ejpmr, 2018,5(10), 14-18

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

BIOAVAILABILITY AND BIOEQUIVALENCE EVALUATION OF LOKMAL® (ARTEMETHER-LUMEFANTRINE 80/480 MG FIXED DOSE TABLET): AN EMZOR® PHARMACEUTICAL PRODUCT

Ezealisiji Kenneth M.*[1], Ifeyinwa Chijioke-Nwauche[2], Igbinaduwa Patrick[1], Chijioke Adonye Nwauche[3]

[1]Department of Pharmaceutical and Medicinal Chemistry, University of Port Harcourt.
[2]Department of Clinical Pharmacy &Management, Faculty of Pharmaceutical Sciences, University of Port Harcourt
[3]Department of Haematology & Blood Transfusion, University of Port Harcourt Teaching Hospital.

***Corresponding Author: Dr. Ezealisiji Kenneth M.**

Department of Pharmaceutical and Medicinal Chemistry, University of Port Harcourt.

Article Received on 23/07/2018 **Article Revised on 13/08/2018** **Article Accepted on 03/09/2018**

ABSTRACT

Present design evaluated the bioavailability and bioequivalence of generic product of artemether - lumefantrine (AL) antimalarial fixed dose tablet which presents as Lokmal® 80/480 mg an Emzor® Pharmaceutical brand formulated as a single dose oral tablet. A non – randomized open label single dose study in sixteen healthy Nigerian male subjects was designed. The volunteers were administered one tablet of the product with a fatty meal and 250 ml of table water after overnight fast. Plasma samples were analyzed for artemether and lumefantrine exposure simultaneously using a validated High Performance Liquid Chromatography System. The 90% confidence interval for the ratio of the geometric means of AUC_{0-t} was compared with the established bioequivalence limit. The adjusted geometric mean C_{max} for artemether and lumefantrine for Lokmal® and Coartem® (standared), was found to be 1.27, 1.29 µg/ml and 33.99, 34.01 µg/ml respectively. The AUC_{0-t} was found to be 4.3, 4.17 µg.h/ml and 791.76, 792.09 µg.h/ml respectively for Lokmal® against Coartem ®. The 90% Confidence interval of the adjusted geometric mean ratio for the basic pharmacokinetic parameters were found to be within limit of specification 80-125% indicating bioequivalence.

KEYWORDS: Bioequivalence, Pharmacokinetics, Chromatography, artemether, lumefantrine.

INTRODUCTION

Malaria is a parasitic disease caused by Plasmodium species and transmitted through the bite of female Anopheles mosquito and has posed a huge global treat in recent times. In 2012, 250 million cases and 750,000 mortality were documented.[1] An overall increase in Plasmodium resistance to malarial medicines poses an outstanding challenge to malarial eradication.[2] The battle against malaria seems unachievable in the presence of fake and substandard drugs especially in developing countries including Nigeria. The understanding of Bioequivalence (BE) and Bioavailability (BA) have been so useful during the last four decades because of their application to new brand-name drugs.[3] These have formed the regulatory requirement for the approval of generic drug products[4] BA and BE have become indispensable for the approval of brand-name and generic in line with World Health Organization Specification.[5] These have given room for the use of brand-name drugs with reduction in cost of development. The need for generic drugs in health care has made it mandatory that the Pharmaceutical quality and in-vivo activity of generic drugs be properly evaluated.[6-8] Coartem® an Artemether/Lumefantrine 80/480 mg fixed dose (AL) have been approved by the World Health Organization since 2001 for use in the treatment of uncomplicated malaria and presents as an oral six-dose regimen. The tablet is administered twice daily over three days in adults. Hence formulation was aimed to enhance compliance and reducing the frequency of dosing. The sponsor has developed a new brand Lokmal® containing Artemether/Lumefantrin 80/480 mg fixed dose tablet which is cost-effective and relatively more available with ease of sourcing for people who cannot afford the innovator brand.

