Bioequivalence study of dihydroartemisinin-piperaquine (DHP) generic formulation in fixed-dose combination, in healthy Indonesian volunteers

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ABSTRACT

Background: Malaria is still considered as a major health problem in the world including in Indonesia, which is regarded as one of the malaria-endemic countries. Since 2006, WHO has recommended the use of artemisinin-based combination therapy (ACT) to treat uncomplicated falciparum malaria. In Indonesia, DHP tablet (combination of dihydroartemisinin and piperaquine) is the first line therapy used in malaria control program, which currently used imported DHP tablet. To produce generic DHP tablet, comparative bioequivalence test between DHP tablet and the drug previously used is needed.

Methods: A single dosed, randomized, double-blinded, one-period, and parallel study design was conducted in the present research. Every twenty-four subjects were assigned into two groups, which are test group and comparison group.

Results: The results showed that even though in vitro comparison of dissolution test has fulfilled the requirements, in vivo test results has not fulfilled the bioequivalence standards. Obtained geometric mean ratios (90% confidence intervals) of the test drug to comparator drug for dihydroartemisinin were 83.30% (67.06%–103.48%) for AUC_{0-inf}, 83.24% (67.10%–103.26%) for AUC_{max}, and 75.67% (61.83%–92.61%) for \( C_{max} \). The geometric mean ratios (90% confidence intervals) of the test drug to comparator drug for piperaquine were 97.31% (76.50%–123.80%) for AUC_{0-inf}, 94.18% (74.18%–119.59%) for AUC_{max}, and 96.47% (71.80%–129.62%) for \( C_{max} \).

Conclusions: Therefore, the pharmacokinetic profile of the test drug is concluded to be bio-inequivalent with the comparator drug.

INTRODUCTION

To this day, malaria remains a crucial health concern worldwide, as well as in Indonesia, which is endemic to malaria. Various malaria drugs have become one of the solutions in controlling malaria. However, Plasmodium resistance to some antimalarial medicine has been one of the most significant challenges in malaria treatment. Therefore, in 2006, WHO recommended the use of Artemisinin Combination Malaria Therapy (ACT) to treat uncomplicated falciparum malaria. In Indonesia, the first line drug currently used in malaria control program is DHP Tablet. Dihydroartemisinin has bigger potency than artemisinin.

Oral administration of DHP Fixed Dose Combination Tablet has a tendency to overcome malaria as well as to prevent resistance of the plasmodium. Chemical and physical properties of dihydroartemisinin are: white needle crystal, bitter, odorless, easily dissolves in chloroform, partially dissolves in methanol and ethanol, dissolves in acetone, does not dissolve in water. Chemical and physical properties of piperaquine phosphate are: yellow-white to pale yellow colored crystal, odorless, slightly bitter, exposure to sun, partially dissolves in water, does not dissolve in dehydrated ethanol and chloroform. Formulation effort is increased absorption of active ingredient so it might achieve therapy concentration.

Fixed dose combination of DHP tablet is the first generic formulation of DHP tablet produced to improve its solubility characteristics so that the new drug might be more easily formulated and more efficiently delivered.
MATERIALS AND METHODS

Subjects
Forty-eight healthy Indonesian male and female volunteers participated in this study. All selected subjects should fulfill the following inclusion criteria: Aged 18 – 55 years with absence of significant disease or clinically significant abnormal values on laboratory evaluation, considered healthy based on medical history and physical examination; Body Mass Index (BMI) within 18 to 25 kg/m²; preferably non-smokers or smoke less than 10 cigarettes per day; no history of drug or alcohol abuse; no participation in any clinical trial within the past 3 months; able to participate in the study, willing to communicate well with the investigators and would provide written informed consent to participate in the study.

Pregnant women, nursing mothers, subjects with known contraindications or hypersensitivity to dihydroartemisinin and piperaquine or allied drugs, subjects who had a history of chronic gastrointestinal problems, liver dysfunction, clinically significant hematological abnormalities, renal insufficiency, and had positive test results for HBsAg, anti-HCV, and anti-HIV were excluded.

