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RESEARCH ARTICLE

COMBINATION PHARMACOKINETICS AND PHARMACODYNAMICS OF DIHYDROARTEMISININ-PIPERAQUINE AND PRIMAQUINE IN PATIENTS WITH UNCOMPLICATED FALCIPARUM MALARIA IN HALMAHERA INDONESIA

***Arend Laurence Mapanawang, Mustofa, Mahardika A Wijayanti, Rina Handayani, Lukman Hakim, Maikel Killing, Aleksander Maengkom, Frangky Mapanawang, Fernandes Sambode, Henderina Maengkom and Sarah Mapanawang**

Doctoral Program of Medical and Health Sciences, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia; STIKES Halmahera Indonesia

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ABSTRACT

Malaria remains one of the deadly diseases in Indonesia. In its attempts to cure malaria, the government has implemented the DHP formulation (dihydroartemisinin-piperaquine) added with primaquine. However, no study has been carried out on the pharmacokinetics and pharmacodynamics of this combination. The purpose of this research is to compare absorption rate constant (Ka), time of maximum concentration observed (T_{max}), elimination half-life ($t_{1/2}$), volume of distribution (VD), clearance (CL), plasma maximum concentration (C_{max}), and area under curve (AUC) of the DHA-piperaquine (DHP) and primaquine combination in 12 uncomplicated falciparum malaria patients and the pharmacological effects. Random clinical tests were conducted with an experimental method to 12 patients from September to December 2014. Blood samples were taken sequentially, starting from day 0 to day 28, and then tested using LC-MS to measure the kinetic concentration. The results showed that the kinetic profile of DHA, piperaquine, and primaquine synergized well with no contradictions recorded as the patients were cured without any side effects. The DHP-primaquine combination (Cmax and Auc) was able to clean the parasite in just two days of treatment and showed that there was a significant relation ($P = 0.001 < 0.05$ AND $0.010 < 0.05$) between the drug content and parasite clearance. The pharmacological effect was APCR, with 100% of treatment success.

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INTRODUCTION

Malaria is one of the deadly diseases in the world (World Health Organization (WHO, 2009). In 2008, it was estimated that 243 million infection cases resulted in nearly 863,000 deaths, which was cited as the highest number of death caused by malaria since its discovery (WHO, 2009& 2010). The malaria infections happen in various parts of the world, specifically in tropical and sub-tropical areas such as most regions in Asia (particularly in South East Asia), America (mainly Latin America) and sub-African Sahara. In Indonesia, around 35% of its population lives in malaria risk areas and it is reported that more than 38,000 people a year have died because of severe malaria caused by *P. falciparum* (Laihad, 2013).

****Corresponding author: Arend Laurence Mapanawang,***
Doctoral Program of Medical and Health Sciences, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia; STIKES Halmahera Indonesia.

The malaria outbreak occurs almost every year in various endemic regions in Indonesia such as East Nusa Tenggara, West Nusa Tenggara, Maluku, North Maluku, Central Borneo, Bangka Belitung, Riau Island, Bengkulu, Jambi, Central Sulawesi, West Sulawesi (Regency of Mamuju and Mamasa), Gorontalo, and Nanggroe Aceh Darussalam. These areas are categorized as the malaria red zones (Departemen Kesehatan, 2011). The failure of ACT therapy is shown by a prolonged clearance time on the malaria patients. This marks the infection caused by resistant parasite strains (Dondorp *et al*., 2009). A study in assessing the efficacy of artensunate-mefloquine between June 2007 and May 2008 was conducted in two different regions: Wang Pha, Thailand where the parasite was sensitive to ACT, and Pailin, Cambodia where the parasite was believed to be more resistant (Dondorp *et al*., 2009). The findings showed that there was a significant extension of parasite clearance period in Pailin compared to Wang Pha. Another research was conducted (Noedl, 2008) in Province of

Battambang, Cambodia, located near Pailin, in a smaller scale that included 60 patients. Both studies found two patients (3.3%) with longer parasite clearance although the metabolite artemisinin content within plasma was adequate. Currently, the program of Indonesian government in its attempts to cure malaria is the implementation of DHP formulation (dihydroartemisinin-piperaquine) added with primaquine. The malaria treatment by implementing DHP combination, as conducted in Papua, still has a 7% of cumulative recrudescence risk of *P. falciparum*.