The above two fixed-dose tablet formulation (Artemether/Lumefantrin 80/480 mg Emzor Pharmaceutical brand) and the innovator brand (Coartem® Norvatis) have been extensively studied for bioavailability and bioequivalence. Following a single oral dose (80/480 mg fixed dose), artemether peak plasma concentration is observed between 2-3 hours.[9] Intake of high fat diet after dosing enhances the absorption of artemether with a resultant increase in bioavailability. Dihydroartemisinine is the metabolite for artemether.[10] The elimination half-life is about two hours [11] while a single oral dose (80/480 mg fixed dose)

lumefantrine peak plasma concentration are reached after about 7-8 hours [12-13] High fat diets are also known to enhance the absorption of Lumefantrine equally with a resultant increase in the relative bioavailability. The principal metabolite for Lumefantrine is desbutyl-Lumefantrine.[14-15] The elimination half life is about 3-4 days in healthy subjects [16]. Present design aims to evaluate the bioavailability and bioequivalence of generic product of artemether - lumefantrine (AL) antimalarial fixed dose tablet which presents as Lokmal® 80/480 mg an Emzor® Pharmaceutical brand formulated as a single dose oral tablet.

MATERIAL AND METHODS

Material

Samples of Lokmal® 80/480 mg fixed dose tablets used were kindly provided by Emzor Pharmaceutical Company Lagos, Nigeria. Innovator brand , Coartem® 80/480 mg fixed dose tablets were procured from Norvatis distributor in Nigeria. Acetonitrile (HPLC) grade, dichloromethane (HPLC) grade were all from Merck (Darmstadt, Germany). Ultra pure water was prepared with a Milli-Q Academic System (Millipore Co, USA).

Study Design

Present method adapted a single dose, open-label; two-way cross-over study in clinically healthy subjects which was carried out under fed condition bearing in mind that oily food enhances Lumefantrine absorption. The study design consisted of 8- day sample collection period, two baseline periods (before and after treatment) followed by a washout period of two weeks. A crossover comparism of Lokmal® 80/480 mg fixed dose tablet versus Coartem® 80/480 mg fixed dose tablet was carried out. In each case a single tablet was taken once and this was done under surveillance with 250 ml of table water after breakfast (high fat diet). Blood sample collection were carried out before and at 0, 0.5, 1, 2, 4, 5, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168 and 192 hours after dosing. Tablets that were given are Lokmal® tablet 80/480 mg; Lot: LOK - 80, Expiry Date 10.2019: MFD.10.2017 and Coartem® tablet 80/480 mg; Lot K0081, Expiry Date 03.2019: MFD.04.2017. Artemether and Lumefantrine in plasma were extracted in dichloromethane and acetonitrile (30/70 %). The acetonitrile helps in the precipitation of plasma protein and aids in the release of drugs from plasma. The extracted active principles were reconstituted with HPLC grade reagent (1 ml of acetonitrile before measuring the plasma concentration using HPLC/DAD/UV. Primary Pharmacokinetic parameters were estimated using Sigma plot 11 software (USA).

Subjects

Sixteen human volunteers (Males, N=16) within the age of between 20 to 24 years with weight not less than 55 kg. Average Body Mass Index (BMI) of 20.5 kg/sq m and clinically healthy as certified by the principal medical investigator (Supervising physician), were qualified to participate in this research study. Subjects that are on medication as at the time of this study were disqualified. Those with history of adverse reaction or hypersensitivity to artemether/lumefantrine, smokers and those on alcohol were excluded from the study. Those with hepatitis or other clinically deleterious conditions were not allowed to take part in this study. The study was conducted at the Centre for Malarial Elimination and Phytomedicine Research, Port Harcourt, Nigeria. The protocol was approved by the University of Port Harcourt Teaching Hospital Ethical Committee. Qualified volunteers were enrolled after signing an informed consent letter. These studies were conducted in accordance with the Helsinki Declaration.[17] with an ethical approval number UPTH/ADM/90/S.II/Vol.XI/525 of 30th Nov.2017. The European Medicines Agency Guidelines for Bioequivalence Studies[18] were also adapted. Subjects were all admitted prior to the baseline evaluation (Zero hours) of blood sample collection).