Subjects underwent a medical assessment within 14 days prior to their first day of treatment. The assessment includes physical examination; vital signs (blood pressure, pulse rate, respiratory rate, and temperature) examination; perform ECG and laboratory tests of liver function (AP, ALT, AST, total/direct bilirubin), renal function (serum creatinine and urea levels), routine hematology (hemoglobin, hematocrit, erythrocyte count, platelet and leucocyte count), blood glucose, routine urinalysis (pH, glucose, protein, and urine sediment); and immunology test for HBsAg, anti-HCV, and anti-HIV. Pregnancy test (for women) was performed at screening and before taking the drug. All subjects signed the informed consent form to participate in the present study, which was approved by the Ethical Committee of NIHRD, Indonesian Ministry of Health.

Study design and procedure
This was a single dose, randomized, double-blinded, and one-period, parallel study under fasting condition. Subjects were randomized according to block randomization with a block size of four. The six permutations obtained from block size four were randomly assigned using Table of Random Numbers from Dixon & Massey, 1969, p. 449, column 24 row 1 (read downward) to receive either the test drug or the reference drug. The test drug used was generic DHP tablet composed of a combination of dihydroartemisinin 40 mg and piperaquine 320 mg in film-coated tablets. The reference drug was DHP-Frimal, which is manufactured by Zhejiang Holley Nanhu under license of Beijing Holley Cotec, China, imported by PT Mersifarma TM, Indonesia. This study was registered by Indonesia Agency of Drug and Food Control Number B-PN.02.03.322.3.09.1 6.4627 dated September 23rd, 2016.

Subjects arrived at the clinical study site (an independent laboratory) a night before drug administration and were requested to fast from any food and drink except mineral water from 09.00 PM. In the morning on the first day of intervention, after an overnight fast, a pre-dose blood sample was taken. Afterwards, either one generic DHP tablet or one DHP-Frimal tablet were given at 07.00 AM with 200 mL of water.

Venous blood samples were drawn 10 mL immediately before taking the drug (as a control), and 5 mL each at 0.17, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, 24, 48 and 72 hours after drug administration. Blood samples were drawn from the forearm vein by using drawing needle 22G and K EDTA vacuum tubes. Blood samples were centrifuged within 30 min to minimize hemolysis, and the separated plasma was stored below -20°C until analyzed.

Pharmacokinetic analyses
The plasma sample was dispensed into an appropriate tube, added nipsol solution as internal standard of dihydroartemisinin and loratadine solution as internal standard of piperaquine. Dihydroartemisinin was extracted from 1000 μL plasma using liquid-liquid extraction with appropriate solvent and piperaquine was extracted from 500 μL plasma using solid phase extraction with appropriate solvent then injected into the analysis platform in designated conditions for each drug. The dihydroartemisinin and piperaquine concentrations in plasma were determined using a fully validated ultra performance liquid chromatography-tandem mass spectrometry detector (UPLC-MS/MS), with respect to adequate selectivity, sensitivity, linearity, accuracy, and precision (both within and between days). Stability of the samples under frozen conditions, at room
temperature, and during the freeze-thaw cycle was also determined. Calibration standards, controls, and samples were processed in batches.

UPLC-MS/MS condition for Dihydroartemisinin column was: ACQUITY UPLC® BEH C18 1.7 μm 2.1x50 mm; mobile phase was acetonitrile: methanol: ammonium formate 10 mM; MRM: m/z Dihydroartemisinin 307.25 > 261.25 and m/z nipasol 181.11 > 139.10. UPLC-MS/MS condition for Piperaquine column was: ACQUITY UPLC® BEH C18 1.7 μm 2.1x50 mm; mobile phase was acetonitrile: methanol: ammonium formate 10 mM; MRM: m/z Piperaquine 535.20 > 288.22 and m/z Loratadine 383.20 > 337.20.

Validation of dihydroartemisinin is shown as the standard calibration curve of dihydroartemisinin, which ranged from 0.51 to 202.69 ng/mL. A linear relationship between concentration and signal intensity was obtained (r = 0.9935 day 1, r = 0.9953 day 2, r = 0.9949 day 3) and the lower limit of quantification (LLOQ) was 0.51 ng/mL. Piperaquine validation is shown as the standard calibration curve of piperaquine as well, ranged from 1.02 to 203.90 ng/mL. A linear relationship between concentration and signal intensity was obtained (r = 0.9969 day 1, r = 0.9991 day 2, r = 0.9982 day 3) and the LLOQ was 1.02 ng/mL.