In relation to this issue, the existing treatment therapy with DHP combination added with primaquine is expected to suppress the previous number of cumulative recrudescence (Hasugian *et al*., 2007). Hence, the treatment therapy as managed and programmed by the government in line with Permenkes (*Peraturan Menteri Kesehatan Republik Indonesia Nomor, 2013*) by coformulation of dihydroartemisinin, piperaquine, and primaquine has been implemented in Halmahera. This implementation is based on the fact that, as reported by Internist Division of Local Public Hospital (RSUD) of Tobelo, one of five main diseases in Halmahera is malaria (Laporan, 2013). The efficacy extent of DHP and primaquine combination application is simply the increasing number of cured malaria patients. Therefore, it is important to conduct pharmacokinetic treatment test on the combination of dihydroartemisinin, piperaquine, and primaquine as well as its pharmacological effects. This research focuses on the pharmacokinetic test and pharmacological effects of DHP-primaquine combination in patients with falciparum malaria disease in Halmahera.

MATERIALS AND METHODS

Site and Time

The research was conducted in RSUD Tobelo, North Halmahera from September to November 2014. This site is chosen based on several considerations: (1) RSUD Tobelo, North Halamhera is located in malaria endemic region so that the hospital has sufficient number of patients suffering from malaria; (2) previous research findings show that Halmahera, North Maluku reveals higher endemicity;

Population and Sample

The population of this research was all in-patients suffering from falciparum malaria that were hospitalized in Internist Division of RSUD Tobelo. Referring to previous studies, there has been no precise regulation concerning the total number of sample for kinetic test, ranging between 7 and 24 samples. Hence, the sampling technique used in this study is consecutive sampling, i.e. patients who meet the research criteria (inclusion and exclusion) were used as samples (Dahlan *et al.,* 2010). From 40 patients, 21 patients met the criteria. After sample data collection, six patients resigned with various reasons, leaving 15 patients to proceed to the experiment. However, after checking process and laboratory data management, three samples were found unfit because the results showed that the two patients had parasite lower than 2000/µl and the other patient had mixed malaria (*P. falciparum* and *P.vivax*). This results in the final number of samples of 12 patients.

Instrument

The instrument used LC-MS used to measure drug concentration within plasma.

Sampling regimen

Blood of each patient was taken for blood smear for hemoglobin assessment. Patients, then, were orally given a 25 mg/kg dihydroartemisinin and 4 mg/kg piperaquine on day 1, 2, and 3 and the primaquine on day 1. The patients were then observed directly for 30 minutes. Patients who vomited were given another dose and observed for the next 30 minutes. The research's doctor evaluated each subject and collected blood for thick and thin blood supply examination on days 0, 1, 2, 3, and 7. Patients, then was asked to return on day 7, 14, and 28 and whenever they feel sick. Physical and temperature examination were recorded, and special attention was given for patients' disease record, side effect, potential condition, and medication record of other ongoing disease treatment. On days 1 and 2, the research's drugs were observed. The research doctor then conducted treatment for other diseases suffered by the patients at the same time as malaria. The research finding is evaluated with WHO clinical response and parasitology standards (WHO, 2005 & 2011).

Table 1. Drug distribution based on patients'body weight

Patients No	> 60 kg	41 – 60 kg	≤ 40 kg	DHP + primaquine	Dosage								
					D-0			D-1			D-2		
					4tb	3tb	2tb	4tb	3tb	2tb	4tb	3tb	2tb
1	63	-	-	4 DHP + 3 Prm	√			√			√		
2	62	-	-	4 DHP + 3 Prm	√			√			√		
3	-	59	-	3 DHP + 2 Prm		√			√			√	
4	-	45	-	3 DHP + 2 Prm		√			√			√	
5	-	45	-	3 DHP + 2 Prm		√			√			√	
6	62	-	-	4 DHP + 3 Prm	√			√			√		
7	-	56	-	3 DHP + 2 Prm		√			√			√	
8	-	-	39	2 DHP + 2 Prm			√			√			√
9	-	55	-	3 DHP + 2 Prm		√			√			√	
10	-	-	40	2 DHP + 2 Prm			√			√			√
11	-	55	-	3 DHP + 2 Prm		√			√			√	
12	-	50	-	3 DHP + 2 Prm		√			√			√	
Total	3	7	2		3	7	2	3	7	2	3	7	2

DHP: Dihydroartemisinin and Piperaquine Prm: Primaquine D: Day tb: Tablet

If there is parasitology and clinical failure, coartem or duo-cotexcin is given, or if there are severe disease symptoms, quinine intravenous is given. The patients who are asymptomatic and parasite free on day 28, with no early treatment failure (EFT), late clinical failure (LCF), or late parasitological failure (LPF) are classified as adequate clinical and parasitological response (ACPR).