Extraction Procedure

Plasma artemether-lumefantrine were prepared by extracting these active pharmacological principle in 2 mls of plasma with dichloromethane and acetonitrile (30/70 % v/v) in a 10 ml glass tube by gentle vortex mixing for 30 sec followed by centrifugation for 5 minutes at 3500 rpm. Organic layer were recovered through a Millex GV 0.45 µm filter and evaporated at 40^{oC} and later reconstituted with HPLC grade acetonitrile (Mobile phase). A 50 µL sample was injected into the HPLC/DAD/UV system.

Bio assay

The bio-analysis was carried out within the Lowest Limit of Quantification (LLOQ) of both components when 1 ml of sample was used. Calibration curves were generated for the two active pharmaceutical principles under investigation and were linear over the concentration range of 0.25 µg/ml to 25 µg/ml and 10 µg/ml to 75 µg/ml for artemether and lumefantrine respectively. The assay adapted, has been validated for use in human Pharmacokinetic study. Percentage recovery, precision and accuracy were also considered.

Statistical analysis

Descriptive statistical analysis of analyte concentrations was carried out also the primary PK parameters considered statistically included mean, standard deviation (SD), % CV, median,minimum and maximum. Concentrations below lower limit of quantification (LLOQ) were treated as zero in summary statistics. An analysis of variance (ANOVA) was performed on the log-transformed data.

RESULTS

Artemether-Lumefantrine Quantification in Human Plasma

The HPLC-DAD/UV analysis employed for artemether-lumefantrine determination afforded the specificity,

sensitivity and high sample through-put necessary for Bioequivalence and Pharmacokinetic studies. Observed retention time for artemether and lumefantrine were 3.5 minutes and 4.2 minutes in both the test and standard sample respectively and there was no recorded interference peak from components of blank plasma residue. Mean extraction recovery values were 96.2 % for artemether and 92.6 % for lumefantrine in both cases for Lokmal and Coartem respectively. Intra-assays accuracy and precision were within the range of 91.8 % to 96.4 % and 8.6 to 2.8 % respectively while Inter-assays accuracy and precision range from 98.0% to 98.6 % and from 3.4 % to 4.8 % respectively.

Subjects withdrawal, baseline characteristics and demographical index

In this study, a total of 16 subjects were enrolled and eight subjects were engaged in each study period comparing the generic Lokmal® 80/480 mg fixed dose with standard drug Coartem® 80/480 fixed dose tablet. The entire subject completed the study in both stages. Two subjects did not complete their breakfast before taking their tablet. The baseline demographics including the average age and body mass index are presented in Table x below.

Table 1: Table of subject demographics.

		80/480 mg (lokmal)			80/480 mg (coartem)		
		Period			Period		
		1	2	Total	1	2	Total
		N=8	N=8	N=16	N=8	N=8	N=16
Age (year)	Mean(SD)	22.3 (9.28)	22.3 (9.28)	22.3 (8.96)	22.3 (9.28)	22.3 (9.28)	22.3 (8.96)
	Range	20.86	20.0	18.58	20.86	20.0	18.58
Height (m)	Mean (SD)	1.74 (8.98)	1.74(9.46)	1.74(8.99)	1.74 (8.98)	1.74(9.46)	1.74(8.99)
	Range	1.60	1.60	1.59	1.60	1.60	1.59
Weight (kg)	Mean (SD)	61.0(9.34)	61.0(9.82)	61.30(8.98)	61.0(9.34)	61.0(9.82)	61.30(8.98)
	Range	58	58	59	58	58	59
BMI (kg/m^2)	Mean (SD)	20.6(8.95)	20.6(9.64)	21.0(8.98)	20.6(8.95)	20.6(9.64)	21.0(8.98)
	Range	19.8	19.8	20.0	19.8	19.8	20.0
Sex	Male	8	8	16	8	8	16
Ethnicity	Nigerian	8	8	16	8	8	16

Bioequivalence and Pharmacokinetics

Following critical studies of pharmacokinetics and bioequivalence of artemether and lumefantrine in this study, it was observed that the mean plasma concentration-time profiles of artemether and lumefantrine formulations (Lokmal and Coartem) were superimposable as shown in the 3-D graphical chart in figure 1&2. Artemether was absorbed rapidly following single fixed dose administration with mean T_{max} of three hours for Lokmal 80/480 mg fixed dose (Emzor Pharmaceuticals brand) and the innovator brand (Coartem 80/480 mg Novartis product). Both formulations gave a mean C_{max} value of 1.27 µg/ml and 1.29 µg/ml respectively. The observed mean AUC_{last} for both formulation was 4.13 and 4.17 µg.h/ml. The $T_{1/2}$ was observed at the range of 2.35 hours respectively while the mean $AUC_{infinity}$ was found to be 5.72 µg.h/ml and 5.90 µg.h/ml respectively.

