COMPARISON OF THE BIOEQUIVALENCE OF THREE ORAL FORMULATIONS OF DIHYDROARTESMININ BASED ON EX VIVO BLOOD SCHIZONTOCIDAL ACTIVITIES AGAINST PLASMODIUM FALCIPARUM

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Abstract. Sera collected at various time intervals from healthy Thai male subjects after the administration of the three oral formulations of dihydroartemisinin (Cotexin® manufactured in the People’s Republic of China, a formulation manufactured by Arenco n.v. Pharmaceutica in Belgium, and a formulation manufactured by the Faculty of Pharmacy of Mahidol University in Thailand) were investigated for their ex vivo blood schizontocidal activities against the K1 strain of Plasmodium falciparum. Blood schizontocidal activities of sera were evaluated using the maximum inhibitory dilution as a parameter. Sera obtained following the administration of the three formulations of dihydroartemisinin showed significantly distinct degree of ex vivo antimalarial activities. The differences may reflect the bioinequivalence between these three formulations of dihydroartemisinin. The ex vivo blood schizontocidal activity profiles generally coincided with plasma concentration-time profiles. Thus, the ex vivo model might be the useful tool for evaluating and comparing the bioequivalence of the interesting drugs especially where high-performance liquid chromatography with reductive electrochemical detection for drug analysis is not available. The effect of inoculum size of P. falciparum was shown in the ex vivo model as presented in the in vitro sensitivity test. To determine the effect of the inoculum size on the drug activity, the ex vivo model might be superior to the in vitro model since the pharmacokinetic profiles can be considered.

INTRODUCTION

Malaria remains a major public health problem worldwide due to the resistance of Plasmodium falciparum to the currently used antimalarial drugs. At present, artemisinin and its derivatives are the most promising antimalarials available for the treatment of malaria in many areas of the world, especially in the Southeast Asian region where multidrug-resistant P. falciparum exists.1,2 Dihydroartemisinin, which is the major active metabolite of all artemisinin derivatives, has proved to be more active against P. falciparum than the parent compounds.3–7 Since dihydroartemisinin is the most potent and least expensive to manufacture, it has the potential to be developed and used as a formulated drug. Dihydroartemisinin was originally produced in China as a tablet formulation and has been shown to be effective in the treatment of multidrug-resistant P. falciparum malaria in this country.8 Recently, dihydroartemisinin has been developed and used in Vietnam, Thailand, and the Netherlands, and has become available for clinical trials.9 The study of the pharmacokinetics of dihydroartemisinin in healthy Vietnamese subjects was reported by Le and others.10 It was found that the observed pharmacokinetics of oral dihydroartemisinin (Cotexin® manufactured in the People’s Republic of China) in this study were different from those previously investigated by Na-Bangchang and others using a dihydroartemisinin formulation manufactured in Belgium.11 The differences may reflect the bioequivalence between these two formulations of dihydroartemisinin. The observed bioequivalence of drugs in various drug formulations has been previously reported.12,13 Results indicated the influence of pharmaceutical preparations and/or formulations on the kinetics of the drugs. Despite the fact that several pharmaceutical formulations of dihydroartemisinin have been generated extensively, their relative bioequivalence has yet to be examined.

In the present study, ex vivo blood schizontocidal activities and bioequivalence of sera collected at various time intervals from 10 healthy Thai male subjects were investigated after the administration of the three oral formulations of dihydroartemisinin (Cotexin® from the People’s Republic of China, a formulation manufactured in Belgium, and a formulation manufactured by the Faculty of Pharmacy of Mahidol University in Thailand). One of the most important factors in determining drug sensitivity in vitro is the inoculum effect.14–20 The term of inoculum effect is defined as an increase in drug concentrations necessary to inhibit parasite growth with high percentages of parasitemia.15 The study of Duraisingh and others underlines the importance of inoculum size on in vitro sensitivity testing with artemisinin and dihydroartemisinin.20 They demonstrated that the 50% inhibition concentration (IC₅₀) of both drugs were increased depending on the increasing inoculum size. Despite the higher drug concentration achieved in vivo with treatment doses, the inoculum effect may occur because the absolute concentrations of parasitized erythrocytes are high. The possibility of an inoculum effect on ex vivo blood schizontocidal activities was also investigated in this study.