Drug distribution

Drug was distributed orally or with water with a dosage of 2-4 tablets depending on the patients' body weight as shown in Table 1. This distribution refers to Permenkes RI, 2013.

Blood collection procedure

- Day 1 collection was done at hour 0 or pre-treatment. Blood samples (5 ml) were taken at hour 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 6, 8, 12, 18, and 24 (samples 1-13).
- Day 2 was done post-treatment once at hour 1.5 (sample 14).
- Day 3 was done post-treatment at hour 1 and 3 (sample 15-16).
- Days 7, 14, and 28 were done once at 8 o'clock in the morning along with the thick and thin blood drop sample collection.

The sample was put in the vacutainer containing EDTA, centrifugalized for 15 minutes by 3,000 rpm, stored at 41 º C in a tube, and then analyzed in laboratory.

Pharmacokinetic analysis

Statistical analysis was done by using parameters such as Ka (absorption rate), T_{max} (time to reach peak content), $t_{1/2}$ (time which half-life of drug is eliminated), VD (distribution volume), CL (clearance), C_{max} (peak content), and AUC (area under the curve). Correlation test analysis is used to prove the relation of drug content that is C_{max} (peak content) with parasite clearance from dihydroartemisinin, piperaquine, and primaquine combination (Nguyen *et al*., 2009; Bin Quok Vu, 2009; Rijken *et al*., 2011; White *et al*., 2006; Liu *et al*., 2006 and Denis *et al*., 2002). In order to identify the artemisinin pharmacokinetics, maximum artemisinin concentration (C_{max}) and time to reach concentration (T_{max}) were taken directly from data of observed time concentration for each individual. Artemisinin elimination rate constant (Ka) was estimated for each individual with long-linear, ordinary-least-squares regression from three to five of terminal concentration-time-datum point.

Terminal half-life ($t_{1/2}$) was counted as 1n2/k. AUC from drug supply to the late sample where artemisinin concentration that could be counted (AUC_{0-t}) was measured with linear trapezoidal method to view declining phase of the curve. AUC from the late time of parasite quantification within the sample until unlimited (AUS_{t-00}) was estimated by dividing last predicted concentration with Ka. AUC from zero to unlimited time (AUC_{0-00}) was counted as total of $AUC_{0-t}$and (AUS_{t-00}). Oral clearance based on blood concentration (CL_s/F) for each subject was derived as dose divided with (AUS_{0-00})[14].

DHA, piperaquine, and primaquine content analysis

The procedure was conducted in health laboratory of DKI Jakarta Province. The blood samples were collected into sterile heparin-lithium, and plasma was separated and stored at 20ºC to avoid photodecomposition. Later on, the tube was wrapped with aluminum foil. For plasma sample (1.0 ml) containing standard internal was added with acetonitrile (2.0 ml). The mixture then was put in the vortex over 15 minutes. After being centrifugalized (200g; 5 minutes), liquid phase was moved to a clean tube, where each of which was added with 2.0 ml of ammonia. The mix was extracted with tumbling mechanical over 15 minutes with disetile-ether (5.0x2). After centrifugal process (1500g; 10 minutes) and separated, organic phase combination was vapored with nitrogen flow at 25ºC. The residual, thus, was reconstructed within methanol (100µl) and injected in HPLC, so that piperaquine and primaquine content was derived. Analysis of dihydroartemisinin content (DHA) in the blood. Plasma was put into centrifugal tube + 100µl of internal standard of water methanol (50:50, v/v), well mixed + 100µl of ether-diclomethane mixture (60:40), mixed with water steam during 1 minute, and shivered over 10 minutes (240 time/minute), and centrifugalized at 3000 rm over 10 minutes. The organic phase was reserved and put into tube test which was dried at 25ºC with nitrogen flow +150µl of mobile phase (methanol-water, 50-50), and put into residue within water steam. Then, 10µlwas taken to be analyzed with LCMS (liquid chromatography-mass spectrometry), so that dihydroartemisinin content of varying time serial was derived.

