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Bioequivalence Study of Zidovudine 100 Mg Capsule with Two-Way Crossover Design

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Research Article

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Abstract

Zidovudine is a synthetic nucleoside analogue, specifically a nucleoside reverse transcriptase inhibitor (NRTI), that inhibits the replication of retroviruses, including HIV.

Study Objective: This study objective was to determine the bioequivalence of Zidovudine 100 mg capsules produced by PT Kimia Farma Tbk compared to Retrovir 100 mg capsules produced by GlaxoSmithKline Pharmaceuticals S.A, in healthy subjects

Methods: The study was conducted in a randomized, single-dose, open-label, two-way crossover design (2 treatments, 2 periods, and 2 sequences) under fasting state with 7 (seven) days washed-out period between each period. The number of subjects who participated and completed the study were 31 of 32 adult male and female. One subject dropped out from 2nd period because of personal reason. The subjects received an explanation of the study and signed informed consent. Subjects fasted for a minimum of 8 hours before receiving the test drug and reference drug. Blood samples were collected 15 times at the following time points: 0 hours (before drug administration), 0.16, 0.33, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 hours after drug administration. Plasma concentrations of the drug were determined by LC-MS/MS method. The pharmacokinetic parameters calculated in this study are AUC0-t, AUC0-inf, Cmax, tmax, and t½, while the statistical interval proposed was 80.00 - 125.00% for AUC0-t and Cmax with 90% Confidence Interval (CI) with $\alpha = 5.00\%$.

Results: The main pharmacokinetic parameters of the test drug Zidovudine (BN: G2 1346 J) compared to reference drug, Retrovir (BN: JN8K) were calculated based on geometric mean ratio and 90% confidence interval (CI). The results for AUC0-t and Cmax were 101.65% (96.77- 106.83%) and 95.63% (84.09-108.76%) respectively, with intra-subject variability (%CV) for AUC0-t and Cmax were 11.49% and 30.47%. Hence, the number of 31 subjects has adequate number for required power of study.

Conclusion: Based on the AUC0-t and Cmax values, Zidovudine 100 mg Capsules Produced by PT. Kimia Farma Tbk is bioequivalent to Retrovir 100 mg Capsules Produced by GlaxoSmithKline Pharmaceuticals S.A.

Keywords: Zidovudine; AUC; Bioequivalence; Comparator; Crossover

Abbreviations: ANOVA: Analysis of Variance; AUC: Area Under Curve; BMI: Body Mass Index; CI: Confidence Interval; Cmax: Maximum Plasma Concentration; CRF: Case Report Form; CV: Coefficient of Variation; EMA: European Medicines Agency; ICH: International Conference on Harmonization; LCMS/MS: Liquid Chromatography Tandem with Mass

Spectrometry; LLOQ: Lower Limit of Quantification; Tmax: Time to Reach Maximum Plasma Concentration.

Introduction

Zidovudine is a synthetic nucleoside analogue, specifically a nucleoside reverse transcriptase inhibitor (NRTI), that inhibits the replication of retroviruses, including HIV. Intracellularly, Zidovudine is phosphorylated into its active metabolite, 5′-triphosphate and zidovudine triphosphate (ZDV-TP). The level of phosphorylation varies depending on the cell type. ZDV- TP inhibits the activity of HIV by inhibiting the reverse transcriptase (RT) enzyme through the terminal DNA chain after separation with a nucleoside analogue. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage that is important for DNA chain elongation, and thus the growth of the DNA virus is halted. ZDP-TP is a weak inhibitor of α-DNA polymerase and mitochondrial γ-polymerase [1-3].

- Absorption After oral administration, Zidovudine is rapidly absorbed from the GI tract. Absorption is delayed by food. Bioavailability: Approximately 64%. Peak plasma concentration is reached within 0.5-1.5 hours.
- Distribution Zidovudine is distributed to CSF, crosses the blood-brain barrier and placenta, enters breast milk, and is found in semen. Its volume of distribution is 1-2.2 L/kg. Its plasma protein binding is 34-38%.
- Metabolism Zidovudine is metabolized intracellularly into active metabolite 5′-triphosphate and undergoes metabolism in the liver, mainly into inactive glucuronide.
- Excretion Zidovudine is excreted in the urine as both an unchanged drug and antimetabolites. The plasma half-life is around 0.5-3 hours [4-6].
- Adverse Event The side effects of Zidovudine include headache, malaise, anorexia, nausea, vomiting [4].
- This study objective to determine the bioequivalence of Zidovudine 100 mg capsules produced by PT Kimia Farma Tbk compared to Retrovir 100 mg capsules produced by GlaxoSmithKline Pharmaceuticals S.A., in healthy subjects.