MATERIALS AND METHODS

Subjects. Ten healthy Thai male subjects (age range = 21–37 years, weight range = 43–64 kg) with no history of liver or kidney disease were recruited into the study. Written informed consent for participation was obtained from all subjects before initiation of the study. Individuals who smoked, drank alcoholic beverages, or were taking medications were excluded, and no other drugs were given during the period of study. On admission, these subjects were hospitalized overnight at the Bangkok Hospital for Tropical Diseases. Each was given a physical examination and the following tests were preformed: monitoring of heart rate, blood pressure, routine blood examinations (hematology and clinical chemistry), a 12-lead electrocardiogram, and urinalysis. The study was re-
viewed and approved by the Ethical Committee of Mahidol University.

**Drug administration and blood sampling.** Blood samples were collected before (0 hour) and 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 10 hours after administration of a single oral dose (300 mg) of the three different formulations of dihydroartemisinin from 10 healthy Thai male subjects. Formulation I was a single oral dose of 300 mg of dihydroartemisinin (20 mg per tablet, Coteclin®; Beijing Cotec New Technology Corp., Beijing Sixth Pharmaceutical Factory, Beijing, People’s Republic of China). Formulation II was a single oral dose of 300 mg of dihydroartemisinin (50 mg per tablet; Arenco n.v. Pharmaceutica, Geel, Belgium). Formulation III was a single oral dose of 300 mg dihydroartemisinin (100 mg per tablet; Dr. Nuttanan Sinschaipanid, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand).

The study was a cross-over, randomized design in which subjects were randomized to receive one of the three different formulations of dihydroartemisinin on three separate occasions. The wash-out period during each occasion of drug administration was seven days. During this study, all subjects were given the same diet and allowed to eat two hours after drug administration. Plasma samples for quantification of dihydroartemisinin concentrations were obtained by centrifugation (1,200 × g for 10 minutes) within five minutes of obtaining the blood sample and stored at −80°C until analysis. Serum samples for assessment of ex vivo antimalarial activities were obtained from the remaining portion of blood by centrifugation (1,200 × g for 10 minutes) immediately after clotting (approximately two hours) and stored at −80°C until analysis.

**Drug analysis.** Dihydroartemisinin concentrations in plasma were measured using high-performance liquid chromatography with reductive electrochemical detection (HPLC-ED) at Department of Medical Technology, Faculty of Allied Health Sciences at Thammasat University according to the method of Na-Bangchang and others.21

**Assessment of ex vivo blood schizontocidal activities of sera.** All serum samples were investigated for their blood schizontocidal activities against the chloroquine-resistant, dihydroartemisinin-sensitive K1 strain of *P. falciparum*. A 50% (v/v) cell suspension in complete RPMI 1640 medium with 1% ring stages of the K1 strain was used as the parasite inoculum. This suspension was then adjusted to a 3% cell suspension with complete RPMI 1640 medium and is referred to as the parasite inoculum.

Assessment of ex vivo blood schizontocidal activities of sera was performed using the in vitro microtechnique according to the method of Rieckmann and others22 with some modifications.23 Briefly, serum samples collected at each interval were serially diluted two-fold and six-fold with normal AB serum to dilutions of 1:2, 1:4, 1:6, 1:8, 1:12, 1:16, 1:24, 1:32, 1:48, 1:64, 1:96, and 1:128, respectively. Throughout the study, the same batch of pooled AB serum was used for the dilution. In the control wells, normal AB serum and serum collected prior to drug administration (hour 0) were used. Eighty microliters of parasite inoculum was added into each well containing 20 μL of undiluted and/or diluted serum samples. The final volume in each well of the microtiter plate was 100 μL. After gentle mixing, the microtiter plates were placed in a candle jar and incubated at 37°C for 48 hours.

**Evaluation of results.** Following serum exposure for 48 hours, thin blood smears were made from each well and stained with Giemsa. The number of infected erythrocytes and the morphology of parasites were examined under a light microscope. The assay was considered successful if at least a 4–5-fold increasing number of infected erythrocytes in the control wells was achieved. The number of infected erythrocytes with normal apparent parasites was counted per 10,000 erythrocytes. The effect of sera containing dihydroartemisinin on the parasite growth was assessed microscopically by both the decrease in parasite density and the viability of the remaining parasites. Ex vivo blood schizontocidal activities of sera were evaluated using the maximum inhibitory dilution (MID) as the parameter indicating antimalarial activity. The MID was the final dilution of sera containing drug at which complete inhibitory effect on parasite growth (at least 95% inhibition, IC95) was observed.

