.02 (1 - 4.1)	1 (0.25 - 2.03)	0.7
CL/F (L/hr)	201 (96 - 341)	327 (111 - 1206)	1.66 (0.66 - 3.98)	<0.01
V/F (L)	750 (220 - 1767)	930 (284 - 2640)	1.28 (0.33 - 3.25)	0.02
T _{1/2} (hr)	2.22 (1.49 - 6.05)	1.93 (1.24 - 3.07)	0.72 (0.33 - 1.44)	<0.01
AUC _{0-last} (hr×ng/mL)	364 (216 - 780)	228 (59 - 674)	0.59 (0.25 - 1.56)	<0.01
AUC _{0-∞} (hr×ng/mL)	379 (224 - 794)	233 (63 - 683)	0.6 (0.25-1.53)	<0.01
Lumefantrine	(N = 28)	(N = 28)		
C _{max} (ng/mL)	10000 (2000 - 18000)	7591 (3000 - 30000)	1.05 (0.4-1.9)	0.6
AUC _{0-last} (hr×ng/mL)	291671 (79000 - 699000)	229605 (77000- 760000)	0.89 (0.31-2.13)	0.4

Artemether CL/F and V/F increased (601 vs 1983L/hr, $p<0.01$) and (4095 vs 7748L, $p<0.01$) while C_{\max} and AUC reduced by 59% (28.5 vs 11.6ng/mL, $p<0.01$) and 72% (123 vs 34hr \times ng/mL $p<0.01$) during co-administration with nevirapine. Pharmacokinetic profiles of artemether with and without nevirapine are shown in in figure 6.1.

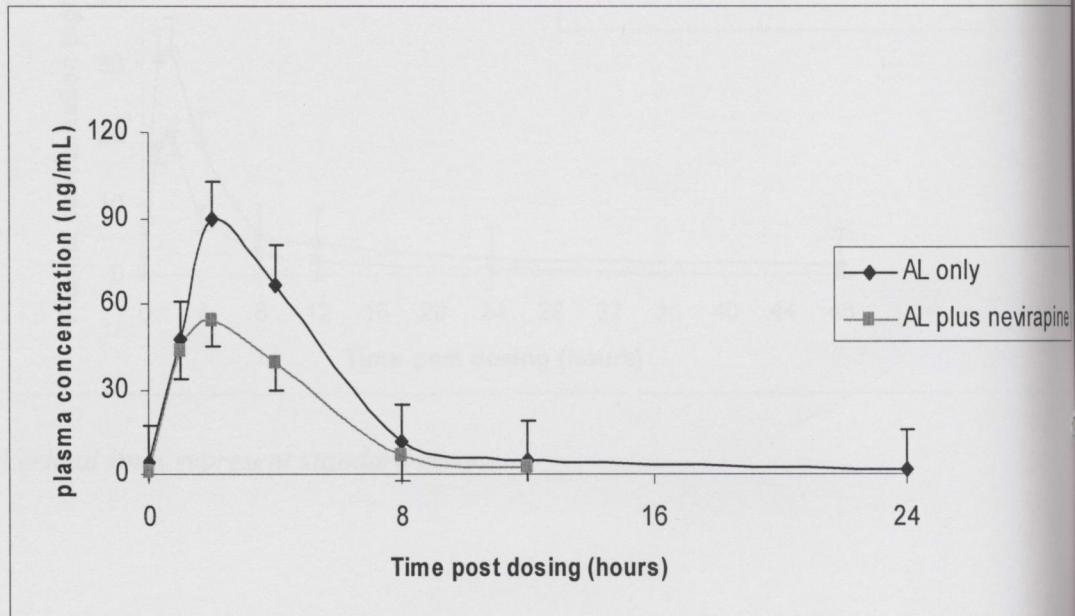
Figure 6.1. Mean (\pm SE) plasma concentration versus time of artemether with and without nevirapine



Vertical lines represent standard error

Dihydroartemisinin CL/F and V/F increased (median: 201 vs 327 L/hr, $p<0.01$) and (750 vs 930 L $p=0.02$) while C_{\max} and AUC reduced by 44% (107 vs 59.2ng/mL $p<0.01$) and 37% (364 vs 228 hr \times ng/mL $p<0.01$) during co-administration with nevirapine. Pharmacokinetic profiles of dihydroartemisinin with and without nevirapine are shown in in figure 6.2.

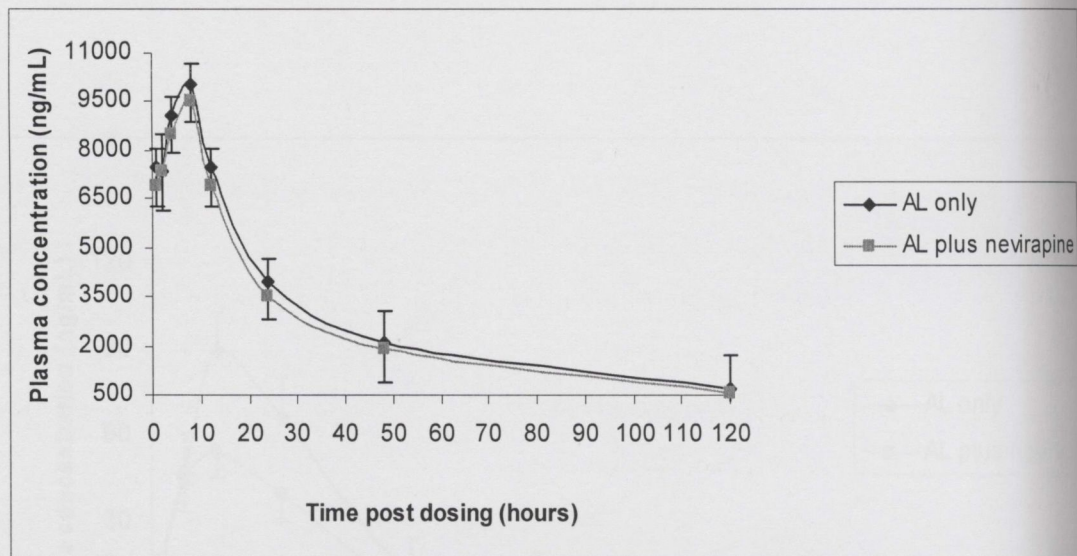
Figure 6.2. Mean (\pm SE) plasma concentration versus time of dihydroartemisinin with and without nevirapine



Vertical lines represent standard error

Lumefantrine C_{\max} and AUC were unaffected by nevirapine (median 10000 vs 7591ng/mL, $p=0.6$ and 291671 vs 229605hr \times ng/mL $p=0.4$) Pharmacokinetic profiles of lumefantrine with and without nevirapine are shown in in figure 6.3.

Figure 6.3. Mean (\pm SE) plasma concentration versus time of lumefantrine with and without nevirapine



Vertical lines represent standard error

There was no difference in day-seven lumefantrine concentrations with and without nevirapine for which the median (IQR) was 453 (252 – 646) compared to 607 (385 – 842) ng/mL, $p=0.1$. Two of 28 (7%) participants had day-seven lumefantrine concentration less than the threshold of 280ng/mL without nevirapine compared to 8 of 28 (28%) with nevirapine. Using the 175ng/mL day-seven threshold; one participant had less than the threshold without nevirapine compared to none with nevirapine.

Nevirapine pharmacokinetics

Nevirapine exposure was reduced during co-administration with AL. Nevirapine C_{max} and AUC reduced by 42% (8620 vs 4958ng/ml, $p<0.01$) and 46% (66329 vs 35728hr \times ng/mL, $p<0.01$). Median (IQR) nevirapine C_{trough} was reduced during co-administration with AL (6406 (3364 – 8455) vs 4382 (2807 – 6188)ng/ml, $p = 0.026$). Two of 28 participants (7%) compared to 7 of 28 (25%) had nevirapine C_{trough} below the MEC of 3000 ng/ml during co-administration of nevirapine without and with AL respectively.

6.5 Discussion

In this study, co-administration of AL with nevirapine, significantly reduced artemether and dihydroartemisinin exposure. Total lumefantrine exposure was non-significantly reduced possibly due to small numbers. The reduction in artemether and lumefantrine exposure is most likely due to induction of CYP3A4 and 2B6 by nevirapine. Like efavirenz, nevirapine activates the hPXR and hCAR, markedly increasing CYP3A4 and 2B6 functional activity (76, 167). The reduction in dihydroartemisinin exposure is likely due to activation of the hPXR and hCAR inducing specific UGT1A isoforms.

Our data contrasts with data from the South-African study in which co-administration of AL with nevirapine resulted in elevated lumefantrine concentration (142). Drug bioavailability varies with factors such as differing genetics, disease and concomitant medication as discussed in chapter one (183) which could explain the differences between the two study findings. Another plausible explanation for the different findings is the difference in study designs. The South African study was performed using a parallel study design which is

prone to bias from inter-individual variability. The cross-over study design facilitated comparison using patients as self-matched controls minimizing inter-individual variability. We further minimized pharmacokinetic variability with restrictive eligibility criteria. The predominantly female study population reflects both the demographics of the HIV/AIDS pandemic and use of nevirapine as first-line treatment for women of child-bearing age. It is unlikely that this influenced our results.

We minimized errors by training study staff, ensuring food intake before AL administration as recommended by the manufacturer (132), providing adequate fat to enhance AL absorption, use of SOPs for all study procedures, accurate labeling of sampling containers, processing of samples within 30 minutes of collection, centrifugation at 3000 revolutions per minute and storage at -80 degrees centigrade which keeps artemether and lumefantrine stable for more than 2 years (146).

From a clinical perspective, our data implies that reduced artemether and dihydroartemisinin exposure due to drug interactions between AL and nevirapine may predispose to slower parasite clearance with slower symptom resolution. However, as discussed in chapter five above, extrapolation of data from our study to clinical relevance should be performed in consideration of changes in drug bioavailability during acute disease states. In both studies presented, we compared AL pharmacokinetics pre and post ART initiation. It is possible that the intervention (ART) and consequent immune reconstitution could have some impact on AL pharmacokinetics that may not arise from the effect of drug interactions but from changes in the pharmacokinetic processes in the body therefore explaining some of the differences observed in AL pharmacokinetics pre and post ART.

Nevirapine exposure was significantly reduced during co-administration with AL, possibly due to the autoinduction of CYP3A4. Artemether also induces CYP3A4 and could have contributed to this effect. In malaria endemic areas, recurrent malaria attacks occur and individuals get re-treated. In the unlikely event that the reduction in nevirapine concentration was due to induction of CYP3A4 by artemether, recurrent co-administration of AL with nevirapine may predispose to intermittent sub-therapeutic nevirapine concentrations predisposing to ART

failure with risk for development of resistance. On this basis, alternative antimalarial regimens may be prescribed for HIV-infected patients receiving nevirapine containing ART until more data become available.

In conclusion, co-administration of AL with nevirapine resulted in significant reduction in artemether, dihydroartemisinin and nevirapine pharmacokinetic exposures which are likely to result in malaria treatment failure.

In chapters 5 and 6 co-administration of AL with efavirenz or nevirapine resulted in reduction of antimalarial plasma concentrations which are likely to predispose patients to malaria treatment failure. This is great concern especially because AL is the first-line treatment for uncomplicated malaria in Uganda. Uncomplicated malaria if not adequately treated complicates into severe malaria. Severe malaria requires prompt treatment with highly efficacious drugs. Therapeutic concentrations of the antimalarial drugs used for severe malaria treatment should be achieved as soon as possible once the diagnosis is made. I therefore set out to investigate the pharmacokinetics of artesunate during treatment of severe malaria. Artesunate is a new antimalarial drug in the Uganda, soon to be introduced to the public sector, with great promise in the treatment of severe malaria. In the next chapter we present a description of the clinical response and pharmacokinetic profile of IV artesunate during treatment of severe malaria in adults.

Chapter 7

Clinical response and pharmacokinetic profile of intravenous artesunate during treatment of severe malaria in Ugandan adults

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7.1 Introduction

Severe malaria a major cause of death particularly in rural areas that are not adequately serviced by the formal health system. However, even under ideal conditions in specialized hospitals, the case fatality rate still remains unacceptably high. It is a medical emergency associated with an immediate threat to life therefore requires prompt treatment with effective therapy. The increasing seriousness of this problem calls for the need to evaluate and embrace new interventions for the management of this disease.

The current first-line treatment for severe malaria in Uganda is intravenous (IV) quinine. Artesunate was recently approved as an alternative to IV quinine. Artesunate is a water soluble artemisinin derivative with faster schizonticidal action and improved clinical outcome compared to quinine. Studies done in Asia comparing IV artesunate with IV quinine showed significant reduction in risk of death, parasite clearance time, and hypoglycaemia with artesunate compared to quinine (107). A recent study in Africa demonstrated superiority of artesunate over quinine (108). Artesunate is increasingly used as the first-line drug in the treatment of severe malaria especially in Asia. In addition to being highly effective, artesunate is devoid of major side-effects and its use does not warrant sophisticated or intensive monitoring. This makes it highly attractive as it can be used even in remote peripheral centres, where the need for rapid schizonticidal drugs is greatest.

Studies on the pharmacokinetics of IV artesunate in healthy volunteers and patients with complicated and severe malaria have been reported previously (113-115, 184). However, translating clinical efficacy and pharmacokinetic data from one human population to another requires confirmation that pharmacology, pharmacokinetics and clinical response are similar to what was found in the first population. Since there were no data on the pharmacokinetics of IV artesunate in treatment of severe malaria in Ugandan patients and the results from the African study on efficacy of artesunate were not yet published, we performed this study to assess the pharmacokinetic profile and clinical response to IV artesunate during treatment of severe malaria in Ugandan adults.

7.2 Study objective

To describe the pharmacokinetic profile and clinical response to IV artesunate during treatment of severe malaria in Ugandan adults.

7.3 Materials and Methods

Study participants

A sample size of 14 participants was selected to describe the pharmacokinetic profile of IV artesunate. This number was adequate for an intensive pharmacokinetic study for which the number of participants recommended is between 10 and 20 (146).

Study participants were adults with severe malaria requiring parenteral therapy. Participants were screened and enrolled consecutively from the patients attending the emergency clinic of Mulago Hospital. Participants were enrolled if they were 18 years of age and above, with a positive blood smear for *P. falciparum* mono-infection, no other obvious cause of the fever or symptoms and at least one laboratory or clinical feature of severe malaria requiring parenteral therapy. Patients with history of antimalarial intake within the last 72 hours, receiving any herbal medication or known inhibitors or inducers of CYP enzymes and pregnant mothers were excluded.

Study design

We performed an open label intensive pharmacokinetic study and monitored participants' clinical response using physical examination and serial blood smears for malaria parasite density.

Study procedures

Participants were admitted to the private ward of Mulago Hospital for treatment and monitoring. They received supportive therapy in accordance with the national severe malaria treatment guidelines. All participants received baseline evaluation including; thorough history, physical examination and laboratory investigations. Blood samples were collected by finger-prick for malaria smears and venipuncture for hematocrit, serum lactate, glucose, renal and liver function tests. All participants received IV artesunate (Guilin Pharmaceutical Factory, Guangxi,

People's Republic of China), for a minimum of 24hours, as 2.4mg/kg at the start, then 1.2mg/kg at 12 hours from start of treatment and 1.2mg/kg/day until they could tolerate oral therapy at which point oral AL was administered as a standard six-dose regimen of Coartem® (Novartis Pharma AG, Basel, Switzerland).

Serial thick blood films and estimation of parasite densities were performed at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 18, 20, and 24hours and every 6hours until 6 hours after parasite clearance. Parasite smears were repeated one week post discharge from hospital. Blood smears were stained with 2% Giemsa stain for thirty minutes, and parasite densities were calculated by counting the number of asexual parasites per 200 white blood cells (WBC) using the patient's WBC count per ul of blood.

Intensive pharmacokinetic sampling of venous blood was performed for artesunate and dihydroartemisinin concentration measurement at 0 (predosing), 5, 10, 15, 30, 45 minutes, 1hr, 1.5hr, 2hr, 3hr, 4hr, 5hr, 6hr, 8hr and 12 hours post dosing. Four mls of blood was collected at each time point in fluoride-oxalate tubes from the arm opposite that used for drug administration. All sampling tubes were chilled prior to blood draws and all samples were chilled immediately after withdraw to prevent artesunate degradation by plasma esterases. Blood was centrifuged within 30 minutes to minimize hemolysis, and the separated plasma was stored below -80 degrees centigrade till analysis.

Artesunate concentration measurement

Artesunate concentration was measured at the Clinical Pharmacology Laboratory, Mahidol-Oxford Tropical Medicine Research Unit, Mahidol University, Bangkok, Thailand. Concentrations of artesunate and dihydroartemisinin were determined by solid-phase extraction and liquid chromatography-tandem mass spectrometry on an API 5000 triple-quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, CA) with a TurboV ionization source operated in the positive ion mode (185). Stable isotope-labeled artesunate (SIL-artesunate) and stable isotope-labeled dihydroartemisinin (SIL-dihydroartemisinin) were used as internal standards. Total assay coefficients of variation for artesunate and dihydroartemisinin were <5% for inter- and intraday precisions. The LLOQ for artesunate and dihydroartemisinin were 1.2 and 2.0 ng/ml, respectively.

Statistical analysis

Participant baseline characteristics were summarized into medians with interquartile range (IQR). For pharmacokinetic analysis, we assumed total conversion of artesunate to dihydroartemisinin. Pharmacokinetic parameters were summarized into medians with range. Parasite density was log transformed and summarized into median with IQR. Parasite clearance time was defined as the time taken to clear all parasites from circulation ie time until the first of two sequential negative thick blood smears.

7.4 Results

Demographics of study participants

A total of 14 adults (9, 64% female) admitted with severe malaria were enrolled. At admission participants, had been ill for a median (IQR) of 7 (3 – 7) days. One participant had been ill for 14 and another 21 days. Some participants had more than 1 feature of severe malaria as follows; 2 (14%) reported severe vomiting, 5 (36%) had jaundice, 2 (14%) had extreme weakness with inability to sit or stand, 10 (71%) were dehydrated, 1 (7%) had hyperpyrexia and (1, 7%) had hemoglobinuria. Mean (range) parasite density at baseline was 22,924 (500 – 79950) parasites/ul. All participants received acetaminophen (paracetamol) for fever and pain relief. No other non-study medications were administered. Baseline clinical and laboratory characteristics of study participants at admission are shown in table 7.1.

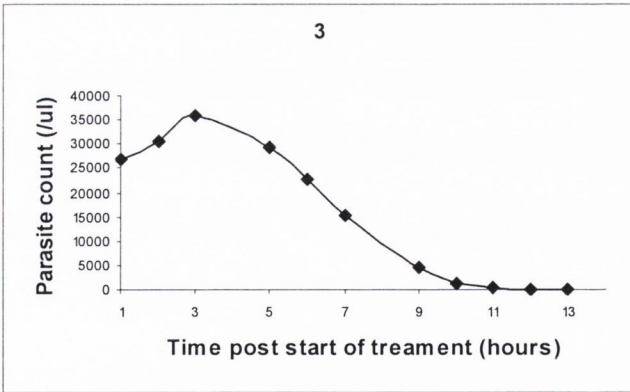
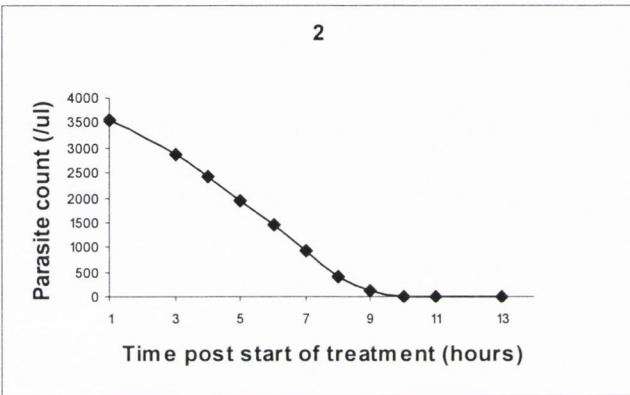
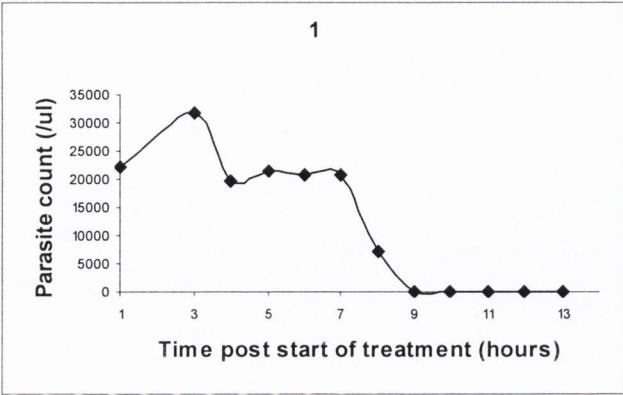
Table 7.1. Clinical characteristics of study participants at admission

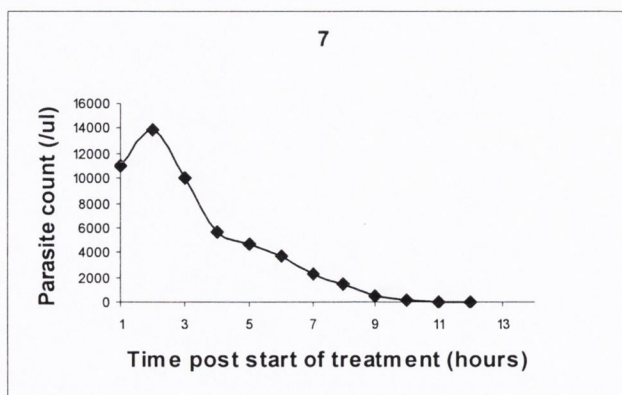
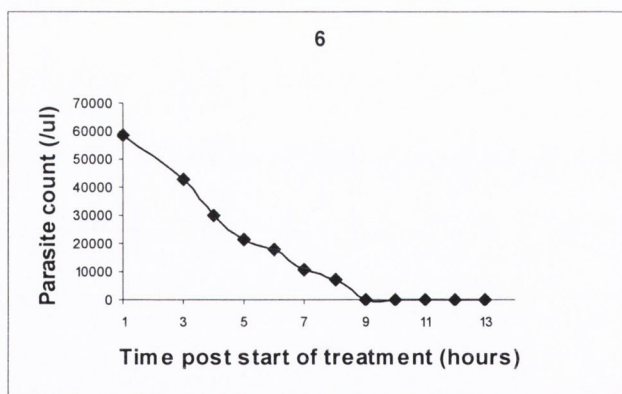
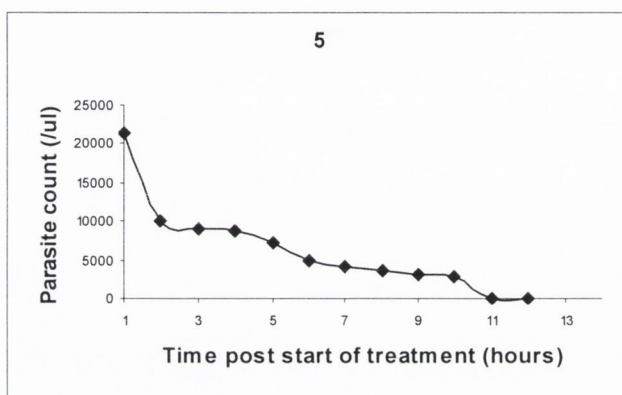
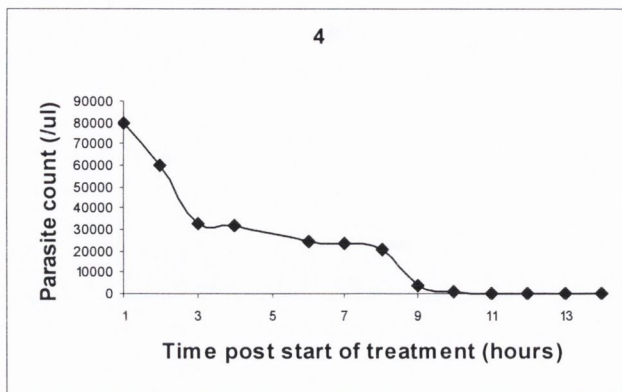
Parameter	Median (IQR)
Age (years)	24 (20 -35)
Weight (kgs)	56.3 (54 – 62.5)
BMI	20 (18.8 – 23.3)
Axillary temperature (°C)	37.1 (36.5 - 38.8)
Respiratory rate (/min)	24 (24 – 26)
Pulse rate (/min)	106 (95 – 113)
Systolic blood pressure (mmHg)	114 (106 - 128)
Diastolic blood pressure (mmHg)	73 (69 – 82)
Hemoglobin (mg/dl)	12.9 (11.2 – 14.2)
Serum creatinine (g/dl)	63.5 (54 – 75)
Serum bilirubin (umol/L)	30 (15.2 – 87.6)
Platelet count (/ml)	121500 (84000 – 183000)
Serum lactate (mmol/L)	3.25 (1.9 – 4.1)
Blood sugar(mmol/L)	4.8 (4.4 – 6.1)

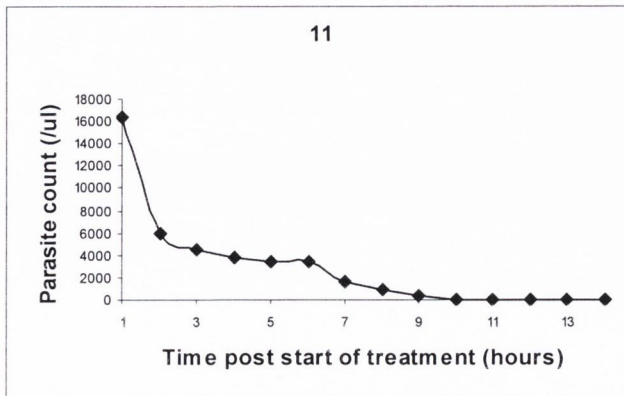
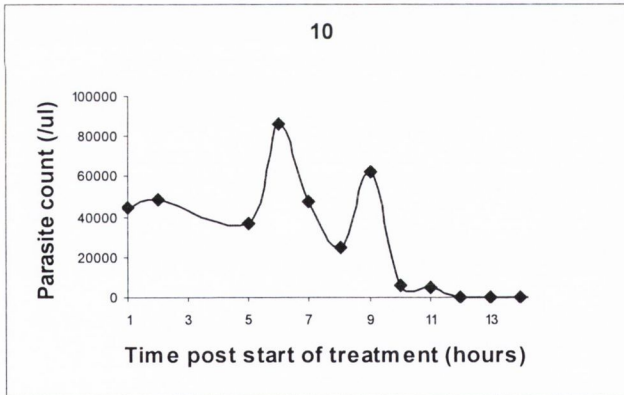
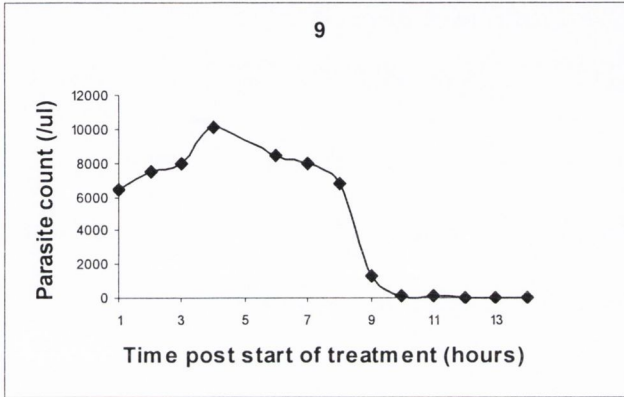
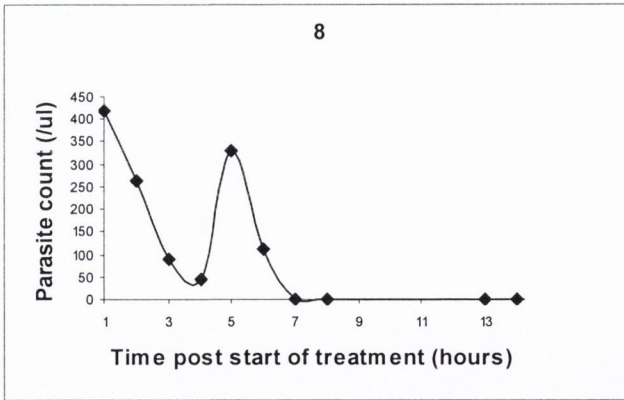
Clinical response

All study participants tolerated artesunate very well and reported very rapid recovery from symptoms; with ability to take oral medication within 24 hours. No immediate adverse events were recorded. None of the participants required additional medication. The mean (range) parasite clearance time was 16.5 (10 – 24) hours. Dynamics of individual parasite clearance are shown in figure 7.1 with geometric mean parasite density plotted against time post start of treatment in figure 7.2. Blood smears for all participants remained negative for malaria parasites at one week post discharge from hospital. Only 1 patient had gametocytes at baseline and these cleared within 4 hours post start of treatment.

