ARTESUNATE AND AMODIAQUINE: TOLERABILITY AND DRUG INTERACTION STUDY IN HEALTHY NORMAL VOLUNTEERS

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List of abbreviations

AQ	amodiaquine
AS	artesunate
AST	serum aspartate transferase
AUC	area under the concentration-time curve
Beta-HCG	beta-human chorionic gonadotrophin
Cmax	maximum plasma concentration
D-AQ	desethylamodiaquine
DHAS	dihydroartemisinin
ECG	electrocardiogram
HPLC	High Performance Liquid Chromatographic method
PK	Pharmacokinetic
Thalf	effective half-life
Tmax	time to maximum plasma concentration
UV	ultraviolet

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1.0 Summary

Resistance of the malaria parasite to treatment is increasing globally. One method of delaying this may be through the use of combination therapy with an artemisinin derivative and a standard antimalarial drug. In this randomised crossover study the anti-malarials amodiaquine (AQ) and artesunate (AS) were given to 15 normal healthy volunteers, both as individual agents and together, over three study phases.

Serial plasma samples were taken for determination of pharmacokinetic (PK) parameters of the drugs: maximum plasma concentration (Cmax), time to achieve Cmax (Tmax) and the area under the concentration time curve (AUC). These were determined for the drug combination and compared to the drugs given individually. The parameters of the major, active metabolites of the parent drugs, dihydroartemisinin (DHAS) and desethylamodiaquine were calculated as well. The tolerability of the combination was assessed in the volunteers over the study period.

Thirteen subjects completed the study. Two subjects were withdrawn; one after generalised flushing was experienced shortly after amodiaquine dosing and the other due to concomitant administration of fluoxetine.

There were no significant changes in the PK parameters of AS when given alone or in combination with AQ. For DHAS, there was a significant decrease in the Cmax (446.2 vs 844.5 ng/ml, p=0.01), a significant prolongation of the half-life of DHAS (2.12h vs 1.67h, p=0.01), and a significant decrease in the AUC to 24 hours (1229 vs 1743 ng.h/ml, p=0.03) with the AS-AQ combination compared to AS alone. The AUC to infinity was not significantly different.

Compared to AQ given alone, there was a significant decrease in the AQ Cmax (22.9 ng/ml vs 30.01 ng/ml, p=0.03) and AUC_{0-24} (114.6 vs 171.1 ng.h/ml, p=0.04) in the AQ-AS combination. Comparing AQ-AS with AQ alone, D-amodiaquine reached its Tmax earlier (2.25 vs 3.63h, p=0.007) and had a shortened half-life (135.6 vs 242.0h, p=0.03). The AUC values at 24 hours and infinity did not change significantly.

The total drug exposure to both parent drugs and metabolites was similar, when these drugs were given alone or together. It is difficult to conclude from the PK data of this study, what impact the PK interactions might have on parasite killing. However, clinical data have shown that AQ-AS is more effective than AQ alone. Therefore, this interaction is of little if any clinical significance. Minor adverse events were common; and four moderately severe adverse events [neutropenia (n=2), hypersensitivity (n=1) and hepatitis (n=1)]. The small sample size of this study precludes a definitive analysis of safety. Further study of safety and efficacy in malaria patients is warranted.

2.0 Introduction

Resistance of *Plasmodium falciparum* to almost all antimalarial drugs has become widespread in Southeast Asia, Africa and South America (Wernsdorfer and Payne, 1991). The only exception to this situation is with the artemisinin derivatives which have recently been introduced for widespread use in Southeast Asia (Price, 2000). Cross-resistance between compounds of the same class magnifies the problem.

Antifolate resistance in both *P.falciparum* and *P.vivax* developed rapidly following the introduction of pyrimethamine in many parts of the tropics (WHO, 1984). The fixed combination of sulfadoxine/pyrimethamine has been very successful, but is no longer effective in many areas of Southeast Asia (White, 1992) and its efficacy is declining in East and West Africa (Garcia-Vidal et al, 1989). Mefloquine resistance was reported even before the drug had been routinely used because of cross resistance with quinine (Brasseur et al, 1990). Resistance to mefloquine has now severely curtailed its efficacy in several parts of Southeast Asia (Nosten et al, 1991; Fontanet et al, 1993). Halofantrine resistance has emerged concomitantly (Basco et al, 1991; Brasseur et al, 1992). When atovaquone was used alone in preliminary clinical trials, 28% of patients had recrudescent infections associated with the development of resistance (Looareesuwan et al, 1996). Quinine and chloroquine have enjoyed longer periods of effective use, but in almost all malaria endemic areas, chloroquine is no longer effective treatment for falciparum malaria (Wernsdorfer, 1991) and *P. vivax* has developed resistance in some parts of Oceania (Murphy et al, 1993). Quinine effectiveness or resistance too, is threatened in some parts of the world (Pukrittayakamee et al, 1994).

In the absence of new and effective methods of vector control and the limited prospects for a rapid development of safe and effective malaria vaccines (Playfair et al, 1990; Nosten et al, 1996), drugs retain a key role in the control of malaria, particularly in preventing death and reducing morbidity. This situation has stimulated research into better and more effective usage of the currently available drugs, the development of new novel antimalarial agents and the introduction of drug combinations. Recently White and Olliaro (1996) reviewed the rationale for combination chemotherapy for malaria. Essentially, while waiting for new drugs to be discovered and hopefully deployed,

appropriate measures such as drug combinations should be taken to safeguard the few compounds available for the treatment of malaria.

3.0 Background

Resistance is the prime determinant of drug's lifespan, and protection of drugs against the development of resistance is a key factor in the fight against malaria. The WHO TDR has initiated research into drug resistance and policies, which will primarily generate accurate data to inform policy makers and reform current policies based on research results.

Drug policies play an important role in the emergence and spread of resistance. Thus far, drugs have been used alone (single agent chemotherapy) and exploited to the point where they become ineffective; then a new drug introduced. Such an attitude assumes unlimited resources and indefinite availability of new drugs. However the pharmaceutical industry does not consider the malaria market profitable, and new drugs are not readily available.

Amongst the various activities that are or will be conducted under this new initiative is research to optimize drug treatments, which includes an array of studies on drug combinations aimed at gaining "extra-mileage" from existing antimalarial drugs. The scientific rationale of this approach is to use a rapidly acting, short half life drug to reduce substantially the parasite biomass; this is readily achieved by an artemisinin derivative. The remaining parasites are then killed by high concentrations of the companion drug (White, 1997). Several artesunate based combinations have been studied in clinical trials, including one using artesunate with amodiaquine. von Seidlein et al, 2000; Dorsey et al, 2002; Marquino et al, 2003)

In three trials of AQ-AS versus AQ alone, 941 children (400 in Kenya, 321 in Senegal, and 220 in Gabon) who were ≤10 years with uncomplicated *Plasmodium falciparum* malaria were randomly assigned amodiaquine (10 mg/kg/day for 3 days) plus artesunate (4 mg/kg per day for 3 days) or amodiaquine (as above) and placebo (for 3 days). The primary endpoints were parasitological cure rates at days 14 and 28. The data were analysed by intention to treat. The day-14 cure rates for amodiaquine-artesunate versus amodiaquine were: 175/192 (91%) versus 140/188 (74%) in Kenya (p<0.0001), 148/160 (93%) versus 147/157 (94%) in Senegal (p=0.7), and 92/94 (98%) versus 86/96 (90%) in Gabon (p=0.02). The corresponding rates for day 28 were: 123/180 (68%) versus

75/183 (41%) in Kenya (p<0.0001), 130/159 (82%) versus 123/156 (79%) in Senegal (p=0.5), and 80/94 (85%) versus 70/98 (71%) in Gabon (p=0.02). Combining the results in an aggregate meta analysis, the combination was superior to monotherapy with an odds ratio of 2·5 (95% CI 1·6–3·8) for Day 14 and 2·2 (1·6–3·0) for Day 28.

Both regimens were well tolerated. Six patients in the amodiaquine-artesunate group and five in the amodiaquine group developed early, drug-induced vomiting, necessitating alternative treatment. Nine of 153 (5.9%) children in whom paired white cell counts from day 0 and 28 were available developed neutropenia (absolute neutrophil count < 1000/microL), three in the combination arm and six with amodiaquine alone. All nine were asymptomatic and afebrile and one had falciparum parasitaemia. Amodiaquine monotherapy has previously been associated with neutropenia, particularly when used as prophylaxis. Neutropenia may be associated with hepatitis (Hatton et al, 1986; Larrey et al, 1986; Neftel et al, 1986). A case report of possible amodiaquine related hepatitis has been published from this study (Orrell et al, 2001).

The results from this trial were encouraging in that the addition of artesunate had a beneficial effect on the cure rates. The result in Senegal was probably due to the high efficacy of AQ alone. The cases of neutropenia call for caution and further safety data to be collected in malaria patients. Amodiaquine-artesunate is a potential combination for use in Africa where the background rate of AQ resistance is not high. Further investigations to assess the potential effect on the evolution of drug resistance, disease transmission, and safety of amodiaquine-artesunate are warranted.

Based on current published pharmacokinetic/metabolic data it is expected that there would be little or no interaction between artesunate and amodiaquine. (Dollery, C. 1991). This study is one of several studies to explore the usefulness of different drug combinations with artesunate for application in the treatment of falciparum malaria. Such approach was endorsed by a WHO informal consultation on the use of artemisinin and its derivatives, Geneva 10-12 June 1998 and given high priority.

4.0 Rationale

These studies are based on the concept primarily developed by White (1997,1998) and others (Peters, 1990) of initial parasite biomass reduction to protect vulnerable antimalarials. Antimalarials with long terminal elimination phases and therefore long residence have a particular propensity to generate resistance.

