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Research Article

PHARMACOKINETIC AND BIOEQUIVALENCE COMPARISON BETWEEN ARTEMETHER TABLETS 80MG: AN OPEN LABEL, BALANCED, RANDOMIZED-SEQUENCE, SINGLE-DOSE, TWO-PERIOD CROSSOVER STUDY IN HEALTHY MALE VOLUNTEERS

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Abstract

Background: This present bioequivalence study was designed to determine the pharmacokinetic, bioavailability and bioequivalence of Artemether (80mg tablets in comparison with COARTEM™ Artemether (4x20mg) tablets after single dose administration under fed conditions in healthy adult male subjects. Therefore the design of an open label, balanced, randomized, two-sequence, single dose, two way crossover study with a wash-out period of at least 7 days was used.

Methods: An open-labeled, balanced, single-dose with food, two-treatment, two-period, two-sequence, randomized crossover study was conducted in 12 healthy male volunteers. Each volunteer received 80mg Artemether tablet of the reference or test drug respectively. On the day of dosing, blood samples were collected before dosing and at various time points up to 216 hours after dosing. Analysis of Artemether concentrations was performed using a validated liquid chromatography with tandem mass spectrometry (LC-MS/MS) method. The pharmacokinetic parameters including C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, $t_{1/2}$ and K_{el} were analyzed using the non-compartmental model. Drug safety and tolerability were assessed.

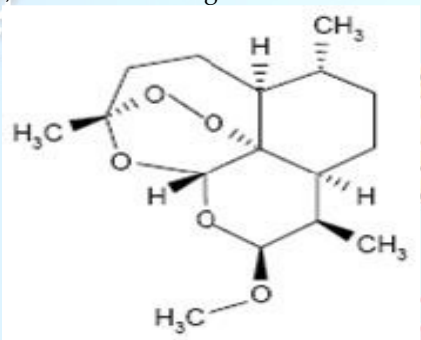
Results: The pharmacokinetic parameters including C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, $t_{1/2}$ and K_{el} were analyzed using the non-compartmental model. Drug safety and tolerability were assessed. The primary pharmacokinetic parameters (C_{max}, AUC_{0-t}, AUC_{0-inf}) 90%CI were within the 80 to 125% interval required for bioequivalence as stipulated in the current regulations of the USFDA acceptance criteria. The geometric mean ratios (Test/Reference) between the two products of 80mg tablets under fed condition 105.7% (81.23%-110.58%) for Cmax ratios, 91.0% (90.43%-108.32%) for AUC0-t ratios, 84.8% (80.45%-104.98%) for AUC0-inf ratios of Artemether, respectively. 12 volunteers had completed both treatment periods. There was no significant difference of the T_{max} parameter between the two formulations ($p > 0.05$). No serious adverse events related to the study drugs were found.

Conclusion: This single dose study found that the test formulation Artemether 80mg tablets are bioequivalent to the reference formulation COARTEM™ Artemether (4x(20mg) tablets in terms of extent and rate of absorption, under fed condition in healthy adult male volunteers according to the USFDA regulatory guidance.

Keywords: Artemether, Bioavailability, Bioequivalence, Intrasubject Variability

INTRODUCTION

Co-Artemether is an oral fixed-dose combination tablet of artemether [a derivative of artemisinin] and lumefantrine, an antimalarial synthesized and developed by the academy of military medical sciences in Beijing, People's Republic of China[1-4]. This combination was registered in China in 1992 for the treatment of plasmodium falciparum malaria, and has been subsequently further developed by Novartis Pharmaceuticals [5-8]. This combination has proved very well tolerated and highly efficacious in children and adults[9-12], even against multi-drug resistant strains of Plasmodium falciparum[13-14]. The chemical name of artemether is [3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR]-decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4, 3-j]-1, 2-benzodioxepine. It has the empirical formula $C_{16}H_{26}O_5$ with a molecular weight of 298.4 g/mol, and the following structural formula:



