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ORIGINAL ARTICLE

Efficacy of Deferasirox through Bioequivalence Study in Indonesian Healthy Volunteer

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ABSTRACT

Background: Deferasirox is an orally bioavailable synthetic, tridentate iron chelator that binds iron at a 2:1 ratio. The generic brand for this drug is still not available in Indonesia. We aimed to compare the efficacy between the generic drug and its innovator (Exjade®) by a study of bioequivalence in Indonesia among healthy volunteers.
Methods: An open-label, single-dose, two-sequence, randomized two-way crossover study with one-week wash-out period was evaluated in 28 enrolled volunteers. Blood samples were collected up to 60 hours following drug administration. Deferasirox level was determined by HPLC method with ultraviolet detector. The pharmacokinetic parameters tested for bioequivalence assessment were AUC_{0-t}, $AUC_{0-\infty}$, and C_{max}.
Results: The 90% confidence intervals obtained by analysis of variance for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were 83.04%-95.53%, 83.18%-98.88%, and 81.67%-105.47%, respectively. These results were all within the range of 80.00-125.00%.
Conclusion: Our results indicated that a single dose of 500 mg Deferasirox tablet demonstrated similar bioequivalence in terms of rate and extent of absorpstion to single dose of Exjade® 500 mg tablet.

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Introduction

Thalassemia is an inherited anemia characterized by defects in synthesis of hemoglobin.[1] Ineffective erythropoiesis and hemolysis in thalassemia major causes a lethal type of transfusion dependent anemia in the patients.[2] Regular blood cell transfusion will eliminate the complications of anemia; however, will lead to iron overload, which has serious clinical sequelae. Iron overload leads to deposition of iron and subsequent dysfunction in organs such as liver, heart, and endocrine glands.[2-4] Iron chelation can be used to promote the excretion of excess iron in body.[5]

There are three classes of iron chelators: Deferoxamine (hexadentate), Deferiprone (bidentate), and Deferasirox (tridentate).[1] Deferoxamine was the first iron chelator used in beta thalassemia major, but due its route of administration (subcutaneously) is associated with a low compliance. Deferiprone and deferasirox are oral iron chelators which are obviously associated with increased patients' compliance.[1, 5]

The once daily dosing of deferasirox is expected to increase treatment adherence of the patients.[6] Deferasirox or 4-(3,5-bis[2-hydroxyphenyl]-1H-1,2,4-triazol-1-yl)-benzoic acid has a good oral bioavailability (reaching 70%) with a low molecular weight of 373 g/mol and is absorbed by the gastrointestinal tract.[1, 7]

Exjade® is the innovator drug of deferasirox. However, the innovator drug usually has a higher cost than generic one. The development of the generic product has become a trend due to the presumed low cost compared with the

innovator product.[8] The quality of generic drugs should be more strictly controlled and need post marketing study in many cases.

Because of the importance of the generic drugs in health care, the establishment of their efficacy through bioequivalence studies is fundamental in successful application of the generic drugs.[9]

The objective of this study was to compare the efficacy between the generic drug of deferasirox and its innovator (Exjade®) by a bioequivalence study in healthy volunteers in Indonesia.

Materials and methods

The protocol study was reviewed by the Committee of the Medical Research Ethics of the Faculty of Medicine, University of Indonesia (Jakarta, Indonesia) and was approved by the National Agency of Drug and Food Control (Jakarta, Indonesia). This study was conducted in compliance with the ethical principles of the Declaration of Helsinki for biomedical research involving human subjects and Good Clinical Practice (GCP). All participants signed a written informed consent after they had been informed of the nature and details of the study in accordance with Indonesian Guidelines for Bioequivalence Studies.

Study Design

This study was an open-label, randomized, single-dose, two-sequence, two-period, two-way crossover design under fasting period with one-week wash-out period between two treatment periods. The treatment periods should be separated by adequate wash-out period to ensure the drug concentrations are below the lower limit of bioanalytical quantification in all subjects at the beginning of the second period.[10] Subjects were randomized to one of the two sequences to receive either formulation according to the randomization scheme. Based on the previous study, the sample size of 24 subjects was sufficient to ensure the power of 80% for correctly concluding bioequivalence under the following assumption: a=0.05, $0.95<\mu T/\mu R<1.05$ and an intra-subject variability of 25%.[11]

The test product of Deferasirox; 500 mg dispersible tablet formulation was manufactured by P.T. Novell Pharmaceutical Laboratories Indonesia (Batch No D17H02) and the reference product was Exjade® dispersible tablet 500 mg, manufactured by Novartis Pharma Stein AG, for Novartis Pharma AG, Switzerland, imported by P.T. Novartis Indonesia (Lot No S0752).