Plasma concentration-time data were analyzed and the parameters were derived as follows: Cmax and tmax were obtained directly from the observed data; the AUC(0→t) was calculated by the trapezoidal method; the AUC(0→inf) was calculated as AUC(0→t) + C0 / k, (C0 was the last quantifiable concentration; k was the terminal elimination rate constant) and was determined by least-squares reggression analysis during the terminal log-linear phase of the concentration-time curve; the t1/2 was calculated as 0.693/k. Comparative dissolusion profiles were conducted prior to BE study of the two products (DHP generic tablet versus DHP-Frimal).

Statistical Analyses

Phoenix® WinNonlin Version 6.4 (Certara L.P., St. Louis, MO, USA) was used to perform the statistical analyses of AUC(0→t), AUC(0→inf) and Cmax using analysis of variance (ANOVA) after transformation of the data into their logarithmic (ln) values. The anti ln of the above confidence intervals was the 90% CI (confidence interval) of the ratios of the test / the comparator geometric means. The power of the study was 80% with α of 5% (2-sided).

RESULTS

Forty-eight healthy Indonesian male and female volunteers were enrolled in the study. Male subjects were almost 2.5 times that of female subjects; 41.7% of them were light to moderate smokers who smoked 1-8 cigarettes per day. The demographic data of subjects in this study are presented in Table 1.

Comparative dissolution of dihydroartemisinin and piperaquine conducted prior to BE study showed that the dissolution profile of generic DHP tablet was similar to that of its comparator (DHP-Frimal), in various pH solution, in which the concentration of dihydroartemisinin and piperaquine were slightly higher in the generic tablet (Figure 1-5). Further pharmacokinetic profiles of the test drug (generic DHP) and its comparator are shown in Figure 6 and 7 below.

The pharmacokinetics profiles (the mean plasma concentration-time) of dihydroartemisinin and piperaquine of all subjects (N=48) after a single dose oral administration of the film-coated generic DHP tablets and DHP-Frimal revealed that the mean plasma concentration of the generic DHP tablet was lower compared to DFP-Frimal. The pharmacokinetic parameters (i.e. AUC(0→t), AUC(0→inf), Cmax, tmax and t1/2) of the test drug and its comparator are presented in Table 2.

The statistical analysis of the pharmacokinetic parameters is presented in Table III. Statistical calculations for AUC and Cmax were based on the ln-transformed data and the calculated 90% confidence intervals of test/comparator geometric means, using analysis of variance (ANOVA). Statistical calculations for t1/2 of dihydroartemisinin and piperaquine after a single dose oral administration of the test drug and the comparator drug was analyzed using Mann-Whitney test. Moreover, statistical calculations for t1/2 of dihydroartemisinin and piperaquine were analyzed using Independent Sample T-test.

The geometric mean ratios (90% CI) of the test drug/comparator drug for dihydroartemisinin were 83.30% (67.06%–103.48%) for AUC(0→t), 83.24% (67.10%–103.26%) for AUC(0→inf) and 75.67% (61.83%–92.61%) for Cmax. The geometric mean ratios (90% CI) of the test drug/comparator drug for piperaquine were 97.31% (76.50%–123.80%) for AUC(0→t), 94.18% (74.18%–119.59%) for AUC(0→inf) and 96.47% (71.80%–129.62%) for Cmax. The test product

| Table 1 | Demographic data of subjects in dihydroartemisinin and piperaquine BE study (N=48) |
|---------|---------------------|----|----|---|
| Gender, n (M, F) | 34 | (M), 14 (F) |
| Age, yr (range) | 19 | – 48 |
| BMI, kg/m² (range) | 18.07 – 25.00 |
| Smoking*, n (%) | 20 | (41.7) |