Study Protocol

The study protocol was reviewed and approved by the Ethics Committee of the Medical Faculty University of Indonesia and the Indonesian Food and Drug Regulatory Authority [7]. The protocol described all details of the project, including design of the study, clinical procedures, bioanalysis of blood samples obtained from the participants, pharmacokinetic and statistical data analysis, bioequivalence evaluation, the informed consent form, and complete documentation and final report issuance.

Ethical Considerations

The protocol and informed consent have been reviewed by an Ethics Committee of the Medical Faculty University of Indonesia which was certified by the Forum for Ethical Review Committee in the Asia and Western Pacific Region (FERCAP). The principal investigator for the study must communicate with the ethics committee regarding any modifications to the protocol, including the conduct, benefits, or risks of the study. Protocol amendments will be made if there are changes to the objectives, study design, sample size, study procedures, or other aspects that alter the study. Amendments has been reviewed by the Ethics Committee and BPOM for approval.

Each subject was given the informed consent form during the screening phase prior to the study. A meeting was arranged by the principal investigator and clinical investigators to explain all details of the study to the subject, including the purposes, risks, advantages, procedures, and the right as a study subject to withdraw at any time during the study, and the compensation in case of any harm caused by the study.

Study Design

The study was conducted in a randomized, single- dose, open-label, two-way crossover design (2 treatments, 2 periods, and 2 sequences) under fasting state with 7 (seven) days washed-out period between each period [8-10]. The subjects were randomly assigned to each dosing sequence of the investigational drug products (test and reference drug). This study was carried out on 31 of 32 adult male and female. One subject dropped out from period 2 (two) because of personal reason.

Inclusion and Exclusion Criteria for Participation in the Study

The subjects were regarded as eligible for participation in this study based on the following inclusion criteria: willing to sign an informed consent; Healthy based on clinical laboratory tests (routine haematology, liver function, kidney function, blood glucose, urinalysis, hepatitis B (HBsAg), hepatitis C (Anti- HCV) and HIV (Anti-HIV), medical history, and physical examination); Male and female subjects (if female, consider the risks for women of childbearing age and perform pregnancy tests); Age between 18-55 years; Normal weight range according to Body Mass Index (BMI) 18-25 kg/m^2); Vital signs within the following ranges: systolic blood pressure 110-129 mmHg, diastolic blood pressure 70-84 mmHg, normal pulse rate 60-90bpm, oxygen saturation (SpO_2) in the normal range of 95- 100%, and normal respiratory rate of 12-20/min.

The exclusion criteria for this study including: Smoking more than 10 cigarettes per day; Pregnant or breastfeeding women (Pregnancy tests was performed during screening and prior to the administration of the investigational or comparator drug); History of kidney or liver disease, or history of allergy, hypersensitivity or contraindication to the investigational bioequivalence drug (Zidovudine); Clinically significant haematological abnormalities; Abnormal electrocardiogram (ECG); Difficulty accessing veins in the left or right arm; History of significant ongoing clinically or medically significant chronic or acute illness; History of drug or alcohol abuse within the past 12 months (1 year) prior to screening for this study; Positive serology test results for Hepatitis B (HBsAg), Hepatitis C (anti-HCV), HIV (anti-HIV). Positive rapid antigen test results for SARS-CoV-2 (if the BE study is conducted during a pandemic); Have history or condition that can affect drug kinetics; Use of drugs or dietary supplements no more than 7 days since the start of the study; participated in previous clinical trials no more than 3 months from the start of the study; and Blood donation or blood loss of more than 300 ml within 3 months from the start of the study.