Artemether is a white, crystalline powder that is freely soluble in acetone, soluble in methanol and ethanol, and practically insoluble in water. Lumefantrine is a yellow, crystalline powder that is freely soluble in N, N-dimethylformamide, chloroform, and ethyl acetate; soluble in dichloromethane; slightly soluble in ethanol and methanol; and insoluble in water. Artemether is active against the erythrocytic stages of plasmodium falciparum [16-18]. Strains of P. falciparum with a moderate decrease in susceptibility to artemether or lumefantrine alone can be selected in vitro or in vivo, but not maintained in the case of artemether. Artemether is rapidly metabolized into an active dihydroartemisinin [19-23]. The anti-malarial activity of artemether and dihydroartemisinin [DHA] has been attributed to endoperoxide moiety. Artemether is absorbed with peak plasma concentrations reached about two hrs after oral drug administration. Artemether highly bound to human serum proteins in vitro [95.4% and 99.7%, respectively]. Dihydroartemisinin is also bound to human serum proteins [47% to 76%]. Protein binding to human plasma proteins is linear. The metabolism of artemether was catalyzed predominantly by CYP3A4/5. Dihydroartemisinin [DHA] is an active of artemether. The metabolism of artemether was also catalyzed to a lesser extent by CYP2B6, CYP2C9 and CYP2C19.Artemether and DHA are cleared from plasma with an elimination half-life of about two hrs.

The rationale of this present bioequivalence study for two formulations of Artemether tablets was examined between generic drug Artemether 80mg tablets as the test product and COARTEM™ Artemether (4x(20mg) as the reference product. This bioequivalence study could give assurance when prescribing less expensive generic drugs as alternatives with similar efficacy and safety.

The study objectives of this present study are to assess the single dose bioequivalence of Artemether (80mg) tablets with COARTEM™ in healthy, adult, human study participants under fed conditions and to monitor the clinical status, adverse events, laboratory investigations and assess relative safety and tolerance of Artemether formulations under fed conditions.

MATERIALS AND METHODS

According to the USFDA Regulatory individual product recommendations, two studies (Fed and Fasting) to be done with Artemether (80mg) tablets to obtain marketing authorization in USA.

Study drugs

Artemether (80mg) tablets and COARTEM™ from Novartis were used as the test and the reference products respectively. Both products were prepared as Artemether tablets equivalent to Artemether (80mg). Both the products were stored at controlled room temperature 25°C (77 °F).

Study population

The study protocol was approved by the Ethics Committee. In addition, the protocol was performed in accordance with the Declaration of Helsinki Principles [24] as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP) [25]. All subjects were given a detailed description of the study and written informed consent was ob-

tained prior to the enrollment.

The sample size was estimated based on, Coefficient of variation (C.V.) of the drug, sufficient statistical power to detect 20% difference with the power of 0.8 in C_{max} and AUC between the test and reference product, Regulatory requirements.

Sample size was based on estimates obtained from reported literature and previous studies. Assuming a formulation ratio (T/R) ranging from 0.95-1.05 a sample of 12 subjects including dropouts would be sufficient to show bioequivalence between the two formulations with a power of at least 80%. Hence sample size of 12 subjects was enrolled in the study.

12 healthy male volunteers between the ages of 18-45 years with a body mass index between 18.5 kg/m^2 and 24.9 kg/m^2, with body weight equal to or not less than 50 kg were assessed to be in good physical condition by a complete medical screening including a medical history, physical examination and laboratory screening test for hematologic and blood biochemistry parameters. Subjects with a history of hypersensitivity to any ingredients in the Artemether/Lumefantrine products and/or related drugs or its constituents or who were taking any medication or alcohol for a 21-day period prior to the study were excluded. Subjects who had a history of cardiovascular, hepatic, renal, gastrointestinal or hematologic disease were excluded from the study.