**Determination of inoculum effect on ex vivo blood schizontocidal activities of sera.** Due to the limited amount of serum samples, sera collected from seven healthy Thai male subjects after the administration of the three different formulations of dihydroartemisinin were selectively used to investigate the inoculum effect. The K1 strain of *P. falciparum* was used in this experiment. A parasite inoculum was prepared as a 3% cell suspension with 1% and 5% ring-stage parasitemias. The methodology and determination of the inoculum effect was performed using the same method used for assessment of ex vivo antimalarial activities, as described earlier in this report.

**Statistical analysis.** Pharmacokinetic characteristics for rate and extent of dihydroartemisinin absorption were the maximum plasma concentrations (Cmax), the time at which maximum plasma concentrations occurred (tmax), and the area under the plasma concentration-time curve (AUC). All pharmacokinetic parameters of dihydroartemisinin were analyzed using model-independent methods for non-normally distributed data.24 The tmax and Cmax were obtained directly from plasma concentration-time profiles. The area under the plasma concentration-time curve from zero time to the last observed time (AUC0-t) or certain specific time points were calculated using the linear trapezoidal rule for ascending data points and by the log trapezoidal rule for descending data points.

Ex vivo blood schizontocidal activity profiles, i.e., maximal MIDs, time to maximum MIDs, and area under the MID pattern (AUD) were analyzed using model-independent methods for non-normally distributed data.24 Maximum MIDs was determined by noting the highest MID (peak antimalarial activity) of sera collected from individual samples following the administration of each oral formulation of dihydroartemisinin. The time at which maximum MIDs observed and the maximum MIDs were obtained directly from ex vivo antimalarial activity profiles (MID patterns). The area under the MID pattern from zero time to the last observed time (AUD0-t) or certain specific time points were calculated using the trapezoidal rule for all data points.

Comparison of both pharmacokinetic parameters (i.e., Cmax, tmax, and AUC) of dihydroartemisinin and ex vivo antimalarial activity profiles (i.e., maximum MIDs, time to maximum MIDs, and AUD) of sera obtained following the administration of dihydroartemisinin between formulations was performed using the Mann-Whitney U test for non-
normally distributed data. The level of statistical significance was set at $P < 0.05$.

The consideration of bioequivalence of the three oral formulations of dihydroartemisinin was based on the two-sided $t$-test approach that included classic 95% confidence intervals.

**RESULTS**

**Morphologic changes of** *P. falciparum* **after exposure to sera.** In the wells containing sera collected before drug administration, as well as in the control (normal AB serum) wells, all parasite stages, i.e., rings, trophozoites, and schizonts, were found to be normal in appearance (Figure 1a). In contrast, no developmental growth of parasites was observed in the test wells after exposure to sera containing dihydroartemisinin at inhibitory level. In wells with complete inhibition, all parasites were inhibited at the ring stage. Most of dead rings had purple-stained pyknotic nuclei with no cytoplasm (Figure 1b). The wells that showed incomplete inhibition (at a higher titer of sera containing dihydroartemisinin) contained both viable and dead parasites. In these wells, only a few normal mature schizonts and rings were detected together with some dead trophozoites and dead schizonts. As for dead trophozoites, the contour of their nucleus could not be distinguished from their cytoplasm (Figure 1c). Dead schizonts had fewer nuclei when compared with normal appearance parasites in the control wells (Figure 1d). The nuclei were fragmented and the contour of cytoplasm was not sharply pronounced. The number of infected erythrocytes with normal-appearing parasites was increased, corresponding to an increase in the dilution of sera containing dihydroartemisinin. In the wells that showed no inhibitory effect, normal parasites were observed, most of them in the ring stage, and the number of infected erythrocytes increased from the initial number at time zero as that seen with the control wells.