A total of twenty-eight healthy volunteers, aged between 18-55 years old with normal body mass index (BMI=18-25 kg/m^2) participated in this study. Volunteers were selected after passing a clinical screening procedure including a physical examination, ECG and clinical laboratory tests (hemoglobin, hematocrit, erythrocyte, leukocyte, platelets, leukocyte differential, blood urea nitrogen, SGPT, SGOT, alkaline phosphatase, total bilirubin, total protein, fasting glucose, albumin, globulin, total cholesterol, urea, creatinine, urinalysis and negative pregnancy test for female volunteers, negative result for drug abuse test, and negative results of Hepatitis B, C and HIV.

We excluded smokers (>5 cigarettes/day), pregnant women or nursing mothers, those who had a history of hepatic, renal, cardiovascular or gastrointestinal disease, myelodysplastic syndrome or other hematological and non-hematological malignancies, platelet counts less than 50×10^9/L, creatinine clearance less than 40 mL per minute, hypersensitivity to the drug, history of alcohol or drug abuse within 12 months prior to the screening, had received any other medication within two weeks and donation or loss more than 450 mL of blood within 3 months prior to the screening of the study.

All volunteers were asked to avoid using other drugs for at least two weeks prior to the study and after its completion. They were also refrained from ingesting alcohol, caffeine, chocolate, tea or soda containing beverages for at least 48 hours prior to the study until its completion.

The volunteers were confined to the clinical unit of Clinisindo Laboratories one night before the study until 24 hours after drug administration to assure the fasting condition (10 hours before drug administration) and to monitor their conditions. On the study day, each volunteer received a single oral dose of one dispersible tablet of 500 mg Deferasirox (the generic) or Exjade®. The receiving tablet was dispersed and stirred in 240 mL of water within a few minutes. Water intake was allowed except for within one hour before and one hour after the dose. No food was allowed until 4 hours after dose administration. Standard meals were served at 4 hours (±1186.28 calories) and 11 hours (±1069.95 calories), snack was served at 8 hours (±270 calories) after drug administration. Total calories were calculated by a nutritionist. The volunteers were not permitted to perform strenuous exercise during the sampling days.

Blood pressure, heart rate, body temperature and adverse events were monitored during the study. Five ml of the venous blood were collected at pre-dose, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48 and 60 hours after drug administration (17 blood samples) in heparinized tubes. After blood separation, plasma was frozen at -20°C until analysis. After one week wash out period, volunteers returned to Clinisindo Laboratories and the blood sample analysis was repeated in the second period in the same manner to complete the crossover design.

Safety Evaluation

Analysis of the safety-related data was considered using adverse events which occurred after initiation of the study treatment and was supported by the following detailed tabulations and analysis.

Analytical Method

The concentration of the deferasirox in plasma was analyzed using HPLC method with ultraviolet detector in the Clinisindo Laboratories (Jakarta, Indonesia).[12] Diazepam will be used as the internal standard. The mobile phase was phosphate buffer 10 mM pH 3.00: acetonitrile (50:50) pumped isocratically at 1 ml/min through a Lichrospher® 100 RP-18 endcapped, 5µm,

250×4 mm i.d. column (Merck, Darmstadt, Germany) heated to 40 °C. The wavelength was set at 248 nm.

Briefly, a 20µl aliquot of internal standard (diazepam, 100.8 µg/mL in methanol:water=1:1) was added to a 200µL of aliquot of plasma sample and 500 µL of acetonitrile. The mixture was vortex mixed for 30 seconds and centrifuged at 3000 rpm for 5 minutes. After mixing, 400 µL buffer KH_2PO_4 1 M pH 3.00 was added to convert the iron chelate back to free drug. Then, the mixture was vortexed for 1 minute and centrifuged at 3000 rpm for 10 min. The supernatant was removed into vials and a volume of 50 µL supernatant was injected into the HPLC system. The analytical method was conveniently validated.[13] The assay was linear over the concentration range of 0.09970–19.94 µg/mL.