Study design

The study was an open-labeled, single-dose, study taken with food, two-treatment, two-period, two-sequence randomized two way crossover with at least one week washout period. Subjects were randomly allocated to two groups by the sequence of product administered [Test-Reference (TR) and Reference-Test (RT) group]. In each period, 1x(80mg) tablet of Artemether of the test or 4x(20mg) tablets of Artemether of reference product was administered 30 minutes after starting a high fat, high calorie breakfast at the same time in the morning before dosing. Subjects were housed 12 hours prior to dosing in the clinical facility from a time adequate to ensure 10 hours supervised fasting before consuming high fat breakfast and were allowed to leave the facility after 24.00 hours post-dose sample in each period. The subjects received a standard meal at about 4.0, 9.0 and 13.0 hours after dosing in each period. During housing, all meal plans were identical for all the periods. Drinking water was not allowed from one hour before dosing till one hour post-dose (except for 240 ± 02 mL of drinking water given for dosing). Before and after that, drinking water was allowed at *ad libitum*. After a minimum of 1 week washout period, the subjects were crossed over to the next treatment following the same procedure as conducted in the 1st period.

Sample collection

During dosing day in each period, 26 blood samples will be collected as per the following schedule:

Pre dose sample(0.00 hr) within 02 hrs prior to drug administration and the others at 0.25, 0.5, 0.75,1.0, 1.25, 1.50, 1.75, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 18.0, 20.0, 24.0, 48.0, 72.0, 96.0, 120.0, 144.0, 168.0, 192.0, 216.0 hr post dose. The total volume collected per study participant in this study will not exceed approximately 321 mL including up to 9 mL for screening, and 7-9 mL for post clinical assessment of lab parameters and 18 mL for discarded blood sample resulting from use of intravenous cannula for 12 hours and 2-9 mL was collected for repeat/additional lab tests, if required. For separating plasma, all blood samples were centrifuged at 3800 RPM for 10 minutes at 4°C ± 2°C.

Centrifugation of all samples was done as early as possible after each sample draw time point. After centrifugation, plasma samples were aliquoted into two sets in properly labeled polypropylene tubes and immediately stored at about -60°C or colder.

Artemether analysis by LC-MS/MS [26,27,28,32-46]

The published LC-MS/MS methods [26-27] were validated according to USFDA regulations [28] for quantification of Artemether from extracted subject plasma samples.

Pharmacokinetic and statistical analysis [29-31]

For the purpose of Average Bioequivalence analysis C_{max}, AUC_{0-t} and AUC_{0-inf} were considered as the primary variables and T_{max}, $t_{1/2}$ and K_{el} were considered as the secondary variables. General

Linear Model for analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for a *p*-value equal to or less than 0.05. 90% confidence interval (CI) for the ratios of geometric mean Test/Reference (T/R) for C_{max}, AUC_{0-t} and AUC_{0-inf} was calculated based on least squares means from the ANOVA of log-transformed data.

The 90% geometric CI of the ratio (T/R) of least squares means from the ANOVA of the log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} should be within 80.00% to 125.00%.

Tolerability assessment

Physical examination and measurement of vital signs (Blood Pressure, Pulse Rate and Oral Temperature) were examined at the time of Check-in, prior to administration of the each study drug (0.00 hr), 1.00, 3.00, 6.00, 12.00, 24.00, 48.0, 72.0, 96.0, 120.0, 144.0, 168.0, 192.0, 216.0hr post dose and during the entire study period. Adverse events were monitored throughout the study and recorded by physicians.

RESULTS

Study population

12 healthy male adults eligible for the study enrollment were randomly divided into 2 groups [Test-Reference (TR) and Reference-Test (RT)] according to the sequence of drug administration. All the subjects had completed both the periods. Thus, this study was balanced in each sequence and the results from 12 volunteers were used for pharmacokinetic and statistical analysis. Table 1 demonstrates the demographic characteristics of the volunteers.