Work procedure

The work procedure of this research can be explained as follows: (Majelis Profesor Riset, 2013)

- Sample transportation procedure that was from Tobelo to Maluku and heading to Jakarta, and the sample was stored in the cool-box during 8 hours to arrive in the health laboratory of Province of DKI Jakarta.
- Content test and sample examination by using LCMS (liquid chromatography-mass spectrometry) was in line with what had given by health laboratory of Province of DKI Jakarta.

RESULTS

Characteristic of research's subject

As shown in Table 2, the total of male and female sample in this research is same (six people). Viewed from age factor, the more dominant age is between 18-25 years old. It indicates that young age is vulnerable to malaria infection in the research site. Based on weight, the more dominant infected by malaria is the weight of 41-60 kg. In addition, based on blood type, in general, the blood type of the research's sample is O.

Patients' investigation

According to measurement results, the highest thick parasite film was at subject number 5 with 128.749/µL, followed by subject number 12 with 104.154/µL, and subject number 2 with 73.692/µL. In contrast, the lowest was at subject number 3 with

2.427/µL. The examination results of thick blood supply revealed that ring-shaped parasites were found in the research's blood sample number 12 in H_0, hence, the therapy or treatment of dihydroartemisinin, piperaquine, and primaquine combination was then administered.

Table 2. Characteristics of Respondents with falciparum malaria (*n* = 12)

Characteristics	Total number	Sample percentage
Gender:		
•Male	6	50.00
•Female	6	50.00
Age:		
•18 – 25 y/o	10	83.34
•26 – 35 y/o	2	16.66
Body Weight:		
•≤ 40 kg	2	16.66
•41 – 60 kg	7	58.34
•≥ 60 kg	3	25.00
Blood group:		
•O	8	66.66
•A	3	25.00
•B	1	8.33
•AB	0	0.00

Treatment and Sample Collection

The patients were given the combination of dihydroartemisinin, piperaquine, and primaquine orally. On day 1, subject 4 and 5 felt dizzy and headache. On day 2, subject 4 vomited and felt nauseous, so the subject received another dosage and was observed for the next 30 minutes. After that, the patient was no longer having complaints so that the examination could continue. On day 3, subjects 3 and 4 dazed. In total, there were nine subjects including subject 1, 2, 6, 7, 8, 9, 10, 11, and 12 who had not felt any clinical symptoms from the drug. Patients involved in the research whose hemoglobin was < 8 g/dl would be treated with oral zinc tablet. However, all samples in this research had HB > 8 g/dl , between 11.1-16.0 g/dl so that the zinc treatment was not necessary.

Pharmacokinetics of DHA, piperaquine, and primaquine

Prior to reviewing the result of dihydroartemisinin, piperaquine, and primaquine combination, firstly the kinetic profile of each dihydroartemisinin, piperaquine, and primaquine is explained.

Dihydroartemisinin on 12 patients

Pharmacokinetics of DHA is absorbed quickly if taken orally where the content in the blood peaked after 2.5 hours. Absorption by perirectal is slower than oral, where its content within blood occurs ± 4 hours after given. The bind of protein plasma is 55%. Its elimination period is 45 minutes through colon and Glucuronides' hepatic (Majelis Profesor Riset, 2013). Since the blood sample collection at hour 0.25, the lowest plasma concentration (C_p) of DHA was subject number 12 (0.50 ng/mL), and the highest was subject number 10 (6.082 ng/mL). The highest peak of DHA C_p occurred at hour 1.00 at subject number 5 (3.279,56 ng/mL). At hour 24, the concentration declined and the lowest was at subject number 3 (1.410 ng/mL). Further, the DHA C_p is seen from the mean of concentration at subject number 12. Hence, since the collection, the mean was 2.724 µg/mL. The highest drug concentration of DHA happened at hour 1.00 or one hour after DHA was distributed where the mean was 385.244 ng/mL, and, later on, it decreased at hour 24 with just 6.411 ng/mL. This data shows that starting from hour 0.25 to 24 the blood contained the lowest drug concentration of DHA on plasma, and it continued to increase and decline afterward. It indicates that the DHA C_p highly reacted at hour 1.00 after the drug distribution.