There is good theoretical basis for the chances of a drug-resistant mutant appearing to be reduced if such drugs are combined with another effective antimalarial with a different mechanism of action. This benefit is expected to be greater when an antimalarial is combined with an artemisinin derivative. Through its rapid onset of action the latter will leave a small residual parasite biomass for the companion drug to eradicate at a time of maximum blood concentrations; similarly, the parasites will never be exposed to the artemisinin derivative alone (given its short half-life). Artemisinin derivatives are also effective in reducing transmissibility and thus the likelihood of resistant mutants being transmitted.

5.0 Objectives

The objectives of this study were:

1. To investigate the tolerability of concomitant oral administration of artesunate and amodiaquine in healthy normal volunteers (HNV).
2. To investigate the pharmacokinetic properties of the same combination. The kinetics of both the parent drugs and the primary active metabolites (dihydro-artesunate and desethylamodiaquine) were determined.

6.0 Study design

6.1 Description

The trial design was a single randomised three-phase crossover model in a total of 15 healthy normal subjects. All the subjects took artesunate, the short half-life drug in the first phase, in order to complete the pharmacokinetics of this drug prior to the administration of amodiaquine, with its long half-life.

All trial drugs were given as a stat dose with 200ml tap water on an empty stomach after an overnight fast. For a week before the study, during the study and during the washout periods the volunteers took no other drugs or alcohol.

Phase one: All subjects were initially administered with oral artesunate (4 mg/kg).

Phases two and three: One week later subjects were randomly allocated to one of the treatment groups:

(i) A single oral dose of sodium artesunate (4 mg/kg) and amodiaquine base (10 mg/kg).

OR

(ii) A single oral dose of amodiaquine (10 mg/kg) alone.

At the end of a 21 day washout period, the subjects were crossed over.

The elimination half-life of artesunate is 0.53hr and that of amodiaquine 5.2 hours. The washout period for this study was based on the elimination half-life of desethylamodiaquine, the active metabolite of amodiaquine, which is between 9 and 18 days (Dollery, C. 1991, Krishna, S et al. 1996, Pussard, E et al 1994).

6.2 Study drug:

The medicines were procured by representatives at the World Health organisation and made available for the study. The artesunate was produced by Sanofi Winthrop as *Arsumax®* 50mg tablets. The amodiaquine was produced by Parke Davis as *Camoquin®* 200mg tablets.

6.3 Randomisation

Packaging and labeling of the drugs was in accordance with a randomisation list drawn up by an independent statistician and identified by the study, phase and volunteer ID. The randomisation plan is tabulated in Appendix B.

6.4 Location of Trial

The study was performed in the Department of Pharmacology, University of Cape Town. All plasma concentrations were assayed in the laboratory of the same department.

6.5 Number of Subjects

Fifteen subjects were enrolled. Four additional subjects were screened; two were excluded due to neutropenia and two elected not to participate in this study.

7.0 STUDY POPULATION:

Fifteen healthy normal volunteers of either sex who met all the inclusion criteria and none of the exclusion criteria were recruited in the 30 days prior to study commencement.

Informed consent was obtained from each subject admitted to the study. All subjects were fluent in English. The consent form is attached as Appendix A.

7.1 Inclusion Criteria

- Age 18-45 years
- Written consent was given after reading the patient information leaflet. Participation was voluntary and volunteers were fully informed of possible side effects. They were advised that they were free to withdraw at any time.
- No significant abnormal findings on history or examination, particularly no prior liver disease, cardiovascular disease (including arrhythmias) or peripheral neuropathy.
- No clinically significant abnormalities on haematology, liver and renal function tests.
- Negative pregnancy test (women).
- Normal electrocardiogram.
- No history of antimalarial ingestion in the preceding two months.
- No other drugs or medications, including over-the counter preparations, ingested in the preceding week.
- Adequate venous access.

7.2 Exclusion Criteria

- Refusal of consent.
- Biological or electrocardiograph anomalies.
- Presence of hepatic, renal and gastrointestinal disorders.
- Smokers (>5/day), abuse of alcohol or recreational drugs
- Presence of malaria parasites on a thick smear
- Subjects having been in a malarial area in the preceding 8 weeks

- Subjects having ingested any drugs, including over the counter preparations, in the preceding week
- Presence of acute or chronic infections

8.0 STUDY PROCEDURES AND SCHEDULES

8.1 Pre-study visit: (visit 1)

In the 30 days prior to the study the volunteers underwent a screening process (Visit 1) to ensure selection of suitable candidates for the study. Fifteen healthy volunteers who fulfilled all the inclusion and none of the exclusion criteria were recruited.

Visit 1 involved a medical history and clinical examination including age, sex, race, evidence of physical illness or current disease, weight, height, blood pressure, heart rate and temperature. Blood was taken for chemistry (AST, ALT, total bilirubin, sodium, potassium, creatinine and glucose), haematology (full blood count and differential) and for a malaria smear. Women were screened for pregnancy by quantitative assay of beta-HCG. Urine was screened for drugs of abuse by semi-quantitative assays including cannabinoids, cocaine, amphetamines, barbiturates, opiates and benzodiazepines. An ECG was performed to exclude conduction abnormalities.

8.2 Peri-study restrictions:

Volunteers were asked to avoid alcohol, caffeine, any medications (without consulting the investigator) and strenuous exercise over the study period. They were required to fast from midnight prior to each study day.

8.2 On-Treatment and Post-Treatment

8.2.1. Study days (visits 2, 4 and 14)

Subjects were admitted to the ward at 7am on each study day after an overnight fast. Their vital signs (heart rate, temperature and blood pressure) were recorded.

All study day venous blood samples (2 or 3 X 5ml each) were drawn by a vacutainer system directly into heparinized tubes for further processing. Specimens in the first

12 hours of each phase were drawn via an indwelling catheter. The remainder were by direct venupuncture.

Study medications were taken with 200ml water at 8am on each study day and under the direct supervision of one of the study staff. An ECG was recorded for each volunteer about 2 hours after the drug dose.

Subjects in **phase one (visit 2 - day 0)**, receiving artesunate alone, had blood samples collected pre-dose and at 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8 and 12 hours after drug administration. All the samples were 2 X 5 ml venous blood each.

Phases two (visit 4 - day 7) and three (visit 14 - day 28): Subjects receiving artesunate plus amodiaquine or amodiaquine alone (Groups I and II). Blood samples were collected at pre-dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hours, and days 4, 5, 7, 10, 14 and day 20 post-medication. Three X 5ml blood samples were collected at each time point for the first 12 hours from those subjects receiving both drugs. All the remaining samples were 2 X 5 ml each. Subjects left the ward after the 12 hours specimen was drawn and the remaining specimens were collected on an outpatient visit basis.

Immediately after collection of blood, the tubes were inverted by hand to ensure thorough mixing with the anticoagulant and placed on melting ice. They were transferred on ice within 60 minutes of collection to the laboratory and centrifuged for 5 minutes at 3 000g. After centrifugation the plasma samples were immediately transferred to separate appropriately labeled plastic cryotubes and frozen at -70°C.

A standard breakfast was given at 10am (2hours after drug administration) on each study day and a standardised lunch at 13h00. After this time food and decaffeinated drinks could be taken freely.

8.2.2. Safety tests:

The following tests were performed 6 days after artesunate administration (visit 3) and 20 days after administration of-medication to groups (I) and (II) respectively, on both

arms of the crossover i.e. visit 13 and 23. Any significant abnormalities would have precluded the volunteer from continuing in the study.

- Clinical examination
- Temperature
- SGOT, SGPT, bilirubin
- Blood urea nitrogen and creatinine
- White blood cell total and differential counts, hemoglobin, red blood cell count, haematocrit and platelet counts

9.0 Methods of evaluation:

9.1 Blood tests for screening and adverse event detection

Haematology, chemical pathology and urine drug assays were carried out by validated laboratories of the University of Cape Town (UCT), according to standard operating procedures. Normal ranges of laboratory variables are presented in Appendix C. ECGs were completed by technicians of the Department of Cardiology, UCT, and read by the principal investigator. Abnormalities were discussed with cardiology consultants.

Results outside the normal ranges, as assessed to be clinically significant by the principal investigator, excluded a volunteer from the study.

9.2 Drug concentration determination

The blood sampling times were described previously. Each volunteer would have had 12 blood samples taken during phase one and 21 samples taken during each of phase 2 and 3. The additional samples in the latter phases were taken for the determination of desethylamodiaquine levels. Desethylamodiaquine has an elimination half-life between 9 and 18 days (Dollery, C. 1991, Krishna, S et al. 1996, Pussard, E et al 1994). The exact times of drug administration and sampling were recorded in the case record forms. Samples were collected into dry lithium heparin tubes and placed immediately onto melting ice. They were centrifuged within 60 minutes of collection and transferred to polypropylene tubes for storage in a dark environment at -70°C. These samples were kept in storage until at least 12 months after study completion.

Amodiaquine was analysed by HPLC-MS with UV detection while artesunate was analysed by HPLC with post-column decomposition followed by UV detection (Appendix E). Both methods are validated. The limit of quantification for the artesunate assay was set at 24ng/ml. Levels below this were halved and reported at 12ng/ml. The limit of quantification for the amodiaquine assay was 5ng/ml.

9.3 Safety criteria and assessment of adverse events

Liver functions and a full blood count were completed at the end of each phase i.e. before an additional dose of a trial drug was given, and at the end of the study. Significant abnormalities would have excluded the volunteer form further drug administration, and would have been followed up until the cause was determined or the values returned to normal.

At each visit the volunteers were asked to report any symptoms of illness or adverse reactions to the medication. Targeted physical examination was conducted if deemed necessary by the principal investigator.

