

Open clinical trial: Plasma levels of artemether and dihydroartemisinin for two commercially available artemether intramuscular injections.

Principal investigator: L. K. Penali.

Abstract

To 23 patients with non-complicated malaria, a single dose of artemether intramuscular (4 mg/kg) was administered. Blood samples were withdrawn over a 24 hour period, at 0, 1, 2, 4, 6, 8, 12 and 24 hours. The blood plasma levels of artemether and its principal metabolite dihydroartemisinin were determined by LC-MS.

The patients were treated randomly with two different preparations of artemether im, ie. Artesiane 80 (Dafra Pharma, Belgium), Paluether 80 (Aventis-Sanofi, France).

No significant differences in pharmacokinetic behaviour were found.

Initial reports from a Thai Group suggested that individual artemether plasma concentration profiles are relatively uniform with high peak concentrations of 500 ng/ml achieved around 4 hours after administration. In these reports a rapid and prompt biotransformation of artemether into dihydroartemisinin was also described.

However, research by others groups has not been able to verify this data and these researchers have instead come up with data that are in line with our study. The reports have shown that the absorption of artemether is slow (7- 8 hours before Tmax) and very variable. Furthermore, in all studies the amount of DHA found is very low, and never more than 10 % of the level of artemether. In theory, this would be expected since there is no first-pass metabolism after im administration.

The plasma levels obtained in this study are above the IC50 reported for artemether and dihydroartemisinin (1-6 nm or 0.3 – 1.8 ng/ml) within one hour and stay like this throughout the observed 24-hour period.

The longer period of elevated plasma levels for artemether after intramuscular artemether might explain the finding that the recrudescence and reinfection after a full artemether im treatment course is less likely than with oral artesunate (Uvira, data on file Dafra).

Study Site: Anonkoua-Kouté (Abidjan), Ivory Coast.

Experimental Procedure: Open Controlled Clinical Trial

Principal Investigator: Dr. O. André Touré

Study Director Dr L. Koné PENALI

Co-Investigators: Dr E. W. BEUGRE
Dr AKA WOGNIN

Data Management and Analysis: Mr T. SERY

Sponsor: DAFRA PHARMA

Pharmaceutical Support: DAFRA PHARMA

External Monitor: F. Herwig Jansen, M.D., Ph.D.
Fhj@dafra.be

Start of the study: July 2004

Duration of study: 1 month

Number of patients included: 23

Collaborating Institutions:

- a) UNITE DE PALUDOLOGIE- INSTITUT PASTEUR D'ABIDJAN
01 BP 490 ABIDJAN 01 – COTE D'IVOIRE

- b) DAFRA PHARMA
Steenweg op Mol 148
2360 Oud-Turnhout
Tel. ++/32/14/61 78 20
Fax ++/32/14/61 78 59
E-mail: info@dafra.be; Fhj@dafra.be

1. INTRODUCTION

Artemether, an artemisinin derivative, is available in various formulations suitable for the treatment of complicated and non-complicated malaria.

We decided to study if there is a difference in plasma levels of the active compounds of two commercially available intramuscular artemether injections and to compare the results with literature. Since the available literature data is primarily based on research on Asian subjects, with this new study we could generate data in an African population.

The two products used were Artesiane (artemether in miglyol) from Dafra and Paluether (artemether in arachis oil) from Aventis-Sanofi.

Next to the present study, we studied the clinical efficacy and tolerability of the Artesiane i.m. injection in the treatment of non-complicated malaria with 30 subjects. This study will be reported separately.

2. OBJECTIVES

2.1. Principal Objective

To determine the plasma levels of artemether and dihydroartemisin after administration of an artemether injection in a single dose of 4 mg/kg in patients with non-complicated malaria.

2.2. Secondary Objectives

- none

3. METHODOLOGY

3.1. Study Site: The study site is Anonkoua-Kouté. Ivory Coast is a country located in West Africa. Malaria is endemic (hypo- to hyperendemic), but great variation exists according to the area. Malaria transmission is mainly seasonal and most of the transmission occurs during the rainy season.