Pharmacokinetic and Statistical Analysis

The bioequivalence was determined using the primary parameters, AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}. The maximum plasma concentration (C_{max}) was obtained from the highest concentration of individual drug plasma concentration. T_{max} was obtained from the time when the maximum concentration (C_{max}) was reached. The area under the plasma concentration time curve up to the last time (t) showing a measurable concentration (C_t) of the analyte (AUC_{0-t}) was determined by using the linear trapezoidal rule.

Whenever possible, at least 4 non-zero observations during the terminal elimination phase were used to calculate the elimination rate constant (K_e). A minimum of 3 observations was used if fewer than 4 observations were available. The $AUC_{0-\infty}$ values were determined by adding the quotient of C_t and the appropriate K_{el} to the corresponding AUC_{0-t}, which was:

$AUC_{0-\infty}=AUC_{0-t}+C_t/K_{el}$

Where C_t is the estimated last plasma concentration.

The apparent elimination half-life ($t_{½}$) of deferasirox in plasma was calculated by using the following equation:$t_{½}=(\ln 2)/K_{el}$

For the parameters of AUC_{0-t}, $AUC_{0-\infty}$ and $C_{max,}$ a multiplicative model was assumed, and analysis of variance (ANOVA) was applied using the respective ln-transformed data. For estimation of bioequivalence the 90% confidence interval of the geometric mean ratio test/reference (T/R) for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were calculated assuming a multiplicative model. The accepted bioequivalence range for these parameters was 80.00-125.00%. All statistical analyses were performed using Microsoft® Excel 2010 and EquivTest® version 2.0 software.

Results

28 volunteers were enrolled and randomized in the study. Two volunteers withdrew from the study due to personal reasons. One volunteer withdrew consent during period I while last blood sample was taken at 24 hours after drug administration and the other withdrew before period II had been started. 26 volunteers (20 men and 6 women) completed both periods of the study (table 1). The safety analysis was performed on all 28 volunteers who had received at least one of the study drugs. However, the pharmacokinetic and statistical analysis were performed only on 26 volunteers who had completed both periods of the study.

Both Deferasirox formulations were well-tolerated at the administered dose and no significant adverse clinical event was observed. 36 adverse events were experienced during the study and none were serious. Most of the adverse events were of mild intensity except one who experienced a moderate adverse event of headache, nausea, and vomiting during period I of the study. The adverse events were resolved by administering Panadol® which contained paracetamol 500 mg (2×1 tablet) and Ondansetron 8 mg (1×1 tablet). This volunteer was not removed from the study because the vomiting event occurred after 2 times the median t_{max} of the drug based on previous bioequivalence studies (2.50 hours).[7] The concomitant medications (others drug or supplements that volunteers were taking beside the investigation drug) was not affecting on the results of the bio-analytical data in plasma concentration-time profile. Other adverse events resolved completely without any concomitant medications. The disposition of the adverse events is shown in table 2.

Table 1: Demographic data for Deferasirox bioequivalence study in 26 volunteers

Parameter	Mean±SD	Value Range
Age (years)	31.4±9.8	19-54
Weight (kg)	58.9±7.6	47.5-76
Height (cm)	164.3±6.5	150-175
Body mass index (kg m^{-2})	21.8±2.0	18-25

Table 2: Disposition of adverse events

Causal relation to study drug	Events	Total
Related	Headache	1
	Nausea	1
	Vomiting	1
Unrelated	Bradycardia	11
	Hypotension	9
	Hypertension	9
	Tachycardia	4
Total		36