Lumefantrine in both formulations under comparison was absorbed with mean T_{max} of 8 hours. Mean C_{max} for the two formulations were 33.99 µg/ml and 34.01 µg/ml respectively. Mean AUC_{last} for Lokmal and Coartem 80/480 mg fixed dose tablet were 791.76 µg.h/ml and 792.09 µg.h/ml the corresponding mean $AUC_{infinity}$ were 802.0 µg.h/ml and 805.0 µg.h/ml respectively. The mean $T_{1/2}$ was observed at 112 hours and 118 hours for both the generic brand and the innovator brand.

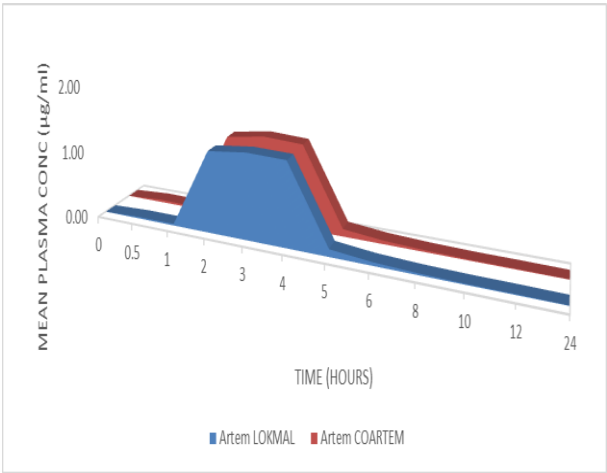

Figure 1: Artemether plasma concentration (mean ± SD) during treatment with Lokmal® and Coartem® for 24 hours.

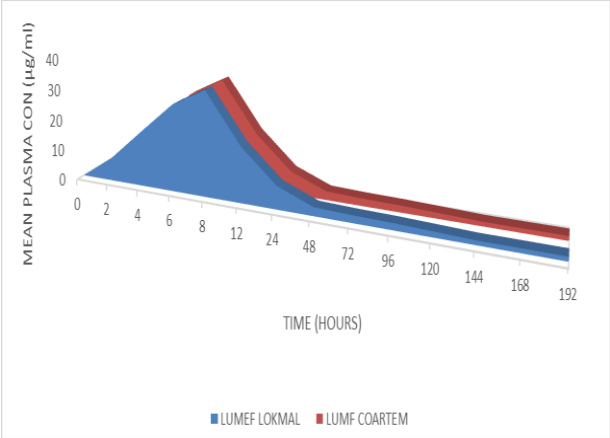

Figure 2: Lumefantrine plasma concentration (mean ± SD) during treatment with Lokmal® and Coartem® for 192 hours.

DISCUSSION

Adjusted geometric means for the primary pharmacokinetic parameters studied and the comparative platform, 90 % CI of C_{max}, AUC_{last} and T_{max} for artemether and Lumefantrine were found within the range in both formulations. Only one case of nausea and weakness was reported amongst the group that were administered Lokmal® brand within the first study period while two cases of mild loss of appetite were reported following administration of Coartem® in the second period of study which is in line with the specification on the drug information leaflet for Coartem®. No significant abnormalities were demonstrated on examination of the subjects at the end of the study. The present work is in agreement with the previous research by Lefe'vre et al which shows that a fatty meal is known to enhance the bioavailability of artemether and lumefantrine compared with fasted state and the PK evaluation for all the formulations did not reveal any noteworthy deviations in the primary PK parameters from their prior studies in healthy subjects, C_{max}, AUC_{last}, and AUC_{inf} were similar between the two treatments (in both comparison) for artemether and lumefantrine.[19] Previous review by Philip Debrah et al. referred to other documentaries which affirms that WHO survey of counterfeit medicine reports from 20 countries between January 1999 and October 2000 reveals that 60 % of all counterfeit medicines finds occurred in poor developing countries because of weak drug regulation control and enforcement, inconsistent supply and scarcity of basic medicines.[20]