Anonkoua-Kouté is a peri urban area of Abidjan, the capital city of Ivory Coast. *P. falciparum* is a predominant species, accounting for more than 95% of malaria cases.

3.2. Study Period: The study was done in July 2004 during the rainy season.

3.3. Study Design:

The study was an open study. The plasma levels for artemether and its principal metabolite dihydroartemisinin were determined.

3. 4. Target Population: Subjects living in the study area and aged from 16 years or more, with an acute uncomplicated *P. falciparum* malaria.

3. 5. Inclusion and Exclusion Criteria

3.5.1. Inclusion criteria:

- Age \geq 16 years
- Weight \geq 35 kg
- To be a resident of Anonkoua-kouté during the study period.
- Axillary temperature \geq 37,5 °C or report of fever within the last 24 hours.
- Monospecific infection with *P. falciparum* between 1000 and 100 000/ μ L blood.
- Declaration of informed consent or permission from participant or parents/guardians for minors.

3.5.2. Exclusion criteria:

- Presence of severe or complicated malaria (according to the WHO 2000).
- Presence of severe concomittant illness or any other illness that required a treatment non-compatible with this study.
- Allergy to study drugs
- Pregnancy (detected clinically, or with β HCG test)
- Use of any component of the study drugs within the last 28 days prior to the inclusion.

3. 6. Sample Size: 23 patients were included.

3. 7. Data Collection Methods: Data were collected directly into the Case Report Form (CRF) for clinical information. For laboratory exams, data were recorded in Source documents and then recorded into the CRF later.

3.8. Study Products: Artesiane[®] im injection was a gift from Dafra pharma in Belgium. It contains 80 mg/ml artemether in miglyol. Paluether[®] im injection was bought in Abidjan. It contains 80 mg/ml artemether in arachis oil.

3. 9. Treatment Procedure: All treatments were administered to the study site's health center by the investigator. Artemether was administrated as 4 mg/kg at time 0, in a single injection in the outer upper quadrant fo the quadriceps muscle.

3.10 Sample storage and shipping

Immediately after collection, the samples were centrifuged and the plasma fraction refrigerated at - 20 °C. At the end of the study all samples were packed and transported by special refrigerated air freight to Belgium. Upon receipt in Belgium, the samples were stored at - 20 °C, until the time of analysis.

3.11 Analysis of plasma levels by LC-MS

A sensitive and selective method was developed for the determination of artemether and its active dihydroartemisinin metabolite in human plasma using artemisinin as internal standard. The method consists of a liquid-liquid extraction with subsequent evaporation of the supernatant to dryness followed by the analysis of the reconstituted sample by liquid chromatography-mass spectrometry (LC-MS) in single ion monitoring mode using atmospheric pressure chemical ionization (APCI) as an interface. Chromatography was performed on a C₁₈ column using gradient mixtures of water-formic acid 0.1 % (A) & acetonitrile-formic acid 0.1% (B) as mobile phases.

The gradient scheme is presented below:

0 - 8 min	45:55 = A: B
8 -10 min	45:55 = A:B → 100 B
10 -16 min	100 B
16 - 18 min	100 B → 45:55 = A:B
18-30	45:55 = A:B

The method was validated over a concentration range of 5–200 ng/ml using 1.0 ml of human plasma per assay. The method was applied to the quantification of artemether and its metabolite in human plasma.

The quantity of artemether and DHA is calculated in relation to the internal artemisinin standard.

Sample preparation:

To 1 ml of a plasma sample 20 µl of internal artemisinin standard solution (10 µg/ml artemisinin) was added, followed by 0.5 ml of a saturated NaCl-solution. The sample is vortexed and extracted with 5 ml isooctane/ethylacetate 7/3. The complete sample is vortexed for 1 minute. The samples are centrifuged at 2000 rpm for 5 minutes. The upper phase is transferred into a second sample tube and evaporated. The residue is dissolved in 200 µl mobile phase (45/55 water-formic acid 0.1 % : acetonitrile–formic acid 0.1%)

3.12. Data Management, Quality Assurance and Quality Control:

All of the study personnel was trained in GCPs (Good Clinical Practices) and GLPs (Good Laboratory Practicies) with respect for conducting the study according to the protocol and the principles of ethics.