*light to moderate smoker: 1-8 cigarettes/day
Table 2  Summary of pharmacokinetic parameters of dihydroartemisinin and piperaquine after a single dose oral administration of dihydroartemisinin 40 mg/piperaquine 320 mg combination in film-coated generic tablet produced by PT X and the comparator drug (DHP-Frimal)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test drug mean (SD)</th>
<th>Comparator drug (DHP-Frimal) mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC_{0-t} (ng.h.mL^{-1})</td>
<td>274.77 (126.18)</td>
<td>322.22 (134.67)</td>
</tr>
<tr>
<td>AUC_{0-inf} (ng.h.mL^{-1})</td>
<td>276.71 (126.52)</td>
<td>325.03 (135.08)</td>
</tr>
<tr>
<td>C_{max} (ng.mL^{-1})</td>
<td>130.54 (56.36)</td>
<td>166.85 (64.19)</td>
</tr>
<tr>
<td>t_{1/2} (h)</td>
<td>1.29 (0.51)</td>
<td>1.70 (1.03)</td>
</tr>
<tr>
<td>t_{max} (h)*</td>
<td>1.00 (0.50 – 2.50)</td>
<td>1.00 (0.50 – 2.50)</td>
</tr>
</tbody>
</table>

*median (range)

Table 3  Statistical calculations for AUC0-t, AUC0-inf, and Cmax of dihydroartemisinin and piperaquine after a single dose oral administration of dihydroartemisinin 40 mg/piperaquine 320 mg combination in film-coated generic tablet produced by PT X and the comparator drug (DHP-Frimal)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% Ratio of Geometric Means (T/R)</th>
<th>90% Confidence Interval (T/R)</th>
<th>% CV</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dihydroartemisinin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-t} (ng.h.mL)</td>
<td>83.30</td>
<td>67.06 – 103.48</td>
<td>47.09</td>
<td>Bio inequivalent*</td>
</tr>
<tr>
<td>AUC_{0-inf} (ng.h.mL^{-1})</td>
<td>83.24</td>
<td>67.10 – 103.26</td>
<td>46.78</td>
<td></td>
</tr>
<tr>
<td>C_{max} (ng.mL)</td>
<td>75.67</td>
<td>61.83 – 92.61</td>
<td>43.56</td>
<td></td>
</tr>
<tr>
<td>Piperaquine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC_{0-t} (ng.h.mL)</td>
<td>97.31</td>
<td>76.50 – 123.80</td>
<td>52.90</td>
<td></td>
</tr>
<tr>
<td>AUC_{0-inf} (ng.h.mL^{-1})</td>
<td>94.18</td>
<td>74.18 – 119.59</td>
<td>52.43</td>
<td></td>
</tr>
<tr>
<td>C_{max} (ng.mL)</td>
<td>96.47</td>
<td>71.80 – 129.62</td>
<td>67.08</td>
<td></td>
</tr>
</tbody>
</table>

*significant (p<0.05)

Figure 1  Dissolution profile of dihydroartemisinin in HCl 0.1 N

Figure 2  Dissolution profile of dihydroartemisinin in pH 4.5

Figure 3  Dissolution profile of dihydroartemisinin in pH 6.8

Figure 4  Dissolution profile of piperaquine in HCl 0.1 N
is considered bioequivalent with the comparator product if the 90% confidence interval of the geometric mean ratios of AUC and C\textsubscript{max} between the test drug and comparator drug (for both dihydroartemisinin and piperaquine) fall within the range of 80.00%–125.00%. Hence, it was inferred that generic formulation of dihydroartemisinin 40 mg/piperaquine 320 mg (DHP) combinations in film-coated generic tablets was bio-inferior to the comparator drug (DHP-Frimal).

Mann-Whitney statistical test calculations for \( t\text{\textsubscript{max}} \) of dihydroartemisinin did not show significant difference between the test and comparator drugs because the \( p \) value was larger than 0.05. However, the \( t\text{\textsubscript{max}} \) of piperaquine of the test and comparator drugs were significantly different because the \( p \) value was less than 0.05.

Based on analysis using independent samples T-test calculations for the \( t\text{\textsubscript{max}} \) of dihydroartemisinin and piperaquine, the differences found between the test drug and the reference drug were not statistically significant (\( p > 0.05 \)). There was no adverse event found during this BE study.

**DISCUSSION**

Normally, the study design applied for bioequivalence study is a crossover trial. However, the half-life of one of the components of the tested drug (piperaquine) is quite long (22 days), which means a washout period up to 100 days (five times of the half-life) will be needed if crossover design is applied.\(^7,8\) Therefore, a parallel design was chosen in this study, with consideration of the possibility of a low risk of the subjects’ dropout.