Table 2: PK Parameter values (summary)

Analyte	PK Parameter	Lokmal® 80/480 mg	Coartem® 80/480 mg
Artemether	C_{max} (µg/ml)	1.27±0.50	1.29±0.18
		[n=16]	[n=16]
	AUC_{last} (µg.h/ml)	4.13±2.10	4.17±1.98
		[n=16]	[n=16]
	$AUC_{infinity}$(µg.h/ml)	5.72±2.30	5.90±2.00
		[n=16]	[n=16]
	T_{max} (h)	3.00	3.00
		[n=16]	[n=16]
	$T_{1/2}$ (h)	2.35±1.00	2.35±1.00
		[n=16]	[n=16]
Lumefantrine	C_{max} (µg/ml)	33.99±12.1	34.01±11.3
		[n=16]	[n=16]
	AUC_{last} (µg.h/ml)	791.76±264.5	792.09±302.8
		[n=16]	[n=16]
	$AUC_{infinity}$(µg.h/ml)	802.0±304.6	805.0±298.9
		[n=16]	[n=16]
	T_{max} (h)	8.00	8.00
		[n=16]	[n=16]
	$T_{1/2}$ (h)	112	118
		[n=16]	[n=16]

CONCLUSION

Pharmacokinetics and statistical evaluations revealed that the two brands of artemether-lumefantrine 80/480 mg fixed dose tablets (Lokmal® and Coartem®) are bioequivalent and can be used interchangeably. The 90 % Confidence Interval (CI) for the basic pharmacokinetic parameter C_{max}, AUC_{last}, $AUC_{infinity}$ and T_{max} are within Limit of Specification (0.80 – 1.25). The bioavailability of both brands is also comparable.

DECLARATIONS

ACKNOWLEDGEMENTS

Present study was supported by Emzor Pharmaceutical Nigeria Limited and the NDDC Professorial Chair for Malaria Elimination and Phytomedicine Research.(Lok/Res Fund/20180034).

Conflict of Interest: The authors declare no conflict of interest and that all authors do not work for, or represent in any way, Emzor Pharmaceuticals Limited.

Authors Contributions
All authors met International Committee of Medical Journal Editors Criteria for authorship.
Ethical approval: Ethical Registration Number: UPTH/ADM/90/S.II/Vol.XI/525 of 30th Nov.2017