**Ex vivo blood schizontocidal activities of sera.** The MID patterns of sera collected at various time intervals from 10 healthy Thai male subjects following the administration of the three oral formulations of dihydroartemisinin are shown in Figure 2. No inhibitory effect was observed in the undiluted sera collected prior to drug administration, as well as in the control wells. In most serum samples, activities of sera containing dihydroartemisinin for all three oral formulations were detectable in the first serum sample (30 minutes after drug administration). As for the MID pattern of Cotecxin®, a complete inhibitory effect was detected starting at 0.5–8 hours in most serum samples. The MIDs of individual samples varied throughout the investigation period, with the range of dilution between 1:1 and 1:16. The highest MID was achieved at three hours with a dilution of 1:16. After the peak of antimalarial activities, decreases in the MIDs were apparent, and no inhibitory effect was observed at 10 hours in all serum samples. Serum samples at a dilution <1:1 (undiluted serum) had no inhibitory effect. The MID pattern of the formulation manufactured by Arenco n.v. Pharmaceutica was generally similar to that of Cotecxin®. There were no differences in either the range of MIDs (1:1–1:16) and the highest MID (1:16). However, the formulation manufactured by Arenco n.v. Pharmaceutica reached the peak of antimalarial activities earlier than that of Cotecxin® (1.5–2 hours versus 3 hours). A highly variable MID pattern was observed for the formulation manufactured by the Faculty of Pharmacy of Mahidol University. The MIDs of individual samples varied throughout the investigation period, with the range of dilution between 1:2 and 1:64. The highest MID was achieved at 1.5 hours with a dilution of 1:64. After the peak of antimalarial activities, decreases in MIDs were observed.

The correlations of *ex vivo* blood schizontocidal activities (MIDs) and plasma concentration-time profiles of dihydroartemisinin in 10 healthy Thai male subjects following the administration of dihydroartemisinin for all three oral formulations are shown in Figure 3. *Ex vivo* blood schizontocidal activities of sera reflected by the MID patterns generally coincided with plasma concentrations of dihydroartemisinin. The MIDs were found to increase and corresponded to an

![Figure 1](image-url)
increase in plasma concentrations of the drug. Maximum MIDs was found within the period of time that concentration of dihydroartemisinin reached a peak in most subjects.

**Comparison of bioequivalence of the three oral formulations of dihydroartemisinin.** Statistical analysis of ex vivo antimalarial activity profiles and pharmacokinetic parameters of all three oral formulations of dihydroartemisinin using the Mann-Whitney U test are summarized in Table 1. Sera obtained following the administration of the formulation manufactured by the Faculty of Pharmacy of Mahidol University showed significantly higher median maximum MIDs than Cotecxin® and the formulation manufactured by Arenco n.v. Pharmaceutica (1:24 versus 1:7 and 1:6) \((P = 0.003)\). The times to maximum MIDs of sera obtained following the administration of dihydroartemisinin for all three formulations were not significantly different. The median AUD value of sera obtained following the administration of the formulation manufactured by the Faculty of Pharmacy of Mahidol University was significantly higher than those of the formulation manufactured by Arenco n.v. Pharmaceutica (54 versus 18) \((P = 0.033)\); however, it was not significantly different from that of Cotecxin®. A significantly higher median \(C_{\text{max}}\) was observed with sera obtained following the administration of the formulation manufactured by the Faculty of Pharmacy of Mahidol University than with Cotecxin® and the formulation manufactured by Arenco n.v. Pharmaceutica (894.5 versus 686.5 and 501 ng/mL) \((P = 0.028\) and 0.00043, respectively). The \(t_{\text{max}}\) of sera obtained following the administration of the formulation manufactured by the Faculty of Pharmacy of Mahidol University was significantly shorter than those of the other two formulations (1.5 hours versus 2 hours and 2 hours) \((P = 0.018\) and 0.022, respectively). A significantly lower median AUD was observed with sera obtained following the administration of the formulation manufactured by Arenco n.v. Pharmaceutica than with Cotecxin® and the formulation manufactured by the Faculty of Pharmacy of Mahidol University (1,597 versus 2,606 and 2,511 ng/mL) \((P = 0.049\) and 0.041, respectively). The median AUD of the dihydroartemisinin formulation manufactured by the Faculty of Pharmacy of Mahidol University was not significantly different from that of Cotecxin®. Statistical analysis did not show significant differences in maximum MID, AUD, \(C_{\text{max}}\), and \(t_{\text{max}}\) between sera obtained following the administration of Cotecxin® and sera obtained following the administration of dihydroartemisinin formulation manufactured by Arenco n.v. Pharmaceutica.

**Inoculum effect of K1 strains of *P. falciparum* on ex vivo blood schizontocidal activities of sera.** An increase in the inoculum size from 1% to 5% parasitemia affected ex vivo blood schizontocidal activities of sera. The MIDs of sera with a 5% parasitemia were lower than those with a 1% parasitemia at nearly all time intervals, with varying degrees depending on the parasites and the drug formulations.