Figure 7.1. Dynamics of individual parasite clearance







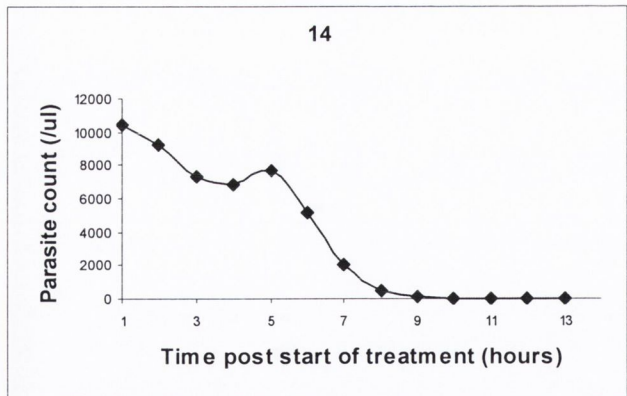
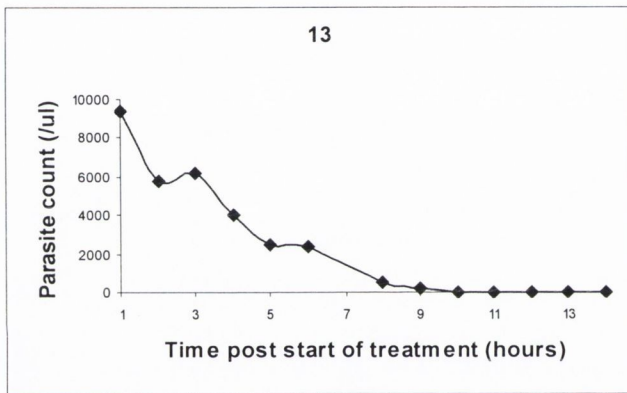
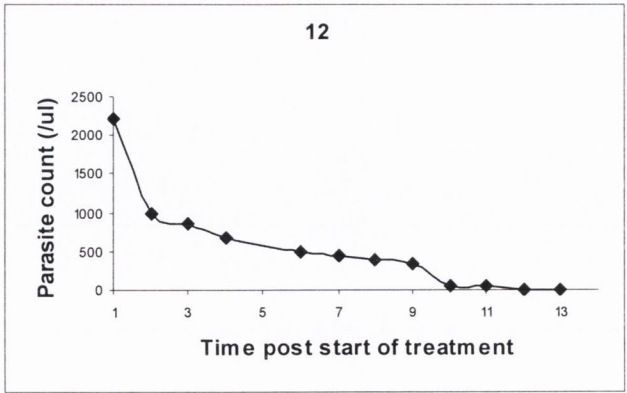
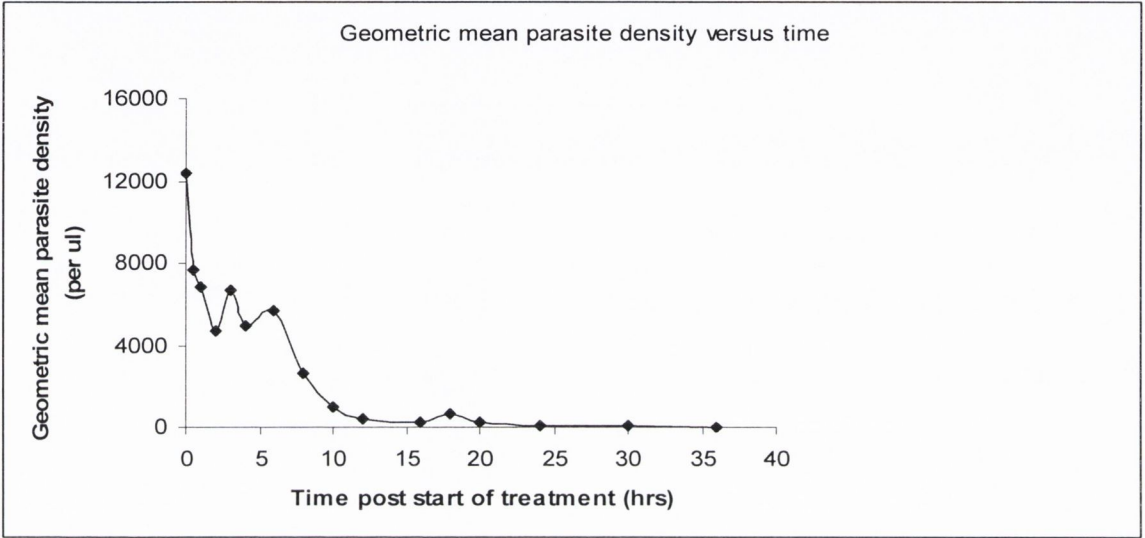


Figure 7.2. Geometric mean parasite density plotted against time post start of treatment



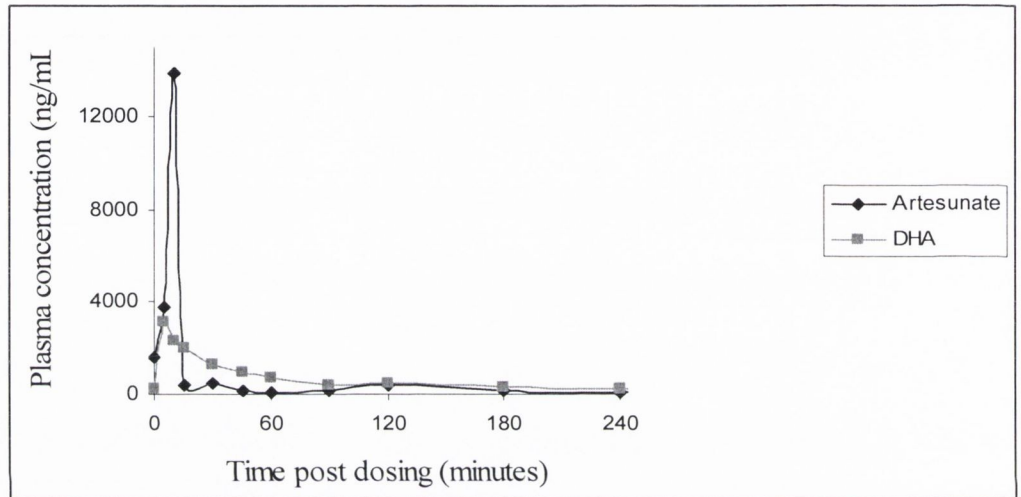
Pharmacokinetics of artesunate and dihydroartemisinin

Pharmacokinetic parameters and profiles for artesunate and dihydroartemisinin are summarized in table 7.2 and figure 7.3. Median (range) artesunate C_{\max} was 3260 (1020-164000) ng/mL, terminal elimination $T_{1/2}$ was 0.25 (0.1-1.8) hours and AUC was 727 (290-111256) hr*ng/mL. Median (range) dihydroartemisinin C_{\max} was 3140 (1670-9530) ng/mL, dihydroartemisinin $T_{1/2}$ was 1.3 (0.8-2.8) hours and dihydroartemisinin AUC was 3492 (2183-6338) hr*ng/mL. There was no correlation between total artesunate or total dihydroartemisinin exposure and parasite clearance times (Spearman's rho correlation coefficient -0.12 and -0.18 respectively).

Table 7.2. Pharmacokinetic parameters of artesunate and dihydroartemisinin

	Artesunate (N=14)	Dihydroartemisinin (N=13)
Parameter	Median (range)	Median (range)
Dose (mg)	140 (111-190)	103 (82 – 140)
C _{max} (ng/mL)	3260 (1020 - 164000)	3140 (1670 - 9530)
T _{max} (hr)	0.09 (0 - 6.07)	0.14 (0 - 6.07)
CL (L/hr)	180 (1 - 652)	32.25 (16 – 55)
V (L)	68.51 (0.18 - 818)	59.73 (26 - 117)
T _{1/2} (hr)	0.25 (0.11 - 1.82)	1.31 (0.89 - 2.87)
AUC _{0-last} (hr×ng/mL)	727 (290 - 111256)	3492 (2183-6338)

Figure 7.3. Mean (\pm SE) plasma concentration versus time of artesunate and dihydroartemisinin



Vertical lines represent standard error

7.5 Discussion

We studied the clinical response to and pharmacokinetics of IV artesunate in adults with severe malaria. Following IV administration, artesunate was detected and measured in plasma very promptly post administration rising to the C_{\max} within 5 minutes. Artesunate was cleared very fast with median elimination $T_{1/2}$ of 0.25 hours equal to 15 minutes, with a range of 0.1 to 1.8 hours. This $T_{1/2}$ is comparable to the range from previous studies among patients with malaria (113, 184), but contrasts with $T_{1/2}$ of 2-5 minutes in previous healthy volunteer studies (186-187). The difference most likely arises from differences in clearance and volume of distribution in diseased and healthy states as well as differences in the rate of IV infusion. In the earlier studies, artesunate was administered at a rate of 2-3 minutes compared to the 3-4 minutes in our study.

The C_{\max} for dihydroartemisinin was achieved within 8.4 minutes post dose administration showing very rapid conversion of artesunate to dihydroartemisinin. The median dihydroartemisinin AUC was similar to that demonstrated in a previous study conducted among patients with severe malaria (184). Both artesunate and dihydroartemisinin AUC varied markedly among participants. This marked variability is similar to data from previous studies (184), however, despite this very large inter-individual variability; all patients had very rapid parasite clearance. The large inter-individual variability is possibly due to inter-individual variability in CYP3A4 activity. Complete comparison of all pharmacokinetic parameters from different studies is hindered by differences in rate of administration or infusion of drug, disease severity of participants, parasitemia, drug content and assay method.

Our median parasite clearance time of 16 hours was much shorter than the 66 hours and 32 hours from previous studies of adults with malaria in western Thailand (113, 184), possibly due to differences in parasite sensitivity to artesunate. All our participants had complete recovery with no adverse events reported. These data support the preferential use of artesunate over quinine in Uganda.

Dihydroartemisinin is said to have more potent antimalarial activity than artesunate. Indeed some studies have attributed the effectiveness of artesunate to its rapid and complete conversion to dihydroartemisinin. A previous study suggested a trend to an association between artesunate and dihydroartemisinin AUC and parasite clearance (114); however we found no correlation between the artesunate and dihydroartemisinin AUC and parasite clearance time. This finding is similar to data by Newton et al, which demonstrated no relationship between artesunate pharmacokinetic parameters and parasitocidal effect (184). It is not clear which artesunate pharmacokinetic parameter best correlates with antimalarial treatment effect, but previous dose finding studies have suggested doses higher than 2mg/kg and 2.4 mg/kg as the minimum initial dose for malaria treatment in view of the considerable inter-individual variability in artesunate pharmacokinetic profile (113, 184).

Compared to artesunate, quinine has a number of disadvantages including poor compliance and a significant adverse event profile such as hypotension, hypoglycemia and GIT intolerance (188-189). The IV quinine infusion is difficult and expensive to institute and needs constant monitoring for arrhythmia and hypoglycemia. Adherence to the 8 hourly regimen of quinine is poor and often patients do not complete the dose increasing the risks for treatment failure and development of drug resistance. Facilities for the IV infusion are inadequate in many health centres and hospitals in Uganda leading to inappropriate methods of quinine administration (190). The ease of administration of IV artesunate plus the lack of a significant side effect profile make it an excellent choice for remote peripheral centres that suffer the greatest burden of severe malaria. Our data contribute to the existing knowledge on the clinical response to and pharmacokinetics of IV artesunate for treatment of severe malaria. These data plus previous data from Asia and Africa strongly suggest that parenteral artesunate should be considered the drug of first choice in treating severe malaria (107-108). However; parenteral artesunate is not yet widely available especially in sub-Saharan Africa where the greatest burden of severe malaria and death occurs and efforts to improve accessibility should be reinforced.

Chapter 8

Summary of Key Findings and Discussion

Summary of key study findings

In chapter 2, the steady-state pharmacokinetic profiles of generic Triomune 40[®], were similar to profiles of branded products in HIV-infected adults at this large HIV treatment clinic in Uganda.

In chapter 3, co-administration of LPV/r with AL to HIV-infected Ugandan adults significantly reduced pharmacokinetic exposure of artemether with increase in lumefantrine exposure (Table 8.1).

In chapter 4, co-administration of LPV/r with AL was not associated with cardiac conduction abnormalities.

In chapter 5, co-administration of efavirenz with AL to HIV-infected Ugandan adults resulted in significant reduction in artemether, dihydroartemisinin and lumefantrine pharmacokinetic exposure (Table 8.1).

In chapter 6, co-administration of nevirapine with AL to HIV-infected Ugandan adults resulted in significant reduction in artemether, dihydroartemisinin and nevirapine exposure (Table 8.1).

In chapter 7, participants promptly attained therapeutic concentrations of artesunate following IV administration with rapid parasite and symptom clearance.

Table 8.1 Summary of the effect of co-administration of AL with LPV/r, efavirenz or nevirapine on pharmacokinetic exposure of AL

ART	Effect on exposure of antimalarial		
	Artemether	Dihydroartemisinin	Lumefantrine
LPV/r	43% reduction	Not affected	386% increase
Efavirenz	77% reduction	75% reduction	55% reduction
Nevirapine	72% reduction	37% reduction	21% reduction

Discussion

HIV and malaria are both preventable and treatable diseases that inflict great impact on sub-Saharan Africa. The two are major threats to economic growth, cause loss of productivity, loss of income on treatment, school and work absenteeism, low food production, famine, low income and low standard of living. In the absence of vaccines for both diseases, countries rely on prevention of new infections and treatment of infected individuals to alter the course of the two diseases and prevent transmission and death. Efforts to prevent HIV and malaria infection have been scaled up in most African countries. Interventions such as cotrimoxazole prophylaxis, insecticide treated mosquito nets, indoor residual spraying, and intermittent preventive treatment during pregnancy are all effective in preventing malaria (127-128, 191-192) and are currently being rolled out in most African countries. Behavior change messages and condoms are widespread for the prevention of HIV; however, case management remains a vital component of HIV and malaria control.

While one of the eight millennium development goals specifically relates to combating HIV/AIDS and malaria, achieving and sustaining malaria and HIV control is central to meeting many of the other millennium development goals such as eradicating poverty and hunger, reducing child mortality and improving maternal health. In the last decade global funding towards malaria and HIV control has increased, with funding from the Global fund to fight HIV, tuberculosis and malaria, the World Bank, US President's Malaria Initiative, Bill and Melinda Gates' Foundation, Multicountry AIDS Program, the Presidents' Emergency Plan for AIDS Relief, and the Elizabeth Glaser Paediatric AIDS Foundation, among others. This funding has greatly accelerated delivery of critical interventions and medicines in many African countries enabling access to life saving but also more expensive strategies; the ACTs and ART.

Maintaining the achievement made so far means sustaining the effectiveness of these life-saving medications. This is a major concern. Currently there is no alternative to the ACTs which are highly effective against malaria and few but expensive alternatives to the current ART available in sub-Saharan Africa for HIV treatment. Global effort should be made to prevent emergence of resistance to these drugs. This thesis provides contribution to the sparse evidence on AL and

ART interactions as part of global efforts to maintaining the ART and ACT effectiveness. We provide data on the pharmacokinetics of generic ART, drug interactions of ART and AL and pharmacokinetic profiles of AL and artesunate, a drug that has brought great hope in preventing deaths from severe malaria.

The first study demonstrated that the generic Triomune 40[®] had similar pharmacokinetic profiles to the branded products. This was very important and useful information at the time since most HIV care centres in Uganda depended on this generic formulation to meet treatment goals. Standard practice in Uganda is for all batches of drugs to undergo *in vitro* dissolution testing at the National Quality Control Laboratory in Kampala, however, while this is very useful in detecting counterfeit drugs, unwanted additives and sub-therapeutic drug levels, it does not address the issue of patient drug levels which clinical pharmacokinetic studies deal with.

We demonstrated that co-administration of AL with LPV/r leads to significant reduction in artemether with significant increase in lumefantrine exposure. Our study confirms results by German et al on AL and LPV/r interactions (141). Increased lumefantrine exposure may be beneficial in malaria treatment, however may be associated with toxicity. Although lumefantrine exposure was significantly elevated; the single dose of AL in our study was not associated with any cardiac conduction abnormalities (162). We however recommend caution and safety monitoring during co-administration of the standard six-dose AL regimen with LPV/r.

Our data illustrates significant drug interactions between AL and efavirenz or nevirapine. Co-administration led to significant reduction in artemether and dihydroartemisinin exposure. Lumefantrine exposure was significantly reduced by co-administration with efavirenz and non-significantly reduced with nevirapine. We attribute these findings to induction of CYP3A4 by efavirenz and nevirapine (21, 176). The effect of efavirenz or nevirapine on AL pharmacokinetics has serious implications for treatment of HIV-malaria co-infected patients with risk for poor treatment outcomes and development of resistance to AL. In view of the increased risk for malaria in HIV-infected individuals (98-99) plus some evidence to suggest poor antimalarial treatment outcomes due to HIV (122-123), these drug interactions are worrying and require urgent attention.

Both artemether and dihydroartemisinin are very potent antimalarial agents which rapidly clear parasites from circulation and are rapidly eliminated from the body (139). Lumefantrine with a much longer $T_{1/2}$ clears residual parasites, preventing recrudescence and is the major determinant of cure. This is beneficial both to the individual receiving treatment and the community. The individual benefits from reduction of malaria associated morbidity such as anaemia while the community benefits from a reduction in the parasite reservoir, reduced transmission and delayed emergence plus spread of drug resistance. Although drugs with long $T_{1/2}$ increase selection pressure for resistance, the residual antimalarial drug in circulation resulting from slow clearance provides additional secondary prophylaxis against new malaria infections. Any drug-interactions that reduce pharmacokinetic exposure deprive individuals and the community of these important advantages.

The reduction in nevirapine exposure during AL co-administration needs further investigation. Nevirapine is widely prescribed as part of first-line ART regimens especially when efavirenz is contra-indicated. Nevirapine has low barrier to development of resistance and cross resistance to efavirenz occurs. In areas with “stable” malaria transmission, meaning where populations are continuously exposed to a fairly constant, high rate of malarial infections, individuals develop partial immunity to malaria with age thus clinical disease is mostly confined to young children. Pregnancy modifies this immunity so increases risk for malaria acquisition. Very young children and pregnant mothers with HIV are at increased risk for malaria and are likely to receive nevirapine containing ART regimens. Repeated malaria infection requiring repeated treatment will predispose to intermittent occurrence of AL and nevirapine drug interactions. The physiological changes that occur during pregnancy may further modify drug bioavailability. Investigation of pharmacokinetic drug interactions in these populations should be considered priority.

Currently there are six ACTs available for treatment of uncomplicated malaria; AL, artesunate plus amodiaquine, artesunate plus mefloquine, artesunate plus sulphadoxine-pyrimethamine, dihydroartemisinin plus piperaquine and artemisinin plus naphthoquine. For all of them; it is the properties of the partner drug that determine the efficacy of the combination, however for all, the short

acting artemisin component and the long acting counterpart undergo CYP mediated metabolism, therefore have potential for drug interactions with ART (138). There is therefore urgent need to investigate drug interactions of ART with the different ACTs. As the malaria endemic countries progress with the scale-up of ART and ACTs, there is urgent need to integrate HIV and malaria care and treatment services and to educate health care workers on these interactions.

Uncomplicated malaria if not treated adequately progresses to severe malaria. Mortality from severe malaria is 100% without treatment, it reduces to 15-20% with treatment (93, 106). Death often occurs within hours post admission thus the primary objective for treatment of severe malaria is to prevent death. This requires provision of prompt and highly effective antimalarial therapy with attainment of therapeutic concentrations as soon as possible. A previous large multi centre trial demonstrated superiority of artesunate over quinine for severe malaria treatment (108). These data plus our data on the pharmacokinetic profile and clinical response of artesunate support the use of artesunate as first-line therapy for severe malaria in Uganda. Because HIV-infected individuals are at increased risk for severe malaria (87, 104), artesunate offers a great alternative to quinine for this special population. Currently artesunate is not available in the Ugandan public health sector mainly due to the high cost of the drug. In view of the excellent efficacy, fewer requirements for clinical monitoring and clinical supplies such as IV infusion sets associated with artesunate use, the cost-effectiveness of artesunate over quinine should be evaluated to support advocacy for expanding access to artesunate in Uganda.

Malaria and HIV are not sub-Saharan Africa's only scourge; there are many communicable and non-communicable diseases that make up the burden of disease. The need for health care is still demanding. Any new advances and discoveries contribute to global efforts to improve health and standard of living. With the hope of eradication of malaria and HIV still uncertain, global and cross-border efforts are needed to maintain effectiveness of currently available strategies. This will greatly contribute to improvement of the lives of those most in need. We will definitely need new strategies, tools and drugs thus any effort at research capacity building contributes significant gain in the fight against HIV and malaria which is a significant contribution to the fight against poverty.

Policy implications and recommendations

1. Generic products play a crucial role in improving access to ART; however, there is need for ongoing surveillance of their quality in target populations.
2. Dosage modification of AL for HIV-malaria co-infected patients receiving LPV/r, efavirenz or nevirapine based ART is advised.
3. Monitoring of AL treatment response among HIV-malaria co-infected patients receiving LPV/r, efavirenz or nevirapine based ART regimens is recommended.
4. Alternative antimalarial regimens other than AL should be prescribed for HIV-malaria co-infected patients receiving nevirapine based ART.
5. Intravenous artesunate should be rolled out for treatment of severe malaria.

Implications for further research

1. Investment in human and material resources to develop pharmacokinetic units in resource-limited settings should be considered a priority.
2. There is urgent need to define the optimal dosage of AL for HIV-malaria co-infected patients receiving ART.
3. Evaluation of pharmacokinetic interactions between ART and the other available ACTs is important.

Future plans

I hold a faculty position as a lecturer at the Department of Medicine of Makerere University College of Health Sciences where I will continue to teach, conduct research, carry on with patient care, mentorship and administrative tasks. I hope to get promoted to the rank of senior lecturer within this year. I will continue with the research career which will require me to write more grant applications for research funding.

I successfully applied for the EDCTP Senior Fellowship which has been awarded to conduct research on severe malaria treatment. This project has started in a rural hospital in Eastern Uganda, where malaria is responsible for the greatest burden of disease. Data from this project will answer questions on the best mode of treatment of severe malaria including both parenteral and follow-on oral treatment. We will evaluate the pharmacokinetics of antimalarial drugs during severe malaria treatment as well as the pharmacokinetic drug interactions

between antimalarial and antiretroviral drugs and the correlation with treatment outcome in populations receiving treatment for malaria.

Non-compartmental pharmacokinetic analysis does not take into consideration the effect of other covariates. We have initiated the application of pharmacokinetic modeling techniques to this data using the WinNonlin software. This is in collaboration with investigators from Mahidol-Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Potentially influential covariates to be considered include age, weight, height, gender, hematocrit, CD4 count and viral load. This approach will incorporate more data when fitting the model controlling for the various factors and therefore provide more reliable parameter estimates than the individual non-compartmental analysis.

“In education it isn't how much you have committed to memory, or even how much you know.

It's being able to differentiate, between what you know and what you don't.

*It's knowing where to go to find out what you need to know
and it's knowing how to use the information you get”*

William Feather

“Learning is the antidote to stagnation, boredom and derailment.”

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Appendix 1: Publications

The following publications are based on the work contained in this thesis

Papers

Byakika-Kibwika P, Lamorde M, Okaba-Kayom V, Mayanja-Kizza H, Katabira E, Hanpithakpong W, Pakker N, Dorlo TP, Tarning J, Lindegardh N, de Vries PJ, Back D, Khoo S, Merry C. Lopinavir/ritonavir significantly influences pharmacokinetic exposure of artemether/lumefantrine in HIV-infected Ugandan adults.

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Nevirapine pharmacokinetics when initiated at 200 mg or 400 mg daily in HIV-1 and tuberculosis co-infected Ugandan adults on rifampicin.

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Suboptimal Nevirapine Steady-State Pharmacokinetics During Intrapartum Compared With Postpartum in HIV-1–Seropositive Ugandan Women.

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Abstracts and Poster Presentations

Pauline Byakika-Kibwika, Mohammed Lamorde, Lillian Nabukeera, Harriet Mayanja-Kizza, Elly Katabira, Warunee Hanpithakpong, Mairin Ryan, Nadine Pakker, Saye Khoo, David Back, Niklas Lindegardh, Joel Tarning, Peter J de Vries and Concepta Merry

Efavirenz significantly affects pharmacokinetic exposure of artemether-lumefantrine in HIV-infected Ugandan adults

19th Conference on Retroviruses and Opportunistic Infections. Seattle, Washington, USA, 2012.

Pauline Byakika-Kibwika, Mohammed Lamorde, Lillian Nabukeera, Harriet Mayanja-Kizza, Elly Katabira, Warunee Hanpithakpong, Mairin Ryan, Nadine Pakker, Saye Khoo, David Back, Niklas Lindegardh, Joel Tarning, Peter J de Vries and Concepta Merry

Efavirenz significantly affects pharmacokinetic exposure of artemether-lumefantrine in HIV-infected Ugandan adults

12th Annual Scientific Conference of the Uganda Society for Health Scientists. Golf Course Hotel, Kampala, 2011.

Pauline Byakika-Kibwika, Mohammed Lamorde, Peter Lwabi, Violet Okaba, Mairin Ryan, Harriet Mayanja-Kizza, Nadine Parker, Marta Boffito, Elly Katabira, Peter de Vries, David Back, Saye Khoo and Concepta Merry.

Co-administration of Artemether-lumefantrine with lopinavir/ritonavir in HIV positive Ugandan adults. 5th Annual Scientific conference of Makerere University College of Health Sciences, 22nd-24th September 2010. Imperial Royale Hotel, Kampala, Uganda

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Steady-State Pharmacokinetic Comparison of Generic and Branded Formulations of Stavudine, Lamivudine and Nevirapine in HIV-Infected Ugandan Adults.
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Steady-State Pharmacokinetic Comparison of Generic and Branded Formulations of Stavudine, Lamivudine and Nevirapine in HIV-Infected Ugandan Adults.
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Lopinavir/ritonavir significantly influences pharmacokinetic exposure of artemether/lumefantrine in HIV-infected Ugandan adults

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Background: Treatment of HIV/malaria-coinfected patients with antiretroviral therapy (ART) and artemisinin-based combination therapy has potential for drug interactions. We investigated the pharmacokinetics of artemether, dihydroartemisinin and lumefantrine after administration of a single dose of 80/480 mg of artemether/lumefantrine to HIV-infected adults, taken with and without lopinavir/ritonavir.

Methods: A two-arm parallel study of 13 HIV-infected ART-naïve adults and 16 HIV-infected adults stable on 400/100 mg of lopinavir/ritonavir plus two nucleoside reverse transcriptase inhibitors (ClinicalTrials.gov, NCT 00619944). Each participant received a single dose of 80/480 mg of artemether/lumefantrine under continuous cardiac function monitoring. Plasma concentrations of artemether, dihydroartemisinin and lumefantrine were measured.

Results: Co-administration of artemether/lumefantrine with lopinavir/ritonavir significantly reduced artemether maximum concentration (C_{max}) and area under the concentration–time curve (AUC) [median (range): 112 (20–362) versus 56 (17–236) ng/mL, $P=0.03$; and 264 (92–1129) versus 151 (38–606) ng·h/mL, $P<0.01$]. Dihydroartemisinin C_{max} and AUC were not affected [66 (10–111) versus 73 (31–224) ng/mL, $P=0.55$; and 213 (68–343) versus 175 (118–262) ng·h/mL $P=0.27$]. Lumefantrine C_{max} and AUC increased during co-administration [2532 (1071–5957) versus 7097 (2396–9462) ng/mL, $P<0.01$; and 41 119 (12 850–125 200) versus 199 678 (71 205–251 015) ng·h/mL, $P<0.01$].

Conclusions: Co-administration of artemether/lumefantrine with lopinavir/ritonavir significantly increases lumefantrine exposure, but decreases artemether exposure. Population pharmacokinetic and pharmacodynamic trials will be highly valuable in evaluating the clinical significance of this interaction and determining whether dosage modifications are indicated.

Keywords: antiretrovirals, antimalarials, drug interactions

Introduction

Malaria and HIV are two infectious diseases causing significant morbidity and mortality worldwide. The two diseases have overlapping geographical distribution in sub-Saharan Africa, where over 90% of the world malaria burden and 67% of the global HIV burden occur.^{1,2} Significant interactions occur between the

two diseases, with HIV increasing the risks for malaria frequency and severity.^{3,4} Infection with malaria stimulates immune mechanisms that activate HIV replication, causing a transient increase in HIV viral load.^{5,6}

Major effort has been made to ensure universal access to antiretroviral therapy (ART), with significant improvement in quality of life and survival of people living with HIV. In 2009,

1.2 million people were initiated on ART, a 30% increase in ART coverage in one year.⁷ Successful treatment of infectious diseases such as HIV and malaria requires adequate drug concentrations at the target site to produce maximal efficacy with minimal toxicity. Drug pharmacokinetics might be influenced by drug–drug interactions. Antiretroviral drugs, specifically the non-nucleoside reverse transcriptase inhibitors and protease inhibitors, are potent inducers and/or inhibitors of cytochrome (CYP) enzymes and transporter proteins, with potential for drug–drug interactions when co-administered with other drugs.^{8,9}

The WHO recommends artemisinin-based combination therapy (ACT) for the treatment of uncomplicated malaria.² The combination of artemether and lumefantrine offers excellent efficacy against susceptible and multidrug-resistant *Plasmodium falciparum*. Both artemether and lumefantrine are metabolized predominantly by CYP3A4.¹⁰ Artemether is metabolized to dihydroartemisinin, predominantly by CYP3A4/5 and to a lesser extent by CYP2B6, CYP2C9, CYP2C19 and possibly CYP2A6.^{8,10–12} Dihydroartemisinin is rapidly converted into inactive metabolites primarily by glucuronidation via uridine diphosphoglucuronyltransferases (UGTs) UGT1A1, UGT1A8/9 and UGT2B7.^{10,12–14} Both artemether and dihydroartemisinin possess potent antimalarial properties, causing a rapid reduction in asexual parasite biomass, with prompt resolution of symptoms.^{15,16}

Lumefantrine is slowly eliminated, mainly metabolized by CYP3A4 to desbutyl-lumefantrine.^{10–12} Lumefantrine eradicates residual malaria parasites thereby preventing recrudescence.^{10,13,14} Total exposure to lumefantrine predicts parasite eradication and is the principal pharmacokinetic correlate of artemether/lumefantrine treatment.¹⁶

Lopinavir and ritonavir are inhibitors of CYP3A4, so co-administration with artemether/lumefantrine may result in increased artemether and lumefantrine plasma concentrations. Elevated lumefantrine plasma concentrations are of particular concern because of the structural similarity to halofantrine, a drug associated with cardiac arrhythmias and sudden death.^{17–19} In a previous study, co-administration of lopinavir/ritonavir with artemether/lumefantrine to healthy volunteers resulted in significantly increased lumefantrine exposure, decreased dihydroartemisinin exposure and a trend towards decreased artemether exposure.²⁰

The aim of the present study was to investigate the pharmacokinetics of artemether, dihydroartemisinin and lumefantrine after administration of a single dose of 80/480 mg of artemether/lumefantrine to HIV-infected adults, taken with and without lopinavir/ritonavir-based ART. To avoid unknown adverse effects, we administered a single dose of artemether/lumefantrine to HIV-infected patients without malaria and vigilantly monitored their cardiac function.