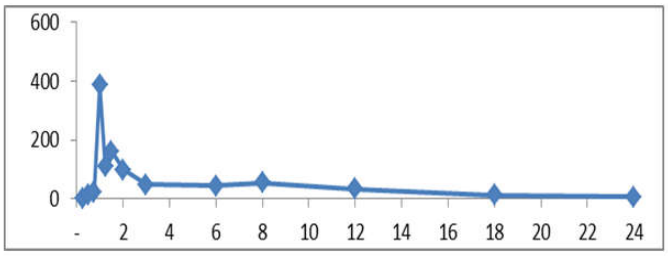

Figure 1. Mean of Drug Concentration of Dihydroartemisinin (DHA) on Plasma (C_p) on Day 1 of Drug Distribution

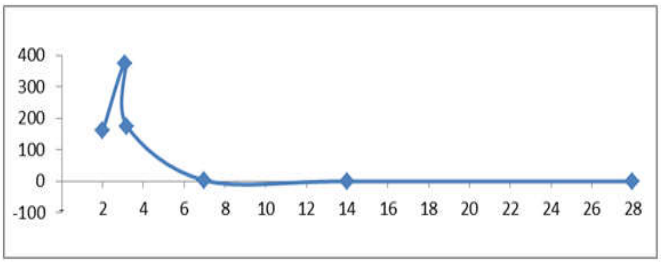

Figure 2. Mean of Drug Concentration of Dihydroartemisinin (DHA) on Plasma (C_p) at Day 2-28

On day 2-28, DHA C_p gradually decreased (Figure 2). Clearly seen on day 7, the DHA C_p on the blood of nine subjects was not found again. Also, overall, it was not found in the patient's blood on day 14 and 28.

Piperaquine in 12 patients

Since the sample collection at hour 0.25, the lowest piperaquine Cp was at subject number 3 (1.99 ng/mL). Meanwhile, the highest was at subject number 9 (58.02 ng/mL). The highest peak of piperaquine C_p happened at hour 1.25 at subject number 11 (1.960,10 ng/mL). At hour 24, the piperaquine Cp and the lowest drug concentration were at subject number 4 (2.55 ng/mL). However, if it is seen from the mean of piperaquine Cp on 12 patients (Figure 3), since the sample collection at hour 0.25, the mean was 26.79 ng/mL. The highest peak of piperaquine Cp happened at hour 1.25 or a quarter hour after piperaquine was distributed to the patients with the mean of 1436.92 ng/mL. In addition, piperaquine Cp declined at hour 24 with the mean of 16.35 ng/mL. This data illustrates that the piperaquine Cp fluctuated. It indicates that the highest reaction of piperaquine Cp was at hour 1.25 after the drug distribution. The highest peak of piperaquine Cp occurred at subject number 11 and the lowest was subject number 4 (at hour 24). Thus, the mean of the highest peak of

drug concentration of piperaquine on plasma (C_p) happened at hour 1.50.

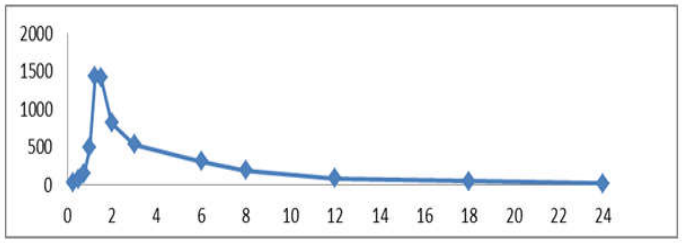

Figure 3. Mean of Drug Concentration of Piperaquine on Plasma (C_p) at Day One of Drug Distribution

On day 2-28, the piperaquine Cp was regularly lower. Seen from the mean of the piperaquine Cp (Figure 4), it can be said that on day 2-28 the mean of the highest piperaquine Cp occurred at hour 3.75 at subject number 4 (1186.19 µg/mL). On day 28, it was still found, but it was decreasing gradually.