Details of all adverse events, including concomitant illnesses, were recorded as adverse events in the case record forms. All concomitant medications taken were not known to have drug interactions with either study drug, and were recorded as appropriate in the case record forms. Use of concomitant medication with an interaction previously described resulted in patients being withdrawn from the study.

9.4 Statistical analysis

The following kinetic parameters were calculated from the artesunate, dihydroartesunate, amodiaquine and desethylamodiaquine concentration profiles of the samples collected for each subject, using a non-compartmental model (WinNonLin 3.3, Pharsight®).

Plasma peak concentration (Cmax)

Time to peak plasma concentration (Tmax)

Apparent half-life (T1/2)

Area under the plasma concentration time curve (AUC) from 0 to 12 hours for artesunate and dihydroartesunate and from 0 hours to 20 days for amodiaquine and desethylamodiaquine.

Area under the plasma concentration-time curve from 0 to infinity.

Values were tested for significance using the paired t-test (two-tailed p value) and Wilcoxon matched pairs signed rank test (two-tailed p-value for normal approximation).

The effects of treatment on the mean of each haematological parameter (Hb, Hct, Platelets, WCC, lymphocytes and neutrophils) were analysed using regression analysis, taking repeated measures within patient into account (generalized estimating equation).

9.5 Laboratory analysis:

Details of the methodology are attached as Appendix E.

10.0 Ethical and legal considerations

This clinical study was conducted in accordance with the principles laid down by the World Health Assembly of 1975 on Ethics in Human Experimentation and the Helsinki Declaration. The study adhered to the standards established for Good Clinical Practices (GCP). The protocol was approved by the University of Cape Town's Ethical Committee and the WHO Secretariat committee for research Involving Human Subjects (SCRIHS). Each subject was be informed of the nature and possible risks of the trials. The Investigator obtained informed written consent from every subject participating in the study. The subject was informed that he/she was at liberty to abstain from participation in the study and that he/she was free to withdraw the consent of participation at anytime. The investigators and study staff observed strict confidentiality of the records. After the initial screening procedures a coded number recorded the identity of the volunteers.

Insurance coverage for the participants over the duration of the study was obtained from Price Forbes, Johannesburg.

11.0 Results

11.1 Study subjects:

Fifteen healthy normal volunteers were selected for the study. This group was largely comprised of medical students or staff of the University of Cape Town and their families.

Demographic characteristics:

The demographics of the study group are detailed in Table 1, together with the dose of amodiaquine and artesunate, in milligrams, that each subject received, with the milligram per kilogram equivalent. The randomisation table presented in Appendix B presents the sequence in which each subject was dosed.

Table 1: Demographics and dosing details for each subject (Appendix H).

Subject	race	age (years)	gender	weight (kg)	height (cm)	AQ dose (mg)	AQ mg/kg	AS dose (mg)	AS mg/kg
A	c	20	f	69	169	800	11.59	300	4.35
B	c	42	m	70	166	800	11.43	300	4.29
C	w	23	m	82	185	800	9.76	350	4.27
D	w	22	m	63	173	600	9.52	300	4.76
E	b	23	f	68	158	800	11.76	300	4.41
F	w	34	m	73	180	800	10.96	300	4.11
G	w	23	m	61	171	600	9.84	250	4.10
H	b	22	f	59	159	600	10.17	250	4.24
J	a	19	f	50	161	600	12.00	200	4.00
K	b	24	f	54	157	600	11.11	250	4.63
L	c	22	m	74	181	800	10.81	300	4.05
M	c	21	m	97	189	1000	10.31	400	4.12
N	a	23	m	68	176	800	11.76	300	4.41
P	w	24	m	64	179	600	9.38	250	3.91
Q	b	24	m	58	159	600	10.34	250	4.31
Means:		24.4		67.33	170.87		10.72		4.26
Std deviation:		5.90		11.61	10.61		0.88		0.23

a = asian, b= black , c = coloured, w = white

The data for 13 out of the 15 subjects was analysed. Subjects G and H were withdrawn after phase two (see Study Completion Status below). The range of weight was 50 to 82 kilograms (mean 67.3 kg) and the range of height 157 to 189 cm (mean 170.9cm). The

mean dose of amodiaquine and artesunate in mg/kg body weight was 10.72 (SD 0.88) and 4.26 (SD 0.23) respectively.

11.2 Study conduct:

Dose and duration of treatment:

In the first phase each subject received 4 mg/kg of artesunate. After a 1 week washout period, half the subjects were given a 10mg/kg dose of amodiaquine alone and the other half a dose of artesunate AND amodiaquine (Phase 2). Three weeks later, the subjects were crossed over (Phase 3). In total, each subject received a dose of artesunate alone, a dose of amodiaquine alone and a dose of the drugs given together, according to the randomisation plan in Appendix B.

Study completion status:

Two volunteers failed screening owing to neutropaenia at baseline. Of the fifteen subjects who commenced the study, 2 were withdrawn during phase 2 and the other 13 completed all 3 phases. Only the data from the 13 complete subjects is included in the analysis.

Subject G was withdrawn due to a possible allergic reaction experienced shortly after dosing with amodiaquine alone. He experienced generalised body flushing, with no systemic symptoms, which lasted for 4 hours and subsided without therapy.

Subject H was withdrawn as she commenced treatment for depression with fluoxetine during phase 2. The fluoxetine may have impacted on the metabolism of amodiaquine.

Concomitant medications:

Three subjects took medication during the study. Subject E took 4 tablets of hyoscine butyl bromide (*Busocpan®*) for abdominal cramps during phase 2. Subject H took fluoxetine (*Prozac®*) during phase 2 for which she was withdrawn from the study (see above). Lastly, subject M took 5 days of amoxicillin (*Amoxcil®*) and metronidazole (*Flagyl®*) for a dental infection, also during phase 2. Neither amoxicillin or metronidazole are likely to interact with artesunate or amodiaquine.

Protocol violations:

Other than subject H who was withdrawn for taking a contra-indicated medication, there were no protocol violations.

Dosing and sampling times:

The investigator or one of the site assistants directly observed the actual swallowing of the dose by each subject during every phase.

The subjects had samples drawn at all the times specified in the protocol (see 8.2.1). Exact times were recorded in the case record forms for each participant. These case record forms are available for review.

11.3 Pharmacokinetic results:

The data from the 13 patients who completed the study were included in the pharmacokinetic analysis. Artesunate and dihydroartesunate (DHAS) results were available in August 2001. Amodiaquine results were available by May 2003. Samples were stored at –70^{0}C in the interim. Appendices D-1, D-2 and D-3 contain the data for each study drug and metabolite by individual as well as summary graphs. Raw concentration vs. time data by individual for AS, DHAS, AQ and D-amodiaquine, at all time points, is available on request from the principal investigator.

Table 2: Mean pharmacokinetic parameters derived for artesunate and dihydroartemisinin with and without amodiaquine.

	Tmax (SEM)	**Cmax (SEM)**	**Thalf (SEM)**	**AUC (0-24) (SEM)**	**AUC (0-inf) (SEM)**
Artesunate alone	0.62 hr (0.08)	231.8 ng/ml (42.99)	0.44 hr (0.08)	135.9 ng.h/ml (24.76)	168.3 ng.h/ml (25.19)
Artesunate with amodiaquine	0.79 hr (1.09)	146.3 ng/ml (32.19)	0.52 hr (0.07)	122.9 ng.h/ml (26.40)	156.6 ng.h/ml (26.63)
Paired t-test (two-tailed p value)	p=0.25 nsd	p=0.12 nsd	p=0.08 nsd	p=0.62 nsd	p=0.25 nsd
Wicoxon matched pairs	p=0.36 nsd	p=0.09 nsd	p=0.06 nsd	p=0.73 nsd	p=0.47 nsd
DHAS alone	0.98 hr (0.18)	844.5 ng/ml (85.83)	1.67 hr (0.11)	1743 ng.h/ml (133.6)	1804 ng.h/ml (129.90)
DHAS with amodiaquine	1.58 hr (0.31)	446.2 ng/ml (66.41)	2.15 hr (0.26)	1229 ng.h/ml (139.50)	1490 ng.h/ml (137.30)
Paired t-test (two-tailed p value)	p=0.16 nsd	**p=0.007 sd**	**p=0.02 sd**	**p=0.02 sd**	p=0.09 nsd
Wicoxon matched pairs	p=0.12 nsd	**p=0.01 sd**	**p=0.01 sd**	**p=0.03 sd**	p=0.15 nsd

nsd = not significantly different sd = significant difference
hr = hour

Table 2 summarises the mean pharmacokinetic parameters for artesunate (AS) and dihydroartesunate). Table 3 summarises the same for amodiaquine (AQ) and desethylamodiaquine (D-AQ).

Table 3: Mean pharmacokinetic parameters derived for amodiaquine and desethylamodiaquine with and without artesunate.