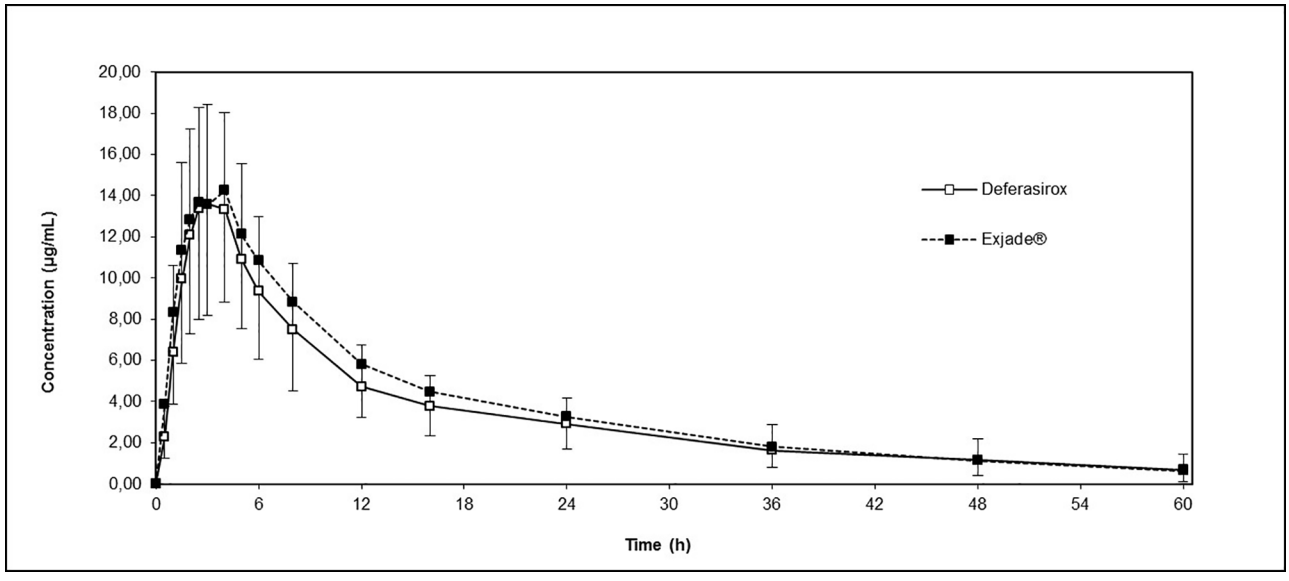

Figure 1: Mean plasma concentration-time profiles of deferasirox after a single dose of two 500 mg deferasirox dispersible tablets of two different formulations.

26 volunteers were available for pharmacokinetic evaluation. The mean Deferasirox concentration versus time profiles for both formulations is shown in figure 1. The pharmacokinetic parameters that are used to assess the bioequivalence of the test formulation versus the reference drug were AUC_{0-t}, $AUC_{0-\infty}$ for the extent of the absorption and C_{max} and t_{max} for the rate of absorption. Descriptive statistics of the pharmacokinetic parameters for Deferasirox and the reference are summarized in table 3 where the arithmetic mean values and the range for the AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and $t_{½}$ values obtained for each formulation are shown. The pharmacokinetic characteristic t_{max} is presented as median values. The mean obtained values for test and reference formulations were 15.03 and 16.14 µg/mL for C_{max}; 201.81 and 226.63 µgxh/mL for AUC_{0-t}; 226.86 and 246.69 µgxh/mL for $AUC_{0-\infty}$. The median t_{max} for both formulations was 3.00 h.

For this study, AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} were defined as the main target parameters in order to assess possible bioequivalence between both preparations. The 90% confidence interval of the relative bioavailability should be within an acceptance interval of 80- 125 %.[10] As shown in table 4, the results of the statistical evaluation for 90% confidence interval of AUC_{0-t}, $AUC_{0-\infty}$, C_{max}, and $t_{½}$ for deferasirox were all within the bioequivalence acceptance limit of 80–125 %, and no significant differences were found for t_{max} as analyzed by Wilcoxon sign rank test (P=0.6120).

The mean ratio of $AUC_{0-t}/AUC_{0-\infty}$ for all individuals on test and reference products were 91.54% and 92.97%, respectively; indicate an adequate sampling time since the extrapolated portion of the total AUC was more than 80%.