Data were collected on standardized data collection forms and standard laboratory notebooks. At the end of the day, lab and clinical personnel checked the completeness, readability range and consistency of the data in the recorded forms and corrected them if necessary. The study was monitored on regular basis by senior investigators.

3.13. Ethical Considerations: Institutional Ethical Committee of Dafra and of the Pasteur Institute, Abidjan, Ivory Coast approved the protocol and the consent form. Individual written consent were obtained from all participants or parents/legal guardians (for minors) prior to the inclusion.

4. RESULTS

4.1 Baseline patient characteristics at time of admittance.

Artesiane:

Patient number	Sexe	Age (years)	Parasitemia (/ μ l blood)
1	F	16	2000
2	F	35	5280
5	F	21	3280
12	M	25	2560
13	F	22	4160
17	M	16	10720
18	F	20	8160
19	M	31	6280
21	M	26	2560
22	F	32	2800
23	F	30	3760
Range	7 F – 4 M	16 - 35	2560 - 10720
Average		25 \pm 7	4687 \pm 2726

Paluether

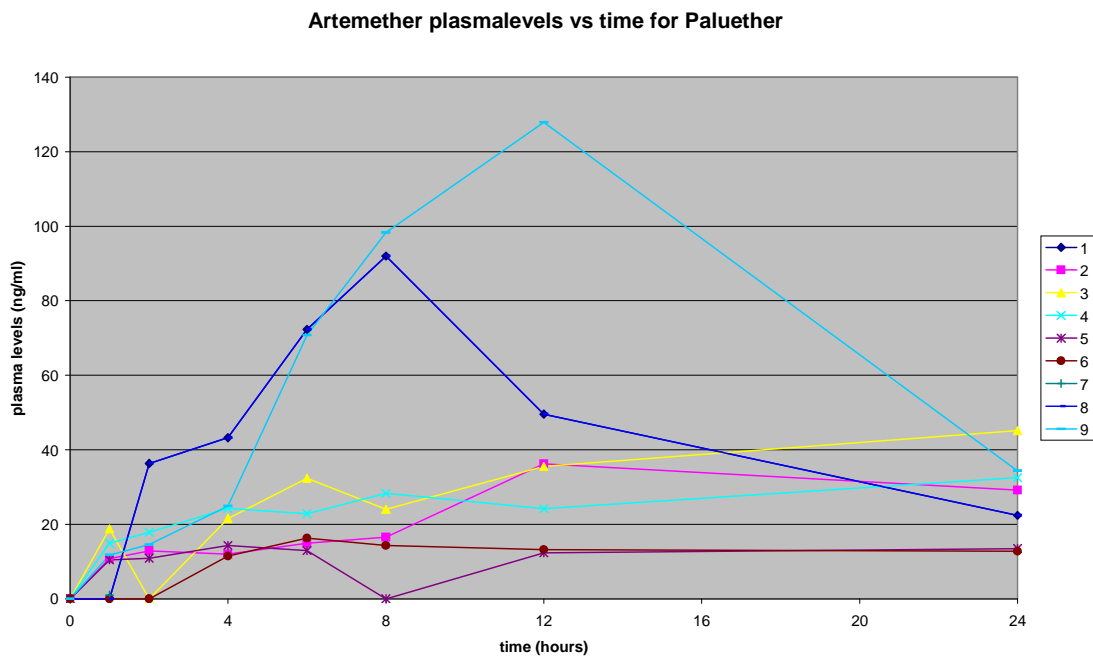
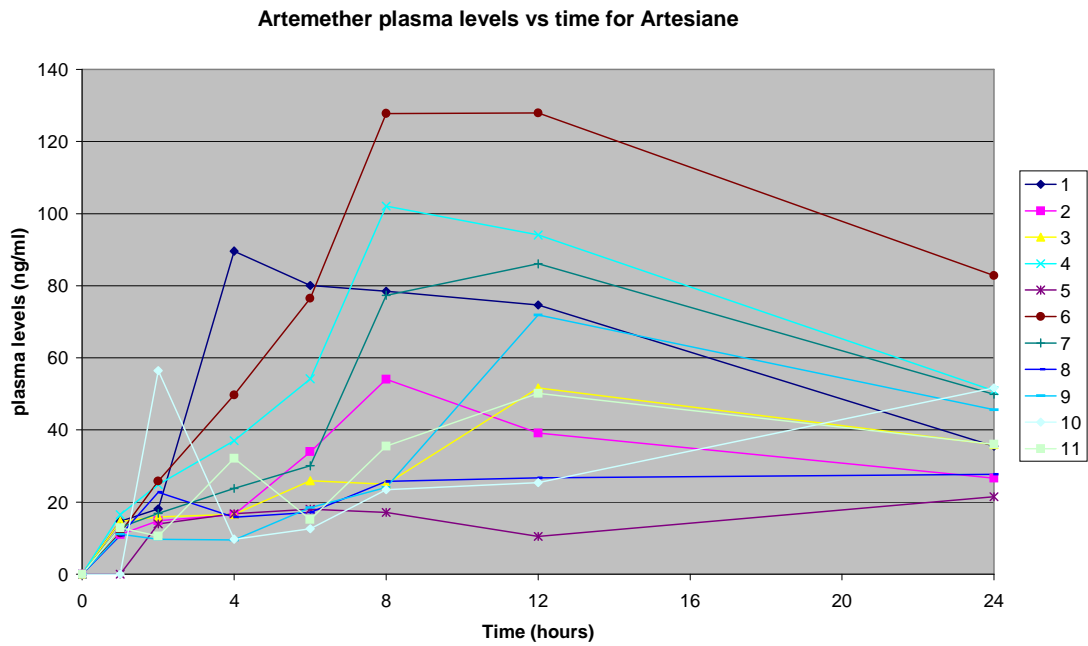
Patient number	Sexe	Age (years)	Parasitemia (/ μ l blood)
3	F	16	31360
4	F	18	14240
8	F	32	56880
9	F	16	12240
10	F	37	6640
11	M	30	5120
15	M	17	7680
16	F	31	2160
20	F	26	5600
Range	7 F – 2 M	16 - 37	2160 - 56880
Average \pm SD		25 \pm 8	15769 \pm 17673

Patients 6 & 7 whom were given artesiane 80 did not show up anymore after drug administration. Patient 14, whom was given paluether, was excluded later on, because the parasitemia was higher than 100.000.

Between the two groups the age and sexe-distribution were similar. The parasite count in the group receiving paluether was higher. (two sample t-test= 0.05)

4.2. Plasma levels of individual patients

The determined plasma levels of artemether obtained with the two different formulations are presented in the following 2 graphs. The plasma levels of dihydroartemisinin stayed very low throughout the study and are not displayed separately.



4.3. Average plasma levels

A graph of the average plasma levels of both formula's is given below. Furthermore the results are presented in tabulated format.