Methods

Study site

The study was conducted between January 2008 and June 2009 at the Infectious Diseases Institute (IDI) and the Uganda Heart Institute, Mulago Hospital, Kampala, Uganda.

Study design and population

This was a two-arm parallel study to assess the pharmacokinetics of a single dose of artemether/lumefantrine co-administered with and without lopinavir/ritonavir-based ART to HIV-infected patients without malaria. Patients were eligible to participate if they were older than 18 years, with no evidence of systemic illness and no indication for medications with known potential for drug interactions with the study drugs. Patients with abnormal cardiac, liver or renal function, positive blood smear for malaria, pregnant mothers and those who reported use of any herbal medication were excluded.

Ethical considerations

The study was approved by the Uganda National HIV/AIDS Research Committee (ARC 056) and the Uganda National Council of Science and Technology (HS 195), and was registered with ClinicalTrials.gov (NCT 00619944). Study procedures were explained to participants in their local languages. Each participant received an information leaflet to take home. All participants provided written informed consent prior to study entry. Study procedures were conducted in accordance with the principles of Good Clinical Practice.

Study procedures

Patients were screened and enrolled consecutively from the cohort of patients attending the IDI. The artemether/lumefantrine plus lopinavir/ritonavir arm consisted of HIV-positive patients stable on 400/100 mg of lopinavir/ritonavir plus two nucleoside reverse transcriptase inhibitors (NRTIs) taken twice daily for at least 1 month. The artemether/lumefantrine arm consisted of HIV-positive ART-naïve patients who had not started ART and were not yet eligible for ART according to national guidelines. Patients in both arms took co-trimoxazole daily for prophylaxis against opportunistic infections. Adherence to study drugs was assessed using self-report and pill count at each clinical visit. On the evening prior to the study day, participants were reminded of their study-day appointment and were given detailed instructions to eat food; those in the lopinavir/ritonavir arm were reminded to administer their ART by 8.00 pm, and arrive at the hospital by 7.00 am in a fasting state.

On the morning of the study day, patients were admitted to the Heart Institute. Blood smears for malaria parasites were performed, and patients found to have positive smears were given a standard six-dose course of artemether/lumefantrine and excluded from further study. A 12-lead electrocardiograph (ECG) monitor was attached for continuous cardiac function monitoring. An indwelling intravenous catheter was inserted following aseptic techniques, and blood samples were drawn for the determination of pre-dose concentrations of artemether, dihydroartemisinin and lumefantrine. A standardized breakfast with added fat to cater for the fat requirement for artemether/lumefantrine absorption was administered.²¹ The intake of breakfast and study drugs was directly observed by study staff.

All patients took a single dose of four tablets, equivalent to 80/480 mg of artemether/lumefantrine (Coartem[®], Novartis Pharma AG, Basel, Switzerland; Batch number: F0660) with water immediately after breakfast. Patients in the lopinavir/ritonavir arm took 400/100 mg of lopinavir/ritonavir (Aluvia[®], Abbott Laboratories, USA) plus two NRTIs with their study artemether/lumefantrine dose. The NRTI combination consisted of zidovudine plus didanosine, or tenofovir plus emtricitabine.

Sampling was performed at 1, 2, 4, 6, 8, 12, 24, 48 and 72 h post-artemether/lumefantrine dosing. An aliquot of 4 mL of blood was collected per sampling time in lithium–heparin tubes. Samples were centrifuged immediately for 10 min; plasma was separated and stored immediately at –70°C until shipment on dry ice to the Clinical Pharmacology Laboratory, Mahidol-Oxford Tropical Medicine Research Unit,

Mahidol University, Bangkok, Thailand for measurement of artemether, dihydroartemisinin and lumefantrine plasma concentrations.

Safety assessment

Medical history, physical examination, routine clinical laboratory tests, ECG and urine screens for pregnancy were performed at screening. On the study day, medical history, physical examination and blood smears for malaria parasites were performed. Standard 12-lead ECGs were recorded at screening, immediately prior to dosing, then continuously for 12 h post-dose of artemether/lumefantrine and once daily for 3 days thereafter. Participants were monitored for adverse events until 2 weeks post-sampling; the onset, duration, severity and relationship to the trial drugs (if any) were noted.

Artemether, dihydroartemisinin and lumefantrine plasma concentration measurement

Artemether and dihydroartemisinin concentrations were measured using solid-phase extraction and liquid chromatography/mass spectrometry.²² Total-assay coefficients of variation for dihydroartemisinin and artemether during analysis were less than 5% at all quality control levels. The lower limit of quantification was 1.4 ng/mL and the limit of detection was 0.5 ng/mL for both drugs.²²

Lumefantrine concentrations were determined using a solid-phase extraction/liquid chromatographic assay with ultraviolet detection.²³ The coefficient of variation was less than 6% at all quality control levels. The lower limit of quantification was 25 ng/mL and the limit of detection was 15 ng/mL.²³

Analytical and pharmacokinetic methods

Non-compartmental analysis was performed using WinNonlin Professional™ software, version 5.2 (Pharsight Corp., Mountain View, CA, USA). Pharmacokinetic parameters included the observed maximum concentration (C_{max}), time to C_{max} (T_{max}), area under the plasma concentration-time curve from zero to the last observation (AUC_{0-last}), area under the plasma concentration-time curve from zero extrapolated to infinity ($AUC_{0-\infty}$), elimination clearance (CL/F), apparent volume of distribution (V/F), elimination half-life ($t_{1/2}$) and absorption lag time (T_{lag}). The trapezoidal rule (linear-up/log-down) was used to estimate AUC. All parameters were calculated using actual blood sampling times. Drug concentrations below the lower limit of quantification of the bioanalytical assays were treated as missing data. The median values and ranges of the pharmacokinetic parameters were recorded for the two groups.

Statistical analysis

Data were analysed using STATA® version 10.0 (StataCorp, College Station, TX, USA). Baseline characteristics were summarized as mean with 95% CI and compared using the independent *t*-test. The Wilcoxon rank-sum test was used to compare pharmacokinetic parameters between the two groups. A *P* value of <0.05 was considered statistically significant.

Results

A total of 36 participants were enrolled, of whom 29 completed the 72 h sampling. Of the seven participants who did not complete sampling, two dropped out before sampling started, one participant had only the first three samples drawn due to difficulty with cannulation and four patients had positive blood smears for malaria on the sampling visit; the latter were given the standard six-dose regimen of artemether/lumefantrine and excluded from further study.

Analyses were performed on data from the 29 participants who completed sampling: 16 [9 (56%) female] in the artemether/lumefantrine plus lopinavir/ritonavir arm, and 13 [9 (69%) female] in the artemether/lumefantrine arm. All participants taking lopinavir/ritonavir-based ART had viral load below the level of detection (400 copies/mL). Mean (95% CI) of the log of viral load was 4.5 (4.0–5.0) copies/mL among the ART-naïve patients. Participants in the two study arms were comparable for all other baseline characteristics measured except haemoglobin, which was significantly higher among patients taking lopinavir/ritonavir-based ART (Table 1). All participants tolerated study drugs very well, with no adverse events reported. ECG parameters for patients in both study arms remained well within normal limits throughout the 72 h follow-up period. These data have been published elsewhere.²⁴

Effect of lopinavir/ritonavir on artemether and dihydroartemisinin pharmacokinetics

Co-administration of artemether/lumefantrine with lopinavir/ritonavir significantly increased artemether CL/F and V/F, by 67% ($P < 0.01$) and 39% ($P = 0.02$), respectively. Artemether C_{max} and AUC_{0-last} were significantly reduced, by 50% ($P = 0.03$) and 43% ($P < 0.01$), respectively (Table 2 and Figure 1a). Dihydroartemisinin CL/F and V/F were not influenced by lopinavir/

Table 1. Baseline characteristics of study participants

Variable	Mean (95% CI)		P value
	artemether/ lumefantrine arm	artemether/lumefantrine plus lopinavir/ritonavir arm	
Age (years)	34.5 (29.9–39.0)	37.6 (34.3–40.9)	0.2
Body weight (kg)	63.8 (58.1–69.5)	64.0 (57.6–70.5)	0.9
Height (cm)	160.1 (155.2–165.0)	165.8 (161.5–170.0)	0.06
Body mass index (kg/m ²)	25.1 (22.2–28.0)	23.4 (21.0–25.8)	0.3
Haemoglobin (mg/dL)	12.6 (11.4–13.8)	14.3 (13.8–14.9)	0.004 ^a
QTc (ms)	411.5 (402.3–420.6)	416.8 (406.0–427.5)	0.4

^aStatistically significant.

Table 2. Comparison of pharmacokinetic parameters of artemether, dihydroartemisinin and lumefantrine

Parameter	Artemether/lumefantrine (N=13), median (range)	Artemether/lumefantrine plus lopinavir/ritonavir (N=16), median (range)	P value
Artemether			
C_{max} (ng/mL)	112 (20–362)	56 (17–236)	0.03
T_{max} (h)	1 (1–4)	2 (1–4)	0.38
CL/F (L/h)	295 (69–817)	492 (129–1805)	<0.01
V/F (L)	1072 (593–2651)	1487 (762–3485)	0.02
$t_{1/2}$ (h)	2 (1–5)	1 (1–6)	0.04
AUC_{0-last} (ng·h/mL)	264 (92–1129)	151 (38–606)	<0.01
$AUC_{0-\infty}$ (ng·h/mL)	271 (97–1150)	162 (44–618)	<0.01
Dihydroartemisinin			
C_{max} (ng/mL)	66 (10–111)	73 (31–224)	0.55
T_{max} (h)	2 (1–4)	2 (1–4)	0.89
CL/F (L/h)	350 (210–942)	424 (280–626)	0.23
V/F (L)	922 (498–4779)	876 (734–1315)	1
$t_{1/2}$ (h)	1 (1–3)	1 (1–2)	0.06
AUC_{0-last} (ng·h/mL)	213 (68–343)	175 (118–262)	0.27
$AUC_{0-\infty}$ (ng·h/mL)	217 (81–363)	180 (121–272)	0.23
Lumefantrine			
T_{lag} (h)	1 (0–4)	1 (0–1)	0.16
C_{max} (ng/mL)	2532 (1071–5957)	7097 (2396–9462)	<0.01
T_{max} (h)	8 (3–12)	8 (4–12)	0.26
CL/F (L/h)	10 (3–32)	1 (1–5)	<0.01
V/F (L)	179 (53–860)	86 (59–219)	0.01
$t_{1/2}$ (h)	23 (6–51)	31 (24–43)	<0.01
AUC_{0-last} (ng·h/mL)	41119 (12850–125200)	199678 (71205–251015)	<0.01
$AUC_{0-\infty}$ (ng·h/mL)	46925 (14559–136297)	267386 (84845–344468)	<0.01

ritonavir co-administration. Similarly dihydroartemisinin C_{max} and AUC_{0-last} were unaffected (Table 2 and Figure 1b).

Effect of lopinavir/ritonavir on lumefantrine pharmacokinetics

Co-administration of artemether/lumefantrine with lopinavir/ritonavir significantly reduced lumefantrine CL/F and V/F, by 90% ($P<0.01$) and 52% ($P=0.01$), respectively. Lumefantrine C_{max} increased significantly by 180% ($P<0.01$) and AUC_{0-last} by 386% ($P<0.01$) (Table 2 and Figure 1c).

Discussion

We investigated the pharmacokinetics of artemether, dihydroartemisinin and lumefantrine after administration of a single dose of 80/480 mg of artemether/lumefantrine to HIV-infected adults, taken with and without lopinavir/ritonavir-based ART. Co-administration of artemether/lumefantrine with lopinavir/ritonavir significantly increased artemether clearance with a consequently significant reduction in artemether exposure. Dihydroartemisinin pharmacokinetic parameters were not affected by lopinavir/ritonavir. Lumefantrine clearance significantly decreased with a consequently significant increase in exposure.

Our data for the direction of the interaction between lopinavir/ritonavir and artemether/lumefantrine show a similar trend to data from a previous study by German *et al.*,²⁰ however, differences in the magnitude of the interaction as well as the effect on dihydroartemisinin were evident between the two studies. The previous study demonstrated a trend towards decreased artemether exposure, significant reduction in dihydroartemisinin exposure and significant increase in lumefantrine exposure following standard six-dose artemether/lumefantrine administration with lopinavir/ritonavir to 13 healthy HIV-seronegative adults.²⁰ The differences in the results from the two studies possibly arise from differences in the study designs and population. German *et al.*²⁰ conducted a sequential cross-over study in which artemether/lumefantrine parameters were compared within the same individuals with and without lopinavir/ritonavir, while we employed a parallel study design with comparison of parameters from different individuals with and without lopinavir/ritonavir. The parallel study design was adequate for the objectives of our study, but has a limitation due to the high inter-individual variability of artemether and dihydroartemisinin. Comparison of pharmacokinetic exposures in the same individuals using the sequential design was not feasible given that lopinavir/ritonavir is used for second-line HIV treatment in our study setting.

In addition, our population was composed of HIV-infected adults of African origin, unlike the HIV-uninfected healthy

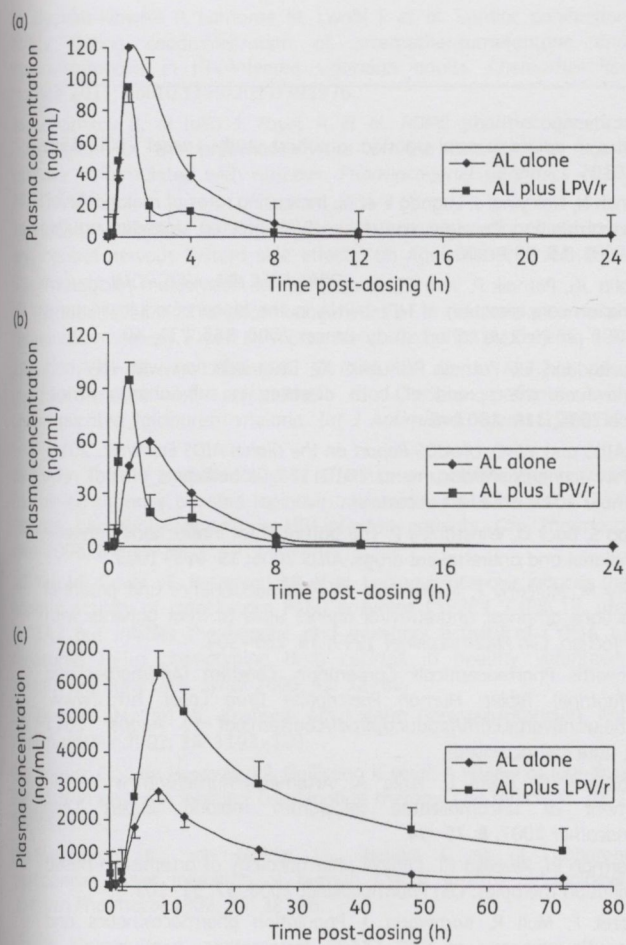


Figure 1. Mean (\pm SEM) plasma concentration versus time of (a) artemether, (b) dihydroartemisinin and (c) lumefantrine for participants taking artemether/lumefantrine alone (AL alone) and artemether/lumefantrine in combination with lopinavir/ritonavir (AL plus LPV/r).

volunteers of primarily white origin in the study by German *et al.*²⁰ Genetic variation may cause inter-individual pharmacokinetic variability due to polymorphisms of genes encoding drug-metabolizing enzymes.^{25–27} In addition, drug pharmacokinetics may differ in healthy volunteers compared with patients with disease.

Further differences in the magnitude of the effects of interaction between our data and the German *et al.*²⁰ data could have arisen from the six-dose compared with the single-dose regimen of artemether/lumefantrine. We administered a single dose of artemether/lumefantrine to avoid any unknown adverse effects of co-administration of artemether/lumefantrine with lopinavir/ritonavir in HIV-infected participants. German *et al.*²⁰ administered the standard six-dose artemether/lumefantrine regimen to healthy volunteers. Artemether undergoes auto-induction of its metabolism, and artemether/dihydroartemisinin ratios after 3 days of treatment with the standard dose are lower than those seen after a single dose.²⁸

In both studies lumefantrine exposure was elevated during co-administration with lopinavir/ritonavir; however, despite the elevated lumefantrine exposure, participants tolerated the study drugs very well, with all reported adverse events consistent with what had previously been reported for artemether/lumefantrine and lopinavir/ritonavir. Our data did not demonstrate evidence of cardiac conduction abnormalities. However, caution and safety monitoring of HIV/malaria-coinfected patients receiving artemether/lumefantrine with lopinavir/ritonavir is advised. It will be important to determine if these effects are additive in the standard six-dose artemether/lumefantrine regimen in HIV/malaria-coinfected patients receiving lopinavir/ritonavir.

Ritonavir-boosted lopinavir influences the activity of several CYP enzymes and drug transporters such as the efflux transporter P-glycoprotein.²⁹ Both lopinavir and ritonavir inhibit intestinal and hepatic CYP3A4 and P-glycoprotein.^{29,30} Inhibition of CYP3A4 or P-glycoprotein expression decreases biotransformation, resulting in an increase in bioavailability of co-administered substrates.^{9,31–33} Previous data demonstrated increased artemether, dihydroartemisinin and lumefantrine exposure in the presence of the CYP3A4 inhibitors ketoconazole and grapefruit juice.^{34,35} Inhibitions of CYP3A4 and P-glycoprotein are likely explanations for the increased lumefantrine exposure in our study.

The reduction in artemether exposure was unexpected, since CYP3A4 is suggested to be the predominant CYP enzyme in the metabolism of artemether.¹⁰ Although artemether is predominantly metabolized via CYP3A4/5, other CYP enzymes (CYP2B6, CYP2C9, CYP2C19 and possibly CYP2A6) are involved.^{10,13,14} Lopinavir/ritonavir was shown to induce CYP1A2, CYP2B6, CYP2C9, and CYP2C19.^{30,36} The observed increased clearance and decreased artemether exposure is likely due to induction of these CYP enzymes by lopinavir/ritonavir.

Dihydroartemisinin is converted into inactive metabolites by UGT1A1, UGT1A8/9 and UGT2B7.^{10,13,14} Induction and inhibition of UGTs by xenobiotics have been described previously, and lopinavir/ritonavir was shown to inhibit UGTs 1A1, 1A3, 1A4, 1A6, 1A9 and 2B7.^{37–39} However, we found no statistical difference in the pharmacokinetic parameters of dihydroartemisinin after lopinavir/ritonavir co-administration compared with administration alone. The reason for this is unclear, but might be due to the small numbers and large inter-individual variability.

Artemether and dihydroartemisinin have very short half-lives and rapidly clear parasites from circulation.¹¹ Both are very potent antimalarial agents, although dihydroartemisinin is more potent.¹⁶ Lumefantrine has a much longer half-life and mainly clears residual parasites, preventing recrudescence. Higher artemether and dihydroartemisinin exposure decreases parasite clearance time,¹³ but the major determinant of radical cure is lumefantrine exposure.⁴⁰ Given that HIV/malaria-coinfected patients present with higher parasite counts,^{3,41} which is an independent predictor of poor treatment response,⁴² reduction in artemether exposure may predispose patients to develop severe malaria due to slower parasite clearance. The clinical relevance of the present findings should be interpreted with caution given that we administered a single artemether/lumefantrine dose while a six-dose artemether/lumefantrine regimen is administered for malaria treatment.

The reduction in artemether exposure by lopinavir/ritonavir after the single artemether/lumefantrine dose may be offset

by the increase in lumefantrine exposure. Previous data revealed that lumefantrine exposure is the key determinant for malaria cure,⁴³ therefore the increase in lumefantrine exposure during lopinavir/ritonavir co-administration may be beneficial for malaria cure. However, rapid clearance of artemether and reduced clearance of lumefantrine may create longer periods of exposure to lumefantrine monotherapy with the risk of development of resistance.

Conclusions

Co-administration of a single dose of artemether/lumefantrine with lopinavir/ritonavir significantly reduced artemether exposure, with a significant increase in lumefantrine exposure. Population pharmacokinetic and pharmacodynamic trials will be highly valuable in evaluating the clinical significance of this interaction and determining whether dosage modifications are indicated.

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Transparency declarations

None to declare.

Author contributions

P. B.-K., M. L., S. K. and C. M. contributed to the design and conduct of the study. P. B.-K., M. L. and V. O.-K. participated in recruitment of patients and data collection. W. H. and N. L. performed the bioanalytical assays. P. B.-K., T. P. C. D., J. T., N. L. and P. J. d. V. analysed and interpreted the pharmacokinetic data. H. M.-K., E. K., N. P., P. J. d. V., D. B., S. K. and C. M., participated in training the study staff, and provided scientific support. P. B.-K. drafted the first version, and all authors reviewed and approved the manuscript for submission.

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Review Article

Artemether-Lumefantrine Combination Therapy for Treatment of Uncomplicated Malaria: The Potential for Complex Interactions with Antiretroviral Drugs in HIV-Infected Individuals

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Treatment of malaria in HIV-infected individuals receiving antiretroviral therapy (ART) poses significant challenges. Artemether-lumefantrine (AL) is one of the artemisinin-based combination therapies recommended for treatment of malaria. The drug combination is highly efficacious against sensitive and multidrug resistant *falciparum* malaria. Both artemether and lumefantrine are metabolized by hepatic cytochrome P450 (CYP450) enzymes which metabolize the protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs) used for HIV treatment. Coadministration of NNRTIs and PIs with AL could potentially cause complex pharmacokinetic drug interactions. NNRTI by inducing CYP450 3A4 enzyme and PIs by inhibiting CYP450 3A4 enzymes could influence both artemether and lumefantrine concentrations and their active metabolites dihydroartemisinin and desbutyl-lumefantrine, predisposing patients to poor treatment response, toxicity, and risk for development of resistance. There are scanty data on these interactions and their consequences. Pharmacokinetic studies to evaluate these interactions in the target populations are urgently needed.

1. Introduction

Human immunodeficiency virus (HIV) and malaria have overlapping geographical distribution. Together, the two diseases account for 4 million deaths a year worldwide [1]. Over 90% of the world malaria burden occurs in sub-Saharan Africa, the region with 67% of the global HIV burden. Given the extensive overlap in geographical distribution of the two diseases, any interaction between the two could have profound public health consequences. In areas with stable malaria transmission, HIV increases risk of malaria infection and clinical malaria especially in individuals with advanced immunosuppression and in areas with unstable malaria

transmission; HIV-infected individuals are at increased risk for severe malaria and death [1, 2].

This vulnerable population requires prompt, safe and effective antimalarial treatment. Current guidelines for malaria treatment advocate use of artemisinin-based combination therapies (ACTs). Treatment of malaria in HIV-infected individuals receiving ART poses significant challenges with gaps in the knowledge of ART and ACT drug-drug interactions and their consequences.

Antiretroviral drugs are among the most therapeutically risky drugs for drug-drug interactions due to the potent inhibition and induction of cytochrome (CYP) enzymes as well as transport proteins. The combination of at least

three drugs for highly active ART increases the risk for drug-drug interactions. The risk of clinically significant interactions involving ART when coadministered with substrates of CYP enzymes is considerable and may result in high concentrations with excessive toxicity or reduced concentrations with reduced efficacy and risk for development of resistance. Clinically significant CYP-mediated drug-drug interactions are more likely to occur with non nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors- (PIs-) based ART regimens because these are substrates, inducers, and/or inhibitors of CYP enzymes which metabolize the majority of drugs and xenobiotics [3]. Nucleoside reverse transcriptase inhibitors do not undergo CYP-mediated metabolism and are less likely to cause CYP-mediated drug interactions. They may cause interactions by influencing absorption, distribution, and elimination of coadministered drugs.

2. Artemether-Lumefantrine Use in Treatment of Malaria

Artemether and lumefantrine have different modes of action and act at different points in the parasite life cycle. Oral formulations of AL are available as tablet and dispersible formulations with similar pharmacokinetic properties [4, 5]. A six-dose regimen of artemether (20 mg) coformulated with lumefantrine (120 mg) is recommended; with first and second doses taken eight hours apart, the third dose is taken 24 hours after the first and the remaining doses 12 hours apart [6, 7]. Food enhances absorption of both artemether and lumefantrine although this effect is more apparent for lumefantrine [8–10]. The typical fat content of African diets has been demonstrated to be adequate for optimal absorption of AL [11], although the loss of appetite, nausea and vomiting in patients with malaria may compromise fat intake. Plasma concentrations of lumefantrine remain high with repeated doses over the 3 day course; however, poor adherence to the 3-day regimen may reduce effectiveness of AL. In multidrug resistant areas, day 7 lumefantrine concentration was a useful surrogate marker for AUC and concentrations of less than 280 ng/mL predicted treatment failure [9, 12].

Efficacy of the 6-dose regimen of AL is consistently greater than 95%, with rapid parasite and symptom clearance and significant gametocidal effect [4, 13–17]. A few cases of treatment failure are recorded after AL treatment; however, these are mostly reinfections [18–21]. This is of particular concern in areas with very intense malaria transmission where antimalarial drugs with longer half-life may offer the advantage of preventing reinfection. It is also of concern in HIV-infected individuals who are at increased risk for malaria infection [22]. Use of cotrimoxazole prophylaxis and insecticide treated bednets markedly reduces the incidence of malaria in HIV-infected individuals and are recommended.

AL is safe and well tolerated. Majority of adverse events are of mild or moderate severity mostly affecting gastrointestinal and nervous systems; however, most are typical of the symptomatology of malaria or concomitant

infections [4, 14–17, 23]. Although lumefantrine possesses similar chemical structure with halofantrine which is known to cause cardiac arrhythmia and sudden death, safety studies have not shown lumefantrine to be cardiotoxic or to prolong QTc interval at therapeutic doses [24, 25].

3. Pharmacology of Artemether

Artemether is derived from the Chinese herb sweet wormwood (*Artemisia annua*). The antimalarial properties of artemether stem from interference with parasite transport proteins, disruption of parasite mitochondrial function, inhibition of angiogenesis, and modulation of host immune function [26]. Artemether is absorbed very rapidly after oral administration reaching peak plasma concentrations within 2 hours after dose [8, 10, 12]. It has a half-life of 1–3 hours. It is metabolized quickly via CYP450 2B6, CYP450 3A4 and possibly CYP450 2A6 [27, 28] to the more potent antimalarial metabolite DHA, which in turn is converted to inactive metabolites primarily by glucuronidation via UGT1A1, 1A8/9 and 2B7 [27]. Artemether induces CYP450 2C19 and 3A4 [28]. DHA reaches maximum plasma concentration within 2-3 hours after dosing. Artemether acts rapidly to clear malaria parasites from circulation. Both artemether and DHA offer potent antimalarial properties causing significant reduction in asexual parasite mass of approximately 10,000 fold (4 log) per reproductive cycle, with prompt resolution of symptoms [29, 30].

4. Pharmacology of Lumefantrine

Lumefantrine is an aryl-amino alcohol [27] that prevents detoxification of hemozoin, such that toxic hemozoin and free radicals induce parasite death [31]. Lumefantrine absorption occurs 2 hours after oral intake reaching peak plasma concentration after 3-4 hours [9]. It has a half life of 3–6 days and is responsible for preventing recurrent malaria parasitemia [32]. It is absorbed and cleared slowly acting to eliminate residual parasites that may remain after artemether and DHA have been cleared from the body and thus prevents recrudescence [8, 31]. Lumefantrine is metabolized by N-debutylation mainly by CYP450 3A4 [27, 28] to desbutyl-lumefantrine with 5–8-fold higher antiparasitic effect than lumefantrine. Lumefantrine inhibits CYP450 2D6 [28].

5. Pharmacology of Antiretroviral Drugs

Current guidelines for treatment of HIV in most resource limited settings recommend combination therapy of 2 NRTIs and 1 NNRTI as initial treatment for ART naïve patients and for patients with treatment failure; 2NRTIs and 1 PI are recommended. The NRTIs are analogues of naturally occurring deoxynucleotides needed to synthesize viral DNA. They are well absorbed after oral administration; however, NRTIs must be converted to their active metabolites, NRTI triphosphates, intracellularly, after endocytosis by addition of three phosphate groups to their deoxyribose moiety, a reaction catalyzed by cellular kinase enzymes. The triphosphate

metabolites compete with the natural deoxynucleotides for incorporation into the viral DNA chain. Their incorporation inhibits formation of phosphodiester bridges and prevents viral DNA synthesis and elongation. Most NRTIs are excreted unchanged through the kidney while zidovudine is excreted via the liver through glucuronidation.