Health Screening

Health screening is conducted prior to the study to evaluate the subject's health condition based on inclusion and exclusion criteria. Subjects were through medical examination within 7 days prior to their first study drug administration day. These include assessment of physical examination, vital signs (i.e., blood pressure, pulse rate, and body temperature), and ECG was conducted by Responsible Physician in Equitrust Lab. Laboratory values of routine haematology, liver function, kidney function, blood glucose, and urinalysis were tested by Clinical Laboratory. Immunology test for HBsAg, HCV, HIV, and Rapid Test Antigen Covid-19 was conducted in Equitrust Lab. During the screening and immunology test (HBsAg, HCV, and HIV) approximately 10 mL of blood samples were drawn from each subject.

Drug Product Administration

This study consists of two periods during 10 hours for each period. At the specified time, the subjects will be asked to come to the Equitrust Lab on a day before drug administration for quarantine. The subjects are requested to report to the investigator regarding any health disturbances experienced and the medication or food supplements taken since their last arrival.

The subjects must fast for 8 hours before taking the investigational drug or reference drug. About 1 hour before taking the drug, the condition of the subjects is checked by a doctor to assess their health (blood pressure, pulse rate, body temperature, oxygen saturation (SpO_2) examination, and respiratory rate). The results of the examination are recorded in the Case Report Form (CRF).

Starting at 7a.m, on the first sampling day, the subjects are given the investigational drug (1 capsule of 100 mg Zidovudine) or the reference drug (1 capsule of 100 mg Retrovir) with 220 mL of water while sitting according to randomization. The subjects are asked to maintain an upright position, either standing or sitting, for 1 hour after drug administration.

After 2 hours, water was provided. No food was allowed until 4 hours after drug administration. Standard meals were reserved for 4 hours (lunch), 8 hours (snack), and 12 hours (dinner) after study drug administration. Subjects were remaining in a sitting position until 4 hours period after drug administration. Subjects were not allowed to exit the clinical facility.

Blood Samples Collection

Blood samples were taken at specific time points to represent the drug absorption, distribution, and elimination phases. Most drugs require 12-18 blood samples, including 1 sample before dosing, 2-3 samples before Cmax, 4-6 samples around Cmax, and 5-8 samples after Cmax [6]. In the Zidovudine test, blood samples were taken for 10 hours. A pre-dose blood sample was taken at 6.30 a.m within 30-minutes prior to drug administration and a 5 mL of blood was collected through venipuncture using a syringe at the following time points: 0 hours (before drug administration); at 0.16, 0.33, 0.50, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 10 hours after drug administration (15 points). Every deviation exceeding the predetermined time is recorded in the Case Report Form (CRF) as a protocol deviation.

The blood samples are collected via venipuncture using a syringe and transferred into CPDA blood collection tubes. Plasma is centrifuged at 3000 rpm for 10 minutes and immediately transferred plasma into clean microtubes 2.0 mL. Plasma separation was carried out in preparation room at room temperature. Then, the plasma is stored in the freezer at a maximum temperature of -20 ºC until Zidovudine analysis is performed. The total amount of blood collected from each subject was 165 mL (including 15 mL for initial health screening).

Statistical Analysis

Pharmacokinetic parameters, including Cmax, AUC0-t, AUC0-inf, tmax, and t1/2, were calculated for each subject and each period using the Ms. Excel program. Bioequivalence

between the test and reference products was determined based on the average ratios of Cmax and AUC0-t with a 90% Confidence Interval (90% CI) of the log or ln-transformed data. The log or ln-transformed values of Cmax and AUC0-t for the two products are analysed using a two-way Analysis of Variance (ANOVA) and R program. The compared factors were the drug products (Test and Reference), drug administration period (I and II), subject, and sequence (TR and RT). The mean differences in Cmax and AUC0-t between the test and reference products were considered bioequivalent if the ratio of the geometric mean (AUC)T/(AUC)R= 1.00 with 90% CI= 80.00-125.00% (α: 0.05) and (Cmax)T/(Cmax)R= 1.00 with 90% CI= 80.00-125.00% (α: 0.05). The study had a power of 80% with a significance level (alpha) of 5% (two-tailed) [7,8,11].