Table 1: Demographic characteristics

Category		Treatment		TOTAL
		Test (T)	Reference (R)	
Age (years)	***Mean ± SD***	22.114± 6.10	22.84 ± 6.00	22.34 ± 6.05
	Range	18.0 – 37.0	19.0 – 37.0	18.0 – 37.0
	Median	22.0	23.0	23.0
	N	12	12	24
Age Groups	< 18	00	00	00
	18 – 40	12	12	24
	41 – 64	00	00	00
	65 – 75	00	00	00
	> 75	00	00	00
Gender	Female	00	00	00
	Male	12	12	24
Race	American	00	00	00
	Hispanic	00	00	00
	Caucasian	00	00	00
	Asian	12	12	24
Height (cm)	Mean ± SD	173.55 ± 5.69	174.28 ± 5.67	174.48 ± 5.67
	Range	157.0 – 174.0	159.0 – 177.0	157.0 – 177.0
	N	12	12	24
Weight (kg)	Mean ± SD	58.96 ± 6.24	61.56 ± 6.43	60.26 ± 6.41
	Range	52.0 – 70.0	52.0 – 77.0	52.0 – 77.0
	N	12	12	24
BMI (kg/m^2)	Mean ± SD	21.86 ± 1.46	22.10 ± 1.79	21.98 ± 1.62
	Range	20.1 – 24.8	20.0 – 24.9	20.0 – 24.9
	N	12	12	24

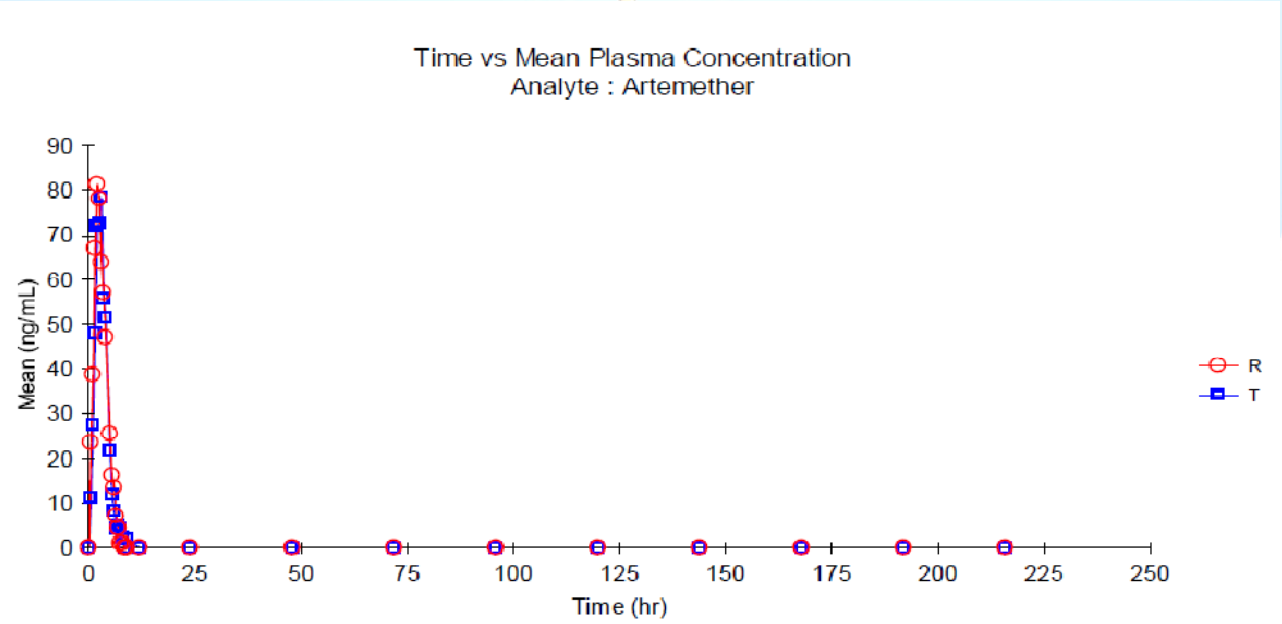
Table 2: Pharmacokinetic Parameters of Artemether for Both Formulations

PK Parameters	Artemether	
	Test	Reference
C_{max} [ng/mL]	99.313	93.935
T_{max} [H]	2.417	2.167
AUC_{0-t} [ng.h/mL]	251.463	276.376
AUC_{0-inf} [ng.h/mL]	281.736	332.363
$T_{1/2}$ [H]	1.407	1.749
K_{el} [H^{-1}]	0.68	0.512