The NNRTIs inhibit reverse transcriptase enzyme by binding directly and noncompetitively to the enzyme at a position in close proximity to the substrate binding site for nucleosides inducing conformational changes that impact enzyme catalytic activities. The resulting complex blocks the catalyst activated binding site which in turn, binds fewer nucleosides, slowing down polymerization significantly [33]. Nevirapine and Efavirenz are the two NNRTIs available for use in most malaria endemic regions [34].

6. Nevirapine

Nevirapine is administered with a dose escalation schedule starting at an adult dose of 200 mg once daily for 2 weeks followed by 200 mg twice daily thereafter because of the potential for adverse events and metabolic autoinduction of CYP450 enzymes. Absorption is not affected by food, acids, or alkali, and more than 90% of the administered dose is absorbed after oral intake [35] with bioavailability of more than 90% and about 60% protein binding.[36] The elimination half life is 25–30 hours. It is distributed throughout the body [35], metabolized by CYP450 3A4 and 2B6, and excreted via the liver and kidneys in the form of glucuronide conjugates of hydroxylated metabolites [35]. It is both a substrate and inducer of CYP450 3A4, and 2B6 [36].

Tolerability to nevirapine in majority of patients is relatively good [37]. The adverse event most commonly observed is a hypersensitivity rash, occurring in about 16% of patients with about 7% experiencing grade 3 or 4 rash with the Steven Johnson syndrome. Hypersensitivity is more common during the first 6 weeks of treatment [35]. The second common adverse event is hepatotoxicity with elevated liver enzymes. Female sex and a high CD4 cell count are associated with higher incidence of nevirapine-induced hypersensitivity [35, 38].

7. Efavirenz

Efavirenz is available as capsules, film-coated tablets and liquid formulation for oral administration. The recommended adult dose is 600 mg od, taken on an empty stomach, preferably at bedtime to diminish possible neuropsychiatric side effects that are enhanced with increased bioavailability in presence of food. It is highly protein bound (>99%), predominantly to albumin [39]. Oral bioavailability is good; reaching peak plasma concentrations within 3–5 hours after dose administration. It has a long serum half-life of 45 hours and reaches steady-state plasma concentrations in 6 to 10 days [40]. Efavirenz is a substrate, inhibitor and inducer of several CYP450 enzymes (2B6, 3A4, 2A6, 2C9, and 2C19) and induces its own metabolism [39]. It is metabolized to inactive hydroxylated metabolites that include 8-hydroxy

and 7-hydroxyefavirenz. Hydroxylated efavirenz metabolites undergo subsequent urinary and biliary excretion after conjugation mainly glucuronidation.

The safety profile of efavirenz is good with minor side effects including skin rash and neuropsychiatric events. The rash is maculopapular, often of mild to moderate intensity (grade 1 or 2), occurring between the first and third week of treatment with incidence up to 34% [40]. It resolves spontaneously within one month or with treatment interruption, after which efavirenz may be reinitiated cautiously. The incidence of grade 3 or 4 rash with Stevens-Johnson syndrome is only 0.1%, and once it occurs, treatment should be stopped immediately. Neuropsychiatric events may occur including dizziness, insomnia, somnolence, impaired concentration, vivid dreams, and nightmares. More severe events like severe depression, suicidal ideation, nonfatal suicidal attempts, aggressive behaviour, paranoid, and manic reactions seldom occur [41]. Hepatotoxicity has been shown to occur during efavirenz treatment [38] with increased risk in patients with chronic viral hepatitis. Other side effects include gynaecomastia, increase in HDL-cholesterol, and elevated triglycerides.

8. Lopinavir/Ritonavir

The PIs prevent viral replication by inhibiting activity of the HIV protease enzyme and preventing HIV from being successfully assembled and released from the infected CD4 cell. HIV-1 protease is an aspartic protease that cleaves both structural and functional proteins from precursor viral polypeptide strands. Inhibition of the protease produces immature, noninfectious virions, thus preventing subsequent cellular infection [42]. Lopinavir coformulated with ritonavir is the most frequently prescribed PI in most malaria endemic regions.

Lopinavir is 3 to 4 times more active against HIV than ritonavir; however, when lopinavir is administered alone, it exhibits poor bioavailability. Lopinavir is metabolized extensively by CYP450 3A4 and coadministration with ritonavir which is a potent inhibitor of CYP450 3A4 results in increased and sustained concentrations of lopinavir [43]. Both drugs are primarily eliminated by the fecal route with urinary excretion accounting for <2% of the eliminated drug [44]. The co-formulation is available in tablet formulation called (Aluvia) for adults, each tablet containing 200 mg lopinavir and 50 mg ritonavir.

The standard dose is 400 mg/100 mg twice daily in treatment experienced patients. Combination therapy with lopinavir/ritonavir containing regimens is well tolerated and effective in suppressing HIV-RNA and increasing CD4⁺ T cell counts [43]. The most frequent side effects are generally mild to moderate and mainly in the gastrointestinal system where diarrhea, nausea, and vomiting may occur. Other side effects are hypertriglyceridemia, hypercholesterolemia, pancreatitis, transient elevations in transaminase levels, insulin resistance, new onset diabetes, and worsening of pre-existing diabetes. Fat redistribution occurs with central obesity, dorsocervical fat enlargement (buffalo hump), peripheral wasting, facial

wasting, breast enlargement, and cushingoid appearance. Less common adverse effects include allergic reaction, asthenia, malaise, headache, myalgias, arthralgias, myocardial infarction, seizures, and lactic acidosis [43].

9. Potential for Pharmacokinetic Interactions between AL and ART

9.1. Effect of ART on AL. Coadministration of NNRTI or PI-based ART with AL could potentially cause drug interactions with effects on the plasma concentrations of artemether and lumefantrine with unknown effects on parasite clearance and adverse effects. There are very scanty data on these interactions and their effects, yet AL and ART continue to be coprescribed in malaria endemic regions. A study that investigated the pharmacokinetics of the standard 6 dose of AL as 80/480 mg twice daily when administered with lopinavir/ritonavir 400/100 mg twice daily in healthy HIV-seronegative volunteers demonstrated 2- to 3-fold increases in lumefantrine AUC and trends towards decreases in artemether C_{max} and AUC with decrease in DHA AUC. The authors concluded that coadministration of AL and lopinavir/ritonavir can be carried out but highlighted the need for formal safety analysis of concomitant therapy [45]. Data from another pharmacokinetics study of HIV-infected participants without malaria, surprisingly demonstrated significantly increased lumefantrine exposure when coadministered with nevirapine although toxicity was not increased [46].

9.2. Effect of AL on ART. It is not known what plasma levels of ART will result if AL is administered with ART; however, since malaria infection occurs as an acute illness requiring a short course of therapy, the effect of AL on ART may only be transient with clinically insignificant results. However, in malaria endemic regions, where individuals are exposed to repeated malaria infections requiring treatment, the effect of drug interactions combined with the transient increase in viral replication and viral load [47] may be similar to effects of suboptimal adherence to ART.

10. Conclusion

There is potential for pharmacokinetic drug interactions between AL and NNRTIs and PIs in HIV-infected patients with malaria. Data on these interactions is sparse. These interactions, if not properly addressed, might have an impact on the Useful Therapeutic Lives (UTL) of the concerned drugs. Results of pharmacokinetic studies evaluating these interactions in depth and their implications are needed.

Disclosure

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Research Article

Cardiac Conduction Safety during Coadministration of Artemether-Lumefantrine and Lopinavir/Ritonavir in HIV-Infected Ugandan Adults

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Background. We aimed to assess cardiac conduction safety of coadministration of the CYP3A4 inhibitor lopinavir/ritonavir (LPV/r) and the CYP3A4 substrate artemether-lumefantrine (AL) in HIV-positive Ugandans. **Methods.** Open-label safety study of HIV-positive adults administered single-dose AL (80/400 mg) alone or with LPV/r (400/100 mg). Cardiac function was monitored using continuous electrocardiograph (ECG). **Results.** Thirty-two patients were enrolled; 16 taking LPV/r-based ART and 16 ART naïve. All took single dose AL. No serious adverse events were observed. ECG parameters in milliseconds remained within normal limits. QTc measurements did not change significantly over 72 hours although were higher in LPV/r arm at 24 (424 versus 406; $P = .02$) and 72 hours (424 versus 408; $P = .004$) after AL intake. **Conclusion.** Coadministration of single dose of AL with LPV/r was safe; however, safety of six-dose AL regimen with LPV/r should be investigated.

1. Introduction

Malaria and HIV infection are leading causes of morbidity and mortality and remain major health problems in endemic regions. Malaria causes about 300–500 million clinical cases annually, 90% of which occur in sub-Saharan Africa [1]. The Joint United Nations Program on HIV/AIDS (UNAIDS) estimated that 29.4 Million Africans are infected with HIV (UNAIDS, December 2002). Together malaria and HIV account for over four million deaths per year.

Studies have demonstrated increased risk for malaria in HIV infected patients especially those with lower CD4 cell counts [2–4]. More evidence suggests transient increase in HIV viral load in patients with acute malaria episodes [5]. A

major challenge to the treatment of malaria in HIV-infected individuals is the potential for pharmacokinetic (PK) drug interactions with concerns regarding safety and efficacy [6].

Due to the widespread resistance to older antimalarial drugs, the World Health Organization now recommends artemisinin combination therapy (ACT) for malaria treatment [7]. Artemether-lumefantrine (AL) is an oral fixed-dose combination tablet of artemether (a derivative of artemisinin) and lumefantrine (a racemic mixture of a synthetic fluorine derivative). The drug combination is highly efficacious against sensitive and multidrug resistant *Plasmodium falciparum*; with the advantage of rapid clearance of parasites by artemether and the slower elimination of residual parasites by lumefantrine [7–9].

Recommendations for antiretroviral therapy (ART) include two nucleoside reverse transcriptase inhibitors (NRTIs) plus a nonnucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). Lopinavir/ritonavir (LPV/r) is an oral fixed-dose combination tablet of LPV (a PI) with low dose ritonavir, a pharmacoenhancer that significantly increases LPV plasma concentrations by cytochrome P450 3A4 (CYP3A4) inhibition.

Concerns over safety monitoring of LPV/r have become more crucial following the recent FDA alert on cardiotoxicity of LPV/r. Safety information on LPV/r includes warnings and precautions regarding QT/QTc interval and PR interval prolongation. According to the revised safety label, LPV/r prolongs the PR interval, and cases of second- or third-degree atrioventricular block have been reported in some patients. Indeed LPV/r should be used with caution in patients who may be at increased risk of developing cardiac conduction abnormalities, such as those with underlying structural heart disease, preexisting conduction system abnormalities, ischemic heart disease, or cardiomyopathies. The effect on the PR interval of coadministration of LPV/r with other drugs that prolong the PR interval has not yet been determined and should be undertaken with caution. Clinical monitoring is recommended especially during coadministration with drugs metabolized by CYP3A [10].

Data from previous studies indicate that artemether and lumefantrine are predominantly metabolized by CYP3A4 [6, 11, 12]. Knowledge of their metabolism suggests potential for PK drug-drug interactions [6]. LPV/r is a potent inhibitor of CYP3A4, therefore, inhibition of CYP3A4 may raise plasma concentrations of artemether and lumefantrine but decrease plasma concentrations of dihydroartemisinin (DHA) the metabolite of artemether. A study that investigated the pharmacokinetics of AL when administered with LPV/r in HIV-uninfected healthy volunteers demonstrated 2 to 3-fold increases in lumefantrine AUC and trends towards decreases in artemether C_{max} and AUC. Formal safety analysis of coadministration was not performed in this study [13]. Increased plasma concentrations of artemether and lumefantrine may enhance toxicity. Lumefantrine has some structural similarity to halofantrine which is cardiotoxic mainly in form of QTc prolongation. Therefore, vigilant evaluation of the cardiac safety of lumefantrine, especially when coadministered with a potent CYP3A4 inhibitor, is warranted [14–17]. We aimed to assess the cardiac safety of coadministration of a single dose of AL (80/480 mg) with LPV/r based ART in HIV-positive Ugandan patients.

2. Materials and Methods

2.1. Ethical Considerations. The study was approved by the Scientific Review Committee of the Infectious Diseases Institute (IDI) of Makerere University, the Uganda National HIV/AIDS Research Committee (ARC 056) and was registered with Uganda National Council of Science and Technology (HS 197) and ClinicalTrials.gov (NCT 00619944). All participants gave written informed consent to participate, and all study procedures were conducted according to Good Clinical Practice (GCP).

2.2. Study Site. The study was conducted between January 2008 and June 2009 at the IDI and the Uganda Heart Institute (UHI) of Mulago National Referral Hospital in Kampala, Uganda. The IDI is a regional centre of excellence for HIV/AIDS treatment, prevention, training and research. To date, over 20,000 HIV-infected patients are registered at the IDI with over 8,000 taking ART. About 10% these are on LPV/r-based second line ART.

2.3. Study Design and Population. This was a two-arm study to assess the safety of coadministration of AL in HIV-positive patients taking LPV/r-based ART and ART naïve patients. Patients were eligible to participate if they were older than 18 years of age, provided written informed consent, had no evidence of systemic illness and required no medications that had known potential for drug interactions with study drugs. Patients with abnormal ECG tracing, abnormal clinical test results, positive blood smear for malaria, pregnant mothers and those who reported use of herbal medication were excluded from the study.

2.4. Study Procedures. Patients were screened and enrolled from the cohort of patients attending the IDI. The LPV/r arm consisted of patients stable on LPV/r 400/100 mg-based ART for at least one month and the ART naïve arm consisted of patients who had not started ART and were not yet eligible for ART according to national guidelines. Patients in both arms took cotrimoxazole daily for prophylaxis against opportunistic infections. Participants had detailed study explanation at enrolment. Adherence to study drugs was assessed using self-report and pill count by the study pharmacist. We collected information on adverse drug events and serious adverse drug events, and a questionnaire on quality of life was administered on each study day. On the evening prior to the study day, participants were reminded of their study day appointment, were given detailed instructions to take their medication and food at 8.00 pm, and told to arrive at the hospital by 7.00 am in a fasting state. On the study day, patients were admitted at the UHI and a 12-lead ECG monitor was attached for continuous cardiac function monitoring. The intake of a standardized breakfast and morning doses of drugs was directly observed by study staff. All patients took a single dose of AL of 80/480 mg with 150 mL of water. Patients in the LPV/r arm took LPV/r (400/100 mg) with their AL dose. ECG monitoring was performed continuously for the first 12 hours after AL intake. Patients were then discharged and returned for the following three mornings ($T = 24, 48, \text{ and } 72$ hours) for a single ECG tracing.

2.5. Safety Assessment. Medical history, physical examination, vital signs, routine clinical laboratory tests, ECGs and urine screens for pregnancy were performed at screening. On the study day, medical history, physical examination, vital signs, and a blood smear for malaria parasites were performed. Adverse events were recorded continuously throughout the trial, and the onset, duration, severity, and relationship to the trial drugs if any were noted. Standard

TABLE 1: shows a comparison of the baseline characteristics of study patients.

Variable	LPV/r arm median (IQR)	ART naïve arm median (IQR)	P value
Age (yrs)	38 (33–41)	34 (28–39)	.2
Weight (kgs)	65 (54–73)	64 (56–71)	.9
Height (cms)	163(158–172)	163 (153–169)	.1
BMI	21 (19–24)	25 (22–31)	.06
Viral load (c/mL)	<400	26756 (5548–181186)	<.01
Hb (g/dL)	14 (14–15.4)	12.2 (12–14)	.003

TABLE 2: shows the mean electrocardiograph (ECG) parameters in milliseconds after AL dosing.

Variable	LPV/r arm mean (SD)	ART naïve arm mean (SD)	P value
Heart rate	69 (8.1)	71 (5.9)	.5
PR	154 (18.4)	169 (15.9)	.02
QRS	87.4 (6.6)	82.8 (6.6)	.06
QTc	421 (20.0)	404 (20.7)	.03

12-lead ECGs were recorded at screening, immediately prior to dosing ($T = 0$ hour), and continuously for 12 hours after dose of AL, then daily for three days. QTc-intervals were calculated using the Bazett formula ($QTc = QT/\sqrt{RR}$) [37, 38] to correct for the influence of heart rate. A senior cardiologist evaluated the PR, QRS, and QT intervals visually on the ECG.

2.6. Statistical Analysis. Demographic and ECG results were entered into EpiData and exported to SPSS version 12.0 and STATA version 8.0 for statistical analysis. Continuous variables were summarized into means, and medians and compared using the Independent *T*-test. A *P*-value < .05 was considered statistically significant.

3. Results

A total of 72 HIV-positive patients (41, 65% females) were screened between January and June 2009; 12 (17%) were excluded because they had other concurrent illnesses that required treatment, 28 (39%) were excluded because they had abnormal ECG tracings and 32 (44%) were enrolled, 16 in each arm. Patients in the two study arms were comparable on majority of baseline characteristics (Table 1); however, patients in the LPV/r arm had significantly higher hemoglobin levels with lower viral load.

There were no serious adverse events during the study period. ECG parameters (heart rate, PR-interval, QRS-complex and QTc) remained well within normal limits in both study arms (Table 2). The mean QRS-complex and QTc interval after AL administration were higher in the LPV/r arm compared to the ART naïve arm (87.4 versus 82.8, $P = .06$ and 421 versus 404, $P = .03$, resp.) but the mean PR-interval was significantly higher in the ART naïve arm (154 versus 169, $P = .02$) (Table 2). Mean (SD) change in

TABLE 3: shows the median QTc interval measurements in milliseconds over 72 hours period after AL dosing.

Time	QTc (ms) median (IQR)		P value
	LPV/r arm	ART naïve arm	
Screening	415 (403–439)	395 (388–425)	.14
12 hours	415 (404–439)	419 (403–427)	.7
24 hours	424 (401–434)	406 (393–411)	.02
48 hours	411 (396–432)	409 (401–419)	.7
72 hours	424 (416–441)	408 (392–417)	.004

QTc interval values from the pre-AL QTc interval values was greater for the ART naïve arm compared to the LPV/r arm (6.7 (15.4) versus -0.8 (13), $P = .17$). The QTc interval measurements for participants in both study arms remained within normal ranges over the 72 hours period (Table 3); with none above the upper limit of normal (450 ms for males and 470 ms for females).

4. Discussion

LPV/r is a potent inhibitor of CYP3A4, therefore, coadministration with AL which is predominantly metabolized by CYP3A4 may potentially result in enhanced pharmacological and toxicological effects. We aimed to assess the cardiac safety of coadministration of a single dose (80/480 mg) of AL in HIV-infected patients taking LPV/r based ART and HIV positive ART naïve patients. Since LPV/r is a potent CYP3A4 inhibitor, only a single dose of AL was given in order to avoid any unknown potential adverse effects of the latter.

We found that HIV-positive patients taking LPV/r had a higher QTc interval prior to administration of AL compared to HIV-positive ART naïve patients, nevertheless, the difference was not statistically significant. It is possible that this could have been a result of the effects of LPV/r on the heart; however, we cannot establish a causal relationship since we did not have QTc measurements for these patients prior to initiation of LPV/r. This however, raises concern especially in view of the recent FDA alert over the effects of LPV/r on the heart. Indeed the label for LPV/r includes warnings and precautions regarding QT/QTc interval and PR interval prolongation [10].

Although the QTc interval for the LPV/r arm was significantly higher than that for the ART naïve arm at 72 hours, the difference could not be attributed to LPV/r coadministration with AL because baseline QTc interval was higher in the LPV/r arm and both study arms had an increment in QTc interval values from baseline which remained well within normal limits (Table 3). It is possible that the increment in the QTc intervals could have been more if patients had received the full six-dose regimen of AL. The LPV/r label clearly states that LPV/r should be avoided in patients using drugs that prolong the QT interval. Since we do not know what levels and effects of lumefantrine would result if the full six-dose AL regimen is coadministered with LPV/r, we suggest close clinical monitoring of HIV-positive patients taking LPV/r with AL concomitantly until more data becomes available.

This is one of the very few studies that have assessed the cardiac safety of coadministration of AL and LPV/r in HIV positive patients. Previous studies have evaluated safety of AL in healthy volunteers and patients with malaria. Bindschedler and others found that the QTc interval remained unchanged after a single dose of AL in healthy males [14]. The difference in results may be explained by the difference in the study populations. Bindschedler and others demonstrated significant exposure dependent increase in the QTc interval in healthy males after halofantrine. It is possible that LPV/r coadministered with a full six-dose regimen of AL may cause increased concentrations of lumefantrine causing an exposure dependent QTc interval prolongation. Previous data showed no evidence of cardiotoxicity during AL treatment in healthy volunteers [18]. However, these were conducted in patients with malaria without coadministration of LPV/r. It is possible that results may be different with concomitant treatment with the full six-dose AL regimen and LPV/r.

5. Conclusion and Recommendation

Our data suggests no evidence of cardiac conduction abnormalities after concomitant treatment with LPV/r and a single dose AL. There is need to assess the safety of the full six-dose regimen of AL in HIV positive patients receiving LPV/r based ART.

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Update on the efficacy, effectiveness and safety of artemether–lumefantrine combination therapy for treatment of uncomplicated malaria

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Abstract: Artemether–lumefantrine is one of the artemisinin-based combination therapies recommended for treatment of uncomplicated *falciparum* malaria. The drug combination is highly efficacious against sensitive and multidrug resistant *falciparum* malaria. It offers the advantage of rapid clearance of parasites by artemether and the slower elimination of residual parasites by lumefantrine. The combination can be used in all populations except pregnant mothers in the first trimester where safety is still uncertain. There are still concerns about safety and pharmacokinetics of the drug combination in children, especially infants, pregnant mothers and drug interactions with mainly non-nucleoside reverse transcriptase inhibitors and protease inhibitors used for HIV therapy.

Keywords: artemether–lumefantrine, efficacy, effectiveness, safety, malaria

Introduction

Malaria is a febrile illness caused by intracellular protozoa of the genus *Plasmodium*, and transmitted by the bite of an infected female mosquito of the genus *Anopheles*. *Plasmodium* species that cause disease in humans include: *P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi*. *P. falciparum* is the most prevalent and most virulent. Worldwide, malaria is one of the most important causes of morbidity and mortality. Approximately 2.2 billion people are exposed to malaria every year of whom about 300 to 500 million develop disease. In 2006, there were 247 million cases of malaria, causing nearly 1 million deaths, mostly among African children.¹ Malaria deaths are responsible for almost 3% of the world's disability-adjusted life years, not counting the considerable and imprecisely quantified burden due to morbidity and disability.² In addition to causing significant morbidity and mortality, malaria significantly contributes to poverty through lost productivity and economic loss on antimalarial treatment. African countries spend US\$12 billion annually on malaria, with individual African families spending up to 25% of their income on malaria prevention and control. Malaria has slowed economic growth in African countries by 1.3% per year. As a result of the compounded effect over 35 years, the gross domestic product for African countries is now up to 32% lower than it would have been in absence of malaria.³

Reduction in malaria-associated morbidity and mortality largely depends on provision of prompt, effective, safe and affordable antimalarial drugs. Resistance to antimalarial drugs poses a significant challenge to malaria control programs in sub-Saharan Africa. Multi-drug resistance to sulfadoxine–pyrimethamine (SP) and chloroquine was described extensively in sub-Saharan Africa. The World Health

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Organization (WHO) recommends use of artemisinin-based combination treatments (ACT) as first-line therapy. The ACTs combine fast-acting artemisinins with another structurally unrelated and more slowly eliminated compound which permits elimination of residual malarial parasites.⁴⁻⁶ Of the 81 countries with endemic *P. falciparum*, 77 have now adopted the WHO recommendation.⁷ Commonly used ACTs are artemether–lumefantrine (AL), amodiaquine–artesunate (AQAS), mefloquine–artesunate, dihydroartemisinin–piperazine (DP) and naphthoquine–artemisinin. In this review we provide an update on efficacy, effectiveness and safety of AL for treatment of uncomplicated malaria.

Pharmacology of artemether–lumefantrine

A 6-dose regimen of artemether (20 mg) co-formulated with lumefantrine (120 mg) is recommended; with first and second doses taken 8 hours apart, the third dose taken 24 hours after the first and the remaining doses 12 hours apart. The 6-dose regimen is superior to the 4-dose regimen.^{8,9} Artemisinin from which artemether is derived is obtained from the Chinese herb sweet wormwood (*Artemisia annua*). Artemisinins have the most potent and rapid onset of antiparasitic activity against all *Plasmodium* species that infect humans.

Artemether acts rapidly with half-life of 1 to 3 hours, whereas lumefantrine has a half-life of 3 to 6 days and is responsible for preventing recurrent parasitemia.¹⁰ Artemether and lumefantrine have different modes of action and act at different points in the parasite life cycle.^{11,12} Artemether interferes with parasite transport proteins, disrupts parasite mitochondrial function, inhibits angiogenesis and modulates host immune function.¹³ Lumefantrine is an aryl-amino alcohol¹⁴ that prevents detoxification of heme, such that toxic heme and free radicals induce parasite death.¹² Oral formulations of AL are available as tablet and dispersible formulations which have similar pharmacokinetic (PK) properties.^{15,16} Artemether and lumefantrine differ in rates of absorption and elimination. Artemether is rapidly absorbed reaching peak plasma concentrations within 2 hours post dose.^{11,17} It is metabolized rapidly by cytochrome P450 (CYP) 2B6, CYP3A4 and possibly CYP2A610 to dihydroartemisinin (DHA) which in turn is converted to inactive metabolites primarily by glucuronidation via UGT1A1, 1A8/9 and 2B7.¹⁴ The metabolite DHA reaches peak plasma concentration within 2 to 3 hours post dosing.¹¹ Both artemether and DHA offer potent antimalarial properties causing significant reduction in asexual parasite mass of approximately 10,000-fold per reproductive cycle, with prompt resolution of symptoms.^{18,19}

Lumefantrine is absorbed and cleared more slowly, acting to eliminate residual parasites that may remain after artemether and DHA have been cleared from the body and thus prevent recrudescence.^{11,12} Lumefantrine is highly lipophilic, thus absorption is enhanced with a fatty meal; its absorption occurs 2 hours after intake reaching peak plasma concentration after 3 to 4 hours²⁰ with an elimination half life of 4 to 10 days.^{20,21}

Food enhances absorption of both artemether and lumefantrine although this effect is more apparent for lumefantrine.^{11,20} Administration of AL with high-fat meal increased bioavailability of both artemether and lumefantrine by 2-fold and 16-fold respectively.¹¹ Premji et al in an evaluation of the typical fat content of African diets noted that total fat intake is 15 to 30 g/day during breast feeding, >10 g/day in the post weaning phase and 30 to 60 g/day in a normal diet and this is adequate for optimal efficacy of lumefantrine.²²

However, the effect of food on AL absorption is of concern because patients with malaria usually have anorexia, vomiting and low food intake. Lumefantrine is metabolized by N-debutylation mainly by CYP3A410 to desbutyl-lumefantrine with 5- to 8-fold higher antiparasitic effect than lumefantrine. The key PK determinant of cure is the area under the concentration time curve (AUC) of the longer-acting lumefantrine.