The 90% Confidence Interval (90% CI) is calculated using the following equation:
(90% CI) diff = Difference ± t0.1(n-2) × SE diff Difference : the mean ln T - the mean ln R n : the number of subjects
α: 0.05

$$SEdiff=[(\frac{1}{2}Mresidual\times(\frac{1}{nRT}))\frac{1}{2}]$$

The 90% CI for the ratio is calculated as follows: (90% CI) ratio = antilog (90% CI) diff × 100%

Assay Methodology and Validation

Prior to the assay of Zidovudine in the sample, bioanalytical method validation was evaluated for selectivity; carry-over effect; calibration curve and Lower Limit of Quantification (LLOQ); precision and accuracy; matrix effect; dilution integrity; and stabilities (i.e., freeze- thawed stability, short-term stability at room temperature, post preparative stability / autosampler batch integrity, stock solution and internal standard stability, and long-term stability). An assay of Zidovudine concentration in plasma was carried out by a fully validated LCMS/MS with LLOQ 10.00 ng/mL. The anticoagulant used during the validation and bioanalytical phase was CPDA (Citrate Phosphate Dextrose Adenine).

Data Quality Assurance

During the bioanalytical phase of the plasma samples, the analysis was monitored by the quality control process include system suitability test, linearity of calibration curve, and quality control samples (Low QC, Medium QC, and High QC) referred to requirement described in European Medicinal Agency (EMA) guideline 2011 [12].

The calibration curve will be conducted with eight concentrations at each batch. The acceptance criteria are %LLOQ deviation + 20.00% and %other concentration deviation + 15.00% with a minimum of 75% of the calibration curve with at least 6 concentration levels must meet these criteria.

QC Samples will be conducted with three concentrations at each batch. The acceptance criteria are %deviation value and RSD of QC samples must be <15% with a minimum of 67% of the total QC samples and a minimum of 50% at each concentration level must meet these criteria.

The investigator has conducted the study in accordance with the protocol requirements, Good Clinical Practice (GCP), and Good Laboratory Practice (GLP) set by the National Agency of Drug and Food Control. The Quality Assurance (QA) department is responsible for conducting periodic audits of the BA/BE study to ensure the integrity of the study, both internally and externally. The QA department keeps written records and signs audit notes, findings, and issues related to the study. Any research issues that arise will be evaluated or audited to ensure the integrity of the study data that will be conveyed to the investigator, and the evaluation results can be used for handling according to the problems faced. A representative from the sponsor's QA will ensure that there are no deviations from the approved protocol or standard operating procedures made in the study.

Result and Discussion

The total number of subjects who participated the study was 32 subjects (21 males and 11 females), where S18 dropped out of the study in the 2nd period because of personal reasons. The demographic data of the subject are tabulated in Table 1.

	MIN	MAX
Age (Year)	23	49
Body Weight (kg)	45	70
Body Height (m)	148	175
BMI (kg/m^2)	18.3	24.7

Table 1: Demographic Data of 32 subjects.

There was one adverse event during this bioequivalence study, i.e. dyspepsia. All of these adverse events were recorded in the CRF and the deviation during the study is reported in protocol deviation point at full study report.

Bioanalytical Result

Total plasma sample from 32 subjects is 945 samples (31 individuals x 15-time points x 2 periods and 1 individual x 15-time points x 1 period). The samples were stored in a freezer at a maximum temperature of - 20ºC until

analysis. The analysis started on Saturday, July 15th, 2023, and was completed on Tuesday, July 25th, 2023. Sample analysis was performed using a validated analytical method which complied to the Guideline on Bioanalytical Method Validation, EMEA 2011. The bioanalysis result of system suitability test, linearity of calibration curve, and quality control samples (Low QC, Medium QC, and High QC) was met the requirements.

Pharmacokinetic Analysis

The pharmacokinetic parameters (AUC0-t, AUC0- inf, Cmax, t½, and tmax) of the test drug and comparator drug were calculated and compared to assessed bioequivalence.

The calculated 90% CI with a = 5.00% for geometric mean of individual and the ratios of AUC0-inf and AUC0-t as well as Cmax for the test drug Zidovudine (BN: G2 1346 J) and reference drug Retrovir (BN: JN8K) were all within 80.00 - 125.00% interval. This was in conformity with the standard guideline for bioequivalence study [3,7].