	Tmax (SEM)	**Cmax (SEM)**	**Thalf (SEM)**	**AUC (0-24) (SEM)**	**AUC (0-inf) (SEM)**
Amodiaquine	2.38 hr (0.33)	30.01 ng/ml (3.10)	4.90 hr (1.17)	171.10 ng.h/ml (29.29)	221.10 ng.h/ml (44.95)
Amodiaquine with artesunate	1.88 hr (0.29)	22.90 ng/ml (2.57)	3.83 hr (0.31)	114.60 ng.h/ml (17.56)	142.30 ng.h/ml (18.45)
Paired t-test (two-tailed p value)	p=0.33 nsd	**p=0.04 sd**	p=0.38 nsd	**p=0.04 sd**	P=0.09 Nsd
Wicoxon matched pairs	p=0.36 nsd	**p=0.03 sd**	p=0.73 nsd	**p=0.04 sd**	P=0.10 nsd
D-amodiaquine	3.63 hr (0.51)	273.40 ng/ml (20.05)	242.00 hr (39.44)	12160 ng.h/ml (920.80)	15300 ng.h/ml (1421.00)
D-amodiaquine with artesunate	2.25 hr (0.29)	303.50 ng/ml (45.76)	135.60 hr (18.06)	10650 ng.h/ml (2066.00)	12610 ng.h/ml (2978.00)
Paired t-test (two-tailed p value)	**p=0.007 sd**	p=0.49 nsd	**p=0.03 sd**	p=0.49 nsd	P=0.43 nsd
Wicoxon matched pairs	**p=0.007 sd**	p=0.52 nsd	**p=0.03 sd**	P=0.23 nsd	P=0.15 nsd

nsd = not significantly different sd = significant difference
hr = hour

The AS half-life of subject F was excluded from this analysis, as this could not be accurately calculated as the levels detected were below the levels of quantification for this assay, and the prolonged half-life calculated (6.9 hours) was an extreme outlier. As the amodiaquine samples for subject N were missing, these could not be included in the

above analysis, although this subject's results were included in the artesunate PK analysis.

There was no significant difference in any of the parameters for the parent drug artesunate when given with or without amodiaquine. For DHAS, there was a 47.2% reduction in Cmax when AS was given in conjunction with amodiaquine. While the Cmax was reduced, the half-life (Thalf) of DHAS was significantly prolonged (by about 30 minutes) in the combination. These changes resulted in a slight decrease in the 24 hour AUC of DHAS when in combination, although the AUC to infinity was not significantly changed.

For the parent drug amodiaquine, a significant reduction in Cmax was noted when given together with AS. This 23.6% reduction in Cmax was reflected in a decrease in the 24-hour AUC. The other parameters remained unchanged. Desethylamodiaquine showed both an earlier Tmax (by 1.38 hours) and a reduced Thalf (by 107 hours) when given with artesunate. The Cmax and AUC (both parameters) remained unchanged.

Graphs of these results are presented in Appendix D-1 (summary graphs for AS, DHAS, AQ and d-AQ) and D-3 (individual graphs).

11.4 Safety:

Table 4: Adverse events.

Subject	Phase	Drug	Adverse event	Duration	Severity	Conc. meds	Study drug involved?	Continue on study?
E	2	AQ	Abdominal cramps	24 hr	mild	y	unlikely	y
M	2	AQ	Dental infection	72 hr	mod	y	unlikely	y
E	2	AQ	Diarrhoea	5 hr	mild	n	yes	y
E	2	AQ	Diarrhoea	24 hr	mild	n	unlikely	y
G	2	AQ	Generalised flushing	4 hr	mod	n	yes	n
A	2	AQ	Headache	6 hr	mild	n	possible	y
E	2	AQ	Headache	5 hr	mild	n	yes	y
P	2	AQ	Headache	12 hr	mild	n	possible	y
B	3	AQ	Headache	12 hr	mild	n	possible	completed
K	3	AQ	Headache	4 hr	mild	n	possible	completed
L	3	AQ	Headache	12 hr	mild	n	yes	completed
E	2	AQ	nausea	5 hr	mild	n	yes	y
G	1	AS	Diarrhoea	14 hr	mild	n	unlikely	y
M	1	AS	Diarrhoea	6 hr	mild	n	unlikely	y
B	1	AS	Headache	12 hr	mod	n	possible	y
B	1	AS	nausea	12hrs	mod	n	possible	y

G	1	AS	nausea	14 hr	mild	n	unlikely	y
J	1	AS	nausea	24 hr	mild	n	unlikely	y
B	1	AS	Vomiting	12 hr	mod	n	possible	y
B	2	ASAQ	Abnormal taste	12 hr	mild	n	possible	y
H	2	ASAQ	Depression	ongoing	mod	y	unlikely	n
B	2	ASAQ	Headache	12 hr	mild	n	possible	y
K	2	ASAQ	Headache	4 hr	mild	n	possible	y
A	3	ASAQ	Headache	3 hr	mild	n	possible	completed
A	3	ASAQ	hepatitis	76 days	severe	n	yes	completed
K	2	ASAQ	nausea	4 hr	mild	n	possible	y
K	2	ASAQ	nausea	10 hr	mild	n	possible	y
A	3	ASAQ	nausea	3 hr	mild	n	possible	completed
M	3	ASAQ	nausea	2hr	mild	n	possible	completed

AS = artesunate Conc. meds = concomitant medication used. y = yes
AQ = amodiaquine mod. = moderate n = no

Table 4 documents all the adverse events experienced during the study. All the events resolved spontaneously. A report has been submitted (attached as Appendix F) for the prolonged severe transaminitis, thought to be due to the study medication, experienced by subject A after phase 3 of the study. Subject A had the highest area under the concentration time curve for desethyl-amodiaquine, close to 3 times the mean, when administered in combination with artesunate in phase 3. There were no other liver function abnormalities detected in any other volunteers. No renal or electrolyte abnormalities were detected in any volunteers.

One subject was withdrawn (subject G) due to generalised flushing of his face and body, which began 2 hours after a dose of amodiaquine alone and continued for 4 hours. Although there were no systemic symptoms associated with this flushing, the risk of this being a hypersensitivity reaction was too great for a second dose of amodiaquine to be risked. Another subject was withdrawn (subject H) due to the need for concomitant administration of fluoxetine for her pre-existing depression. The depression had been an intermittent problem for which she had sought therapy in the past, and was unlikely to have been due to the study medication. A further subject developed and intercurrent and probably unrelated dental infection.

Overall, ten out of 15 subjects (66%) reported adverse events. This frequency was 8/15 (53%) within 3 weeks of administration of amodiaquine alone, 4/15 (26.7%) within 6 days of administration of artesunate alone and 5/15 (33.3%) within 20 days of the combination. Gastrointestinal problems were the most commonly reported adverse

events (16 out of 29 adverse events, 55%), with headaches being the second most often reported (10 out of 29 events, 34%). The frequency of adverse events was similar across all three treatments (p>0.10).

Diarrhoea and nausea were the most common adverse events when AS was taken alone. Headache and diarrhoea were prominent with amodiaquine alone. Headache and nausea were the adverse events occurring most frequently with the combined medication dosing.

The effects of treatment on haematological parameters are summarized in Table 5 (Appendix G). Two volunteers developed a potentially clinically significant leukopenia (WCC < 4 x 10^{-9}) and neutropenia (< 1.8 x 10^{-9}/L), one after administration of amodiaquine monotherapy (Subject E) and the other after administration of artesunate plus amodiaquine (Subject K); neither were associated with any clinical infection. In subject E, the leukopenia (but not neutropenia) also persisted 3 weeks following exposure to amodiaquine plus artesunate. None of the other observed changes in haematological parameters were outside the normal range. These show that each of the treatments was associated with statistically significant decreases in white cell counts, and absolute neutrophil and lymphocyte counts. Artesunate, when administered alone, or in combination with amodiaquine, was associated with a statistically significant decrease in haemoglobin, but not haematocrit. The decrease in platelet count observed was statistically significant for artesunate and amodiaquine, when administered as monotherapy, but not in combination (p=0.06). Repeated exposure to artesunate and to amodiaquine demonstrated a slight cumulative effect, but this was not statistically significant (p > 0.10). These haematological parameters were not significantly different between treatment groups (p > 0.10).

Table 5: Effect of treatments on baseline values – beta=estimate of average change in baseline values due to treatment.

Haematological parameters	**Sodium Artesunate**	**Sodium Artesunate + Amodiaquine**	**Amodiaquine**
	Beta(standard error) p-value	Beta(standard error) p-value	Beta(standard error) p-value
Haemoglobin	-0.634 (0.2928) *p = .030*	-1.060 (0.5655) *p = 0.061*	-0.718 (0.5507) p = 0.193
Haematocrit	-0.006 (0.0098) p = 0.530	-0.013 (0.0189) p = 0.493	-0.003 (0.0184) p = 0.864
Platelet count	*-30.656 (10.9197)* *p = 0.005*	-38.3626 (21.0894) p = 0.069	*-50.8388 (20.5369)* *p = 0.013*
White cell count	*-1.635 (0.5)* p = 0.001	*-3.168 (0.9657)* *p = 0.001*	*-3.414 (0.9404)* *p = 0.000*
Neutrophils	*-0.998 (0.3604)* *p = 0.006*	*-1.682 (0.696)* *p = 0.016*	*-1.935 (0.6778)* *p = 0.004*
Lymphocytes	*-0.540 (0.2661)* *p = 0.042*	*-1.142 (0.5139)* *p = 0.026*	*-1.130 (0.5)* *p = 0.024*

Two of the moderately severe adverse events (hepatitis and hypersensitivity) were reported to the medicines regulatory authority in South Africa, the Medicines Control Council.

12.0 Discussion and conclusions

Pharmacokinetic profiles:

This study has shown that there was a PK interaction between amodiaquine and artesunate. The clinical significance of this can be put into context from extensive clinical trials data which show that the combination is more effective than AQ alone (Adjuik et al 2001).

Of the 2 drugs and 2 major metabolites measured in this study only the rapidly metabolized parent drug artesunate experienced no changes in any of its pharmacokinetic parameters consequent to the addition of amodiaquine. The bulk of the artesunate would be metabolized by 1 hour after dosing; well before the Tmax of amodiaquine (at 2.38 hours) was reached.

The kinetic profile of the major, and active, metabolite of artesunate, dihydroartemisinin was altered by co-administration with amodiaquine. The Cmax was significantly reduced, and the half-life was prolonged. Overall, there was a small, but significant decrease in total drug exposure as measured by the 24-hour AUC, although this difference was lost when AUC to infinity was examined.