The MID patterns of sera collected from healthy Thai male subjects following the administration of the three oral formulations of dihydroartemisinin against the K1 strain of *Plasmodium falciparum* using 1% and 5% parasitemias are shown in Figure 4. When a 1% parasitemia was used, the MIDs of sera obtained following the administration of Cotecxin® and the formulation manufactured by Arenco n.v. Pharmaceutica were

![Figure 2](attachment:figure2.png)

**FIGURE 2.** Patterns of maximum inhibitory dilution (MID) of sera collected after the administration of the three oral formulations of dihydroartemisinin against the K1 strain of *Plasmodium falciparum*. The dots below the dilution 1:1 represent undiluted serum samples that had no inhibitory effect. \(h = \) hours.
in the range of dilutions between 1:1 and 1:16. The highest MID of sera obtained following the administration of both formulations was 1:16. The MIDs of sera obtained following the administration of the formulation manufactured by the Faculty of Pharmacy of Mahidol University were in the range of dilutions between 1:1 and 1:64 and the highest MID was 1:64. When inoculum size of the K1 strain was increased from 1% to 5% parasitemia, the MIDs decreased at nearly all time intervals in most serum samples with all three formulations of dihydroartemisinin, especially during the peak of antimalarial activities. The highest MID of sera obtained following the administration of Cotecxin® and the formulation manufactured by Arenco n.v. Pharmaceutica decreased from a dilution of 1:16 to a dilution of 1:4, a four-fold decrease. The highest MID of sera obtained following the administration of Cotecxin® and the formulation manufactured by the Faculty of Pharmacy of Mahidol University decreased from a dilution of 1:64 to a dilution of 1:8, an eight-fold decrease. Two serum samples obtained following the administration of Cotecxin® and the formulation manufactured by the Faculty of Pharmacy, Mahidol University had no inhibitory effect at all time intervals, as shown by dots below the dilution 1:1.

Statistical analysis of ex vivo antimalarial activity (MIDs) profiles of sera against 1% and 5% parasitemias of the K1 strain (Table 2) showed that the MIDs of sera with a 5% parasitemia were significantly lower than those of sera with a 1% parasitemia with all three oral formulations of dihydroartemisinin (P < 0.05).

### Table 1

<table>
<thead>
<tr>
<th>Ex vivo antimalarial activity profiles and pharmacokinetic parameters</th>
<th>Dihydroartemisinin (300 mg)</th>
<th>Formulation manufactured by Arenco</th>
<th>Formulation manufactured by Faculty of Pharmacy, Mahidol University</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Maximum MIDs</strong></td>
<td>1:7 (1:2–1:16)</td>
<td>1:6 (1:2–1:16)</td>
<td>1:24† (1:8–1:64)</td>
</tr>
<tr>
<td><strong>Time to maximum MIDs (hours)</strong></td>
<td>1.75 (1–3)</td>
<td>1.5 (1.5–2)</td>
<td>1.5 (1.5–2)</td>
</tr>
<tr>
<td><strong>AUD</strong></td>
<td>25.5 (6–54)</td>
<td>18 (7–52)</td>
<td>54† (16.5–139)</td>
</tr>
<tr>
<td><strong>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</strong></td>
<td>686.5 (232–703)</td>
<td>501 (211–740)</td>
<td>894.5§ (555–1,020)</td>
</tr>
<tr>
<td><strong>t&lt;sub&gt;max&lt;/sub&gt; (hours)</strong></td>
<td>2 (1.5–2)</td>
<td>2 (1.5–2)</td>
<td>1.5§ (1.5–2)</td>
</tr>
<tr>
<td><strong>AUC (ng/hour/mL)</strong></td>
<td>2,606# (450–2,907)</td>
<td>1,597 (556–2,172)</td>
<td>2,511** (1,186–3,559)</td>
</tr>
</tbody>
</table>