The Summary of pharmacokinetic parameters of the study shown in Table 2. Meanwhile, the main statistical calculations for AUC0-t and Cmax parameter of study Zidovudine was obtained from 31 subjects after oral administration of the test drug and reference drug shown in Table 3. The means of plasma concentration vs. time profiles after a single dose of oral administration of investigational products are shown in Figure 1.

Parameter	Test Drug		Comparator drug	
	Arithmetic Mean	Standard Deviation	Arithmetic Mean	Standard Deviation
AUC0-t (ng.h.mL-1)	1079.35	367.87	1047.66	316.63
AUC0-inf (ng.h.mL-1)	1099.53	370.12	1068.47	319.67
Cmax (ng.mL-1)	862.99	380.21	879.49	324.66
t½ (h)	0.96	0.11	0.94	0.16
tmax (h)*	0.48 (0.33-1.00)		0.50 (0.33-1.00)	

*mean (range)

Table 2: The Summary of pharmacokinetic parameters.

Parameter	% Ratio of Geometric Means (T/R)	90% Confidence Interval (T/R)		% CV	Power (%)
		Lower Limit	Upper Limit		
AUC0-t	101.68	96.77	106.83	11.5	99
Cmax	95.63	84.09	108.76	30.5	99

Bioequivalence criteria (90%CI): 80.00 – 125.00 %

Table 3: Statistical calculations for AUC0-t and Cmax parameter of Zidovudine after a single-dose Oral Administration of Test and Reference Drug.

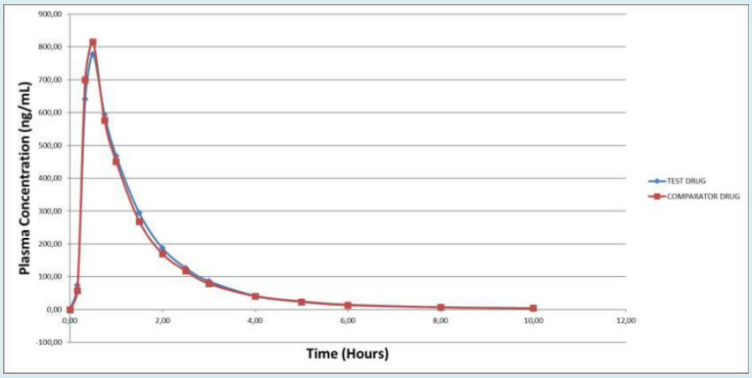

Figure 1: Geometric Means of Plasma Concentration vs Time Profiles After Dosing of Test Drug (t): Zidovudine and Reference Drug (R): Retrovir.

Conclusion

This study aims to determine the bioequivalence of Zidovudine 100 mg capsules produced by PT Kimia Farma Tbk compared to Retrovir 100 mg capsules produced by GlaxoSmithKline Pharmaceuticals S.A, in healthy subjects.

The study was conducted in a randomized, single- dose, open-label, two-way crossover design (2 treatments, 2 periods, and 2 sequences) under fasting state with 7 (seven) days washed-out period between each period. The number of subjects who participated and completed the study were 31 of 32 adult male and female. One subject dropped out from 2nd period because of personal reason. The subjects received an explanation of the study and signed informed consent. The primary parameters to assess the bioequivalence between the test and reference drug of Zidovudine are AUC0-t and Cmax. Based on the bioequivalence criteria of a 90% confidence interval, the ratio of the geometric mean values (AUC)T/(AUC)R=1.00 with 90% CI=(80-125)% (α: 0.05) and (Cmax)T/(Cmax)R=1.00 with 90% CI=(80-125)% (α: 0.05) were used.

The geometric mean ratios (90% confidence intervals) of the test drug/ comparator drug for Zidovudine were 101.65% (96.77- 106.83%) for AUC0-t and 95.63% (84.09-108.76%) for Cmax. Based on the AUC0- t and Cmax values, Zidovudine 100 mg capsules produced by PT Kimia Farma Tbk is bioequivalent to Retrovir 100 mg capsules produced by GlaxoSmithKline Pharmaceuticals S.A. In this study, the intra-subject coefficient of variation (%CV) obtained from ANOVA was 11.49% for AUC0-t and 30.47% for Cmax.

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