Efficacy and effectiveness of AL

Efficacy of the 6 dose regimen of AL judged by elimination of malaria parasites using the 28-day polymerase chain reaction (PCR)-corrected cure rates and resolution of symptoms, has been demonstrated in semi-immune and non-immune populations in Asia and Africa to be consistently greater than 95%, with rapid parasite and symptom clearance and significant gametocidal effect.^{15,23-27} Many studies in Africa and Asia have demonstrated AL to be as efficacious as other ACTs when used in pediatric and adult populations with differing immunity. PCR-corrected day 28 and day 42 cure rates range between 91% and 100% using evaluable patient analysis.²⁸⁻⁶² Correction by PCR enables differentiation between recurrence and recrudescence of the initial infection from re-infection. A few cases of treatment failure were recorded after AL treatment, but these were mostly re-infections.^{29,32,33,38} This is of particular concern in areas with very intense malaria transmission where antimalarial drugs with longer half-life may offer the advantage of preventing re-infection. Lumefantrine with an estimated elimination half-life of 4 to 10 days offers post-treatment antimalarial prophylaxis of up to 4 weeks. Studies showed

both AL and DP to be highly efficacious for treatment of uncomplicated malaria, although DP was superior to AL at preventing new malaria infections.^{33,34,38,50} In addition to excellent efficacy and effectiveness, AL has demonstrated significant gametocidal effects.^{34,42,48,51} A meta-analysis of 32 randomized trials showed AL to be one of the most effective ACTs with 28-day parasitological cure rates of 97.4%.⁶³

Effectiveness of AL may be influenced by poor adherence to the 3-day, 6-dose regimen and the food requirements for AL absorption. Clinical and parasitological responses to AL were similar with both supervised and unsupervised treatment in Uganda.⁶⁴ The supervised treatment arm received AL with fatty food while the unsupervised arm received AL as outpatient treatment with nutritional advice. Unsupervised treatment resulted in lower concentrations of lumefantrine with increased risk of early reinfection.^{4,64} In Uganda and Nigeria adherence to correct AL dose and duration prescribed to febrile children by community medicine distributors was greater than 80% and crude parasitological failure rates varied from 3.7% in Uganda to 41.8% in Nigeria and PCR-adjusted parasitological cure rate was 90.9% in Nigeria and 97.2% in Uganda.⁶ Differences in crude rates may be due to differences in re-infection rates. A recent study of uncomplicated malaria in Uganda showed adherence to AL was 94.5% compared to that of quinine of 85.4% with high unadjusted cure rates of AL of 96% vs 64% for quinine.⁶⁵

In multidrug-resistant areas, day 7 lumefantrine concentration was a useful surrogate marker for AUC and concentrations of less than 280 ng/mL predicted treatment failure.^{17,20} However, results from areas with lumefantrine-sensitive parasites showed no treatment failures despite day 7 concentrations less than 280 ng/mL in 45% of all patients, and re-infections occurred among patients with day 7 concentrations below 400 ng/mL and those who received a lower dose of lumefantrine per kilogram body weight.⁴

Safety of AL

Safety and tolerability of AL has been assessed in clinical trials in Asia and Africa. Most adverse events are mild or moderate, mostly affecting gastrointestinal and nervous systems; however, most are typical of the symptomatology of malaria or concomitant infections.^{15,24–27,66} Serious adverse events were unlikely and were unrelated or most unlikely to be related to study medication.^{15,29–31,33,34,36,38,39,41–43,46–51,53,54,67} Two meta-analyses concluded that AL is well tolerated, with mild or moderate adverse events mostly affecting gastrointestinal and nervous systems. Ototoxicity associated with AL has been reported recently in a few cases,^{68,69} however, this was

not confirmed in a study that investigated hearing sensation following AL treatment.³¹ Lumefantrine possesses a similar chemical structure to halofantrine which is known to cause cardiac arrhythmia; however, safety studies have not shown lumefantrine to be cardiotoxic or to prolong QTc interval.^{67,70} Other studies and a review of 15 trials concluded that AL did not cause hematological adverse events, although pre-clinical trials suggested the repeated exposure to AL may affect blood cell counts.⁷¹

Safety assessment has been conducted during treatment of single episodes of malaria. Safety concerns become more important when AL is administered over the counter, which commonly results in overdiagnosis and overtreatment of malaria, and when patients get recurrent infections requiring repeated treatment. Overdiagnosis of malaria is common in malaria-endemic areas.⁷² There are no standard guidelines for evaluating drug safety and tolerability in antimalarial trials.⁶⁴ Establishing systems for pharmacovigilance in areas where AL is frequently prescribed is of utmost importance and several challenges exist.⁷³

AL use in children

Vomiting, which may be due to disease-related nausea or taste of the medication, may influence drug intake especially in children. A more palatable dispersible formulation of AL is now available and has been shown to be as efficacious as the currently used crushed tablet in infants and children, and with similar safety and PK profile.¹⁵ Pediatric dosing of AL is deduced from adult-based regimens adjusted for body weight, with little consideration for maturational effects on drug absorption and metabolism. Although diet and nutritional status are important determinants of PK processes, drug responses and toxicity, there are few relevant data for AL in this patient group. In resource-constrained areas, children may not be weighed at each clinic visit and dosing in such settings is usually based on age as a proxy measure for weight. Besides research on therapeutic dose levels based on body weight, there is urgent need for evidence-based translation of weight based dosing regimens to regimens that can be based on age, as the majority of fevers in malaria endemic areas are treated with over-the-counter antimalarial drugs without involvement of the formal health sector. Age-based dose regimens are more practical than weight-based regimens, but will inevitably result in a greater proportion of children receiving either too much or too little drug. This is a particular concern with lumefantrine, which has a narrow therapeutic margin between effective and toxic concentrations. This dosing consideration is especially important in malnourished,

pre-school children and during onset of puberty when physiological variations in bodyweight by age are greatest. Earlier experience with SP and DP suggests that lack of clear guidance on age-based dosing as part of the regulatory process contributes to considerable variation in recommended age-based dose regimens,^{74,75} potentially resulting in poor, but widely used regimens, particularly for young rapidly growing children who bear the brunt of the malaria burden. Different age-based regimens are already being used in countries that have recently switched to ACTs. These concerns apply also to young infants <6 months old or of <5 kg body weight. Most ACTs are contra-indicated in this group because of lack of safety data, even though these children are at considerable risk. In western Kenya 50% of infants not protected by insecticide-treated mosquito nets had their first infection by 3 months.⁷⁶ In southern Mozambique, an estimated 9% of out-patient visits for uncomplicated malaria in children less than 5 years of age are children aged <6 months. Infants in endemic areas have the highest burden of severe malarial anemia, blood transfusions and death.^{77,78} Thus, programmatically implemented ACTs will end up being widely used in children <6 months even though the label does not provide guidance for this age group.

Malaria and AL use in pregnancy

Pregnant women with malaria, symptomatic and asymptomatic alike, should be treated without delay with effective and safe antimalarial drugs in order to reduce risks for adverse outcomes for both mother and fetus.⁷⁹ AL is a very attractive alternative because it is highly effective, acts rapidly and is well tolerated. However, there is insufficient information on safety and efficacy of ACTs in pregnancy, including exposure in the first trimester.^{79,80} Early data indicated that artemisinins were embryotoxic and potentially teratogenic in several animal species without maternal toxic effects or impaired fertility, and more recent studies have confirmed these findings.⁷⁹

Artemisinin derivatives have shown embryo-toxic effects in animal reproductive toxicology studies.⁸¹ The mechanism of embryo-toxicity is thought to occur through depletion of embryonic erythroblasts causing severe anemia and cell damage and death due to hypoxia.⁸¹ The most sensitive time window for embryo-toxicity in humans is between weeks 4 to 10. From these data ACTs are not indicated for malaria treatment in the first trimester of pregnancy unless no alternatives exist. There is increasing experience with artemisinin derivatives in second and third trimesters with no evidence of adverse outcomes in more than 1000 prospectively followed pregnancies.^{82,83} WHO Malaria Treatment Guidelines of 2006

recommend use of ACTs in pregnant women in the second and third trimester of gestation. None of the studies on AL use in pregnancy have reported increased risk of serious maternal adverse events, adverse birth outcomes or neuro-developmental deficits. However all these studies were underpowered to detect rare adverse outcomes.⁸⁴ Data from Sudan from a cohort of women who reported use of artemisinins in first trimester and were followed up until delivery and their babies followed up till 1 year of age showed that most delivered apparently healthy babies at full term with no congenital malformations and no maternal deaths, and none of the babies died during their first year of life.⁸⁵ A prospective observational study was conducted recently in Zambia which evaluated safety of AL and SP in pregnant women who received AL and SP to treat symptomatic *falciparum* malaria. Data from 1001 pregnant women and fetuses/newborns indicated that the incidence of perinatal death, spontaneous abortion, neonatal mortality, premature delivery, stillbirth and low birth weight is similar after pregnancy exposure to AL compared to SP.⁸⁶

Pregnancy has been associated with reduced plasma concentrations of AL which have a significant impact on treatment outcome since plasma concentrations of lumefantrine, after elimination of artemether, are an important determinant of cure.^{87,88} A study that evaluated PK of AL in pregnant women with recrudescence uncomplicated multidrug resistant *falciparum* malaria demonstrated that pregnant women in second and third trimester had lower concentrations of artemether, dihydroartemisinin and lumefantrine, and elimination of lumefantrine was more rapid than reported previously in non-pregnant adults.^{87,89} Another study that compared artesunate monotherapy to AL for treatment of uncomplicated *falciparum* malaria in second and third trimesters demonstrated that the standard 6-dose AL regimen was well tolerated and safe but efficacy was inferior to that of 7-day artesunate monotherapy and was unsatisfactory for general deployment in this geographic area. PK parameters measured in this study showed low drug concentrations in later pregnancy which could possibly explain the poor treatment outcomes.⁸⁹ There is need for further studies to determine the optimum dose regimen and efficacy of AL in pregnancy.

AL use in HIV-infected populations

Human immunodeficiency virus (HIV)-infected individuals are at high risk for acquiring malaria parasitemia, with the risk increasing as immunity declines.⁹⁰⁻⁹³ Evidence for this interaction is more consistent in pregnant women of all gravities.⁹⁴⁻⁹⁶ HIV-1 infected pregnant women have a higher prevalence of peripheral parasitemia and placental malaria^{95,96}

and their infants experience higher postnatal mortality when both diseases are present.^{97,98} Therefore, offering adequate and efficacious antimalarial treatment and prevention is extremely important for this high risk group. Little is known about efficacy and safety of antimalarial drugs in HIV-infected individuals and much less on interaction between antimalarial and antiretroviral (ARV) drugs, and reliable data are urgently needed. Few studies have examined the effect of HIV infection on response to antimalarial treatment and these have yielded conflicting results.^{99–103} Most studies have shown that HIV-infected individuals have higher risk of experiencing antimalarial treatment failure due to re-infections.^{101,103} Birku et al demonstrated decreased clearance of parasites by artemisinin treatment in HIV-infected patients with malaria.¹⁰⁴ In Zambia, HIV-infected adult patients with CD4 counts of 300/ μ L and below had higher risk of getting recrudescence malaria than HIV-infected patients with higher CD4 counts and HIV-uninfected patients.¹⁰³ Recent studies, however, suggest that the threshold for an increased risk of malaria treatment failure (new infections or recrudescence) probably lies at 400 CD4 cells/ μ L.^{105,106} Following the latest WHO guidelines for sub-Saharan Africa this malaria vulnerable population should be protected by cotrimoxazole prophylaxis or highly active ARV therapy (HAART). There are concerns about safety of AL treatment in HIV-infected patients concomitantly receiving HAART. The standard first-line HAART regimens in many sub-Saharan countries where malaria is endemic are made up of a non-nucleoside reverse transcriptase inhibitor (NNRTI) backbone with 2 nucleoside reverse transcriptase inhibitors (NRTI). The second-line HAART regimen is made up of a protease inhibitor (PI) backbone and 2 NRTIs. Knowledge of the metabolism of ARVs and AL suggests that there is potential for PK drug–drug interactions.¹⁰⁷ For example, PIs like lopinavir/ritonavir (LPV/r) are among the most potent inhibitors of cytochrome P450 (typically CYP 3A4) metabolism, while NNRTIs (efavirenz and nevirapine) are also substrates of cytochrome P450 and usually these two induce but occasionally efavirenz inhibits some P450 isoforms. Although poorly studied the risk of clinically significant interactions involving AL and ARVs is considerable¹⁰⁸ and may result in high concentrations with excessive toxicity or reduced concentrations with reduced efficacy and risk for development of resistance to AL. The potential for interactions between ARVs and antimalarials have been shown in a study of healthy volunteers where AQAS was co-administered with the NNRTI efavirenz. In the first 2 study participants, the AUC for AQAS increased by 100% to 300% and alanine and

aspartate transferase levels increased markedly above the upper limit of normal, suggesting hepatotoxicity. This led to recommendations that AQAS should be avoided in patients receiving EFV. In a recent study of uncomplicated malaria in Uganda, treatment of HIV-infected children with AQAS was associated with markedly higher risk of neutropenia compared with treatment of HIV-uninfected children. The risk of neutropenia was higher in participants with concurrent ARV use, especially zidovudine, and in those with a history of repeated doses of AQAS.¹⁰⁹ These clinical observations demonstrate the need for thorough examination of the nature of interaction between ARVs and ACTs. An interaction is expected between lumefantrine and both EFV and PIs that could potentially lead to increased levels of lumefantrine (Figure 1); no data are available. The potential interactions with NVP are less clear but co-administration could reduce lumefantrine levels. A study that investigated the PKs of AL when administered with LPV/r in HIV-uninfected healthy volunteers demonstrated that the PK of lumefantrine is influenced by LPV/r, resulting in 2- to 3-fold increases in lumefantrine AUC, and trends towards decreases in artemether maximum concentration (C_{max}) and AUC were noted during co-administration. Decreases in DHA AUC were observed during co-administration without changes in DHA: artemether AUC ratios. The authors concluded that co-administration of AL and LPV/r can be carried out for patients co-infected with malaria and HIV.¹¹⁰ This study did not address safety concerns with co-administration, which need to be considered in future studies among individuals living in malaria-endemic regions.

AL use in patients with co-morbidity

Treatment of tuberculosis is often a minimum of 6 months including 2 months of intense rifampicin-based treatment. Patients may concomitantly develop malaria requiring treatment with AL. There are currently no published data on interactions of rifampicin and AL. Rifampicin is a potent inducer of hepatic cytochrome and may influence the PKs of AL since both drugs are metabolized by CYP 450.¹¹¹ Theoretically co-administration of rifampicin with AL may result in decreased concentrations of AL resulting in decreased efficacy (Figure 1). Data on these PK drug interactions are very scarce, thus the need for more studies. One study evaluated effects of concomitant administration of AL with a potent CYP 3A4 inhibitor. Artemether, DHA, and lumefantrine PKs were altered by ketoconazole. AUC and C_{max} increased for all 3 compounds and terminal half-life increased for artemether and DHA. None of the changes in

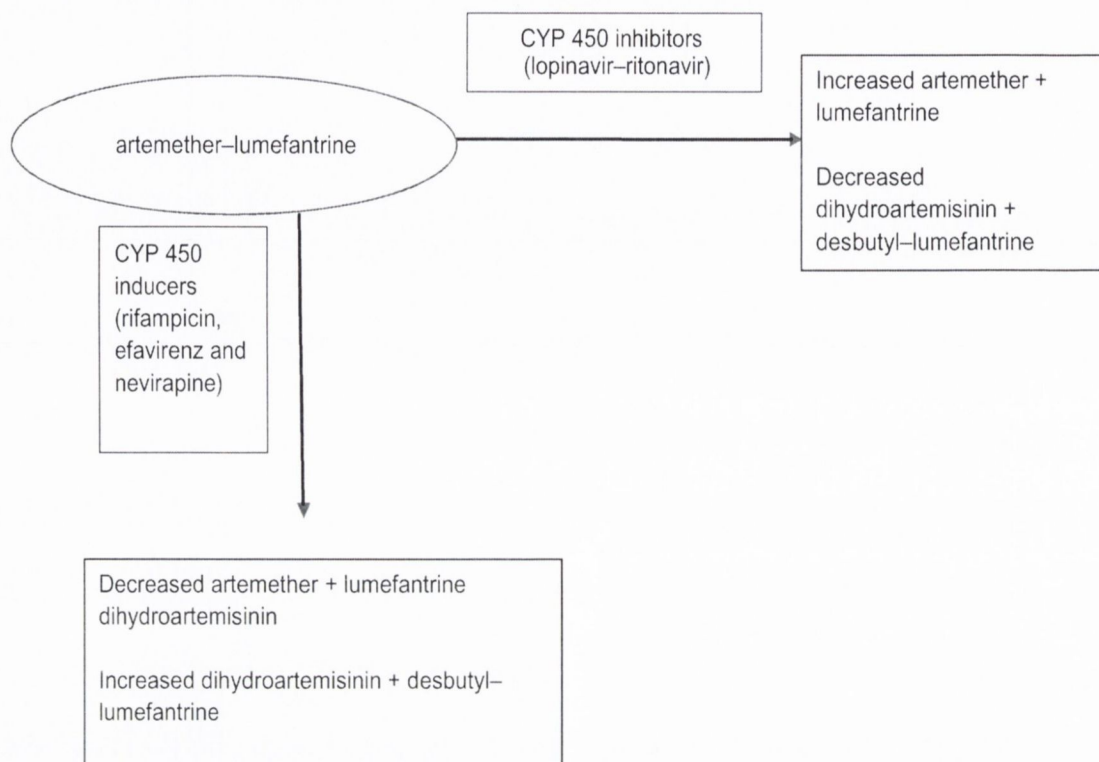


Figure 1 Summary of potential pharmacokinetic interactions between artemether-lumefantrine and commonly prescribed inducers and inhibitors of CYP 450.

PK parameters were greater than the changes observed in healthy volunteers taking AL with a high-fat meal. There was no increase in observed side effects or electrocardiographic changes. The authors concluded that dosage adjustments of AL do not appear to be necessary with concomitant ketoconazole administration.¹¹²

AL resistance

Antimalarial drug resistance has been defined as “the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended, but within the limits of tolerance of the subject.” This definition was later modified to specify that the drug in question must gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action.¹¹³ Antimalarial drug resistance is heightened in individuals with lower immunity, such as children less than 5 years, pregnant women, non-immune immigrants to malarious areas, malnourished individuals and HIV-infected patients.¹¹³ Reduced immunity allows the survival of a residuum of parasites that are able to survive treatment, and as such reduced immunity may

further increase the development, intensification and spread of resistant strains.

Resistance to artemisinin has not been confirmed although reduced sensitivity has been reported in China and Vietnam.^{114,115} Treatment failures occurring after AL treatment are thought to be due to poor absorption with reduced concentrations.^{116,117} AL selects for the *P. falciparum* multidrug resistance gene (PfMDR1) N86, the chloroquine-susceptible allele which has been proposed as a marker for lumefantrine resistance.¹¹⁸ In Tanzania, treatment with AL was associated with selection of newly infecting parasites containing the *pfmdr1* 86N allele,¹¹⁸ which has been associated with decreased in vitro sensitivity to artemisinin and lumefantrine.¹¹⁹

Factors that lead to development, intensification and distribution of antimalarial drug resistance can broadly be classified as: factors leading to treatment failure (incorrect dosing regimen, non-compliance, substandard drugs and misdiagnosis), human behavior, parasite and vector biology, and drug PKs.¹¹³ In sub-Saharan Africa antimalarial drugs are readily available outside public health services, in pharmacies, drug shops and private practitioners' clinics.

Quality of antimalarials is a serious concern and counterfeits may be found in some of these units. In Southeast Asia half of the samples of artemisinins obtained from most countries were counterfeit.^{11,120} In sub-Saharan Africa substandard antimalarials were found in 7 countries.^{121,122}

Conclusion

There is increasing evidence of very high efficacy and effectiveness of AL for treatment of uncomplicated malaria. Continued health education on correct use of AL and surveillance of effectiveness is necessary to prevent and detect emergence of drug resistance. There is need to develop strong systems for pharmacovigilance to increase the evidence base on safety of AL especially in pregnant mothers and infants weighing less than 5 kg. PK studies especially on drug interactions with ARV drugs are urgently needed.

Disclosures

None of the authors declare conflicts of interest.

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Steady-state pharmacokinetic comparison of generic and branded formulations of stavudine, lamivudine and nevirapine in HIV-infected Ugandan adults

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Background: We aimed to compare the steady-state pharmacokinetic parameters and tolerability of Triomune 40[®] (stavudine 40 mg, lamivudine 150 mg and nevirapine 200 mg) and branded formulations of these drugs in HIV-infected Ugandans.

Methods: This includes a randomized, open-label, cross-over study of HIV-infected patients stable on therapy for 1 month. Patients were randomized to generic or branded formulation. Plasma pharmacokinetics were assessed after 1 month. The following day, alternate formulation was administered, and 1 month later, drug pharmacokinetics were re-assessed. Plasma pharmacokinetics were determined using HPLC–UV detection. Similarity between steady-state pharmacokinetic parameters was assessed using the US Food and Drug Administration standards for bioequivalency testing. Tolerability was assessed using questionnaires.

Results: Sixteen (10 females) patients completed the study. Median (IQR) age, weight and CD4 count were 37 (33.7–40) years, 65 (63.4–66) kg and 292 (220.7–344.5) cells/mm³, respectively. All patients received co-trimoxazole. The geometric mean ratio (90% CI) for stavudine, lamivudine and nevirapine was 0.92 (0.78–1.08), 1.11 (0.95–1.30) and 0.84 (0.64–1.11), respectively, for C_{max} , and 0.83 (0.70–0.97), 1.06 (0.94–1.20) and 0.88 (0.71–1.10), respectively, for AUC. Stavudine plasma concentrations were significantly lower for the generic formulation. Pharmacokinetic parameter inter-individual variability ranged from 29% to 99%. There were no differences in tolerability for the two formulations.

Conclusions: Pharmacokinetic profiles of generic and branded drugs were similar. Differences particularly with regard to stavudine were demonstrated. Surveillance of the quality of generic antiretroviral drugs in the target populations is needed. Capacity building for pharmacokinetic research in resource-limited settings is a priority.

Keywords: antiretroviral drugs, PK, Uganda

Introduction

An estimated 1 million HIV-infected adults and children live in Uganda with an average HIV prevalence of 6.2%.¹ There are 105 000 people with HIV on antiretroviral therapy (ART),

constituting ~50% of those who need it.² The major constraint for a widespread use of ART in Uganda, as in many other African countries, has been the high cost of medication. Potent ART became widely available due to initiatives including the Multicountry AIDS Programme, the President's Emergency Plan

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for AIDS Relief (PEPFAR), the Global Fund to Fight AIDS, Tuberculosis and Malaria, the World Health Organization's 3 by 5 Initiative, the Elizabeth Glaser Pediatric AIDS Foundation and Médecins sans Frontières, among others.³ Many of these programmes rely on the less expensive generic ART formulations to attain the treatment goals.³

First-line ART in Uganda consists of zidovudine or stavudine plus lamivudine with either nevirapine or efavirenz. However, the majority of the Ugandan HIV-infected patients are treated with the three-drugs-in-one fixed dose combination (FDC) of generically manufactured stavudine, lamivudine and nevirapine.⁴ Importantly, despite the introduction of generic FDC tablets into HIV clinical practice, data on the quality of generic drugs in HIV-infected adults in Uganda are lacking. Debate is ongoing as to whether generic antiretroviral (ARV) drug formulations are truly the equivalent of brand formulations and whether they should be approved by a regulatory agency before administration to patients in developing countries. In this context, five ARV agents have been removed from clinical use by the WHO because bioequivalence to the trade formulations was not established.⁵ Following new bioequivalence studies, two of these agents have been reinstated.

Although the amount of drug in the generic tablet may be similar to brand formulations, simple chemical studies conducted *in vitro* do not guarantee optimal drug dissolution and absorption in humans. Documentation of bioequivalence in the target population is therefore important. Bioequivalence is a term in pharmacokinetics used to assess the expected *in vivo* biological equivalence of two different preparations of a drug. The US Food and Drug Administration has defined bioequivalence as 'the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study'.⁶ However, bioequivalence is used to compare two formulations of a single dose of drug given to healthy volunteers. Intensive pharmacokinetic studies can be performed faster than expensive randomized clinical trials, which may delay ARV use where required. We performed a randomized, open-label, cross-over intensive pharmacokinetic study to compare the steady-state pharmacokinetic parameters of stavudine, lamivudine and nevirapine in Triomune 40[®] with the branded products using the US Food and Drug Administration standards for bioequivalency testing. In addition, the safety and tolerability of the formulations were assessed.

Methods

Study site

The study was conducted between January and September 2007 at the Infectious Diseases Institute (IDI) at Mulago Hospital in Kampala, Uganda. The IDI is a regional centre of excellence for HIV/AIDS treatment, prevention, training and research. To date, over 12 000 HIV-infected patients are registered at the IDI with over 4000 already taking ART. Over 70% of these are on Triomune therapy.⁷

Study design and population

This was a randomized, open-label, cross-over intensive pharmacokinetic study of generic and trade formulations of stavudine,

lamivudine and nevirapine in patients with HIV-1 infection stable on ART for at least 1 month prior to pharmacokinetic sampling.

Patients were eligible to participate if they were older than 18 years of age and able to provide written informed consent. Patients weighing <60 kg, showing abnormal clinical test results and treated with known inhibitors or inducers of cytochrome P450 metabolism or herbal medications, were excluded.

Ethical considerations

The study was approved by the IDI Scientific Review Committee, and the National HIV/AIDS Research Committee (ARC 047) and was registered with Uganda National Council of Science and Technology and ClinicalTrials.gov (NCT 00455585). All participants gave written informed consent to participate, and all study procedures were conducted according to Good Clinical Practice.

Study procedures

Patients were electronically randomized to either Triomune 40; batch no. G57662 (Cipla, Goa, India) or the patented version of the drugs: Zerit[®]; batch no. 0059 (Bristol Myers Squibb, Princeton, NJ, USA), Epivir[®]; batch no. R270222 (GlaxoSmithKline, Research Triangle Park, NC, USA) and Viramune[®]; batch no. 506396A (Boehringer Ingelheim, Columbus, OH, USA). A regimen of stavudine 40 mg, lamivudine 150 mg and nevirapine 200 mg was taken twice daily. Participants took one tablet twice daily while on the generic formulation (Triomune 40) and one Zerit[®] capsule, one Epivir[®] tablet and one Viramune[®] tablet, twice daily while on the branded formulation. Participants had detailed study explanation at enrolment. On each study day, adherence to study drugs was assessed using self report and pill count by the study pharmacist. In addition, we collected information on adverse drug effects and serious adverse drug effects, and a questionnaire on quality of life was administered on each study day. All participants took their drugs for a month prior to pharmacokinetic sampling. On the evening prior to pharmacokinetic sampling, participants were reminded of their study day appointment and given detailed instructions to take their medication and food by 8.00 pm and arrive at the hospital by 7.00 am in the fasting state.

On study day 1, patients were admitted in the fasting state, an indwelling intravenous catheter was inserted following aseptic techniques and blood samples were drawn for the determination of pre-dose concentrations. The intake of a standardized breakfast and morning doses of drugs was directly observed by study staff. Pharmacokinetic sampling was performed at 2, 4, 6, 8, 10 and 12 h post-dosing. Blood (4 mL) was collected into ethylene diamine tetra-acetic acid tubes each time. Before discharge from the unit, patients were switched to brand formulations from generic and vice versa and were given the drugs to take at home. After 1 month, study subjects were readmitted, and plasma pharmacokinetic sampling was repeated. All patients resumed their pre-study treatment at the end of the second pharmacokinetic day. Blood samples were centrifuged immediately after collection; plasma was removed and stored at -20°C until shipment.

Analytical and pharmacokinetic methods

Drug concentration measurement was performed by standard HPLC with UV detection at the Department of Infectious Diseases, HIV Pharmacology Laboratory, University of Turin, Italy. The lower limit of quantification was 25, 25 and 50 ng/mL for stavudine, lamivudine

Triumune 40[®] steady-state pharmacokinetics in Ugandans

and nevirapine and the limit of detection was 5, 5 and 10 ng/mL, respectively.

The calculated pharmacokinetic parameters for stavudine, lamivudine and nevirapine were the trough plasma concentration (C_{trough}) defined as the 12 h concentration after the observed dose, the maximum observed plasma concentration (C_{max}) and the area under the plasma concentration–time curve (AUC) from 0 to 12 h and the half-life. All pharmacokinetic parameters were calculated using actual blood sampling times and non-compartmental modelling techniques (WinNonlin Professional[™] software, version 4.1; Pharsight Corp., Mountain View, CA, USA).

Statistical analysis

A sample size of 12 was calculated to have 80% power to detect a difference in means of nevirapine C_{max} and AUC between branded and generic formulations based on the definition of bioequivalence using paired *t*-test with a significance level of 0.05. We anticipated a drop-out rate of 30%; therefore, we planned to enrol 16 subjects for 12 to complete all pharmacokinetic assessments.

Descriptive statistics, including mean and standard deviation (SD), were calculated for stavudine, lamivudine and nevirapine pharmacokinetic parameters.

Within-subject changes in drug pharmacokinetic parameters were evaluated by calculating geometric mean ratios (GMRs) and 90% confidence intervals (CIs). The concentrations measured during the branded formulation administration were used as reference. The CIs were determined using logarithms of the individual geometric mean values; the calculated values were then expressed as linear values. Steady-state pharmacokinetic parameters were considered similar if the 90% CI for the C_{max} and the AUC fell within the range of 0.8–1.25.⁶ Inter-individual variability in drug pharmacokinetic parameters was expressed as a coefficient of variation [(SD/mean) × 100].

Results

A total of 27 HIV-infected subjects (16 females) were screened between January and March 2007; 18 were eligible and were enrolled, 1 was discontinued because she missed study appointments and 1 was excluded from the pharmacokinetic analysis because he did not complete pharmacokinetic sampling. A total of 16 (10 females) completed all pharmacokinetic phases. The clinical and demographic characteristics of the 16 subjects who completed the study are illustrated in Table 1.

Both the branded and generic formulations of stavudine, lamivudine and nevirapine were well tolerated and no differences were reported. There were no adverse drug reactions, abnormal

laboratory findings or drop-outs due to toxicity during the study. None of the participants who completed the study reported any missed dose during the study period. Patients preferred the FDC formulation (generic) because of dosing convenience.

Mean [\pm standard error (SE)] concentration–time profile for the three drugs is shown in Figure 1. The mean (\pm SD) stavudine, lamivudine and nevirapine steady-state pharmacokinetic parameters measured during the administration of branded and generic formulations are reported in Table 2. Mean (SD) nevirapine exposure (AUC_{0-12}) while on branded and generic drugs was 75 192.7 ng·h/mL (29 294.9) and 64 338.3 ng·h/mL (19 944.6), respectively. GMR of the test (generic) to reference (brand) of C_{max} and AUC values and 90% CI are given for each drug in Table 2. While no significant differences in lamivudine and nevirapine C_{max} and AUC were observed [GMR (90% CI): 1.11 (0.95–1.30) and 0.84 (0.64–1.11) for C_{max} , and 1.06 (0.94–1.20) and 0.88 (0.71–1.10) for AUC], stavudine plasma concentrations were significantly lower for the generic formulation for C_{max} 0.92 (0.78–1.08) and AUC 0.83 (0.70–0.97).

Only the 90% CI for lamivudine AUC GMR at steady-state was within 0.80 and 1.25. All the other 90% CIs were outside the predefined limits.