Table 3: Bioequivalence Parameters for Artemether and Lumefantrine

Parameter	Artemether		
	C_{max}	AUC_t	AUC_{inf}
90% CI Lower Limit	81.23	90.43	80.45
90% CI Upper Limit	110.58	108.32	104.98
T/R Ratio (%)	105.7	91.0	84.8
Power	1	0.93	0.99
Intra Subject Variability	8.34	2.5	6.5
Inter Subject Variability	22.44	34.23	31.23
ANOVA (p-Value)			
Sequence	0.2	0.2	0.2
Period	0.7	0.5	0.8
Treatment	0.4	0.4	0.2

Fig 2: Time vs. Mean Plasma Concentration Graph of Artemether



Bioanalysis and pharmacokinetics [26,27,28,32-46]

Chromatograms were acquired on a TSQ tandem mass spectrometry (Thermo Finnigan, Sanjose, CA, USA) equipped with Electrospray ionization (ESI) and connected to a PC runs with the standard software Xcalibur 2.0.7 and LC Quan 2.5.6. Mass spectroscopic detection was performed on a Triple quadrapole instrument (Thermo, TSQ Quantum Discovery Max. The calibration curve was con-

structed by weighted $1/x^2$ least-square linear regression analysis of the peak area ratio (drug/ISTD) vs. the concentration of drug. The method is sensitive enough in the range of 10 to 800 ng/mL for Artemether.

Bioequivalence analysis

Ninety percent confidence interval of geometric mean ratios of bioavailability parameters between the test and reference formulation are presented in Table 3. The statistical analysis obtained from this study showed that the point estimate (90% CI) of the geometric mean ratio (GMR) (T/R) of C_{max}, AUC_{0-t} and AUC_{0-inf} was entirely within the equivalence criteria (80.00-125.00%) which was 105.7% (81.23%-110.58%) for Cmax ratios, 91.0% (90.43%-108.32%) for AUC0-t ratios , 84.8% (80.45%-104.98%) for AUC0-inf ratios of Artemether, respectively.

In addition, no significant difference of the T_{max} parameter between the two studied formulations was observed ($p > 0.05$). Therefore, it was concluded that the two formulations of Artemether were bioequivalent in terms of rate and extent of absorption for the drug. The mean plasma concentration vs time profiles were given in Fig 2.

Tolerability

Almost all volunteers taking both formulations were noted for mild adverse events. Most common events were drowsiness, nausea and loss of appetite. However, no subject had any severe adverse event or withdrew from the study because of an adverse event.

DISCUSSION

An open-labeled, single-dose with food, two-treatment, two-period, two-sequence randomized two way crossover design in 12 healthy adult volunteers was considered appropriate and standard for bioequivalence evaluation of the generic and the reference products. The study simulates real life conditions including the influence of meals as well as circadian effects on the performance of the product. For a safety reason, co-administration of the drug with food can reduce nausea, a common side effect of Artemether.

In general, the pharmacokinetic parameters for both formulations were similar to the pharmacokinetic parameters of Artemether in previous published data. This study demonstrated that 90% CI of the logarithmic transformed of parameters C_{max}, AUC_{0-t} and AUC_{0-inf} were contained in 80.00-125.00%. In addition, no significant differences of the T_{max} values between the two formulations were observed ($p > 0.05$). Therefore, the two formulations of Artemether are considered bioequivalent in terms of the rate and extent of absorption. Moreover, both formulations were well tolerated. Hence, the test and reference (COARTEM™) formulations of Artemether (80mg) are bioequivalent.

CONCLUSION

This single dose study found that the test formulation Artemether (80mg) tablets is bioequivalent to the reference formulation COARTEM™ Artemether [4*(20mg)] tablets in terms of the extent and the rate of absorption, under fed condition in healthy adult male volunteers according to the USFDA regulatory guidance.

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