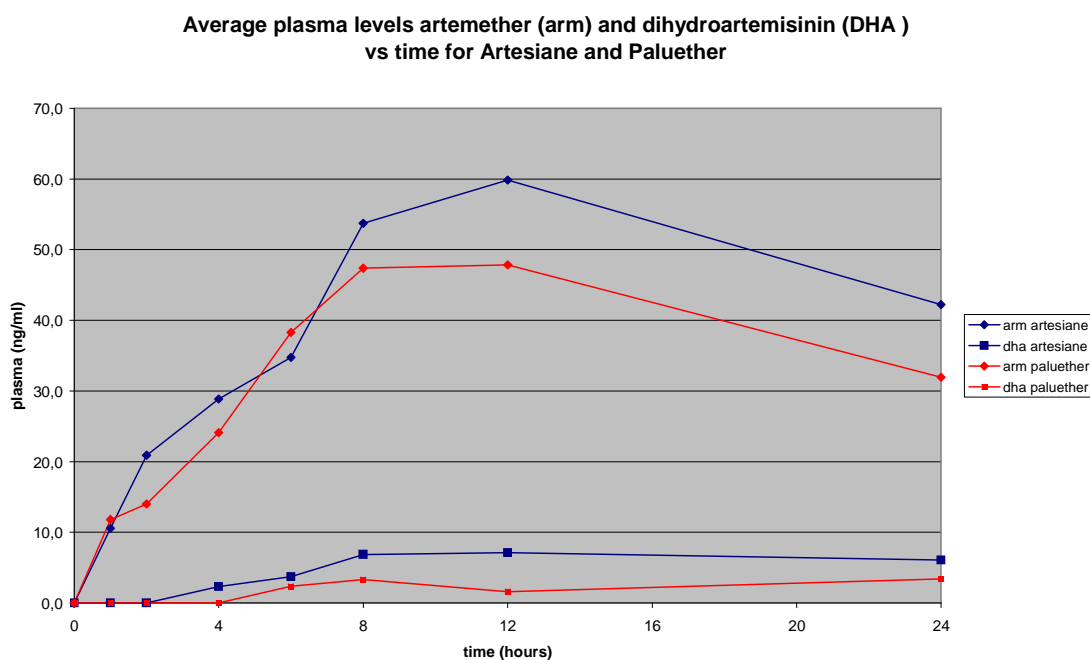


Table: Plasma levels of artemether and dihydroartemisin (\pm SD)

Timepoint	Artesiane [®]		Paluether [®]	
	Artemether (ng/ml)	DHA (ng/ml)	Artemether (ng/ml)	DHA (ng/ml)
t = 0	0.0	0.0	0.0	0.0
t = 1	10.6 \pm 5.5	0.0	11.8 \pm 8.2	0.0
t = 2	20.9 \pm 12.9	0.0	14.0 \pm 10.8	0.0
t = 4	28.9 \pm 23.6	2.3 \pm 7.7	24.1 \pm 11.6	0.0
t = 6	34.8 \pm 24.5	3.7 \pm 9.1	38.3 \pm 24.6	2.4 \pm 4.7
t = 8	53.7 \pm 37.5	6.9 \pm 13.1	47.4 \pm 38.1	3.3 \pm 6.6
t = 12	59.8 \pm 34.9	7.1 \pm 13.4	47.8 \pm 37.7	1.6 \pm 4.9
t = 24	42.2 \pm 16.7	6.1 \pm 10.6	31.9 \pm 14.6	3.4 \pm 6.9

The graph and the table indicate that the two formulations give a similar result.

4.4. Tmax

The Tmax for both formulations is around 12 hours, but due to the limited amount of sampling points it can not be excluded that the real Tmax is slightly later.

4.5 Cmax

The Cmax for artemether and its metabolite dihydroartemisinin for both formula's are presented in the table below:

Preparation	C max (µg/ml)
Artesiane	Artemether: 59.8 (18 – 128)
	DHA: 7 (0 – 40)
Paluether	Artemether: 47.8 (14 – 128)
	DHA: 3 (0 – 18)

The table shows that the Cmax is comparable.

5. Discussion and conclusion

To 23 patients, suffering from non-complicated malaria, a single dose of artemether intramuscular (4 mg/kg) was administered. Blood sampling was done at 0, 1, 2, 4, 6, 8, 12 and 24 hours. The plasma levels of artemether and its principal metabolite dihydroartemisinin were determined by LC-MS.

Summarizing, in this trial no differences were observed between the two formulations.