* Values are medians (ranges). MID = maximum inhibitory dilution; AUD = area under the MID pattern; AUC = area under the plasma concentration time curve; CI = confidence interval.
† Significantly different from Cotecxin® (P = 0.003, 95% CI = 0.002, 0.003) and formulation produced by Arenco (P = 0.003, 95% CI = 0.001, 0.003).
‡ Significantly different from formulation produced by Arenco (P = 0.033, 95% CI = 0.026, 0.033).
§ Significantly different from Cotecxin® (P = 0.028, 95% CI = 0.023, 0.030) and formulation produced by Arenco (P = 0.000, 95% CI = 0.000, 0.000).
¶ Significantly different from formulation produced by Arenco (P = 0.009, 95% CI = 0.007, 0.011).
# Significantly different from formulation produced by Arenco (P = 0.049, 95% CI = 0.047, 0.055).
** Significantly different from formulation produced by Arenco (P = 0.041, 95% CI = 0.043, 0.051).
DISCUSSION

Dihydroartemisinin was effective against all asexual blood stages of *P. falciparum*. This observation was consistent with those of previous reports.\textsuperscript{25–28} It was found that all three formulations of dihydroartemisinin caused similar morphologic changes in *P. falciparum*. Ring, trophozoite, and schizont stages showed abnormalities in both nuclei and cytoplasm, as shown in Figures 1b–d. The *ex vivo* blood schizontocidal activities of sera obtained following the administration of the three oral formulations of dihydroartemisinin observed in the present study were consistent with those previously reported, in which the rapid onset and short duration of activity were observed.\textsuperscript{11,29,30} As shown in Figure 2, the inhibitory dilution (MID) of sera collected after the administration of the three oral formulations of dihydroartemisinin against the K1 strain of *Plasmodium falciparum* at 1% (left) and 5% (right) parasitemia. The dots below the dilution 1:1 represent undiluted serum samples that had no inhibitory effect. h = hours.

\textbf{TABLE 2} *Ex vivo* blood schizontocidal activity (MIDs) profiles of sera obtained following the administration of the three oral formulations of dihydroartemisinin against the K1 strain of *Plasmodium falciparum* with 1% and 5% parasitemias

<table>
<thead>
<tr>
<th>% Parasitemia of <em>P. falciparum</em></th>
<th>Formulation manufactured by Arenco</th>
<th>Formulation manufactured by Faculty of Pharmacy, Mahidol University</th>
</tr>
</thead>
<tbody>
<tr>
<td>1%</td>
<td>8 (2–16)</td>
<td>8 (4–16)</td>
</tr>
<tr>
<td>5%</td>
<td>2† (2–4)</td>
<td>2‡ (2–4)</td>
</tr>
</tbody>
</table>

* Data are medians (ranges). MID = maximum inhibitory dilution; CI = confidence interval.
† Significantly different from 1% parasitemia (*P* = 0.003, 95% CI = 0.002, 0.004).
‡ Significantly different from 1% parasitemia (*P* = 0.001, 95% CI = 0.000, 0.002).
tory effect of sera obtained following the administration of dihydroartemisinin in all three oral formulations were detectable in the first serum sampling (30 minutes after drug administration). From 0.5 to 4 hours, the MIDs were highly variable, especially during the peak antimalarial activities. The lesser variable MIDs were found at 6–10 hours in most serum samples. This indicates the rapid decrease in \textit{ex vivo} blood schizontocidal activities of dihydroartemisinin. Such a high variation in MIDs during the peak antimalarial activities might be affected by the variability among individual subjects in the process of drug absorption from the gastrointestinal tract, resulting in a difference in achieving the peak drug concentration in the blood.\cite{11}

The \textit{ex vivo} blood schizontocidal activity profiles of sera collected from healthy Thai male subjects after the administration of the three oral formulations of dihydroartemisinin observed in this study generally coincided with the plasma concentration-time profiles of dihydroartemisinin measured using HPLC-ED. Dihydroartemisinin concentrations in plasma were detectable in the first sampling (30 minutes after drug administration) with all three oral formulations. The peak antimalarial activities of sera were observed during the time to reach the peak plasma concentrations of dihydroartemisinin with all three oral formulations.