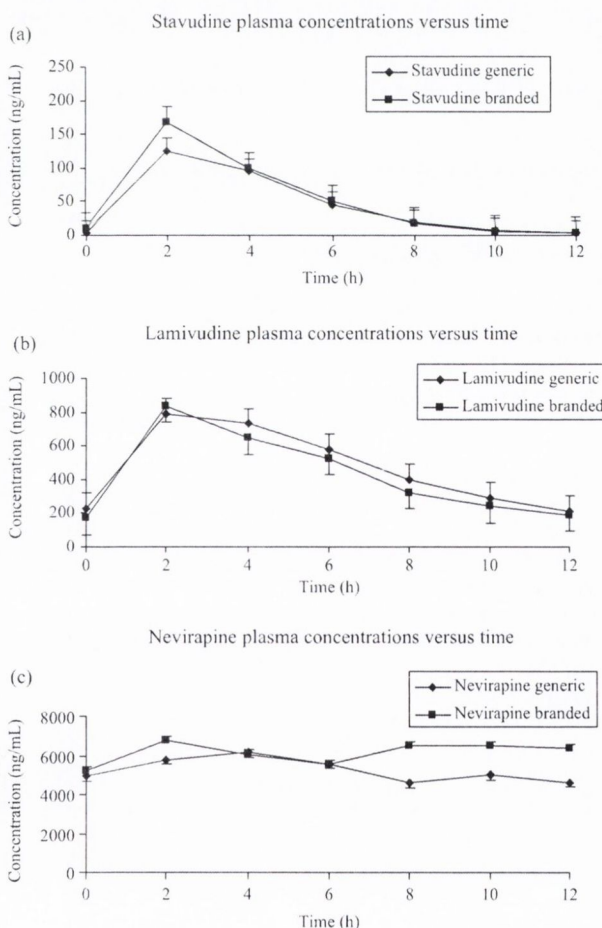


Figure 1. Mean (\pm SE) plasma concentration versus time of (a) stavudine, (b) lamivudine and (c) nevirapine of 16 subjects during the intake of branded and generic formulations.

Table 1. Baseline clinical and demographic characteristics of the study patients according to the randomization arm

	Brand → generic (Arm 1)	Generic → brand (Arm 2)
Age (years)	37.4 (33–40)	36.8 (34–40)
Weight (kg)	64.7 (63–67)	64.5 (63.5–66)
CD4 count (cells/mm ³)	324.4 (255–343)	305.3 (220–349)
Females (%)	56	71

Median (IQR) unless otherwise specified.

Table 2. Pharmacokinetic parameters of branded and generic stavudine, lamivudine and nevirapine

Parameter	Stavudine	Lamivudine	Nevirapine
C_{max} (ng/mL) ^a			
branded ^b	203.5 (\pm 127.7)	855.2 (\pm 276.6)	8594.3 (\pm 3699.0)
CV (%)	62.8	32.3	43.0
generic ^b	210.3 (\pm 208.4)	966.8 (\pm 279.6)	7017.3 (\pm 2757.8)
CV (%)	99.1	29.0	39.3
GMR (90% CI)	0.92 (0.78–1.08)	1.11 (0.95–1.30)	0.84 (0.64–1.11)
AUC _{0–12} (ng·h/mL) ^a			
branded	685.6 (\pm 219.6)	5522.7 (\pm 2009.8)	75 192.7 (\pm 29 294.9)
CV (%)	32.0	36.4	38.9
generic	579.8 (\pm 231.2)	6039.0 (\pm 2370.8)	64 338.3 (\pm 19 944.6)
CV (%)	39.9	39.3	31.0
GMR (90% CI)	0.83 (0.70–0.97)	1.06 (0.94–1.2)	0.88 (0.71–1.10)

GMR, geometric mean ratio; CI, confidence interval; C_{max} , maximum plasma concentration; AUC, area under the curve for concentration–time; CV, coefficient of variation.

^aMean (\pm SD).

^bNumber of samples: branded drugs (stavudine, lamivudine and nevirapine) = 110 each, generic = 112.

Stavudine, lamivudine and nevirapine pharmacokinetic parameter inter-individual variability ranged from 29% to 99% (Table 2).

Median nevirapine C_{trough} was 3615.0 ng/mL (range, 1236–6138) while on the generic formulation and 4479.5 ng/mL (range, 1070–8375) while on Viramune®. Eleven of the 16 subjects for the generic and 10 of 16 for the branded had nevirapine concentrations above the suggested minimum effective concentration (MEC) of 3000 ng/mL.⁸ Only one subject had C_{trough} below the MEC while on both formulations.

Discussion

This study was designed to compare the steady-state pharmacokinetic parameters of stavudine, lamivudine and nevirapine in Triomune 40 and their branded products and to assess the safety and tolerability of the different formulations in HIV-infected Ugandan adults. With the exception of lamivudine, steady-state pharmacokinetic parameters of Triomune 40 did not fall within the limits of the US Food and Drug Administration standards for bioequivalency testing.⁶ However, pharmacokinetic profiles were similar, and there was no difference in tolerability between the two regimens. Evidence for bioequivalence of Triomune 40 to branded products was based on a single-dose study in healthy volunteers.⁹ Since adequate drug concentrations must be maintained for long-term suppression of HIV, it is arguable that steady-state pharmacokinetic studies are of greater clinical significance than single-dose studies.

Although the mean nevirapine pharmacokinetics were similar for the two formulations, there was considerable inter- and intra-subject variability. Steady-state C_{trough} concentrations were above the alleged MEC of 3000 ng/mL⁸ for 11 of 16 subjects for the generic and 10 of 16 for the branded. Although it is not possible to relate plasma concentrations to adverse events,⁸ it is of interest to note that 3 of 16 for the generic and 9 of 16 for the branded had peak concentrations >8000 ng/mL. Nevirapine exposure in

our study was lower than that reported in a recent study from Malawi.¹⁰ The metabolism of nevirapine is partly dependent on CYP2B6 activity, and there is evidence that the functional single nucleotide polymorphism (516G > T) is associated with increased levels of nevirapine. Penzak *et al.*¹¹ reported a 17% prevalence of this mutation in a small Ugandan study. It is possible that a higher prevalence of this and/or other polymorphisms in patients in the Malawi study could account for the different findings and support a theory of wide variability in the distribution of polymorphisms within the African populations.

We found a statistically significant decrease in stavudine concentrations when subjects received Triomune 40. Stavudine and lamivudine are pro-drugs that require intracellular phosphorylation to the active form. Intracellular concentrations rather than plasma concentrations correlate with virological suppression.¹² The 17% reductions in the plasma concentrations (AUC) of stavudine reported in this study may not be clinically significant in terms of achievement and maintenance of virological response. Our findings contrast to those of an 8 h pharmacokinetic study in Malawi reporting a 12% increase in stavudine concentrations while patients received Triomune 40.¹⁰ The differences in stavudine exposure in the two studies cannot be explained by a change in manufacturing practices arising from the reports of the Malawi study because the Triomune 40 tablets used in our study were produced before those data were known. We did not conduct *in vitro* analysis of the respective quantities of stavudine, lamivudine and nevirapine levels in generic and branded drugs prior to administration as this was beyond the scope of the present study. However, regulatory authorities require and routinely conduct such studies prior to drug registration in Uganda.

We conducted this study using Triomune 40 that contains 40 mg of stavudine in each tablet. Treatment guidelines for stavudine-containing regimens have been updated in an attempt to minimize toxicity possibly related to high plasma concentrations. Based on data revealing similar viral suppression rates and fewer adverse events with lower doses of stavudine, the World Health Organization recently adopted stavudine 30 mg

twice daily as the standard of care irrespective of body weight in adults.¹³ Currently, the majority of Ugandan HIV-infected patients are treated with stavudine 30 mg-containing regimens.¹⁴ Uganda and other resource-limited countries depend on cheaper generic agents, and these have been widely utilized in the rapid scaling-up of ART. Overall, clinical outcome data with Triomune use in Africa are encouraging. Laurent *et al.*¹⁵ reported safety and efficacy data in a Cameroonian study in which 80% of the participants had viral loads <400 copies at 24 weeks of therapy. Data from the cohort at our centre revealed high virological suppression rates of up to 86% at 12 months of therapy with over two-thirds of the patients on Triomune.⁷

In conclusion, our data show that although the steady-state pharmacokinetic parameters for Triomune 40 did not fall within the limits set by the US Food and Drug Administration for bioequivalency testing, the pharmacokinetic profiles of generic and branded drugs were similar. Differences particularly with regard to stavudine were demonstrated. While the crucial role generic products have played in the progress of ART is recognized, the need for ongoing surveillance of their quality in target populations must be emphasized. Steady-state pharmacokinetic studies need to be considered as a component of regulatory procedures for ARV drug approval in developing countries. Investment in human and material resources to develop pharmacokinetic units in resource-limited settings must be considered a priority.

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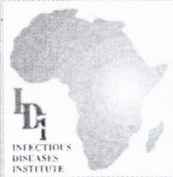
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Transparency declarations

None to declare.

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Quarterly Newsletter of the AIDS Treatment Information Centre, Infectious Diseases Institute, Makerere University

Interaction between Malaria and HIV

By Dr Pauline Byakika-Kibwika (M.B.Ch.B, MSc. CEB, MMed)

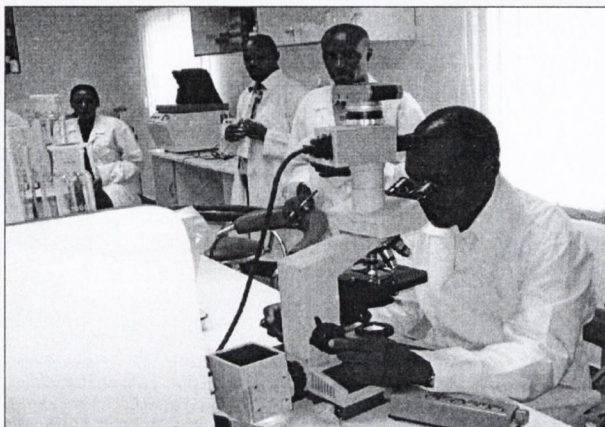
Malaria and HIV infection contribute to significant morbidity and mortality. Worldwide, approximately 2,200 million people are exposed to malaria annually. Of these, about 300-500 million develop clinical malaria, with over 90% occurring in sub-Saharan Africa. Malaria kills between 1.5 - 2.7 million people annually in sub-Saharan Africa¹.

Currently 40 million people worldwide are living with HIV/AIDS. 70% of these are in sub-Saharan Africa, where about 29 million people are infected (UNAIDS 2002).

Given the extensive overlap in the geographical distribution of the two diseases in sub-Saharan Africa, even a small interaction between the two could have profound public health consequences.

This interaction is plausible when the immunological mechanisms involved in both diseases are considered. Humoral and cell mediated immunity are critical to development of an effective immune response to malaria. CD4 T lymphocytes modulate the production of antimalarial antibodies and production of cytokines, like tumour necrosis factor alpha (TNF α), which are directed against merozoites and cellular immune-recruitment to control or modulate clinical infection. The antibodies produced block parasites from invading red blood cells (RBCs), block malaria toxins as well as adherence of red blood cells to endothelium. Activated CD4 and CD8 cells coordinate the cell mediated immune response and facilitate antibody dependent cytotoxicity of infected RBCs, which results in death of intra-erythrocytic parasites.

HIV, on the other hand, infects CD4 lymphocytes, causing depletion of the CD4+ T lymphocytes through mechanisms like cytolysis, syncytial formation, and apoptosis. The loss of CD4+ cells results in diminished T helper 1 (Th1) and T helper 2



Trainees examine malaria parasites in a lab

(Th2) responses. Th2 depletion prevents B cell activation with resultant humoral immunodeficiency while Th1 deficiency causes impaired cellular immune response due to diminished interferon gamma and TNF α production, which impairs the killing of intracellular pathogens. There are several ways that malaria and HIV could potentially interact, with effects on incidence, prevalence, transmission, clinical manifestations, treatment outcomes, drug interactions and toxicity.

Effect of HIV on malaria incidence, parasitemia, clinical presentation and treatment outcome

HIV and non-pregnant adults: Early in the HIV pandemic, several studies reported no convincing association between HIV and malaria². A systematic review published in 1998 supported this conclusion. However, the authors pointed out several limitations of these earlier studies such as inadequate sample size, short follow-up periods and inability to stratify patients by level of immunity. In contrast, more recent evidence has shown a clear association between HIV infection and an increased risk of malaria, with HIV-infected patients having over twice the incidence of clinical malaria compared to HIV-uninfected individuals^{3,4}. Additionally, a "dose-response" relationship, of decreasing CD4 counts associated with increasing rates of clinical malaria and higher parasitemia has been demonstrated.

HIV and malaria in pregnancy: The association is stronger and more consistent among pregnant women of all parities. HIV-1 infected pregnant women have a higher prevalence of peripheral and placental malaria and higher parasite densities with more adverse birth outcomes than non HIV-infected women. A recent review on HIV-malaria co-infection in pregnancy showed that the gravidity-related pattern of malaria in pregnancy is

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From the Editor

Malaria remains one of the worst enemies for populations in the tropical resource limited settings. Clinical malaria is diagnosed in 300-500 million people worldwide per year but 90% of these are in sub-Saharan Africa.

The interaction between HIV infection and malaria is pertinent. The body's defense system against malaria involves CD4 T-lymphocytes, which instigate the production of antibodies and cytokines. On the other hand the human immunodeficiency virus utilises the CD4 T-lymphocytes for replication. This results in a drop of the CD4 T-lymphocyte count and thus reduced immunity.

In this issue of *ATIC News*, our

lead article exposes the impact of the interaction between malaria and HIV/AIDS, and thus the need for appropriate anti-malarial and antiretroviral therapy. We also point out some important drug interactions between the new first line anti-malarial drug combination Coartem and other commonly used drugs. However, information on the interaction between anti-retroviral drugs and Coartem are not yet published, but are in the pipeline for studies.

We encourage our fellow health-care workers to contact ATIC in case of difficult AIDS and Malaria cases.

Thank you and enjoy this quarter of 2007

Editorial Committee

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Drug interaction - updates from CROI

Several hundred abstracts and posters were presented at the 14th Conference on Retroviruses and Opportunistic Infections (CROI) in Los Angeles, California, USA. **Marta Boffito, MD PhD**, captured key studies on drug-drug interactions involving ARVs.

Identification of Drug Interactions Involving ART in New York City HIV Specialty Clinics

Overall 1 in 5 patients receiving ART had a potential drug interaction. 20% of these interactions could have been associated with reduced antiretroviral drug plasma concentrations, which may compromise virologic response. Therefore, identifying potential drug interactions promptly is fundamental and may prevent drug resistance from developing, especially if multiple providers may be prescribing therapy.

Pharmacokinetic Interaction between Efavirenz and Diltiazem or Itraconazole after Multiple-dose Administration in Adult Healthy Subjects

In this study efavirenz was shown to significantly decrease the exposures of diltiazem (DTZ), itraconazole (ITR) and its active metabolite (hydroxy ITR [HITR]). Therefore, when combined with efavirenz, DTZ dose adjustment should be guided by clinical response. In terms of the interactions with ITR, currently there are no data using higher doses of ITR in combination with efavirenz, thus, no dose recommendation can be made and use of alternate treatment may be necessary for optimal antifungal therapy. Finally the study medications were generally safe and well-tolerated when administered alone or in combination.

Pharmacokinetics and 12 Weeks Efficacy of Nevirapine, 400 mg vs 600 mg per day in HIV-infected Patients with Active TB Receiving Rifampicin: A Multicenter Study

Thirty HIV-infected Thai adults with CD4 count < 200 cells/mm³ and active tuberculosis were randomised to receiving a rifampicin 2-6 weeks and a nevirapine 400 (arm 1) or 600 mg (arm 2) per day plus zidovudine and lamivudine. A nevirapine lead-in 2 weeks period was performed, as per standard of care, in both groups at 200 and 400 mg/day, respectively. Plasma nevirapine concentrations were measured at week 2, 4 and 12. In patients treated concomitantly with nevirapine and rifampicin, as many as 80% in the 400 mg arm had suboptimal nevirapine concentrations at 2 weeks after the lead in period, whereas nevirapine 600 mg/day was associated with a high rate of nevirapine hypersensitivity. Therefore, nevirapine 400 mg/day may be sufficient for Asian HIV-infected patients receiving rifampicin, but a 200mg nevirapine lead-in period should be avoided. In addition, zidovudine should also be avoided during the first 3 months of advanced HIV/TB. Rifampicin-ZDV co-administration results in a significant reduction of the ZDV concentration in plasma. This may result in a partial or total loss in the ZDV efficacy (www.hiv-druginteractions.org). While, short-term efficacy is comparable in this study, a long-term efficacy study is under way.

Drug Interaction between Antimalarial Drugs and Efavirenz

An artemisinin-based combination therapy including artesunate (AS) plus amodiaquine (AQ) is now approved for first-line treatment of malaria in 15 African countries. The present study was aimed at investigating the pharmacokinetics of AQ and its active metabolite desethylamodiaquine (DEAQ) in the

Co-artem, the frontline weapon

By Francis Kalemeera (BSc, B Pharm, MPS)

Coartem is an antimalarial drug combination containing artemether and lumefantrine [AL]. One tablet contains 20mg and 120mg of artemether and lumefantrine respectively. Artemether is a sesquiterpene lactone derived from artemisinin, a natural substance from *Artemisia annua*. Lumefantrine is a synthetic racemic fluorine mixture.

Indications

Coartem (AL) is used for the treatment of uncomplicated malaria and has been found to be safe and effective against *Plasmodium falciparum* and mixed infections including *P. falciparum*. Coartem is effective against drug sensitive and drug resistant *P. falciparum* and it is thus recommended in areas where the parasites may be resistant to other antimalarials, including sulphadoxine-pyrimethamine and chloroquine, the commonly used drugs in resource limited settings^{1,2}.

Mechanism of action

Artemether and lumefantrine both act in the food vacuole of the malarial parasite. It is thought that there, they inhibit the conversion of haem (toxic product from haemoglobin breakdown) to haemozoin, malaria pigment. While lumefantrine is thought to interfere with the polymerization process, artemether generates reactive metabolites as a result of the interaction between its peroxide bridge and haem iron.

Pharmacokinetics

Artemether's absorption is fairly rapid, reaching peak plasma concentrations after two hours. Lumefantrine is a highly lipophilic compound whose absorption starts after a lag time of two hours and peak plasma concentrations are seen after 6-8 hours. Coartem should be taken with food as food enhances the absorption of both artemether and lumefantrine.

Artemether and lumefantrine are both highly bound to serum proteins in vitro (95.4% and 99.7% respectively). Artemether is rapidly metabolised to its biologically active metabolite, dihydroartemisinin through the enzyme CYP3A4. Artemether also has the capacity to induce CYP2C19 and CYP3A4. Lumefantrine is metabolised mainly by

	Day 1	Day 2	Day 3
Adults and children \geq 35kg	4 tabs stat then 4 tabs after 8hr	4 tabs b.d	4 tabs b.d
Children: 25 to <35kg	3 tabs stat then 3 tabs after 8hr	3 tabs b.d	3 tabs b.d
Children: 15 to <25kg	2 tabs stat then 2 tabs after 8hr	2 tabs b.d	2 tabs b.d
Children: 5 to <15kg	1 tab stat then 1 tab after 8hr	1 tab b.d	1 tab b.d

*b.d: twice daily

CYP3A4. In vitro, lumefantrine significantly inhibits the activity of CYP2D6 at therapeutic concentrations.

Warnings and precautions

- For severe manifestations of malaria including cerebral malaria, pulmonary oedema and renal failure, other effective drugs such as quinine are recommended.
- If a patient deteriorates while on Coartem, alternate therapy should be started without delay. In such a case, however, the patient should be closely monitored (with an ECG) since lumefantrine and quinine may lead to cardiotoxicity.
- If a patient has been treated with halofantrine, Coartem should not be administered earlier than one month after the last dose of halofantrine.
- If Coartem is given to patients after mefloquine, close monitoring of food intake is important. Mefloquine induces a reduction in bile production. Patients are thus advised to eat at dosing times, which compensates for the associated decrease in bioavailability.
- Caution is advised when Coartem is given with some drugs that are inducers, substrates or inhibitors of the Cytochrome P450 isoenzyme 3A4

Pregnancy and lactation

Coartem is contraindicated in pregnant mothers during the first trimester of pregnancy. Women of child bearing potential should be advised to practice contraception while on Coartem. Coartem should not be given to breastfeeding women until after 28 days. However Coartem may be used in the above mentioned women if the benefit to mother outweighs risk to the fetus.

Side effects

Very common: Headache, dizziness, abdominal pain, anorexia
Common: Sleep disorder, palpitation, cough, diarrhoea, vomiting, nausea, pruritus, rash, arthralgia, myalgia, asthenia, fatigue
Uncommon: Somnolence, involuntary muscle contractions, paraesthesia, hypoesthesia, abnormal gait, ataxia
Very rare: Hypersensitivity

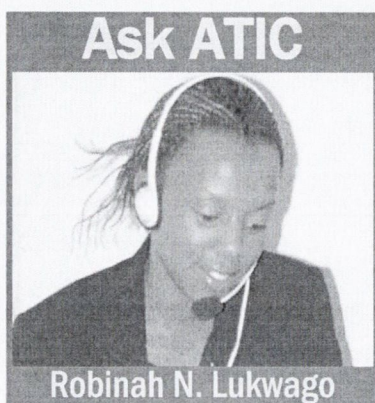
Contraindications

The side-effects, pharmacokinetics and pharmacodynamics of Coartem have significant impact on the decisions made for the use of Coartem. It is contraindicated in patients hypersensitive to the ingredients, first trimester of pregnancy and electrolyte imbalance. Due to the inhibition on CYP2D6 it is contraindicated in patients on drugs metabolised by this isoenzyme, amitriptyline, clomipramine, etc. Coartem is contraindicated in patients taking drugs that are known to prolong the QTc interval such as Antiarrhythmics of classes IA and III, neuroleptics, antidepressant agents, certain antibiotics including some agents of the following classes: macrolides, Fluoroquinolones, imidazole, and triazole antifungal agents.

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Q: Should we continue to use sulfadoxine-pyrimethamine for Intermittent Preventive therapy (IPT) for malaria in pregnancy given that there is resistance to Fansidar?

F.G, Kisoro, Uganda

A: The alarming increase of sulfadoxine-pyrimethamine (SP) resistance in Africa has raised concerns about its use as IPT for malaria. Pharmacokinetic modeling suggests that the suppressive prophylactic effect of SP, assuming similar pharmacokinetic profiles as in non-pregnant adults, may last approximately 2–3 months in areas with sensitive parasites. The period of effective post-treatment prophylaxis then progressively shortens with increasing drug resistance, compromising the efficacy of the two-dose regimen given at 3-month intervals. SP resistance is linked to mutations in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps*) genes. Parasites with four mutations in the *dhfr* gene, including the 164L mutation, are fully resistant. Such parasites have already been observed in Malawi, Uganda, and western Kenya though their rate of spread cannot be predicted. It might be slowed if SP use in the general population is not widespread and is limited to IPT. Semi-immune pregnant women respond better to failing antimalarials than symptomatic young children. Meta-analysis of two trials in primigravidae and secundigravidae has shown that the protective efficacy of two-dose IPTp-SP against placental malaria remains high (52%, 95% CI 32–67), even in areas where the treatment failure rate by day 14 in symptomatic children is between 20% and 40%. This is the basis for the continued use of SP for IPT. However there is no data on IPTp with SP efficacy in areas with high SP resistance (more than 40% treatment failure by day 14 in children). This reveals a priority area for research in Uganda that will help inform the policy making process on whether we should continue to use fansidar for IPT in Uganda.

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Updates from CROI

From Page 2

presence of efavirenz in healthy volunteers. The study was terminated after the first 2 subjects developed asymptomatic but significant elevations in liver transaminases following completion of the study. Addition of efavirenz to AQ resulted in total exposure increases of 114 and 302% for AQ and total exposure decreases of 23.7 and 8.5% for DEAQ for the first 2 subjects, respectively. Efavirenz exposure was at or above historical results for healthy volunteers. Other infectious or metabolic aetiologies were excluded as causes of transaminase elevations. Liver function monitoring is needed in individuals requiring AQ/AS treatment for malaria in the setting of chronic efavirenz therapy.

Plasma Concentrations of Efavirenz and Lopinavir in Children with and without Rifampicin-based Anti-TB Treatment

The study evaluated the plasma concentrations of efavirenz or lopinavir in children taking efavirenz or lopinavir/ritonavir plus 2 nucleoside reverse transcriptase inhibitors with and without rifampicin-based tuberculosis treatment. Standard recommended doses of all investigated agents were used and extra ritonavir was given during tuberculosis treatment to children receiving lopinavir/ritonavir. Rifampicin did not significantly reduce efavirenz concentrations. However, it was noted that 50% of children had an efavirenz minimum concentration below the minimum recommended concentration. This raises concern that a substantial proportion of children may be at risk of the rapid emergence of efavirenz-resistant mutations and treatment failure, suggesting that efavirenz doses should be reevaluated, especially because therapeutic drug monitoring is seldom available in developing countries. Lopinavir minimum concentration was similar between the 2 groups. In all the 28 children studied, lopinavir minimum concentration was above the minimum therapeutic level of 1 mg/L. This study confirmed that additional ritonavir can be used to delay lopinavir elimination, thus overcoming the reduction of lopinavir concentrations caused by rifampicin. However, concern remains in terms of toxicity when higher ritonavir doses are administered.

Abacavir Plasma Pharmacokinetics in the Absence and the Presence of Atazanavir/Ritonavir or Lopinavir/Ritonavir) and Vice Versa in HIV+ Patients

The aim of this study was to investigate abacavir plasma pharmacokinetics in the absence and presence of atazanavir/ritonavir or lopinavir/ritonavir and vice versa in HIV-infected patients. No changes in atazanavir, lopinavir, and ritonavir exposures were observed following addition of abacavir to the regimens containing these protease inhibitors. However, mild (17%) and moderate (32%) decreases in abacavir plasma exposure were observed following addition of atazanavir/ritonavir or lopinavir/ritonavir, respectively. The mechanism of interaction, the impact on intracellular triphosphates and the clinical implications remain unclear and should be investigated further.

Effect of Rifampin on Pharmacokinetics and Safety of Twice-daily Atazanavir: ACTG Protocol A5213

In order to test the hypothesis that adequate plasma concentrations of atazanavir can be maintained if given at higher than approved doses (300 mg and 400 mg twice daily, un-boosted) with concomitant rifampicin, steady state pharmacokinetics and safety of atazanavir and rifampicin were determined in healthy volunteers. However, although safe and generally well tolerated, atazanavir 300 mg or 400 mg every 12 hours did not maintain adequate plasma exposure to effectively treat HIV infection when co-administered with rifampicin 600 mg every 24 hours. Therefore, co-administration of atazanavir and rifampicin must be avoided and further study is needed to investigate the role of ritonavir in limiting the inducing effect of rifampicin on atazanavir exposure.

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Can Septrin be given with antimalarials?

By Robinah.N.Lukwago (B. Pharm)

Approximately one million pregnancies per year are thought to be complicated by co-infection with malaria and HIV in sub-Saharan Africa¹. Maternal malaria infection has been associated with maternal anaemia, infant low birth weight and maternal and infant mortality. Maternal HIV infection has also been associated with maternal anaemia and low birth weight and with increased risk of maternal malaria². HIV-associated risk of maternal malaria affects women of all gravidities, thus attenuating or even eliminating the decrease in malaria parasitaemia normally seen in HIV-negative multigravidae³. The prevalence of maternal anaemia and incidence of low birth weight are both higher in pregnancies affected by HIV/malaria co-infection than in pregnancies affected by malaria or HIV alone. In the presence of co-infection, anaemia prevalence and low birth weight incidence may both exceed 35% in some subgroups⁴. Maternal malaria/HIV co-infection may also increase the incidence of mother-to-child transmission of HIV, perhaps because malaria infection is known to increase HIV viral load, although published evidence has been inconsistent^{4,5}.

WHO now recommends insecticide-treated bednet use and intermittent preventive treatment for all pregnant women living in areas of stable *Plasmodium falciparum* transmission in Africa, along with antenatal HIV testing and antiretroviral therapy if indicated^{6,7}. Sulfadoxine-pyrimethamine (Fansidar) is generally regarded as the preferred antimalarial medication for intermittent preventive treatment, although its effectiveness is now threatened by rising levels of drug resistance^{8,9}. Daily prophylaxis with Co-trimoxazole (Septrin) has been recommended for all HIV-infected pregnant women in sub-Saharan Africa¹⁰. Thus, opportunistic infection prophylaxis with Septrin and malaria prevention with fansidar involve two similar sulfa drugs for HIV-infected pregnant women, which may pose problems in view of the potential risk of increased adverse drug reactions (ADR's).

Many HIV-infected people are intolerant of Septrin because of its sulfonamide component^{11,12}. The risk of adverse reactions to Septrin in HIV-infected people has been estimated at 26.3 per 100 per-



A patient suffering from a severe adverse drug reaction

son-years, increasing substantially with advancing immunosuppression. The likelihood of adverse reactions also appears to vary by sex and race, and may be higher in women¹¹. Studies have shown that concurrent administration of fansidar and septrin has been associated with a substantially increased incidence of severe adverse reactions in HIV-infected patients, and is therefore not recommended⁴.

Because Fansidar is not as effective against bacterial pathogens^{13,14} Septrin might be used to prevent both bacterial infections and malaria. Septrin has been used effectively to treat malaria in children, and daily use of

Septrin has been used effectively to treat malaria in children, and daily use of Septrin by non-pregnant HIV-infected adults has been associated with reductions of over 70% in the incidence of febrile malaria parasitaemic syndromes.