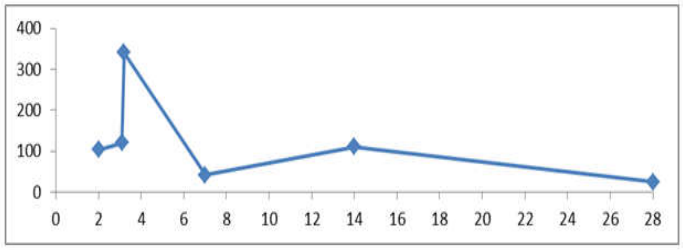

Figure 4. Mean of Drug Concentration of Piperaquine on Plasma (C_p) at Day 2-28

Primaquine in 12 patients

The lowest drug concentration of primaquine on plasma (primaquine C_p) was at subject number 6 (1.47 ng/mL), while the highest was at subject number 11 (38.18 ng/mL). The highest primaquine C_p occurred at hour 1.00 at subject number 5 (484.82 ng/mL). At hour 24, the lowest primaquine C_p was at subject number 6 and 7 (1.86 ng/mL). As can be seen in Figure 5, the mean of primaquine C_p was 10.61ng/ml. The highest peak of primaquine C_p occurred at hour 1.00 after primaquine was distributed with the mean of 279.80 ng/mL, and it decreased at hour 24 by the mean of 7.77 ng/mL. Starting from hour 0.25 to 24, it was fluctuating but then declined.

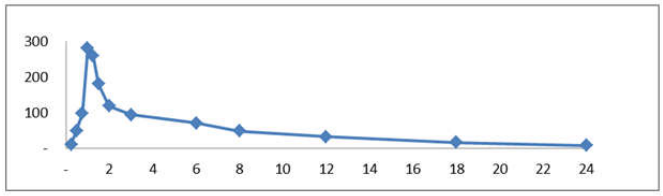

Figure 5. Mean of Drug Concentration of Primaquine on Plasma (C_p) at Day One of Drug Distribution

On day 2-28, it can be said that primaquine C_p was regularly lower. The highest point of primaquine C_p happened on day 3 at subject number 10 (389.15 ng/mL). It happened because there was an increase caused by polymorphism. It shows that the primaquine C_p from the blood of total of 11 patients was not established, excluding subject number 11.

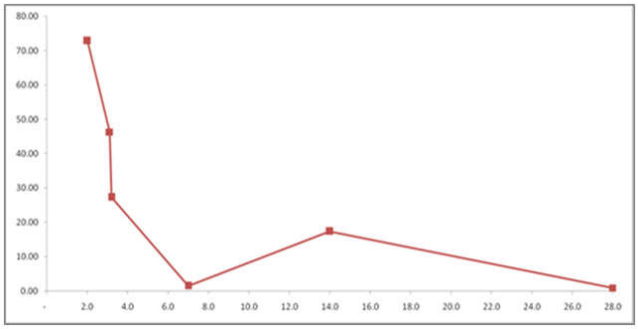

Figure 6. Mean of Drug Concentration of Primaquine on Plasma (C_p) at Day 2-28

Pharmacokinetics of DHA, piperaquine, and primaquine combination

Based on the profile of each DHA, piperaquine, and primaquine, the combination of those three drugs can be described in the treatment of falciparum malaria patients. Since the sample collection at hour 0.25, the mean of drug concentration of DHA on 12 patients' plasma (C_p) was 2.72 ng/mL. The peak occurred on day 1 that was at hour 1.00 or one hour after drug distribution by its C_p mean of 385.24 ng/mL. Meanwhile, at hour 24, the C_p mean was 6.41 ng/mL. Then, the ultimate point of piperaquine C_p, with three drugs combination, was on day 1 after a quarter hour of drug distribution (hour 1.25) by 1,436.71 ng/mL, but at hour 24, the C_p mean was 16.35 µg/mL. Moreover, the peak of primaquine C_p was similar with the peak of DHA that was on day 1 of drug distribution and at hour 1.00 or where its mean was 279.80 ng/mL, and at hour 24 when the C_p mean was 7.77 ng/mL.