On reviewing the PK parameters of the parent drug amodiaquine and its active metabolite desethylamodiaquine, it appears that the combination with AS resulted in a more rapid metabolism of AQ to D-AQ, as noted by a reduction in Cmax and a decrease in AUC of amodiaquine. An earlier Tmax and shorter Thalf for D-AQ (with AS), together with an unchanged Cmax and AUC confirm the more rapid conversion to D-AQ, without loss of total drug exposure.

The significance of these changes in PK parameters on parasite killing is doubtful. Parasite killing is related to the time that the drug concentrations remain above the minimal parasiticidal concentration. For DHAS, the active metabolite of AS, the Cmax is probably the more important PK parameter because the half-life of AS is very short. Thus, the significantly lower Cmax observed for the combination therapy could theoretically reduce to some extent the rate of parasite killing. For DAQ, the active

metabolite of AQ with a long half life, the important PK parameters are the elimination half-life and AUC; these are surrogate measures for the time when DAQ levels are still above the MPC. In combination therapy, the artemisinin is given to kill most of the parasites quickly. The AQ is given to kill the residual parasites. Clinical data from the AQ-AS trials in Africa, showed the combination to be superior to AQ alone. Therefore, although reductions in the Cmax values may theoretically reduce the rate of parasite killing, this is not bourne out in practice. The shortened half life of DAQ might theoretically provide less selective drug pressure for the development of resistant parasites if this shortened half life were observed in malaria treated patients over three days.

Safety:

Overall, the drugs were well tolerated. Reported adverse events occurred throughout the study and were generally mild and transient. There were four adverse events of concern. The asymptomatic hepatitis was probably due to the amodiaquine either alone or in combination and started after two doses were administered (see Appendix F). Hepatitis is a well described complication of amodiaquine in healthy, travellers and was one reason why it was withdrawn from prophylactic use. Moderate neutropenia also occurred in two subjects and this appeared to start to recover. Neutropenia has been observed in healthy travellers when amodiaquine was used as malaria prophylaxis and as treatment for rheumatoid arthritis (Hatton et al, 1986; Larrey et al, 1986; Neftel et al, 1986). Agranulocytosis usually developed between 5 to 14 weeks of prophylaxis and occurred with hepatitis in some travellers. Based on UK data, the risk of developing agranulocytosis was estimated as 1 in 2,200 with a risk of death of 1 in 31,300, and 1:15,650 for a serious hepatic reaction (Phillips-Howard, 1990). Neutropenia has also been reported with amodiaquine use in rheumatoid arthritis (Bepler, 1959). One subject was allergic to amodiaquine that was manifest as generalised flushing. The small sample size of this study cannot provide a proper safety analysis. Never the less, the adverse effects observed call for further research into possible AQ induced toxicity. This is supported by the finding of neutropenia in African children with falciparum malaria (Adjuik et al, 2002).

The findings of our study suggest that monitoring of liver function tests should also be carried out both during dosing and during follow up, as hepatitis might be delayed. as the drugs are cleared at 28 days, should be considered. As with other drugs, warnings should be given about potential allergy, and that AQ should not be used in those who are allergic to AQ. In keeping also with other drugs, some of the minor adverse events observed in this study might limit patient adherence when used for three days as malaria treatment.

Conclusion:

The pharamacokinetic analysis in this randomized study, in which the last two phases were crossed over shows that artesunate and amodiaquine may be effectively given in combination. Amodiaquine was more rapidly metabolized to desethylamodiaquine when administered with artesunate, as compared to when the drugs are given separately. However, the total drug exposure (area under the plasma concentration time curve) to both study drugs was similar when administered alone or together.

Artesunate and amodiaquine could be added to the armamentarium of drugs to combat resistant *Plasmodium falciparum* malaria, provided adequate safety precautions are taken.

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Appendix A: Study Protocol

ARTESUNATE AND AMODIAQUINE: TOLERABILITY AND DRUG INTERACTION STUDY IN HEALTHY NORMAL VOLUNTEERS

Product:	1. Sodium Artesunate 2. Amodiaquine
Design:	Single dose three-phase crossover pharmacokinetic study
Study site:	Department of Pharmacology, University of Cape Town, Cape Town, South Africa
Principal Investigator:	Dr Catherine Orrell
Co-investigators:	1. Professor Peter Folb 2. Dr. Karen Barnes

Data handling and data analysis:

Clinical and pharmacokinetic Study: Dr. C. Orrell

Sponsor and pharmaceutical support:

UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), Geneva, Switzerland.

Clinician:	Dr. C. Orrell
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1.0 Summary

Resistance of the malaria parasite to treatment is increasing globally. One method of delaying this may be through the use of combination therapy. In this randomised crossover study the anti-malarials amodiaquine and artesunate are given to 20 normal healthy volunteers, both as individual agents and together, over three study phases. The pharmacokinetic parameters of maximum plasma concentration (Cmax), time to achieve Cmax (Tmax) and the area under the curve (AUC) will be determined for the drug combination as compared to the drugs individually. The parameters of the active metabolites of the parent drugs, dihydro-artemesinin and desethylamodiaquine will be calculated as well. The tolerability of the combination will be assessed in the volunteers over the study period.

2.0 Introduction

Resistance of *Plasmodium falciparum* to almost all synthetic antimalarial drugs has become widespread in Southeast Asia, Africa and South America (Wernsdorfer and Payne, 1991). The only exception to this situation is with the artemisinin derivatives which have recently been introduced for widespread use in Southeast Asia (Anonymous, 1994). Cross-resistance between compounds of the same class magnifies the problem. Antifolate resistance in both *P.falciparum* and *P.vivax* developed rapidly following the introduction of pyrimethamine in many parts of the tropics (WHO, 1984). The fixed combination of sulfadoxine/pyrimethamine has been very successful, but is no longer effective in many areas of Southeast Asia (White, 1992) and its efficacy is fading in East and West Africa (Lege-Oguntoye et al, 1990; Garcia-Vidal et al, 1989). Mefloquine resistance was reported even before the drug had been routinely used (Brasseur et al, 1990) and has now severely curtailed the usefulness of the drug in some parts of Southeast Asia (Nosten et al, 1991; Fontanet et al, 1993). Halofantrine resistance has emerged concomitantly (Basco et al, 1991; Brasseur et al, 1993). When atovaquone was used alone in preliminary clinical trials, 28% of patients had recrudescent infections associated with the development of resistance (Looareesuwan et al, 1996). Quinine and chloroquine have enjoyed longer periods of effective use, but in large parts of the world, chloroquine is no longer effective treatment for falciparum malaria (Wernsdorfer, 1991) and *P. vivax* has developed resistance in some parts of Oceania (Murphy et al, 1993). Quinine, too, is threatened in some parts of the world (Pukrittayakamee et al, 1994).

In the absence of new and effective methods of vector control and the limited prospects for a rapid development of safe and effective malaria vaccines (Playfair et al, 1990; Nosten et al, 1996), drugs retain a key role in the control of malaria, particularly in preventing death and reducing morbidity. This situation has stimulated research into better and more effective usage of the currently available drugs, the development of new novel antimalarial agents and the introduction of drug combinations. Recently White and Olliaro (1996) reviewed the rationale for combination chemotherapy for malaria. Essentially, while waiting for new drugs to be discovered and hopefully deployed, appropriate measures such as drug combinations should be taken to safeguard the few compounds available for the treatment of malaria.

3.0 Background

Resistance is the prime determinant of drug's lifespan, and protection of drugs against the development of resistance is a key factor in the fight against malaria. The TDR has initiated a new line of research on drug resistance and policies, which will primarily generate accurate data to inform policy makers and reform current policies based on research results.

Drug policies play and important role in the emergence and spread of resistance. Thus far, drugs have been used alone (single agent chemotherapy) and exploited to the point where they become ineffective; then a new drug introduced. Such an attitude assumes unlimited resources and indefinite availability of new drugs. However the pharmaceutical industry does not consider the malaria market profitable, and new drugs are not readily available.

Amongst the various activities that are or will be conducted under this new initiative is research to optimize drug treatments, which includes an array of studies on drug combinations aimed at gaining "extra-mileage" from existing antimalarial drugs.

One combination to be studied is that of artesunate with amodiaquine. To date no clinical study has been conducted to investigate the effectiveness and safety of this particular combination. Based on current published pharmacokinetic/metabolic data it is expected that there would be little or no interaction between them (Dollery, C. 1991) This study is among the first of several studies to explore the usefulness of different drug combinations with artesunate for application in the treatment of falciparum malaria.

Such approach was endorsed by a WHO informal consultation on the use of artemisinin and its derivatives, Geneva 10-12 June 1998 and given high priority.

4.0 Rationale

These studies are based on the concept primarily developed by White (1997,1998) and others (Peters, 1990) of initial parasite biomass reduction to protect vulnerable antimalarials. Antimalarials with long terminal elimination phases and therefore long residence in the organism have a particular propensity to generate resistance.

There is good theoretical basis for the chances of a drug-resistant mutant appearing to be reduced if such drugs are combined with an artemisinin-type compound. Through its rapid onset of action the latter will leave a small residual parasite biomass for the companion drug to eradicate at a time of maximum blood concentrations; similarly, the parasites will never be exposed to the artemisinin derivative alone (given its short half-life). Artemisinin-type compounds are also effective in reducing transmissibility and thus the likelihood of resistant mutants being transmitted.

5.0 Objectives

The objectives of this study are:

3. To investigate the tolerability of concomitant oral administration of artesunate and amodiaquine in healthy normal volunteers (HNV).
4. To investigate the pharmacokinetic properties of the same combination. The kinetics of both the parent drugs and the primary active metabolites (dihydro-artesunate and desethylamodiaquine) will be determined.