Discussion

The negative perception regarding the different efficacy, safety, and quality of the generic drug because of the lower price than the brand drug was debatable. Even though the price was cheaper, the quality was actually

Table 3: Mean pharmacokinetic characteristic for Deferasirox after administration for the two formulation

Parameter	Test Formulation	Reference Formulation
Arithmetic mean C_{max} (µg/mL)	15.03	16.14
Range	5.77–27.24	5.53–29.54
Arithmetic mean AUC_{0-t} (µgxh/mL)	201.81	226.63
Range	112.11–400.36	112.70–571.02
Arithmetic mean $AUC_{0-\infty}$ (µgxh/mL)	226.86	246.69
Range	114.37–475.80	118.68–594.92
Arithmetic mean $t_{½}$ (h)	16.50	15.77
Range	7.33–40.38	7.27–37.03
Median t_{max} (h)	3.00	3.00
Range	1.50–5.00	1.00–6.00
Mean $AUC_{0-t}/AUC_{0-\infty}$ (%)	91.54	92.97

Table 4: Statistical evaluation of AUC_{0-t}, $AUC_{0-\infty}$, C_{max} and $t_{½}$ for deferasirox of two formulations from 26 volunteers.

	AUC_{0-t} (%)	$AUC_{0-\infty}$ (%)	C_{max} (%)	$t_{½}$ (%)
Ratio	89.07	90.69	92.81	100.34
90% Geometric CI	83.04–95.53	83.18–98.88	81.67–105.47	86.89–115.88
Intra-subject CV	14.70	18.14	26.85	30.25

the same as the brand drug. The generic drug was cheaper since there was no need to do the same research that the innovator drug required a long time with a very large cost.

Innovator drugs are drugs with active substance that were first discovered by the pharmaceutical industry (new chemical entity/NCE). This NCE product protected by the patent requires an assessment of the efficacy, safety, and quality during different phases of clinical trials. On the other hand, for medicinal products that are "copy products" such as generic drugs, they need another quality standard, which is a bioequivalence with innovator drug as a comparison.[14]

Two drugs are called bioequivalent if both of them have similar pharmaceutical equivalence or pharmaceutical alternative. Two drugs are having pharmaceutical equivalence if they contain the same active substance in the same amount and the same dosage form. Generic drug is an example of pharmaceutical equivalent. The generic drugs must be proven to be bioequivalent to the innovator drugs, based on the theory that only bioequivalent drugs are expected to produce the same efficacy, quality, and safety as the innovator drug so that it can be interchangeable.[14]

This study was about comparing two formulations of Deferasirox with the same amount and dosage form (1 dispersible tablet of 500 mg Deferasirox). If the result was bioequivalent, these two formulations are expected to be pharmaceutical equivalent as well. Pharmaceutical equivalent drugs are considered to be therapeutically equivalent and can be expected to have the same clinical efficacy and safety.[14]

The safety evaluation report showed that both Deferasirox formulations were well-tolerated at the administered dose and no significant adverse clinical events were observed. The moderate adverse event occurred in the volunteer who had received the reference formulation. It might be happened because the dispersible tablets contain lactose and sodium lauryl sulfate which were thought to cause gastrointestinal side effects.[15] However, those adverse events resolved by drugs which did not affect Deferasirox concentration in the plasma.

There was a previous systematic review and meta-analysis that compared clinical results of some generic brands that were bioequivalent with their innovators. The results showed no evidence that the brand drugs were necessarily superior to the generic ones.[16] In Iraq and Iran, the generic brand of Deferasirox was compared to its innovator (Exjade®) clinically on Iraqi and Iranian thalassemia patients.[8, 17] However, those studies required a long time, a high cost, and a large number of subjects. The result showed a promising efficacy and safety in comparison with the original brand of Exjade®.[8, 17] Based on previous studies, we conducted a bioequivalence study of Deferasirox to assure its efficacy for use with lower prices in patients with thalassemia major in Indonesia.

Conclusion

The generic drug of Deferasirox showed a bioequivalent result similar to its innovator (Exjade®). Thus, it can be assumed that the two formulations were therapeutically equivalent with the same clinical efficacy and safety. It would be promising to use this generic formulation as iron chelation in patients with thalassemia major in Indonesia.

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Conflict of Interest: Asti Swari Paramanindita, Tri Rahayu Wijayanti, Windy Lusthom, Budi Prasaja, Evan Widjaja, Monika Sandra are employees of the contract organization P.T. Clinisindo Laboratories, Jakarta, Indonesia.

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