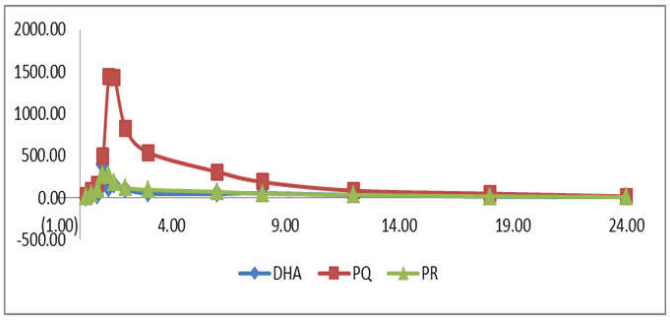

Figure 7. Mean of Dihydroartemisinin (DHA), Piperaquine (PQ), and Primaquine (PR) on Day One on Plasma (C_p)

Figure shows that the peak of the piperaquine C_p and primaquine C_p was similar at hour 1.00. By this similarity, the combination works more effectively in curing falciparum malaria disease in the patients' blood. On day 2-28, the results of sample check showed that the highest concentration mean of DHA on plasma (C_p) happened on day 3 (373.41 ng/mL). Then, on day 14 and 28, DHA had not been spotted anymore in the patient's blood. In contrast, on day 2-28 of examination, the highest concentration mean of piperaquine was on day 3.2 (340.09 ng/mL) and it was still active on day 28 in the patient's blood (24.57 ng/mL). Meanwhile, during the primaquine

examination on day 2-28, the highest mean of plasma (C_p) was on day 3 (389.15 ng/mL). It happened since the significant drug concentration on plasma (C_p) was gained from subject number 10 compared to the other patients. Nevertheless, the drug concentration of primaquine with this combination was no longer detected in the patient's blood on day 14 and 28. It was shown by the lower mean of primaquine on plasma (C_p), which was 0.86 ng/mL. Mean of drug concentration (C_p) in each falciparum malaria patient's plasma by DHA, piperaquine, and primaquine is shown in Figure 8.

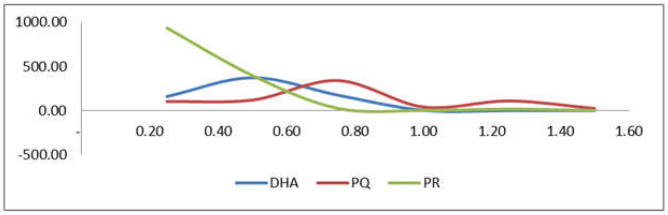

Figure 8. Mean of Dihydroartemisinin (DHA), Piperaquine, and Primaquine Combination on Plasma (C_p) at Day 2-28

On day 2, it is seen that primaquine had the higher mean of C_p than DHA and piperaquine. The highest C_p mean of piperaquine was on day 3.2, and on day 28 tended to be low and even the drug concentration of DHA was no longer detected on plasma (C_p).

Kinetic profile

This kinetic profile of drug combination is viewed from several parameters, such a Ka (absorption velocity, T_{max} (time to reach ultimate concentration), $t_{1/2}$ (time which half-life eliminated), drug VD (distribution volume), CL (clearance), C_{max}(peak concentration), and AUC (area of under curve) [14]. The profile of DHA, piperaquine, and primaquine in this study, compared to the profiles of other similar studies, is shown in Table 3.