Comparative dissolution test (in vitro pharmacokinetics) showed that both formulations were identical (similar) for the dihydroartemisinin component and piperaquine, even the concentration of dihydroartemisinin obtained in the generic formula (test drug) is higher compared to that of comparator product. Generally, comparative dissolution test results (in vitro pharmacokinetics) were able to predict the bioequivalence (in vivo pharmacokinetics) about 80\%.\(^9\) In contrary, bioequivalence (in vivo pharmacokinetics) test results of the present study implies that the generic DHP tablet is bio-inferior to DHP-Frimal. The 90% confidence intervals of the test/comparator ratios for both AUC and \( C\text{\textsubscript{max}} \) of dihydroartemisinin and piperaquine were outside the acceptance range for bioequivalence. There are several possible reasons why these two DHP products are bio-inferior.

The first possibility is in terms of study design, which was parallel design in this study. One of the disadvantages of this design is that there is great variation in intra- and inter-individual subject. This was evident in this study where the mean percentage of coefficient of variation (CV) obtained for dihydroartemisinin is 45.81\%, and the mean percentage of coefficient of variation for piperaquine is 57.47\%. The consequences of the high % CV is the number of subjects should be larger, considering the calculation of the number of

**Figure 5** Dissolution profile of piperaquine in pH 4.5 acetate

**Figure 6** Mean plasma concentration-time profiles of dihydroartemisinin in human subjects (N = 48) after a single dose oral administration of dihydroartemisinin 40 mg/piperaquine 320 mg combination in film-coated generic tablet produced by PT X and the comparator drug (DHP-Frimal). (Vertical bars indicate standard deviation)

**Figure 7** Mean plasma concentration-time profiles of piperaquine in human subjects (N = 48) after a single dose oral administration of dihydroartemisinin 40 mg/piperaquine 320 mg combination in film-coated generic tablet produced by PT X. and the comparator drug (DHP-Frimal). (Vertical bars indicate standard deviation)
samples using CV 23.5%. The results of this study is similar with a study which used crossover design in 24 healthy Vietnamese subjects, the two tested drugs containing dihydroartemisinin and piperaquine were found to be bio-inequivalent.10

The second possibility is related to the physical properties of both or one component of the active ingredients of the drug, which are the solubility and the permeability of the substance to plasma membrane. Dihydroartemisinin is a compound that is practically insoluble in water, but soluble in non-polar solvents, such as chloroform and acetone. Instead, piperaquine is practically insoluble in non-polar solvents (such as chloroform), and slightly soluble in water. Biopharmaceutical (in vitro) test showed the dissolution of dihydroartemisinin component of the test drug (generic tablet) was comparable with its comparator drug on gastric pH (acidic) and intestinal pH (alkaline), with the concentration of dihydroartemisinin of the test drug was slightly higher. On the other hand, for piperaquine component of the test drug, the concentration obtained on the acidic environment (gastric) is lower than its comparator, and on the basic environment (intestine) the concentration could not be measured because it does not provide absorption.

Based on bioequivalence (in vivo) test as well as biopharmaceutical test (in vitro), according to the biopharmaceutical classification system (BCS), the test drug formulation (generic tablet) is categorized as class IV, i.e. formulation with low solubility and permeability. This means that the formulation cannot be well dissolved and absorbed. To increase the permeability and solubility, the tablet needs to be reformulated. The solubility can be improved partly by changing the compound of the active substance into the crystalline form, reducing the particle size or changing its shape into complex compounds, or by adding co-solvent and surfactant in order to obtain good solubility. Permeability of a compound can be improved, among others, by making modifications so that the active substance is not easily ionized in the intestinal or gastric fluids. Surfactant (e.g. Tween and Span) can also increase the permeability of a drug compound and enable the drug to pass through the cell membrane.11,12,13

**CONCLUSION**

Based on the results of the single dose study above, it was concluded that the generic formulation of dihydroartemisinin 40 mg/piperaquine 320 mg (DHP) combinations in film-coated generic formula tablets was bio-inequivalent to the comparator drug (DHP-Frimal, manufactured by Zhejiang Holley Holley Nanhu under license of Beijing Holley Cotec, China, imported by PT Mersifarma TM, Indonesia)

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