The present study has demonstrated the applicability of the \textit{ex vivo} model in evaluating the bioequivalence of the three oral formulations of dihydroartemisinin based on antimalarial activity. The results, in conjunction with previous findings, suggest that the pharmaceutical preparations and/or formulations have an effect on antimalarial activity and pharmacokinetic profiles of the drug.\cite{12,13,32} Evaluation of bioequivalence of various drug formulations in the previous studies relied on statistical analysis of their pharmacokinetic parameters. In the present study, bioequivalence of the three oral formulations of dihydroartemisinin was based on statistical analysis of \textit{ex vivo} blood schizontocidal activity profiles using the Mann-Whitney U test. The results observed in this study clearly indicate that the formulation manufactured by Arenco n.v. Pharmaceutica was not bioequivalent to Cotecxin\textsuperscript{®} and to the formulation manufactured by the Faculty of Pharmacy of Mahidol University. As shown in Table 1, the formulation manufactured by Arenco n.v. Pharmaceutica showed significantly lower \textit{ex vivo} antimalarial activity profiles (maximum MIDs, time to maximum MIDs, and AUD) and pharmacokinetic parameters (C\textsubscript{max}, t\textsubscript{max}, and AUC) than Cotecxin\textsuperscript{®} and the formulation manufactured by the Faculty of Pharmacy of Mahidol University. These results indicate the lower rate (represented by C\textsubscript{max} and t\textsubscript{max}) and extent (represented by AUC) of absorption of the dihydroartemisinin formulation manufactured by Arenco n.v. Pharmaceutica when compared with the other two oral formulations. The dihydroartemisinin formulation manufactured by the Faculty of Pharmacy of Mahidol University showed significantly higher maximum MIDs, C\textsubscript{max}, and t\textsubscript{max} values than those of Cotecxin\textsuperscript{®}. However, the median AUD and AUC values observed with the dihydroartemisinin formulation manufactured by the Faculty of Pharmacy of Mahidol University were not significantly different from those of Cotecxin\textsuperscript{®}. These results indicate that dihydroartemisinin formulation manufactured by the Faculty of Pharmacy of Mahidol University showed a greater rate of drug absorption than that of Cotecxin\textsuperscript{®}; however, the extent of drug absorption of these two formulation was not significantly different.

The differences in \textit{ex vivo} antimalarial activities and pharmacokinetic parameters of these three oral formulations of dihydroartemisinin are probably due to the differences in tablet excipients or crystal modification of each formulation. The kinetics of dihydroartemisinin release (rate and extent of dissolution) from each formulation as it passes through the gastrointestinal tract might reflect the rate and extent of drug absorption. Modification of the pharmaceutical formulation of dihydroartemisinin to improve drug dissolution, while providing high and sustained plasma concentration, e.g., a sustained release formulation, could be an alternative approach to achieve immediate and prolonged therapeutic concentration of dihydroartemisinin in blood circulation.\cite{33} The \textit{ex vivo} model used for the determination of antimalarial activities of sera observed in this study has been shown to be an useful alternative tool for evaluating and/or comparing the bioequivalence of interesting drugs, especially in laboratories where facilities for drug analysis by HPLC-ED are limited. Additionally, the determination of bioequivalence based on antimalarial activity using \textit{ex vivo} experiment is much simpler and cheaper than investigating pharmacokinetic characteristics of the drug using the HPLC-ED method.

The present study clearly demonstrated the influence of inoculum sizes of \textit{P. falciparum} on \textit{ex vivo} blood schizontocidal activities of sera obtained following the administration of the three oral formulations of dihydroartemisinin. By increasing inoculum sizes from 1% to 5% parasitemia, the MIDs of sera were decreased at most time intervals with varying degrees depending on the parasite strains and the drug formulations. The observation of an inoculum effect in the present study was consistent with the effect reported in \textit{in vitro} studies.\cite{14,15,16,17,18,19,20} The results of the inoculum effect on antimalarial activities of sera containing dihydroartemisinin have emphasized the importance of inoculum size on the \textit{ex vivo} drug sensitivity. These findings might have a critical role in considering drug regimens for patients who have high parasitemia levels, if the hypothesis of an inoculum effect is shown to be true. At the present time, it is still uncertain whether drug regimens for patients who have high parasitemia levels should be adjusted. Many other factors, which are important \textit{in vivo} but cannot be investigated \textit{ex vivo} or \textit{in vitro}, may contribute to and/or have an influence on evaluating the inoculum effect. Such factors, as reported by Gluzman and others, include the role of cell-mediated and humoral immunity and the mechanical role of the spleen in removing parasite from the blood.\cite{15} Whether the inoculum effect occurs \textit{in vivo} has yet to be examined. The \textit{ex vivo} model used for determination of inoculum effect on dihydroartemisinin activity observed in the present study might be superior to the \textit{in vitro} model since an importance factor (pharmacokinetics) was considered. Nonetheless, the determination of \textit{ex vivo} drug sensitivity of malarial parasites should be conducted using standardized parasitemias to avoid the effect of inoculum size.

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