Septrin by non-pregnant HIV-infected adults has been associated with reductions of over 70% in the incidence of febrile malaria parasitaemic syndromes^{15,16,17}. However, no published data yet describe the effectiveness of daily Septrin for the prevention of malaria and its consequences (specifically maternal anaemia, placental parasitaemia, and low birth weight) during pregnancy. Nevertheless, WHO now recommends

daily Septrin as an alternative to intermittent preventive treatment with Fansidar for immunocompromised HIV-infected women¹.

Operational constraints resulting from late diagnosis may limit the use of daily Septrin for malaria prophylaxis. Women who are not diagnosed with HIV until after the first antenatal visit may not present for HIV care until late pregnancy, especially where HIV care is not offered at the antenatal clinic itself. In many settings, prescription

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Management of patients with fever

By Umaru Ssekabira (M.B.Ch.B, MSc CEB)

Introduction

Presumptive treatment of patients with fever as malaria is widely advocated in Africa. In resource limited settings like Uganda, febrile episodes are commonly treated with an anti-malarial, often in the absence of a blood smear or even when the smear is negative.

Over-treatment of malaria was acceptable and even promoted in the era of inexpensive and safe chloroquine monotherapy. In the new era of artemisinin-based combination therapy (ACT), presumptive treatment becomes economically and clinically less acceptable. All clinicians need to appreciate the potential benefits of withholding antimalarial treatment from patients with a negative blood smear for malaria parasites which include:

- Clinicians are more likely to focus on the true cause of fever.
- The true cause of fever may be managed in a timely manner instead of being delayed by unnecessary antimalarial treatment.
- It reduces the number of unnecessary antimalarials given which is more cost-effective.
- Targeting antimalarial treatment to those patients who have malaria may limit the development and spread of drug resistance.
- It reduces the risk of adverse events due to unnecessary antimalarials.

The reasons why health workers treat patients with a negative blood smear with antimalarials include:

- Belief that a febrile patient may still have malaria even when the blood smear is negative
- The need to adhere to the MOH treatment guidelines which recommend treatment of fever with an antimalarial even when the blood smear is negative.
- Inability to make any other definitive diagnosis.

In a study done at Mulago Hospital, out of 1,602 patients whose blood smears for malaria parasites were negative at the first visit,

only 12 (0.8%) patient's progressed to uncomplicated malaria within seven days. This suggested that the majority of patients whose blood smears were negative did not have malaria.

Interpretation of a negative blood smear in a patient with fever

The purpose of doing a blood smear is ideally to confirm diagnosis and guide treatment decisions. A negative blood smear in a patient with fever may mean the following:

- The patient has been exposed to a partially effective antimalarial or inadequate doses of an effective drug
- The patient may have malaria but parasites not seen because of low parasite count or technical error
- The patient may not have malaria but another disease that presents as malaria

Possible causes of fever in a patient with a negative malaria smear

For children

- Respiratory tract infections: common cold, pneumonia, tuberculosis and sinusitis
- Otitis media
- Viral infections: measles, mumps, rubella, chicken pox and HIV

- Urinary tract infections
- Gastroenteritis
- Meningitis
- Septicemia

For adults:

- Bacterial infections: meningitis, tuberculosis, typhoid and sepsis
- Parasitic infections: toxoplasmosis, filariasis and amoebiasis
- Viral: HIV, infectious mononucleosis, yellow fever
- Tumors: lymphomas
- Drug reactions

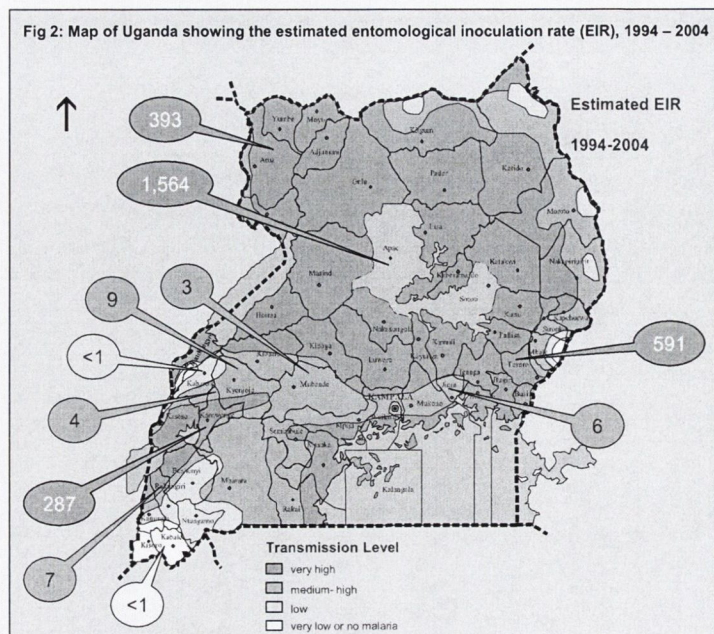
Management of a patient with fever but a negative smear

Assuming that the test has been done with a well maintained microscope and stains, the clinician should re-assess the history, clinical examination findings and the laboratory results. If the health facility and the laboratory can do more investigations the clinician should investigate for other causes of the fever basing on the history and clinical findings.

Further investigations in febrile illness with negative blood slide

Below is an outline of investigations that may be carried out in a patient who presents with fever but a negative smear:

- Blood films (Repeat a malaria smear, comment on the morphology white blood cell and red blood



er but no malaria parasites seen

cell distribution)

- White blood cell count
- Urinalysis
- Stool analysis
- Chest x-ray
- Sputum microscopy
- Blood culture & sensitivity
- Serology
- HIV, Hepatitis
- Biopsy

Blood film examination can be an important tool in excluding important causes of fever like leukemia and sickle cell anaemia. Bacterial infections are commonly associated with increased neutrophils while viral infections are associated with increased lymphocytes. Some cancers, for example leukemia and lymphoma, are also associated with markedly raised white cell count. There are also situations that are associated with abnormally low white cell counts. Examples include viral infections like HIV and conditions that depress the bone marrow.

When all the above is finalised, the clinician should manage the patient according to the algorithm in Figure 1.

To determine the probability that a patient has malaria, consider the following factors:

- Age: Children less than five years are at highest risk.
- Immune status: Pregnant women and HIV-positive patients are at higher risk.
- Transmission intensity: risk is directly proportional to the entomological inoculation rate (EIR), which is a measure of the frequency of infection and is defined as the number of infective bites by anopheles mosquito per year.

Situations where antimalarials can be given

If a patient has previously taken antimalarials, he/she should be asked about what drug was taken, at what dosage, and whether or not they vomited drugs given orally.

If inappropriate drugs or dosages were given, then give the recommended antimalarial drug in the correct doses. The current malaria treatment policy recommends use of artemether/lumefantrine as the first

line drug for uncomplicated malaria amongst patients who are five months and above and are not pregnant.

If a patient received an appropriate drug but did not complete treatment, he/she should be encouraged to complete the treatment.

If the reassessment does not lead to identification of the actual cause of the fever, it's advisable that the patient be given an anti-pyretic and be followed up in two days or advised to return earlier than that if the condition worsens. If there is an identified cause of fever then the clinician should give treatment appropriate for that illness (refer to the national standard treatment guideline for Ministry of health 2005).

Edition

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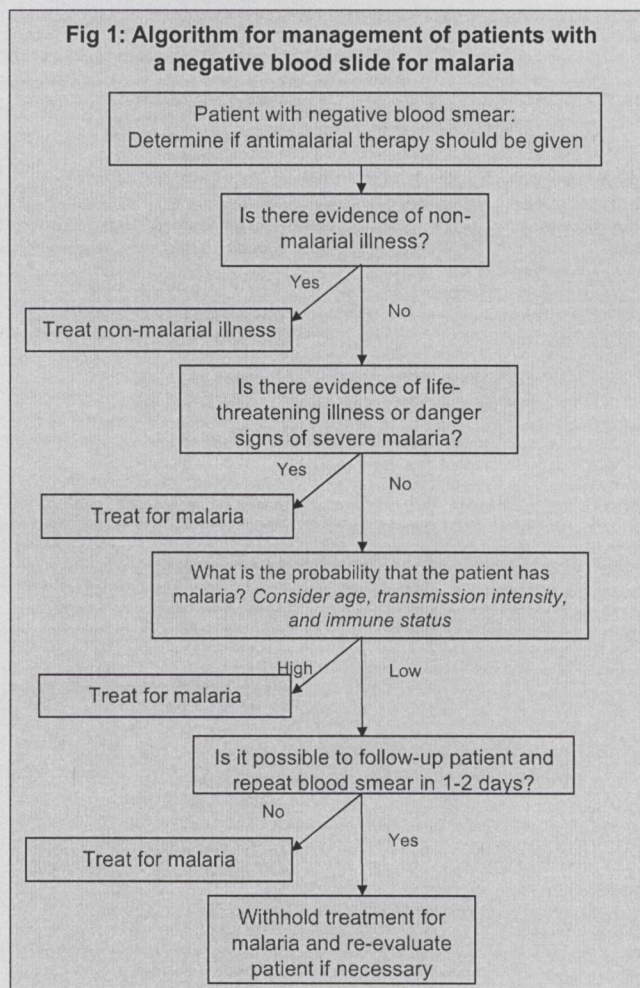
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Seprin vs antiretrovirals

of Seprin may also be contingent on clinical and/or laboratory staging, which may introduce further delays in initiation of Seprin prophylaxis. If Seprin is not begun until the third trimester, malaria-related maternal anaemia and fetal growth retardation may already have developed.

Therefore given that both malaria and HIV/AIDS are leading infectious diseases in our resource limited setting, it is important that studies are conducted to generate evidence on the efficacy of Seprin prophylaxis on reducing rates of placental malaria and anemia in HIV-infected pregnant women.

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Circumcision Checks HIV

Review by Mohammed Lamorde (MBBS)

On December 12, 2006 the Data and Safety Monitoring Board halted the circumcision study in Rakai, Southwestern Uganda, due to efficacy. Interim results of the randomised controlled trial in Rakai revealed 51% fewer HIV infections in a group of circumcised men compared to an uncircumcised control group over a period of two years. With these results male circumcision becomes the first new, proven HIV prevention method in over a decade.

The Rakai circumcision study was initiated following observational studies suggesting lower rates of HIV transmission in areas with high rates of male circumcision. The foreskin is vulnerable to tears and ulcers (e.g. secondary to sexually transmitted infections) and this can create an entry point for HIV. Following circumcision the new skin is keratinized, reducing its vulnerability to HIV.

The Rakai trial enrolled 4,996 men aged 15 - 49 and randomized them into two groups. The intervention arm (n=2464) was offered circumcision within two weeks of enrolment. For the control arm (n=2522) circumcision was to be offered after a delay of two years. Informed consent was obtained.

Adult male circumcision was found to be safe when performed by trained healthcare workers under aseptic conditions. The benefit of circumcision was not obvious in the first six months but became progressively more protective after that time. There were also 50% fewer episodes of genital ulceration among circumcised study participants. The trial results are supported by findings in studies in Kenya and South Africa which also found similar rates of protection in circumcised participants.

The study was done by the Rakai Health Sciences Programme, a partnership involving the Uganda Virus Research Institute, Makerere University Institute of Public Health, Columbia University and Johns Hopkins University.

The investigators caution that circumcision offers only partial protection and must be used as a part of a comprehensive HIV prevention strategy including education, limiting sexual partners and condom use.

The writer is a PK Medical Doctor, Infectious Diseases Institute

Teamwork is key in malaria care

By Jimmy Andama

The Integrated Management of Malaria course at IDI provides an excellent opportunity to build teams and improve the quality of services in health care settings. All staff of Omugo Health Centre IV in Arua District and members of the District Health Management Team (DHT) were trained as a team on management of patients with malaria. The training was attended jointly by clinicians, lab personnel, records staff and members of the DHT. This training was unique because earlier malaria management training only targeted specific categories of health workers independently. The records staff had never been thought about as an important and relevant category of staff in the management of patients.

The course is offered by Joint Uganda Malaria Training Programme (JUMP), Uganda Malaria Surveillance Programme, Makerere University-University of California San Francisco collaboration and Ministry of Health.

As part of the training we were facilitated to develop a health facility work plan aimed at improving the quality of care that we offer to patients suspected to have malaria. We have implemented this plan and it has contributed to the overall improvement in our health care delivery systems. These include:

- Since the training took place, there has been a general improvement in management of fever, particularly malaria, through the use of a more team-based approach. There is increased utilisation of the laboratory. This indicates that clini-

cians try to confirm malaria before deciding the treatment to prescribe. The number of malaria blood slide smears that are positive has decreased, indicating that there is improvement in malaria management especially the preventive aspects which we started emphasising after the training.

- Irrational use of the antimalarial containing artemether and lumefantrine (Coartem) has greatly reduced and thus the total number of doses of coartem dispensed to patients has reduced. Before the training, most fever patients were being given antimalarials despite a negative blood slide.

- Distribution of insecticide-treated mosquito nets to pregnant mothers at antenatal care (ANC) has reduced the number of malaria cases in pregnancy and most mothers tested for malaria, test negative.

- A health education programme on malaria prevention and control was started within the health unit and the community including schools.
- Data on malaria cases is being collected in time, kept securely, analysed and fed back given to every staff. This data is being utilised to plan for ordering of supplies.

- As a result of prompt and proper management of patients at the outpatient department, there have been fewer patients admitted with severe malaria.
- There has been increased team work in the management of patients at the centre, resulting in the better patient management. The staff have formed a task force that is supposed to hold a meeting every month to review issues



The author (R) in class with other trainees

pertaining to malaria. This task force makes a presentation about their work in every general staff meeting.

Challenges

- The new approach has increased the work load in the laboratory, since we prefer that every fever be investigated.

- The national drug supply system has remained poor, leading to stockouts of vital drugs.

- Because of the improved services, staff are overwhelmed by the influx of patients from outside our usual catchment area.

Conclusion

This course has turned our performance around in a very dramatic way. It has helped to re-align us in the true medical professional path, and I would like to suggest that it be rolled out country wide so that every Ugandan Health worker is reached thus contributing to the effort to significantly reduce the burden of malaria in this country.

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A group photo of trainees attending a course at IDI on integrated management of malaria

Interaction between Malaria and HIV

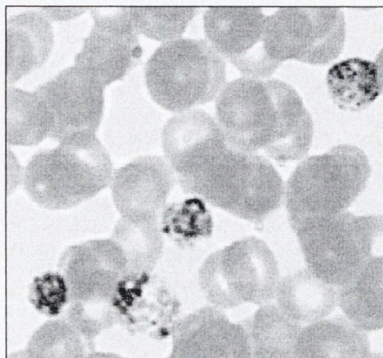
Continued from Page 1

altered by HIV so that the burden is shifted from primigravidae to all pregnant mothers⁵.

HIV and severity of malaria: Whether HIV infected individuals are at risk for severe malaria is still inconclusive. Few studies have examined the effect of HIV on the severity of malaria and these have shown that HIV-infected adults in regions of unstable malaria transmission are at increased risk for severe and complicated malaria and death⁶.

HIV and malaria diagnosis and treatment outcome: Diagnosis of malaria in many resource-limited settings is based on presence of fever. Drugs are commonly prescribed without laboratory confirmation. Given that HIV infected individuals may present with fevers due to other opportunistic infections, absence of laboratory confirmation of malaria may cause over-estimation of the malaria burden and inappropriate administration of antimalarial drugs. A study done in Tanzania found that 95% of all patients presenting with fever were treated with quinine and yet only 46% of them had a positive blood smear for malaria⁷. This misdiagnosis puts patients at risk of death from other causes of fever that may not be treated as well as risk of adverse effects of drugs that they do not require.

Regarding treatment outcome, some studies have suggested that HIV-infected individuals may have inferior responses to antimalarial therapy because of impaired host immunity. This could result from increased susceptibility to new malaria infections, or because of recrudescence of infection. Early studies did not show association between HIV infection and antimalarial treatment response⁸. However, more recent studies suggest that HIV infected patients may be at higher risk of treatment failure. A study done in Ethiopia showed delayed clearance of parasitemia in HIV infected adults treated with artemisinin for uncomplicated malaria⁹. A Ugandan study showed increased risk of re-infection rather than recrudescence among HIV infected patients after antimalarial treatment¹⁰ while another study in Zambia showed that HIV infected patients with malaria and CD4 count <300/ul have higher risk of a recrudescence



Deadly allies: Malaria parasites (L) and the Human Immunodeficiency Virus (R)

cent infection¹¹. Conversely another study in Uganda found that the HIV positive patients who took routine cotrimoxazole prophylaxis had reduced risk of treatment failure when they were treated with sulphadoxine-pyrimethamine (SP)¹².

HIV and malaria transmission and prevention: Given that HIV infection increases the risk of malaria with increased parasitemia and treatment failure, we can hypothesise that even malaria transmission increases with increased disease burden on health care facilities. However, no studies have been conducted to support this association. Health care providers need to be more rigorous in preventing malaria in the HIV infected population. Routine cotrimoxazole prophylaxis has been shown to reduce the incidence of malaria¹³. Other malaria control measures include insecticide treated bed nets (ITNs), indoor residual spraying and intermittent preventive treatment (IPT) with SP in pregnancy. A study in Uganda reported a reduction in febrile parasitemia of 76% with cotrimoxazole prophylaxis, 92% with cotrimoxazole and antiretroviral treatment (ART) and 95% when ITNs were added to cotrimoxazole and ART¹⁴. Another study demonstrated that the risk of malaria

among HIV-infected children receiving cotrimoxazole alone was decreased by 35% while the risk of malaria acquisition in individuals receiving CTX and ITNs was decreased by 97%¹⁵. Use of IPT with SP in pregnancy has been shown to be effective in reducing the burden of malaria in pregnancy¹⁶. Pregnant mothers require two doses of SP in the second and third trimester; however, a clinical trial in western Kenya showed that HIV-infected mothers require at least three doses to achieve a reduction of placental parasitemia similar to that seen in HIV-negative women receiving two doses of SP¹⁶. A recent study in Malawi confirmed that monthly SP (median three doses) was more effective at reducing rates of placental parasitemia than two-dose regimens, in women with and without HIV. However, IPT with SP may not be administered to HIV positive pregnant mothers on routine cotrimoxazole prophylaxis.

Effect of malaria on HIV: The immune response to malaria may increase the pool of lymphocytes available for HIV infection. Malaria antigens and pigments released during the burst of RBCs, stimulate cytokines like TNF alpha and G-CSF, which activate HIV replication, thus increasing viral load. Malarial episodes transiently increase viral load, and thus could theoretically have an impact on HIV disease progression and transmission. Reports from Malawi and Uganda showed a rise in viral load at the time of malaria infection and this reduced with effective antimalarial treatment. In areas where malaria infection is endemic, recurrent infection occurs. However, the effect that this may have on HIV disease progression is not known. Repeated

Reports from Malawi and Uganda showed a rise in viral load at the time of malaria infection and this reduced with effective antimalarial treatment. However, the effect that this may have on HIV disease progression is not known.

Continued on Page 11

Interaction between HIV and Malaria

Continued from Page 10

malaria infections may accelerate HIV disease progression, thus the need for rigorous malaria prevention and treatment in HIV positive individuals. High viral loads have been shown to be associated with increased potential for HIV transmission.

Antiretroviral drugs, by boosting immunity, reduce risk for opportunistic infections including malaria. Some ARVS have been shown to possess antimalarial properties *in vitro*²⁷. With increasing rates of antimalarial drug resistance, the World Health Organisation recommends the use of artemisinin-based combination therapy (ACT). However, there is no data on ACT interaction with antiretroviral drugs. The potential for interaction between ARVS and antimalarials should not be overlooked because these drugs follow similar processes when administered and are metabolised by the same cytochrome family of enzymes¹⁸. This interaction could result in increased or reduced plasma levels of either drug, with increased risk of toxicity or development of resistance respectively.

In summary, Malaria and HIV interact, leading to effects on the incidence, prevalence, clinical manifestations, treatment outcomes, drug interactions and toxicity. There are still gaps in knowledge on this interaction calling for research.

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Updates from CROI

From page 4

Drug-drug Interaction between Lopinavir/Ritonavir and Rosuvastatin

Hyperlipidemia is a common complication in HIV-infected persons on antiretroviral therapy but few HMG CoA reductase inhibitors (also known as statins) are used in this population because of the potential for drug interactions. Rosuvastatin is not a substrate for cytochrome P450 3A4. Thus, drug interactions between rosuvastatin and protease inhibitors seemed unlikely. Nevertheless, van der Lee et al [CROI 2006] showed a 1.5-2 fold increase in rosuvastatin (10 mg once daily) trough concentrations in HIV-infected subjects on lopinavir/ritonavir and advised to monitor possible adverse events when rosuvastatin is co-administered with protease inhibitors. The healthy volunteer study presented this year, however, showed that in the presence of lopinavir/ritonavir rosuvastatin (20 mg once daily) area under the curve (indicating total plasma exposure) and maximum concentrations were unexpectedly increased 2.1- and 4.7-fold and concluded that the co-administration should be avoided and studies to elucidate the mechanism for this interaction are needed.

The Effect of Atazanavir and Atazanavir/Ritonavir on UGT1A4 Using Lamotrigine as a Phenotypic Probe

There are two major categories of metabolism reactions called Phase I and Phase II; and drug interactions may involve drugs metabolised through both phases. Glucuronidation is the most important Phase II reaction and antiretrovirals may be eliminated following glucuronidation and may impact the activity of the reaction. For example, recently, it has been shown that lopinavir/ritonavir induces glucuronidation using lamotrigine as phenotypic probe for UGT1A4 (reduction in lamotrigine exposure of 55%). Atazanavir is known to inhibit glucuronidation through UGT1A1, leading to asymptomatic hyperbilirubinemia. The objective of this study was to evaluate the effect of atazanavir and atazanavir/ritonavir on UGT1A4 using lamotrigine as phenotypic probe. While atazanavir alone did not significantly influence glucuronidation of single-dose lamotrigine, atazanavir/ritonavir resulted in moderately decreased exposure (32% decrease) to lamotrigine.

Effect of Famotidine 20- and 40-mg Dosing Regimens on the Bioavailability of Atazanavir with Ritonavir in Combination with Tenofovir in Healthy Subjects

Atazanavir absorption is pH dependent. Previously, a reduction of 18 to 28% in atazanavir plasma exposure was observed when the H₂-receptor antagonist, famotidine 40 mg every 12 hours was administered with atazanavir/ritonavir 300/100 mg in healthy volunteers. Tenofovir also decreases atazanavir exposures when coadministered with atazanavir/ritonavir by approximately 25 to 30%. The effect of lower doses of famotidine and tenofovir when simultaneously co-administered with atazanavir/ritonavir has not been studied. The objective of this study was to evaluate dosing strategies for famotidine to maintain atazanavir exposure when coadministered with tenofovir, in the presence of ritonavir. When famotidine 20 mg twice daily was administered with atazanavir/ritonavir and tenofovir, an estimated 20% decrease in atazanavir minimum concentrations was observed. When famotidine 40 mg was administered once daily, 12 hours apart from atazanavir/ritonavir, atazanavir minimum concentration was 23% lower relative to the control treatment. Famotidine 40 mg administered twice daily temporally separated from atazanavir/ritonavir and tenofovir (10 hours before and 2 hours after) resulted in decreases in atazanavir maximum concentrations, area under the curve and minimum concentrations of 26%, 21% and 28%, respectively.

Effects of Minocycline and Valproic Acid Co-administration on Atazanavir Plasma Concentrations

There is interest in studying the effects of both valproic acid and minocycline as adjunctive therapy for the treatment of HIV-associated cognitive impairment. The purpose of this study was to determine whether minocycline alone or in combination with valproic acid influenced atazanavir plasma concentrations in patients receiving atazanavir plus ritonavir. Minocycline coadministration resulted in decreased atazanavir exposure (area under the curve 33% decrease, minimum concentration 50% decrease), and there was no evidence that the addition of valproic acid mediated this effect.

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In the news

lopinavir/ritonavir tablets

Lopinavir/ritonavir capsules (Kaletra) should be stored in the refrigerator to maintain their stability. However this has been a challenge for many patients in the developing world who have limited access to refrigeration facilities. The development of a new dosage formulation of Lopinavir/ritonavir that does not have to be stored in the fridge has therefore been received with enthusiasm in many parts of the developing world. The new formulation is called Aluvia. It is the same high-quality, non-refrigerated product as Kaletra capsules manufactured by Abbot. Lopinavir/ritonavir is a recommended second-line treatment for HIV infection in the developing world by the World Health Organisation. Protease inhibitors, such as lopinavir/ritonavir, are important treatment options in the fight against HIV when first-line regimens fail. Abbott is developing a pediatric version of the lopinavir/ritonavir tablet to provide greater dosing flexibility for physicians to treat children living with HIV.

Source: www.abbott.com

Pharmacologists to meet

A four day networking meeting for clinical pharmacologists working in the field of HIV/AIDS, tuberculosis and malaria is being organised by the Infectious Diseases Institute and the Department of Pharmacology, Makerere University. The meeting will focus on the development of Clinical Pharmacology in Africa and is supported by the European-Developing Countries Clinical Trials Partnership (EDCTP). Participants will come from Uganda, South Africa, Nigeria, Ireland, United Kingdom and USA.

Health care related factors associated with severe malaria in children in Kampala, Uganda

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Abstract

Background: Severe malaria is responsible for the high load of malaria mortality. It is not clearly understood why some malaria episodes progress to severe malaria.

Objective: To determine factors associated with severe malaria in children aged 6 months to 5 years living in Kampala.

Methods: Over a 6-month period, 100 children with severe malaria were matched by age and place of residence with 100 children with non-severe malaria. We collected health care information from care takers.

Results: Mean duration of illness before getting antimalarial treatment was shorter for controls than cases (8hours vs. 20hours, $p=0.015$). Children with severe malaria were less likely to have been treated with sulphadoxine-pyrimethamine in the preceding 2 weeks (OR 0.2, 95% CI 0.04-0.85, $p=0.016$). Odds of severe malaria were higher in those who reported lack of protective measures (mosquito coils (OR = 20.63, 95% CI 1.5-283.3, $p=0.02$ and insecticide sprays OR 10.93, 95% CI 1.13-105.64, $p=0.03$), although few reported their use.

Conclusions: Early anti-malarial treatment and use of barriers against mosquitoes prevent severe malaria in children. There is need to increase the use of barriers against mosquito bites and to scale up prompt treatment and community-based interventions to reduce the incidence of severe malaria in children.

Keywords: health care, severe malaria, children, Uganda

Running head: Factors associated with severe malaria in children

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Introduction

It is estimated that in sub-Saharan Africa, between 1.5 – 2.7 million people die annually due to malaria of whom about 1 million are children below five years of age¹. Severe malaria occurs in one in every 100 clinical cases of malaria among African children, often within 48 hours of the onset of fever². Previous studies identified parasite, host genetic, and immunologic factors, associated with development of severe malaria³⁻⁹, however, there is limited data on health care related factors associated with severe malaria in resource limited settings.

Inaccessibility to basic health facilities because of geographical or economic reasons often presents major challenges preventing prompt access to early diagnosis and effective antimalarial treatment. Home based management of malaria in the form of self-

treatment is often opted for after self-diagnosis based on presumptive symptoms of malaria. We performed a case control study to determine health care related factors associated with severe malaria in children.

Materials and Methods

Study site

The study was conducted in Mulago Hospital, the national referral and teaching hospital of Uganda. An area within 20km radius of the hospital was defined as the catchment community for both cases and controls. Malaria is meso-endemic in this area, occurring perennially with peaks during the 2 rainy seasons.

Population

A case of severe malaria was defined as a child aged 6 months to 5 years, with *P. falciparum* asexual parasitaemia, plus either cerebral malaria (*P. falciparum* malaria with manifestations of cerebral dysfunction including any degree of impaired consciousness, delirium, abnormal neurological signs, and focal or generalized convulsions), prostration or severe

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malarial anemia (*P.falciparum* malaria with hemoglobin less than 5mg/dl) with no other confirmed cause of the symptoms. All children with cerebral malaria had lumbar puncture and cerebro-spinal fluid analyzed to exclude meningitis.

A control was defined as a child aged 6 months to 5 years, who presented with fever or history of fever in the preceding 24 hours, with a positive blood smear for *P. falciparum*, a parasite count of at least 2000/ul with no other cause for the fever as well as not satisfying the criteria for severe malaria. Following recruitment of each case, a control from the same residential area and having a birth date within 6 months of the case' birth date was recruited. All the children were from areas surrounding Mulago Hospital and were all recruited during the same study period.

Calculation of sample size was done using data from a previous study done in Jinja hospital¹². Substitution into the formula for comparative studies (Schlesselman, 1974); yielded a sample size of 100 cases and 100 controls.

Study procedures

The caretakers were interviewed to establish symptom history, health seeking behavior, number of fever episodes in the past one year period, and use of protective measures against mosquito bites. A finger prick blood sample was taken for parasitological and hematological examinations and each child had a physical examination performed by the study physician. All children were treated according to the Uganda National Treatment guidelines.

Laboratory Tests

Thin and thick blood smears were stained with 2% Giemsa stain for thirty minutes, and parasite densities were calculated by counting the number of asexual parasites per 200 white blood cells (WBC) assuming a WBC count of 8,000/ul of blood. Complete blood counts and hemoglobin estimation were done using the Coulter method.