6.0 Study design

6.1 Description

The trial design will be a single randomised three-phase crossover model in a total of 12 healthy normal subjects. All trial drugs will be given as a single bolus with 200ml tap water on an empty stomach after an overnight fast. For a week before the study, during the study and during the washout periods the volunteers will take no other drugs or alcohol.

Phase one: All subjects will be administered initially with oral artesunate (4 mg/kg).

Phases two and three: One week later subjects will be randomly allocated to one of the treatment groups:

(i) A single oral dose of sodium artesunate (4 mg/kg) and amodiaquine base (10 mg/kg).

OR

(ii) A single oral dose of amodiaquine (10 mg/kg).

At the end of a 21 day washout period, the subjects will be crossed over.

The elimination half-life of artesunate is 0.53hr and that of amodiaquine 5.2 hours. The washout period for this study is based on the elimination half-life of desethylamodiaquine, the active metabolite of amodiaquine, which is between 9 and 18 days (Dollery, C. 1991, Krishna, S et al. 1996, Pussard, E et al 1994).

6.2 Location of Trial

The study will be performed in the Department of Pharmacology, University of Cape Town. All laboratory assays will be completed in the laboratory of the same department.

6.3 Number of Subjects

The proposed number of subjects is 12.

7.0 STUDY POPULATION:

7.1 Inclusion Criteria

Volunteers will be recruited by means of advertisements placed at the University of Cape Town and Groote Schuur Hospital. Volunteers must meet all the following criteria:

- Age 18-45 years
- Written consent given after reading the patient information leaflet. Participation must be voluntary and volunteers will be fully informed of possible side effects. They will be advised that they are free to withdraw at any time.
- No significant abnormal findings on history or examination, particularly no prior liver disease, cardiovascular disease (including arrythmias) or peripheral neuropathy.
- Clinically significant abnormalities on haematology, liver and renal function tests.
- Negative pregnancy test (women).
- Normal electrocardiogram.
- No history of antimalarial ingestion (chloroquine, amodiaquine, quinine, halofantrine, pyrimethamine-sulfadoxine associated or not to mefloquine) in the preceding two months.

- No other drugs or medications, including over-the counter preparations, ingested in the preceding week.
- Adequate venous access.

7.2 Exclusion Criteria

- Refusal of consent.
- Biological or electrocardiograph anomalies.
- Presence of hepatic, renal and gastrointestinal disorders.
- Smokers (>5/day), abuse of alcohol or recreational drugs
- Presence of malaria parasites on a thick smear
- Subjects having been in a malarial area in the preceding 8 weeks
- Subjects having ingested drugs in the preceding week
- Presence of acute or chronic infections

8.0 ASSIGNMENT TO TREATMENT

8.1 Registration Procedures

All the subjects volunteering to the study will be evaluated for their eligibility to the study and assigned a unique study number. Recruitment will continue until 15 volunteers meeting all entry criteria are accrued. All the volunteers must be registered before entering the study. Only volunteers 1 to 12 will participate in the study. The remaining 3 volunteers (12-15) will be replacements in case of withdrawal of any of the selected volunteers prior to the commencement of the study.

9.0 STUDY DRUG

The medication to be used in this study is amodiaquine tablet (Parke-Davis 200mg base/tablet) and artesunate tablet (Sanofi 50 mg capsules).

9.1 Acquisition:

Both of these medications are not registered with the South African Medicines Control Council, but are used by other centres worldwide. Artesunate is registered in Thailand and amodiaquine in a number of countries, including the United Kingdom, France and Spain.

The study medications will be procured by representatives at the World Health Organisation and made available for the purposes of this study. An application is being made to the Registrar of Medicines at the Department of National Health for permission to conduct the study. **The study will not be conducted unless prior permission is granted for the use of both drugs**.

9.2 Supply, labeling and packaging:

Packaging and labeling of the drugs will be in accordance with a randomisation list drawn up by an independent statistician and identified by the study, phase and volunteer ID.

9.3 Storage, dispensing and disposal

Drugs will be stored in a temperature regulated, limited access area. An inventory of all the drugs (and batch numbers) received and administered will be kept. Drugs remaining at the end on the trial will be returned to the sponsors.

10.0 TREATMENT PLAN

10.1 Dose/Route of Administration

For all three phases a single dose of the appropriate drug/s will be taken orally with 200ml tap water after an overnight fast. The time of administration will be recorded at "Time 0". Study personnel will ensure that the oral medication was taken as scheduled.

10.2 Randomisation

The randomisation list for phases two and three will be prepared and kept by the Director of the Clinical Trial Section. Randomisation will be conducted on the basis of sealed and opaque envelopes provided by the study personnel mentioned above.

10.3 Study Endpoints

1. **Completion of the study**
2. **Patient withdrawn**:

a. Patients will have the right to withdraw from the study at any time for any reason.

b. The investigator will also have the right to withdraw patients from the study in the event of intercurrent illness, adverse events, treatment failure after a prescribed procedure, protocol violations, for administrative or other reasons.

It is understood by all concerned that an excessive rate of withdrawal of patients should be avoided. Should a patient decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. A complete final evaluation at the time of the patient's withdrawal should be made with an explanation of why the patients is withdrawing from the study.

If the reasons for removal from a patients from the study is an adverse event or an abnormal laboratory test result, the principal specific event or test will also be recorded on the Case Report Form.

Volunteers who elect to withdraw or who are withdrawn will not be replaced after the first drug administration.

10.4 Concomitant Therapy

The patients participating in this study should not take other medications for the period of the study and 1 week prior to its commencement. No antimalarials should have been taken in the preceding 8 weeks. No alcohol should be taken 48 hours before and after drug administration.

During the initial 5 hours after drug dosing in all three phases meals will be standardised.

10.5 Drug Compliance

Treatment will be fully randomised. The investigator will administer medication.

11.0 STUDY SCHEDULES

11.1 Pre-Treatment

The following will be performed prior to, and within a month of, commencement of the study drug administrations:

- History
- Clinical examination
- Body weight (only at study entry)
- Height (only at study entry)
- Temperature
- SGOT, SGPT, bilirubin
- Blood urea nitrogen and creatinine
- White blood cell total and differential counts, red blood cell and platelet counts, haemoglobin
- Electrocardiogram

11.2 On-Treatment and Post-Treatment

11.2.1 Safety tests:

The following tests will be performed 6 days after artesunate administration and also on day 20 post-medication of group (i) and (ii) respectively, on both arms of the crossover.

- Clinical examination
- Temperature
- SGOT, SGPT, bilirubin
- Blood urea nitrogen and creatinine
- White blood cell total and differential counts, red blood cell and platelet counts, haemoglobin
- Electrocardiogram (will be done on days 0, 7 and 28)

11.2.2 Collection of Plasma Samples

Blood samples (2 X 5ml each) will be drawn from by a vacutainer system directly into heparinized tubes for further processing. Specimens in the first 12 hours of each phase will be drawn via an indwelling catheter. The others will be by direct venupuncture.

Subjects in **phase one (day 0)**, receiving artesunate alone, will have blood samples collected at pre-dose, 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8 and 12 hours after drug administration.

Phases two (day 7) and three (day 28): Subjects receiving artesunate plus amodiaquine or amodiaquine alone (Groups I and II). Blood samples will be collected at pre-dose, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 48 and 72 hours, and days 4, 5, 7, 10, 14 and day 20 post-medication.

Immediately after collection of blood, the tube will be inverted by hand to ensure thorough mixing with the anticoagulant and centrifuged for 5 minutes at 3 000g. After centrifugation the plasma samples will be immediately transferred to separate appropriately labeled plastic cryotubes frozen at -70°C.

11.2.3 Recording of Results

Subject Records and Laboratory Sheets will be filled in for each volunteer and signed by the Principal Investigator. Copies of the Record Sheets will be made available to the Trial Monitor. The completed case records and laboratory sheets will be made available on request, to TDR, WHO, World Health Organization Headquarters, Geneva. Similarly, the results of the plasma concentration and pharmacokinetic analysis will be forwarded to TDR, WHO, Geneva. A final detailed report of the study will be submitted within six months following completion of the study to TDR, WHO. All documentation will be retained

12.0 DATA HANDLING AND PHARMACOKINETIC ANALYSIS

12.1 Analysis of Plasma Samples

Amodiaquine will be analysed by HPLC with UV detection while artesunate will be analysed by HPLC equipped with an electrochemical detector (Navaratnam et al. 1997). When reporting the results, within-day and day-to-day variations in keeping with the stipulations of GLP must be included.

12.2 Pharmacokinetic Analysis

All data will be analysed using the non-liner least square regression programme. TOPFIT (Heinzel et al. 1993) for estimation of pharmacokinetic parameters (AUC, MRT, T1/2, Cl and Vd). Maximum concentration (Cmax) and time to reach Cmax (Tmax) will be the observed values.

13.0 TOLERABILITY ASSESSMENT

13.1 Adverse Reactions

All adverse reactions during the study must be recorded with the date and hour when they occurred and the date the abnormal findings disappeared. These may include G.I. (vomiting, diarrhoea, etc). CNS, cardiovascular, dermatological, haematological and other changes. For each adverse event, even if not serious, the following information will be entered in the CRF:

- description of adverse event,
- onset date and type (sudden, gradual, unknown),
- duration,
- severity (1 > = mild 2 - moderate, 3 - severe, 4 = unknown),
- cause-effect relationship with the drug (according to the modified Karch and Lasagna's criteria),
- outcome,
- Measures taken (symptomatic treatment, discontinuation of treatment).

Should a serious and/or unexpected reaction occur, the Monitor should be immediately informed by telephone or fax. The South African Medicines Control Council must be informed within 24 hours. The study director will also be informed within a day.