DISCUSSION

Kinetic profile of DHA, piperaquine, and primaquine is depicted by the drug concentration of plasma (Cp). The highest point of DHA Cp happened at hour 1 or one hour after drug distribution at subject number 5 (3,279.56 ng/mL and 0.50 ng/mL). The lowest drug concentration occurred at hour 0.25 at subject number 12 (0,50 ng/mL). This data indicates that the drug distribution of DHA to the malaria patients is relatively fast, i.e. one hour after drug distribution. The speed of drug concentration within plasma (Cp) provides better effect to the malaria patients. In the following examination day (day 2-28), it was seen that starting from day 7 the DHA concentration was not found in the patient's plasma (Cp) and on day 14-18 it was not detected anymore. This shows that DHA is one of effective treatment combinations to cure malaria since it gives clinical or laboratory refinement showing the disappearance of parasite and gametocyte in the patient's blood (Rijken *et al*., 2011; White, 2007; Liu *et al*., 2006 and Denis *et al*., 2012). Meanwhile, the highest point of piperaquine C_p happened at hour 1.25, which is slower than DHA. The piperaquine concentration still existed in days 2-28. However, the concentration peak of both DHA and piperaquine was almost at the same hour: DHA at hour 1.00 and piperaquine at hour 1.25. It means that both drugs complete each other. Similarly, primaquine also showed a similarity with both DHA and piperaquine. The highest concentration of primaquine within the patient's plasma (Cp) was at hour 1.00, similar to that of DHA, at subject number 5 (484, 82 µg/mL) and the lowest was at hour 24 at subject number 6 and 7 (1, 86 µg/mL). Thus, from the similarity of the three-drug combination of DHA, piperaquine, and primaquine in terms of the concentration peak, these drugs are the reference combination for curing malaria as conducted in Halmahera. They have similar peak concentration at the same hour so that these drugs within the patient's plasma (Cp) react faster, which may help the patients recover more quickly (Hoglund *et al*., 2012; Hung *et al*., 2004; Hietala *et al*., 2007 and Karunajeewa *et al*., 2008). The kinetic profile of DHA, piperaquine and primaquine can be seen from the mean of drug absorption acceleration (Ka). In the research, it was shown that the Ka of DHA was 2.24 hours, piperaquine 2.63 hours, and primaquine 2.63 hours. As a result, the mean of those drugs was between 2 – 3 hours. Though there was a slight difference, the absorption acceleration of the three drugs was similar. It means that the treatment is effective in curing the patients. According to the C_{max} (peak concentration), the peak concentration of DHA was 495.80 µg/mL, the piperaquine was 1.576,05 µg/mL and primaquine was 135.67 µg/mL. It shows that there was a difference of peak concentration. The difference means that these drugs provide better therapy within the patient's plasma (Cp) suffering from malaria (McGready *et al*., 2011; Shargel, 2004; Tarning *et al*., 2008). Further, based on the mean of T_{max} (time to reach peak concentration), DHA was 1.25 hours; piperaquine 1.33 hours; and primaquine 1.08 hours. It shows that these drugs have the same T_{max} where it indicates that drug concentration in the systemic circulation reaches the same peak of 2.01 – 2.77 hours.

For the $t_{1/2}$ (half-time of drug to show time required in changing total of drug within the body as half of previous amount), the research reveals that the mean of $t_{1/2}$ elimination of DHA was 9.62 hours; piperaquine 7.37 hours; and primaquine 6.22 hours. In short, DHA had the longest $t_{1/2}$ while primaquine the shortest. In the AUC (area under the curve), DHA was 538,04 µg hour/Ml; piperaquine 3,312.39 µg hour/mL; and primaquine 1,2374.24 µg hour/mL. This result shows that the AUC mean of piperaquine in the systemic circulation was higher than DHA and primaquine and the AUC mean of DHA was lower than piperaquine and primaquine. Based on the VD (volume distribution), the VD mean of DHA was 1,193.60 liters; piperaquine 1,481.93 liters; and primaquine 67.99 liters. It demonstrates that these drugs have different VD from each other and indicates that the distribution of primaquine within the patient's body liquid required smaller volume than DHA and piperaquine. Then, in terms of Cl (clearance) parameter, the mean DHA was 582.12 liters/hour; piperaquine 371.15 liters/hour; and primaquine 20.78 liters/hour. It shows that these drugs had different ability to clear the drug within blood volume where DHA had the highest mean of clearance within blood and primaquine had the lowest. In summary, the kinetic profile of DHA, piperaquine, and primaquine from 12 uncomplicated falciparum malaria patients synergizes perfectly and complements each other within the patient's blood so that the patients were cured without any side

effect. The drug combination is able to clear the parasite within the patients' blood. It demonstrates that there is a relation between the drug combination of DHA, piperaquine, and primaquine and the parasite clearance. It is also shown by the significant measurement value of $p = 0.041 < 0.05$. The pharmacological effect of the drug combination is APCR (adequate clinical and parasitological response). The rate of treatment success is 100% >95% (Sharma, 2006; Mynt *et al*., 2007 and Nambozi *et al*., 2011). After the drug distribution, the parasite within the patient's blood on day 1 was already negative with the exception of patient number 1. Then, on day 2, all patients' blood was clear from the parasite. This means that the treatment length is one day shorter than the treatment standard of three days.

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Disclosures

We declare that there is no conflict of interests, be it financial and/or business, to any parties.

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