Initial reports from a Thai Group (Karbwang, 1997 & 1998) suggested that individual artemether plasma concentration profiles are relatively uniform with high peak concentrations of 500 ng/ml achieved around 4 hours after administration. In these reports a rapid and prompt biotransformation of artemether into dihydroartemisinin was also described.

However, research by others groups (Silamut, 2002; Mithwani, 2003; Teja-Isavadharm, 1996) has not been able to verify this data and has instead come up with data that are in line with our study. The reports showed that the absorption of artemether is slow (7- 8 hours before Tmax) and very variable. Furthermore in all studies the amount of DHA found is very low, and never more than 10 % of the level of artemether. In theory, this would be expected since there is no first-pass metabolism after i.m. administration.

The plasma levels obtained in this study are above the IC50 reported for artemether and dihydroartemisinin (1-6 nm or 0.3 – 1.8 ng/ml) within an hour and stay like this throughout the observed 24-hour period.

The longer period of elevated plasma levels of artemether after intramuscular artemether might explain the finding that the recrudescence and reinfection after a full artemether im treatment course is less likely than with oral artesunate (Uvira, data on file Dafra)

Although the carrier oil in which the artemether is dissolved for both commercial formulations is different, miglyol or arachis oil, no difference in plasma levels of artemether were detected. This indicated that most probably that artemether itself is the limiting factor in absorption from the site of injection and not the formulation. This is in contrast with artemether where it was suggested that the sesame oil used in the formula makes that tmax is extended (Looareesuwan, 2002)

Summarizing, the present study confirms the findings obtained in Asia in African adults.

6. ACKNOWLEDGMENTS

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We are grateful to Dafra Pharma, Belgium for providing the drugs, financial and technical support.

7. LITERATURE CITED

Alin et al (1990) In vitro activity of artemisinin, its derivatives and pyronaridine against different strains of *Plasmodium falciparum*. Trans. R. Soc. Trop. Med 84, 635-637.

Dai. et al (1994) A comparative study of artemether injection and artesunate tablets in *P.falciparum* malaria patients in Vietnam. In house report Dafra Pharma ltd.

Huda et al (2003) A comparative trial of artemether and quinine in children with severe malaria. Downloaded from www. Indianpediatrics.net on 16-01-2004.

Karbwang et al (1997) Pharmacokinetics and bioavailability of oral and intramuscular artemether. Eur. J. Clin. Pharmacol 52, 307-310

Karbwang et al (1998) Pharmacokinetics of intramuscular artemether in patients with severe *falciparum* malaria with or without acute renal failure. Br. J. Clin. Pharmacol 45, 597-602.

Looareesuwan et al (2002) Dose-finding and efficacy study for i.m. artemotil (beta-arteether) and comparison with i.m. artemether in acute uncomplicated *P. falciparum* malaria. Br.J.Clin.Pharmacol. 53, 492-500.

Mithwani et al (2003) Population pharmacokinetics of artemether and dihydroartemisinin following single intramuscular dosing of artemether in African children with severe falciparum malaria. Br. J. Clin. Pharmacol. 57, 146-152

Silamut et al (2003) Artemether bioavailability after oral or intramuscular administration in uncomplicated falciparum malaria. Antimicrob. Agents Chemother. 47, 3795-3798.

Souppart et al (2002) Development and validation of a high-performance liquid chromatography-mass spectrometry assay for the determination of artemether and its metabolite dihydroartemisinin in human plasma. J. Chromatogr. B. Analyt Technol Biomed Life Sci 774, 195-203.

Teja-Isavadharm et al (1996) Comparative bioavailability of oral, rectal, and intramuscular artemether in healthy subjects: use of simultaneous measurement by high performance liquid chromatography and bioassay. Br. J. Clin. Pharmacol. 42, 599-604.

Taylor et al (1998) Intramuscular artemether vs intravenous quinine: an open randomized trial in Malawian children with cerebral malaria. Trop.M. Int. Health. 3, 3-8.