13.2 Tolerability Analysis

Descriptive statistics will be used to summarise baseline values and demography. Pre- and post-treatment values (haematology and blood chemistry) will be compared as follows:

- baseline vs. last assessment; all subjects and by group

- pre-artesunate vs pre-treatment with AQ or AQ + AS (one week)
- pre-treatment with AQ or AQ + AS vs. post-treatment (day 21 and day 42 post-treatment)

For changes over time of continuous variables, changes from baseline will be assessed by using the t-test for paired observations within groups. Between group comparison will use the one-way ANOVA with post-hoc multiple comparison made using the Tukey's honest significance difference (HSD).

14.0 DRUG INVENTORY

Drug supply and Drug Inventory Records will be kept by the investigator as to the disposition of study drug for each subject. The Investigator will prepare and maintain the following:

(i) Individual patient cases records
(ii) Study logbook
(iii) Drug Disposition and accountability
(iv) Serious and/or unexpected adverse event forms

15.0 MONITORING OF THE STUDY

The investigators will permit clinical monitors designated to inspect all case report forms, all medical records, laboratory work sheets and to assess the status of drug storage, dispensing and retrieval at anytime during the study. The inspections are for the purpose of verifying the adherence to the protocol.

16.0 ETHICAL AND LEGAL CONSIDERATIONS

This clinical study will be conducted in accordance with the principles laid down by the World Health Assembly of 1975 on Ethics in Human Experimentation and the Helsinki Declaration. The study will adhere to the standards established for Good Clinical Practices (GCP). The protocol will be submitted for review and approval by the University of Cape Town's Ethical Committee and the WHO Secretariat committee for research Involving Human Subjects (SCRIHS). Each subject will be informed of the nature and possible risks of the trials. The Investigator will obtain informed written consent from every subject participating in the study. The subject must be informed that he is at liberty to abstain from participation in the study and that he is free to withdraw the consent of participation at anytime. The Investigator may order the appropriate medical exclusion of a subject from a study. The investigators and study staff will observe confidentiality of the records. After the initial screening procedures a coded number will record the identity of the volunteers.

The drug manufacturers (Sanofi and Parke Davis) will be responsible for obtaining full insurance coverage for the study.

17.0. REPORTS AND PUBLICATIONS

It is agreed that publication of the results of this study will be permitted. It is understood that there will be, prior to the submission of the manuscript, agreement on the data and their interpretation with the Secretariat of TDR/WHO.

18.0 REVIEW OF TOLERABILITY AND SAFETY OF STUDY MEDICATIONS

(i) Amodiaquine

Introduction:

Amodiaquine (AQ) is a schizontocidal anti-malarial. Structurally it is a convenor of chloroquine. Its mechanism of action is not clearly understood (Dollery, C. 1991). It is postulated that AQ, a basic compound, accumulates is the malarial lysosomes, raising the pH and thereby causing a loss of lysosomal function. Without this the parasites are unable to digest haemoglobin (Dollery, C. 1991). Despite its structural similarity to chloroquine there is evidence that it may be an effective treatment even in areas of chloroquine resistance (Olliaro, P. 1998).

The major metabolite of amodiaquine is desethylamodiaquine. It is an active antimalarial with pharmacokinetic properties similar to chloroquine (Krishna, K. 1996). The elimination half-life of amodiaquine is short (~5 hours) and that of desethylamodiaquine is between 9 and 18 days (Dollery, C. 1991, Krishna, S et al. 1996, Pussard, E et al 1994).

Toxicology:

Amodiaquine has been widely used to prevent and treat Falciparum malaria since its release in the 1950s. Amodiaquine has similar side effects to those of chloroquine. However it was removed from use as a prophylactic agent in the 1980s due to increased incidence of agranulocytosis and the association with hepatitis when given over a prolonged period (Martindale, 1998, Olliaro et al 1996). In 1990 the World health Organisation withdrew the drug from malaria-control treatment programmes. This decision was not fully accepted by the 19th Expert Committee on Malaria, which stated that "amodiaquine can be used for treatment if the risk of infection outweighs the potential for adverse drug reactions". The review by Olliaro (1996) attached to this protocol shows that amodiaquine may be useful in areas with low-grade chloroquine

resistance and that the chance of severe adverse effects is not dissimilar to that with chloroquine or sulphadoxine-pyrimethamine (White 1996).

Most of the cases of neutropaenia reported with amodiaquine occurred when it had been used in anti-inflammatory doses for rheumatoid arthritis. In 1986 a cluster of 23 cases were reported during use for malaria prophylaxis, of which 7 were fatal. In these cases the drug had been used at a dose of 400mg weekly for between 3 and 24 weeks, with an average of 9 weeks. The manufacturers report 28 cases of agranulocytosis (9 fatal). Toxicity does not seem to be related to direct bone marrow damage by the drug or metabolites, but rather due to an immunological reaction to quinone imine. Quinone imine can be produced from both amodiaquine and desethylamodiaquine by auto-oxidation, among other processes (Dollery, C. 1991, Martindale, 1998, Olliaro, P.1996).

There have been a number of reports linking the prophylactic use of amodiaquine with hepatotoxicity. The manufacturers report 14 cases (3 fatal). Some of the cases of agranulocytosis also showed evidence of liver disease (Martindale, 1998). Animal studies using high doses of amodiaquine noted fatty change and central necrosis in the liver (Dollery, C. 1991).

At high doses, opacities of the lens, cornea or both have been noted. In addition to these deposits in the eye, amodiaquine may also increase pigmentation of the nail beds and skin when given in high doses. Pruritus and skin rashes have been reported. Long term administration may result in GIT side effects such as anorexia, nausea, cramps, diarrhoea and vomiting (Dollery, C. 1991).

The most commonly reported adverse events are nausea, vomiting and pruritus (Olliaro, P.1996)

Unlike chloroquine, amodiaquine appears not to cause cardiovascular symptoms during acute overdose. However, few cases of overdose have been reported (Martindale, 1998). Other symptoms following a large dose of amodiaquine include syncope, spasticity, convulsions and involuntary movements (Martindale, 1998).

The safety of amodiaquine in pregnancy has not been established.

It seems that serious and fatal adverse drug events have only been described during prophylaxis after repeated administration. This data only comes from manufacturer and trial based sources (Martindale, 1998, Olliaro, P. 1998). Treatment appears to be safer as a lower total dose is administered. Multiple treatments, in endemic areas, may however produce toxicity (Olliaro, P. 1996). Data given to the UK Committee on the Safety of Medicines predicts that the risk of adverse reactions to amodiaquine may be 1:1700 for serious reactions, 1 in 2200 for blood disorders, 1 in 15 650 for severe hepatic disorders and 1 in 15 650 for fatal reactions (Martindale, 1998). The risk of fatal disorders for amodiaquine is equivalent to that with suphadoxine-pyrimethamine (fansidar) (Olliaro, P. 1996).

Amodiaquine may still prove to be a useful antimalarial with graded, slow and well monitored re-introduction (White 1996).

(ii) Artesunate

Introduction:

Artesunate and derivatives are drugs that have been used for many years in China (Qinghaosu). They have come into use outside China largely by necessity to treat multiple drug resistant malaria (Hien 1994, Looareesuwan 1994, Nosten 1994, Meshnick 1996). As a result, they have largely not undergone comprehensive routine development and testing. Important clinical questions about their use and safety have yet to be answered (Ribeiro, IR, appendix 2).

Artesunate is a pro-drug for its active metabolite dihydroartesunate. Biotransformation occurs rapidly and almost all artesunate is eliminated within 1 hour. The elimation half-life of dihydro-artesunate is only a few hours (Barradel, 1995, de Vries et al, 1996).

Toxicology:

The data described in this section reflect the findings reported from individual trials, and thus may not reflect the overall incidence of the adverse effects associated with artesunate.

Neurotoxicity has been identified to be of major importance for artemisinin derivatives in animal studies (Brewer et al, 1994). To date there have been no clinical drug-related

reports of neurotoxicity in patients receiving artesunate. Therefore, the relevance of evidence of neuropathic lesions at high doses in animal models is unknown (Anonymous, 1994).

A dose-ranging study with intravenously administered artesunate revealed that the dose-limiting adverse effect of artesunate in 18 healthy volunteers was a reduction in reticulocyte count (Barradell and Fitton, 1995). Reticulocyte count returned to baseline after 7 days' treatment with all 5 treatment regimens.

Overviews of clinical studies of artemisinin derivatives conducted in Thailand or at the Myanmar-Thai border, in which more than 1,000 patients received artesunate as monotherapy, or in various combinations typically including mefloquine, concluded that these agents were well tolerated and had insignificant adverse effects (Looareesuwan, 1994; Nosten, 1994). Similarly, an overview of the treatment of malaria in China reported a single episode of skin rash as the only adverse effect associated with oral, intramuscular or intravenous artesunate therapy in more than 400 patients (Li et al, 1994).

Other adverse effects reported in trials in smaller numbers of patients receiving a total oral dose of 600 to 1,200 mg of artesunate included dizziness, itching and vomiting (Bunnag et al, 1991; Karbwang et al, 1994). Other effects such as abdominal pain, flatulence, headache, body ache, diarrhoea, tinnitus and increased hair loss (Bunnag et al, 1991), convulsions (Karbwang et al, 1994), slight reduction in neutrophil count (Bunnag et al, 1991) and macular rash (Looaresuwan et al, 1992) have also been reported. The possibility that these adverse effects may be disease-rather than treatment-related cannot be ruled out. The observation that there were no adverse effects in any patient was also common in other clinical trials (Hien et al, 1992a, Hien et al, 1992b).

Although there are fewer data than in adults, artemisinin derivatives appear to be well tolerated in children (Hien and white, 1993). For pregnant women there is little information, but long term follow-up of the offspring of 17 women who received either artesunate or artemether during pregnancy has not revealed any abnormalities to cause concern (Barradell and Fitton, 1995).