REFERENCES

1. Lurdes C S, Cândida F A, Sandra M X, Margarida T, Raquel L and António C S . Severe imported malaria in an intensive care unit: a review of 59 cases, *Malaria Journal 2012;* **11(1)**: 96.
2. Mohammed H. B**,** Ishtiaq A**,** Faheem M**and** Muhammad A.Z. Quinazolinone Derivatives as a Potential Class of Compounds in Malaria Drug Discovery, International Journal of Pharmacology, 2017; 13(7): 818-831.
3. Upendra C.G, Vijay R.P, Pravin P.A, Pravin D.C . Study on requirements of bioequivalence for registration of Pharmaceutical products in USA, Europe and Canada; Saudi Pharm. Journal, 2013; 22(5): 391-402**.**
4. Kasselheim A.S, Alexander S.M, Margaret R.S, Brookhart M.A, Niteesh K.C, William H.S,. Clinical Equivalence of Generic and Brand-Name Drugs used in Cardiovascular Disease: A Systematic Review and Meta-analysis; JAMA 300: 2008; (21): 2514-2526.
5. Alfredo G.A, John G. Bioequivalence Requirements in the European Union: Critical Discussion; AAPS J. 2012; 14(4): 738-748.
6. Miyamoto E. Quality evaluation of Pharmaceutical products and medical supplies: Proper use; Journal of the Pharm. Soc of Japan; 2014; 138(8): 867-877.
7. Pooja M.V, Manish N.S, Mira K.D . An evaluation of drug promotional literatures published in scientific medical journals; J Pharm Bioallied Sci. 2016; 8(3): 248-252.
8. Doris J.K, Karen S.H, Robin L.C. Evaluation of a Required Senior Research Project in a Doctor of Pharmacy Curriculum; AM J Pharm duc: 2011; 75(1): 5.
9. Pauline B.K, Mohammed L, Harriet M.K, Saye K, Concepta M, Jean-Pierre V . Artemether-Lumefantrine Combination Therapy for Treatment of Uncomplicated Malaria: The Potential for Complex Interactions with Antiretroviral Drugs in HIV-Infected Individuals**,** Malar Res Treat. 703730. doi: 10.4061/2011/703730.
10. Liusheng H, Alexander O, David G, and Francesca T A. Determination of artemether and dihydroartemisinin in human plasma with a new hydrogen peroxide stabilization method, Bioanalysis; 2013; 5(12): doi: 10.4155/bio.13.91
11. Abdoulaye D and Gilbert L. Understanding the pharmacokinetics of Coartem®, Malar J. 2009; 8 (Suppl 1): S4. doi: 10.1186/1475-2875-8-S1-S4.
12. Norah M, Markus J, Lars L G, Celestino O, Paul W, Margarita M, Muhammad N, Olof B, Urban H. Comparable Lumefantrine Oral Bioavailability when Co-administered With Oil-Fortified Maize Porridge or Milk in Healthy Volunteers 2013; 113(1): 66-72.
13. Isha T, K. S, Rama R, Sheelendra P S & Muhammad A. Assessment of pharmacokinetic compatibility of short acting CDRI candidate trioxane derivative, 99–411, with long acting prescription antimalarials, lumefantrine and piperaquine, Scientific Reports 2015; (5): number: 17264 doi:10.1038/srep17264.
14. Rina P. M. W, Sam S, Kenneth F. I, Peter M. S, Ivo M, and Timothy M. E. D . Desbutyl-Lumefantrine Is a Metabolite of Lumefantrine with Potent *In Vitro*Antimalarial Activity That May Influence Artemether-Lumefantrine Treatment Outcome**,** Antimicrob Agents Chemother. 2011; 55(3): 1194–1198. doi: 10.1128/AAC.01312-10
15. Joanna G,Sylvain P,Thomas M,Carine B,Dany S,Emilie P,Thierry B,Blaise G,Laurent A.D . LC–MS/MS method for the simultaneous analysis of seven antimalarials and two active metabolites in dried blood spots for applications in field trials: Analytical and clinical validation, Journal of Pharmaceutical and Biomedical Analysis : 2018; 154: 263-277.
16. Ronan J Jacques Le Bras M R. Pitfalls in new artemisinin-containing antimalarial drug development, Trends in Parasitology: 2011; 27(2): p82–90.
17. Ehab I.T and Fathy I. A. Pharmacokinetics and Bioequivalence Study of Two Proton Pump Inhibitor Products, British Journal of Pharm. Res, 2016; ISSN: 2231-2919; 13(6): 1-8.
18. Chrysa D & Vangelis K . Bioequivalence studies in Europe before and after 2010 Journal of Clinical Research and Regulatory Affairs, 2014; 32(1): 9-21.
19. Gilbert L, Prafulla B, Jay P.J, Sampath K, Yi C, Hardik D and Daniel S. S. Evaluation of two novel tablet formulations of artemether-lumefantrine (Coartem®) for bioequivalence in a randomized, open-label**,** two-period study, *Malaria Journal 2013;* **12**: 312 /doi.org/10.1186/1475-2875-12-312.
20. Philip D, Henry N, Katja K.M, Patrick A.K, Birgitte B, Joseph A.S, Irene A.K, Patrick O.D, Samuel A, Eskild P, and Tore F.H . Artemether-Lumefantrine Concentrations in Tablets and Powders from Ghana Measured by a New High-Performance Liquid Chromatography Method, Am. J. Med. Hyg, 2016; 95(1): 158-161.