Statistical analysis

Data were recorded on standardized case report forms, reviewed daily for accuracy and completeness, and entered into EpiInfo version 6.04® (Centers for Disease Control and Prevention, Atlanta, GA) and analysis was done using SPSS 10.0 statistical software. Odds ratios (OR) with 95% confidence intervals were calculated. Categorical variables were analyzed

using Chi square test and Fisher's exact test was used where cell numbers were expected to be less than 5. The Independent T test was used for comparison of continuous variables that were normally distributed. Non-normally distributed variables were log transformed before applying the Independent T test and multiple regression analysis. Only variables found to have a statistically significant association with severe malaria at bivariate analysis were entered into the multiple regression model using Conditional Logistic Regression to identify independent predictors for severe malaria controlling for other factors. The Cox Regression procedure was used to fit a Conditional logit model. This was done by creating a failure time variable and a censoring indicator variable. A strata variable was created to specify the variable that determined the stratification. The Enter method was used to get the final model of independent risk factors for severe malaria using a p value of 0.05 as the cut off level for significance.

Ethical considerations

The study was approved by the Makerere University Faculty Research and Ethics Committee and was conducted according to Good Clinical Practice standards²¹. All parents/guardians of the children gave written informed consent.

Results

Between January and March 2002, 130 children with severe malaria were screened, of these, 100 children were enrolled consecutively and matched with 100 children with non severe malaria. Thirty children with severe malaria were not enrolled because they either lived outside the catchment community for this study, had malaria with meningitis, or their guardians did not consent to participate.

Among the children with severe malaria 44 (44%) had severe anaemia, (13, 13%) had cerebral malaria and (17, 17%) had prostration. Some children had more than one complication; 11 had cerebral malaria with severe anaemia, 8 had cerebral malaria with prostration, 4 had severe anaemia with prostration, and 3 had severe anaemia, cerebral malaria with prostration. Mean age of participants was 24 (SD 15.9) months and over 30% of the participants were in the age group 6-12 months. Ninety six percent and 97% of the cases and controls were female. Mean (range) number of episodes of fever per child within the previous year was 3 for both cases and controls. Most children (93% cases and 92% controls) had normal haemoglobin type

(haemoglobin AA). Two percent of the cases and 5% of the controls were carriers of the sickle cell gene (haemoglobin AS). None of the controls had hemoglobin SS compared to 3% of cases who had

sickle cell anaemia. Mean malaria parasite density was significantly higher among cases, (175,514/ul vs. 73,052/ul $p=0.002$) as indicated in table 1.

Table 1: Comparison of characteristics of cases and controls

Variable	Cases N=100	Controls N=100	P value
Mean age in months (SD)	25 (15.9)	24 (15)	0.49
% female	39	45	0.39
Mean age of caretaker in years (SD)	25.6 (6.8)	24.8 (6.7)	0.44
% with a mother as caretaker of child	91	91	1.0
Caretaker's marital status	72	81	0.13
% of caretakers with at least primary level education	89	86	0.36
Mean number of malaria episodes in previous year (range)	3 (0-10)	3 (0-20)	0.60
Haemoglobin AA	93 (93%)	92 (92%)	0.213
Haemoglobin AS	2 (2%)	5 (5%)	
Haemoglobin SS	3 (3%)	0	
Mean parasite density	175,514	73,052	0.002

Sociodemographic characteristics of caretakers of cases and controls were comparable as shown in table 1. Most caretakers (132, 66%) administered some form of medicine to the children at home before visiting hospital. Chloroquine was the commonest antimalarial taken at home; (51% cases and 52% controls). Other drugs administered included quinine, sulphadoxine-pyrimethamine (SP), amodiaquine, herbal medication, antibiotics and antipyretics. Duration of illness before receiving antimalarial treatment was significantly shorter for the controls (8 vs. 20 hours, $p=0.015$). Children with severe malaria were less likely to have been treated with SP in the preceding two weeks (OR 0.2 95% CI 0.04-0.85 $p=0.016$).

Most caretakers (89, 89%) knew that mosquitoes transmit malaria although not all of them used protective measures against mosquito bites. Bed nets were the most frequently used protective measure (80, 40%) however, the majority of caretakers did not know if their nets were insecticide treated. Insecticide sprays and mosquito coils were the other methods used, however, very few caretakers reported their use (insecticide sprays; 17, 8.5% and mosquito coils; 10, 5%). Adjusted odds ratios of having severe malaria were higher in those who did not use any protection against mosquito bites (mosquito coils; OR=20.6, 95% CI 1.5-283.3, $P=0.02$, insecticide sprays; OR 10.9, 95% CI 1.1-105.6, $p=0.03$, bednets OR 2.27, 95%CI 0.95-5.36 $p = 0.06$) (table 2).

Table 2: Factors associated with severe malaria after multiple regression analysis

Factor	OR	CI	P value
Log (duration of illness prior to getting antimalarial treatment in hours)	2.23	1.07-4.65	0.03
Log (duration of illness prior to presentation at the hospital in days)	0.51	0.09-2.7	0.43
Lack of use of insecticide sprays	10.93	1.13-105.64	0.03
Lack of use of bed nets	2.27	0.95-5.36	0.06
Lack of use of mosquito coils	20.63	1.50-283.3	0.02
Log (parasite density/ul of blood)	2.0	1.04-3.87	0.03

Discussion

We performed a case control study to determine factors associated with development of severe malaria in children. Our findings show that high

parasite density and longer duration of illness before administering antimalarial treatment plus lack of use of protective measures against mosquito bites

significantly increased risks for severe malaria in children.

Our findings are consistent with findings from previous studies performed in malaria endemic areas¹⁰⁻¹³. We found very limited use of barriers against mosquito bites in our study population. This could be explained by low socio-economic status of our study population although information to determine socio-economic status was not collected. Although two previous studies concluded that socio-economic factors were not the major determinants for severe malaria in children^{3,5}, socio-economic status determines access to insecticide treated nets, insecticide coils and access to health facilities.

Sixty six percent of all the children in our study received some form of treatment within 24 hours of recognition of illness as either herbal medication or monotherapy like chloroquine or amodiaquine which is inappropriate for malaria treatment. This reflects insufficient knowledge of diagnosis and treatment of malaria in the community. Indeed Nsungwa-Sabiti et al in a study in Uganda found a number of fever illness classifications in the community all of which could be biomedical malaria¹⁴ but were defined otherwise. A study done in 4 districts of Uganda found high rates of self-medication as first action when the children fell sick¹⁵. In another study in Kabarole district, Uganda, many mothers gave local herbs which they thought were effective against malaria and so delayed taking their children to health facilities¹⁶. One study found that although treatment initiation was promptly done, over half the times it was inappropriate¹⁷.

Uganda was the first country to scale up Home Based Management of Fever/Malaria (HBMF) in 2002¹⁸. This HBMF strategy in rural Uganda was evaluated and revealed an improvement in the accumulated proportions of patients treated¹⁹. Our finding of reduced risk of severe malaria among children treated with SP in the preceding two weeks suggests that use of a longer acting effective antimalarial reduces risk for severe malaria. Use of artemisinin based combination therapy (ACT) as part of HBMF could reduce the incidence of severe malaria in children. Ajayi et al showed that ACTs can be successfully integrated into the HBMF strategy in a study conducted in Ghana, Nigeria and Uganda²⁰. This could be combined with appropriate training of mothers on recognition of symptoms and prompt treatment of malaria.

The case control study design is prone to information and selection bias as well as bias resulting

from confounding factors. However, we minimised bias by training the interviewers how to administer the questionnaire. Blinding of interviewers was not possible because the clinical picture of the cases was evidently different from that of the controls. Selection bias was minimised by restricting both cases and controls to individuals living within a 20 Km radius of the hospital. Confounding was minimised by matching cases and controls by age and area of residence. It is possible that some of the controls later on developed severe malaria; however, we were unable to identify these because we did not follow them up.

In conclusion, our results demonstrate the need for scale up of health education to promote use of barriers against mosquito bites, prompt anti-malarial treatment and community-based interventions against malaria as part of a national program to reduce the incidence of severe malaria in children.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

Acknowledgement

We thank the study team of doctors, nurses, laboratory technicians and the administrative staff of Makerere University-University of California San Francisco Malaria Research Collaboration. We sincerely thank all the children and parents/caretakers who participated in the study. Financial support was provided by the Fogarty International Centre grant for training malaria research in Uganda through the Makerere University-University of California San Francisco Malaria Research Collaboration.

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Effect of HIV-1 infection on malaria treatment outcome in Ugandan patients

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Abstract

Background: Malaria and HIV-1 infection cause significant morbidity and mortality in sub-Saharan Africa. HIV-1 increases risk for malaria with the risk increasing as immunity declines. The effect of HIV-1 infection on antimalarial treatment outcome is still inconclusive.

Objective: To compare antimalarial treatment outcome among HIV-1 positive and negative patients with acute uncomplicated *falciparum* malaria treated with chloroquine plus sulfadoxine-pyrimethamine (CQ+SP).

Methods: Ninety eight HIV-1 positive patients aged 18 months or older with acute uncomplicated *falciparum* malaria were treated with CQ+SP and followed for 28 days to monitor outcome. Treatment outcome of HIV-1 positive patients was compared to that of 193 HIV-1 negative historical controls. The primary study outcome for both groups was treatment failure.

Results: HIV-1 positive patients older than 5 years of age were less likely to have treatment failure compared to HIV-1 negative patients in the same age group (RR 0.59 95% CI 0.4- 0.8, $p < 0.001$) and HIV-1 positive patients on routine cotrimoxazole prophylaxis were less likely to have treatment failure following CQ+SP treatment compared to HIV negative patients (RR 0.6 95% CI 0.43-0.92, $p = 0.006$). There was no difference in treatment outcome according to HIV-1 status for children younger than 5 years of age.

Conclusions: Adherence to cotrimoxazole prophylaxis should be reinforced in HIV positive patients and it should be reassessed if these patients present with acute episodes of malaria.

Key words: malaria, HIV, Uganda, antimalarial treatment response

Running head: Effect of HIV infection on antimalarial treatment outcome

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Introduction

In Sub Saharan Africa, malaria and HIV infections are endemic and responsible for significant morbidity and mortality. Malaria causes about 300-500 million clinical cases annually, 90% of which occur in sub-Saharan Africa¹. The Joint United Nations Program on HIV/AIDS (UNAIDS) estimated that 29.4 Million Africans are infected with HIV (UNAIDS, December 2002).

Two recent studies on the effect of HIV-1 infection on malaria incidence have provided strong evidence for an increased risk of malaria among HIV-1 positive patients^{2,4}. Because HIV-1 infection impairs cell-mediated immunity, some authors have argued that HIV-1 infected individuals may be at higher risk for or suffer poor outcomes to malaria infection. Only a few studies have examined the effect of HIV-1 infection on response to antimalarial treatment and these have yielded conflicting results⁵⁻¹³. Therefore the effect of HIV-1 infection on antimalarial treatment response is inconclusive. The objective of this study was to compare

the treatment outcome among HIV-1 positive and negative patients with acute uncomplicated *falciparum* malaria treated with chloroquine plus sulfadoxine-pyrimethamine (CQ+SP) and followed for 28 days.

Materials and Methods

Study site

The study was conducted between November 2004 and June 2005 in Mulago Hospital, Kampala, Uganda. The HIV-1 positive patients were recruited from the paediatric and adult infectious diseases clinics and the HIV-negative patients were recruited from the outpatients' clinic.

Ethical consideration

The study was approved by the Makerere University Faculty Research and Ethics Committee and was conducted according to Good Clinical Practice standards. All participants gave written informed consent.

Population

HIV-1 positive patients

Consecutive HIV-1 positive patients with symptoms of acute uncomplicated *falciparum* malaria and a positive screening thick blood smear (stained with 10%

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Leishman's stain for 10 min) were referred for study enrolment. Patients were enrolled if they met the following inclusion criteria:¹ age 18 months and above, ² an elevated temperature at presentation ($\geq 37.5^{\circ}\text{C}$ axillary) or a history of fever in the previous 24 hours, ³ *P. falciparum* mono-infection with $\geq 2,000$ asexual parasites/ul, ⁴ absence of other causes of fever (based on the clinical judgment of the study physician), ⁵ absence of severe malaria (WHO, 2000) or danger signs (inability to stand or drink, recent convulsions, lethargy, or persistent vomiting in children less than five), ⁶ no history of an allergic reaction to sulphonamides, ⁷ willingness of the patient or an adult guardian to provide written informed consent, and ⁸ residence within the city of Kampala.

Patients enrolled in the study were evaluated by a study physician for symptoms and their duration, medication history with emphasis on use of cotrimoxazole prophylaxis, duration of use and adherence measured by self report. Weight was measured in kilograms and temperature was measured using an electronic axillary thermometer. Blood was collected by venipuncture on the day of enrolment and by finger-prick on follow-up days.

HIV-1 negative patients

HIV-1 negative historical controls were selected from an earlier antimalarial study conducted in the outpatients' clinic from December 2003 to August 2004. Selection criteria and follow-up schedule were similar to that for HIV-1 positive patients. A database containing information on patient's age, sex, weight, baseline temperature, pre-treatment parasite density, and treatment outcome was created. New random numbers were computer-generated and linked to original patient study numbers in the database and on filter paper blood samples to identify the corresponding data and blood sample for each patient. The original identification numbers were deleted from the database and the filter papers. HIV-1 testing was performed on dry filter paper blood samples. All participants in this study gave written informed consent to future use of biological specimens and the Faculty Ethics and Research Committee approved HIV-1 testing of the de-linked samples. HIV-1 testing of historical controls was performed on filter paper blood samples using two enzyme linked immunosorbent assays (ELISA) in parallel (Vironostika HIV-1 Plus O Microelisa System,

BioMerieux, Inc. Durham, NC, U.S.A. and Genetic Systems rLAV EIA Bio-Rad Laboratories, Hercules, CA, USA). Patients were classified as HIV-1 positive if both enzyme immunoassays were positive and HIV-1 negative if both were negative. Western blots (Genetic Systems HIV-1 Western Blot, Bio-Rad Laboratories, Hercules, CA, USA) were performed on discordant samples, and the results were classified as positive, negative, or indeterminate. Indeterminate Western blot results were repeated and subsequently classified as positive or negative. Only HIV-negative subjects were selected as historical controls.

Treatment and follow-up

Both HIV-1 positive and negative patients were treated with 25 mg/kg of CQ (Avloclor, ZENECA, 10 mg/kg on days 0 and 1, 5 mg/kg on day 2) plus a single dose of 1.25 mg/kg pyrimethamine and 25 mg/kg sulfadoxine (Fansidar, Roche) on day 0. All doses were directly observed and if a patient vomited within thirty minutes of dosing, the medication was re-administered. Paracetamol was administered to all patients. Patients were followed on days 1, 2, 3, 7, 14, 21 and 28 and follow-up consisted of a brief history, clinical examination and a blood smear for malaria on each day. Patients were encouraged to come back to the clinic at any time if they felt ill, and they then received a full evaluation including examination of a blood smear. Patients who met criteria for clinical failure with CQ+SP were treated with intravenous or oral quinine (10 mg salt/kg every 8 hr for 7 days). If patients did not return for scheduled follow-up, they were visited and assessed at home. If the home health visitor could not locate patients, they were classified as lost to follow-up. Patients were excluded from the study for the following reasons: (1) administration of antimalarial drugs outside the study protocol, (2) emergence of another febrile illness which would interfere with classification of malaria treatment outcome, (3) movement away from the study area, or (4) withdrawal of informed consent.

Laboratory Tests

Thin blood smears (obtained on day 0) and thick blood smears (obtained on days 0, 3, 7, 14, 21, and 28) were stained with 2% Giemsa stain for thirty minutes, and parasite densities were calculated by counting the number of asexual parasites per 200 white blood cells (WBC) assuming a WBC count of 8,000/ul of blood.

Outcome Measurements

Treatment outcome over 28 days of follow-up was classified according to the WHO Treatment Outcome Classification (WHO, 2002). Patients were classified as Early Treatment Failure (ETF) if they developed danger signs or severe malaria on or before day 3, had fever and a day 2 parasite density greater than that on day 0, had fever and parasitemia on day 3, or had a day 3 parasite density $\geq 25\%$ of that on day 0. Late Clinical Failure (LCF) was defined as parasitemia after day 3 with a documented temperature $> 37.5^{\circ}\text{C}$ (axillary), danger signs, or severe malaria. Late Parasitological Failure (LPF) was defined as presence of parasitemia on day 28 and temperature $< 37.5^{\circ}\text{C}$ (axillary), without previously meeting any of the criteria of early or late treatment failure. All others were classified as Adequate Clinical and Parasitological Response (ACPR).

Statistical Analysis

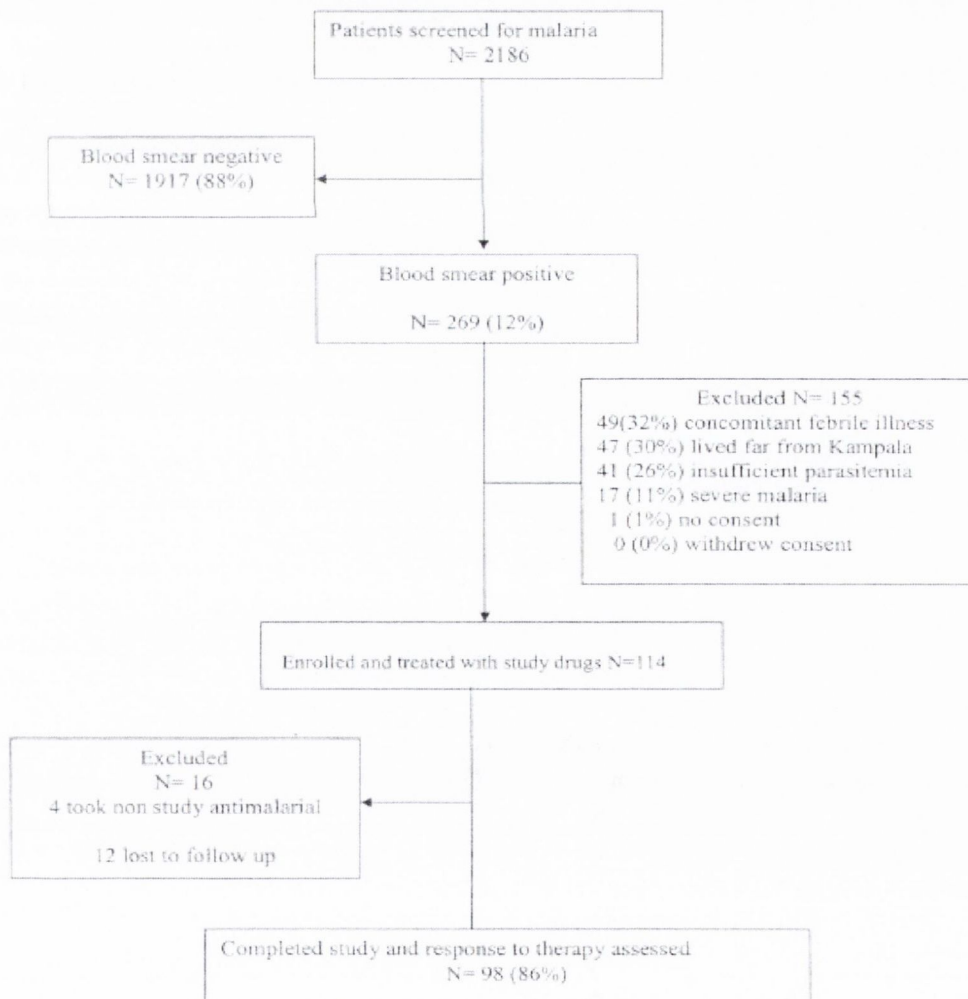
Data were recorded on standardized case report forms, reviewed daily for accuracy and completeness, and

entered into EpiInfo version 6.04[®] (Centers for Disease Control and Prevention, Atlanta, GA). Clinical treatment success was defined as an ACPR response and clinical treatment failure defined as either ETF or LCF or LPF. Data were summarized using frequencies, medians, and means. Analysis for malaria parasite density was done on log-transformed parasite density values. Continuous variables were compared using the Independent T-test. Association between HIV-1 infection and treatment outcome was determined by estimating relative risks and 95% confidence intervals (95% CI) using cross tabulation. A two sided p value < 0.05 was considered statistically significant.

Results

A total of 2186 HIV-1 positive patients with fever and axillary temperature $\geq 37.5^{\circ}\text{C}$ were screened for malaria; 269 (12%) had a positive malaria blood smear and were referred for study inclusion, 114 fulfilled the inclusion criteria and were enrolled into the study (figure 1).

Figure 1: Study profile of HIV-1 positive patients



The primary reasons for exclusion were: presence of concomitant febrile illness (49, 32%), residence outside the city of Kampala (47, 30%), insufficient parasitemia (41, 26%), severe malaria (17, 11%) and lack of consent (1, 1%). Of the 114 HIV-1 positive patients 16 were not included in the analysis because of either loss to follow

up or use of additional antimalarial medication outside the study protocol.

From the historical cohort of 213 patients; 193 (90%) were HIV-1 negative and included as the comparison group, 8 (4%) were HIV-1 positive and 12 (6%) had discordant HIV test results.

Table 1: Baseline characteristics of patients who completed the study

Characteristic	HIV status			
	HIV-1 negative		HIV-1 positive	
	Less than 5 years	5 years and above	Less than 5 years	5 years and above
Percent female	46%	55%	44%	66%
Median age in years (IQR)	3.0 (2-4)	9 (6-12)	4 (3-4)	25 (10-34)
Mean temperature °C (SD)	38.3 (1.2)	37.7 (1.1)	38.5 (0.9)	38.0 (1.0)
Mean Parasite density per ul (SD)	58,828 (74388)	49,897 (65116)	53,250 (67666)	63,032 (107614)
Mean log parasite density (SD)	4.4 (0.65)	4.3 (0.62)	4.3 (0.69)	4.4 (0.6)
Median weight (IQR)	13 (10-15)	24 (19-35)	13.1 (37.7-39.3)	45.5 (24.6-54)

The baseline characteristics of patients who completed the study and were included in the analysis are shown in Table 1. Baseline characteristics of HIV-1 positive and negative patients less than 5 years of age were comparable. HIV-1 positive patients older than 5 years were older than the HIV-1 negative patients in the same age group, they also weighed more ($p < 0.001$), presented with higher temperature ($p = 0.009$) and tended towards higher parasite density ($p = 0.2$) compared to the HIV-1 negative patients in the same age group.

Comparison of treatment outcome

Thirty three (34%) of the HIV-1 positive patients had CQ+SP treatment failure compared to 119 (62%) of the HIV-1 negative patients (RR 0.54 95% CI 0.4-0.7 $p < 0.001$).

Among patients younger than 5 years, 4 (44%) of the HIV-1 positive patients had treatment failure compared to 45 (76%) of the HIV-1 negative patients (RR 0.58 95%CI 0.2-1.2 $p = 0.103$). Among patients 5 years and older; 29 (33%) of the HIV-1 positive patients had treatment failure compared to 74 (55%) of the HIV-1 negative patients (RR 0.59 95% CI 0.4-0.8, $p < 0.001$) (Table 2).

Table 2 Comparison of treatment outcome among HIV-1 positive and negative patients

Treatment outcome	Age; below 5 years		Age; 5 years and above	
	HIV-1 negative (N=59)	HIV-1 positive (N=9)	HIV-1 negative (N=134)	HIV-1 positive (N=89)
Failure n (%)	45 (76)	4 (44)	74 (55)	29 (33)
ACPR n (%)	14 (24)	5 (56)	60 (45)	60 (67)
	P=0.103		P=0.001	

Table 3 Comparison of treatment outcome among HIV-1 positive patients on cotrimoxazole and HIV-1 negative patients

Treatment outcome	HIV-1 positive on cotrimoxazole	HIV-1 negative, not on cotrimoxazole
Failure n, (%)	18 (39)	119 (62)
ACPR n, (%)	28 (61)	74 (38)
	RR = 0.6, 95% CI = 0.43 – 0.9, P = 0.006	

Comparison of treatment outcome of HIV-1 positive patients on cotrimoxazole with that of HIV-1 negative patients (Table 3) showed that 18 (39%) of the HIV-1 positive patients on cotrimoxazole had treatment failure compared to 119 (62%) of the HIV-1 negative patients (RR 0.6 95% CI 0.43-0.9, $p = 0.006$).

Discussion

We compared CQ+SP treatment outcome for acute uncomplicated *falciparum* malaria among HIV-1 positive and negative patients over a 28 day period. We found that HIV-1 positive patients older than 5 years of age were less likely to have treatment failure compared to HIV-1 negative patients in the same age group. There was no difference in treatment outcome according to HIV-1 status among patients younger than 5 years of age; however, the sample size for this age group was very small.

Very few studies have examined the effect of HIV infection on response to antimalarial treatment outcome. Two studies done in Zaire found that there was no significant difference in the level of treatment failure among HIV positive and negative children on day 7 following treatment with quinine⁷. A few studies carried out in other areas have suggested a decreased response to antimalarial treatment in HIV infected patients. One of these was a retrospective study done in Uganda which suggested that co-infection with HIV-1 may render CQ less effective therapy for malaria in children⁶. However, this study used a single antimalarial drug with high resistance levels, on a very small sample size of children. Another study from Ethiopia, suggested decreased clearance of *P.falciparum* in HIV-1 positive patients after treatment with artemisinin¹⁰.

Our findings are similar to results from a few previous studies which showed that HIV-1 infection has no significant impact on malaria treatment outcome in children^{6,9, 11-14}. The study done in Uganda found that HIV-1 infection increased the susceptibility for new malaria infections but not recrudescence in adults, and there was no increased risk of malaria among HIV-1 infected children¹⁴. The recent study from Zambia found that HIV-1 infection was not a risk factor for recrudescence or reinfection, although patients with a CD4 cell count <300cells/ul were more likely to have recurrent parasitemia, recrudescence and new infection¹³.

Response to antimalarial therapy is dependent on the abilities of both antimalarial drugs and host immune responses to inhibit infecting parasites^{15, 16}. Malaria-specific immunity is acquired with repeated exposure to malaria parasites and this immunity increases with age

¹⁷⁻²⁰. Similarly, response to antimalarial therapy improves as the level of acquired immunity increases¹⁸. Although the immunological consequences of HIV infection are well established, the interaction between HIV infection and *P. falciparum*, both widely co-distributed in sub-Saharan Africa is not fully understood²¹. The immunosuppression caused by HIV infection might be associated with the failure to protect against malarial infection and the development of clinical disease²² but not treatment outcome. An older study suggested that some components of the specific immune responses to *falciparum* parasites may not be modified, despite the decrease in CD4 counts with HIV infection²¹.

We classified patients into categories according to age with five years as the cut off age. Previous studies have shown that age is a predictor of antimalarial treatment response. A study on predictors of chloroquine treatment failure showed that patients under the age of five were more likely to fail therapy and fail early in the course of treatment²³. This categorization of patients was also supported by data that shows that antimalarial immunity increases with age, and effectiveness of antimalarial drugs is affected by the immune status of the host^{24, 25}.

The HIV-1 positive patients in our study were relatively older than the HIV negative patients. It is possible that the advantage of older age and therefore greater acquired immunity balanced out the relative disadvantage of HIV infection and reduced immunity among the HIV positive patients.

We also found that HIV-1 positive patients on routine cotrimoxazole prophylaxis were less likely to have treatment failure following CQ+SP treatment compared to HIV-1 negative patients. Daily cotrimoxazole prophylaxis has been shown to provide a beneficial effect in preventing malaria, and death in HIV-1 positive patients.²⁶. Cotrimoxazole is 99.5% effective in preventing malaria while effectiveness with SP is 95%, and both have about 80% therapeutic efficacy for the treatment of malaria²⁷. However, cross-resistance between cotrimoxazole and SP is a potential concern when cotrimoxazole prophylaxis is used in areas where SP is used for treatment of malaria. This cross resistance has been shown to occur between cotrimoxazole and SP (28-30), although analysis of malaria parasites from children in Mali who had received at least one month of cotrimoxazole prophylaxis detected no resistance-conferring mutations. Similarly, a study in Uganda³¹ found no significant difference between either the proportion of malarial episodes with resistant organisms or the incidence of SP-resistant malaria before and after cotrimoxazole prophylaxis was introduced.

Our study was done in a specialized HIV clinic, where comprehensive HIV care is provided, including health education, antiretroviral therapy and malaria preventive materials such as insecticide-treated bed nets. It is possible that patients at these clinics have developed better health-related behavior and higher rates of self-treatment compared to HIV-1 negative patients in the general population. In addition, some protease inhibitors used in the treatment of HIV infection may also be effective in the treatment or prevention of malaria.

Use of a historical cohort was a limitation of this study; however, this may not have significantly affected our study results because the HIV-1 positive cohort which was recruited latter had lower risk of treatment failure. We could have also introduced measurement bias in this study because the two groups of patients were followed up at different times and so there could have been differences in measurements as well as missing data in the historical database. We were unable to perform genotyping to distinguish re-infection from recrudescence. The study done in Uganda showed that HIV positive adults had higher risk for re-infection⁵ and the Zambian study found that patients with a CD4 cell count <300cells/ul were more likely to have recurrent parasitemia, recrudescence and new infection¹¹.

In conclusion, our findings show that the HIV-1 positive patients older than 5 years of age were less likely to have treatment failure compared to the HIV-1 negative patients in the same age group and use of daily cotrimoxazole prophylaxis by the HIV positive patients was associated with reduced risk of CQ+SP treatment failure. Adherence to cotrimoxazole prophylaxis should be reinforced in HIV positive patients and it should be reassessed if these patients present with acute attacks of malaria.

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