In one trial combination therapy with artesunate and mefloquine caused a slightly higher incidence of vomiting than did treatment with either individual drug, but this difference was not statistically significant (Looaresuwan et al, 1992). In larger studies, other investigators found the combination of artesunate and mefloquine was associated with a lower incidence of vomiting than mefloquine alone (Nosten et al, 1994; Luxemburger et al, 1994). Nausea and dizziness were more frequent in patients receiving mefloquine alone than in those receiving it in combination with artesunate (Luxemburger et al, 1994). These apparent differences in the incidence of 'adverse effects' may actually reflect the more rapid resolution of the symptoms of malaria in patients treated with the combination therapy (Looareesuwan et al, 1994), rather than the true incidence of drug-related adverse effects with the different treatments.

In general, to date the artemisinin compounds have proven remarkable non-toxic in clinical practice. There has been no dose-related predictable adverse effect from therapy, even though a few clinicians remain concerned over central nervous system toxicity based on animal data.

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Appendix one

Safety Of Artemisinin And Its Derivatives

A Systematic Review Of Published And Unpublished Clinical Trials

IR Ribeiro & P Olliaro

1. Background and objectives of the systematic review

Artemisinin derivatives have come into use outside China largely by necessity to treat multiple drug resistant malaria (Hien 1994, Looareesuwan 1994, Nosten 1994, Meshnick 1996). As a result, they have largely not undergone consistent and rational development and testing. Important clinical questions about their use and safety have yet to be answered.

In particular, in sufficient doses and, given under specified testing conditions in experimental animals, artemisinin and its congeners induce a dose-dependent injury which results in significant toxic neuronal injury, largely confined to the brainstem, and functional impairment of auditory, vestibular, cerebellar, motor and reticular activating systems. The observations have been confirmed in rats, dogs and monkeys, and they are suspected but not proven in mice and hamsters. In sufficiently high doses in dogs, Brewer et al have described a progressive syndrome of clinical neurological defects with cardiorespiratory collapse and death. Although the applicability to humans and clinical relevance of these studies have not yet been established, hitherto there have been remarkably few adverse effects reported with the use of artemisinin derivatives in man. A comprehensive assessment of benefits and risks is, however, needed to inform drug policy decisions on the rational use of these drugs.

The aim of this review is to summarize the existing evidence of safety of artemisinin derivatives, alone and in combination with other antimalarials, in different epidemiological settings.

2. Methods

The inclusion criteria, search strategy, methods and comparisons were pre-specified in a study protocol.

- Studies considered included comparative and non-comparative studies, patient series, case reports and phase I studies in healthy normal volunteers with either artemisinin, dihydroartemisinin, artesunate or artemether, given by any route, either alone or in combination with other antimalarials.
- Outcome measures:
    - reports of adverse events: number of adverse events, type (using the simplified WHO classification by body system), severity, including death and neurological sequelae.

- clinical laboratory evaluation: number of patients with abnormal results, comparison of mean values (before and after treatment), paired t-test for raw data, and, possibly, shift tables (pre- and post- treatment values, normal versus abnormal results).

- Search strategy: the trials register of the Cochrane Infectious Diseases Group was searched for any trial or reference to a relevant trial (published, in-press or in progress). Organizations and individual researchers working in the field were contacted for unpublished data, confidential reports and raw data of published trials. Citations of existing reviews on artemisinin drugs and of all trials identified by the above methods were also scrutinised.
- Methods of the review: all trials identified were entered in a database register. The inclusion criteria were applied to all identified trials and those qualifying for analysis were retained. Data for the pre-specified outcome measures was abstracted on to a collection form and entered into two databases (RevMan 3.0 and FilemakerPro) for subsequent analysis.

3. Results

3.1 Assessment of Data Quality

One hundred and eighty eight studies were identified, of which 108 fulfilled the entry criteria. One hundred and two of the 188 were published and 6 were unpublished. Individual patient data was available for 3 of the published trials. A total of 9,241 patients included in these studies received artemisinin derivatives (3,526 artemether, 2,481 artemisinin, 3,133 artesunate, 51 dihidroartemisinin and 50 artemether) (Table 1). Fourteen studies did not show any evidence of tolerability assessment and were, therefore, excluded from further analysis. Among the remaining studies, there were 42 in uncomplicated malaria, 27 in severe malaria (11 cerebral malaria), 8 in moderately severe malaria, 17 in unspecified malaria, and 6 in healthy normal volunteers. Six thousand and eighty seven (6,087) patients were enrolled in comparative randomised trials, 1,321 patients in comparative non-randomised trials (or those with non-specified randomisation status), 1,401 in non-comparative trials and patients series and 135 in studies with healthy normal volunteers.

A total of 10 studies with 815 patients treated with artemisinin, 10 studies with 301 patients treated with artesunate, and 9 studies with 388 patients treated with artemether had no reported adverse reactions to the prescribed treatments. In addition, five studies with a total of 700 patients treated with artemisinin, 6 studies with 346 patients treated with artesunate and 9 studies with 395 patients treated with artemether had incomplete information or vague statements precluding a calculation of the number of adverse events. Among the latter studies, five had no adverse reactions ascribed to an artemisinin-derivative.

An additional report (Price et al, submitted for publication) contains retrospective analyses of prospectively retrieved studies in 3,645 patients with acute falciparum malaria. Of these, 2915 are published and included in the present report. The additional 1,730 patients are not included because incompatible data were available at the time of the preparation of this report.

3.2 Studies on healthy normal volunteers

No adverse reactions were reported in three of the volunteer studies (2 with artemisinin and 1 with artesunate).

- *Intravenous artesunate*: 3/32 subjects complained of bitter taste (2 subjects in the 4 mg/kg total dose group and 1 in the 8 mg/kg total dose group) and 5/32 complained of pain at injection site (2 in the 8 mg/kg dose group, 1 in each of 12 mg/kg, 15 mg/kg, and 16.88 mg/kg dose groups). Clinical laboratory evaluation was unremarkable except for the reduction of reticulocyte counts in 7 of the 32 volunteers. Mild reticulocytopenia was seen in only 1 patient in the 12 mg/kg dose group and 2 patients in the 16.88 mg/kg dose group. Reticulocyte levels in the remaining patients stayed within normal limits.
- *Artemisinin per rectum*: 2/18 subjects had fever documented (1 on 4200 mg and 1 on 5100 mg total dose). Two subjects complained of slight burning sensation in the anus. Elevated SGPT was seen in 1 subject receving 5100 mg and 1 receiving 6000 mg total dose.
- *Intramuscular artemether*: 7 of 17 volunteers had transient mild fevers (1 on 12 mg/kg over 5 days, 1 on 9.6 mg/kg, 1 on 7.2 mg/kg and 4 on 12 mg/kg over 3 days). A transient decrease in mean reticulocyte count was seen in the 9.6 and 7.2 mg/kg dose groups. As seen in most subjects receiving artesunate, values remained within normal limits. A slight rise in SGOT was seen in subjects receiving 12 mg/kg over 3 days (2/4 subjects on day 7 and ¼ subjects on day 14).

3.3 Comparative studies, randomised and non-randomised

Several different regimens and dosages of artemisinin-type compounds have been used and compared. The most commonly reported adverse events were gastrointestinal in origin (nausea, vomiting, diarrhoea, abdominal pain). Those reported were mild in severity with no severe, life-threatening adverse event related to the use of artemisinin derivatives. There was no association between the artemisinin-type compounds' dosages and routes and the incidence of adverse events.

Artemisinin derivatives were well tolerated in comparison with quinine and mefloquine. There was no significant difference in the incidence of major side effects (nausea, vomiting, diarrhea, abdominal pain, pruritus, pain/abscess at injection site) between the three compounds.

3.4 Non-comparative studies

A total of 919 patients were enrolled in non-comparative trials with the various artemisinin derivatives. Headaches were the most frequent adverse events, reported in 7% of patients, followed by nausea (4.7%), abdominal pain (3.4%), tenesmus (3%), vomiting (2.6%) and diarrhoea (1%). Dizziness, rash, tinnitus, burning sensation in the anus, transient fever, and excessive salivation have all been reported (but in less than 1% of patients).

3.5 Safety measures

3.5.1 Clinical laboratory evaluation

- Haematological evaluation was performed in 4,062 patients treated with artemisinin derivatives. Neutropenia was documented in 52 of these patients. A decline in neutrophil counts was also mentioned 2 other studies, but the number of cases was not given. A reduction in reticulocyte count was reported in 25 patients, however also seen in an unspecified number of patients in 2 other studies. Eosinophilia was

reported in 40 patients (from a single study). Anaemia was observed in 8 patients, 4 of which requiring blood transfusions.

- Biochemical testing was done in 3,893 patients. Elevated transaminases was reported in 36 patients and also mentioned in 2 other trials (without the number of cases). Elevated total bilirubin was reported in one study.
- Urine Analysis was conducted in 564 patients had urine analysis done. No abnormalities were noted in the majority of studies. Culture-negative pyuria was reported in 20 patients (an increased incidence when compared with quinine). Blackwater (hemoglobinuria) has also been described in 7 patients treated with artemether.

3.5.2 Electrocardiography (EKG)

EKGs were performed in 2,638 patients. Thirty patients (1.1%) had episodes of bradycardia, mostly reported as mild and transient (or with no comments). Thirty-two patients (1.2%) had a significant prolongation in the QT interval.Two further studies mentioned a few cases of bradycardia, but giving no precise numbers. First degree AV block was seen in 3 patients from three different studies. One patient was reported to have atrial extra-systoles and 5 patients T-wave abnormalities. None of the abnormalities mentioned required treatment or had associated clinical changes.

Two patient series focused on electrocardiographic monitoring and changes occurring during severe malaria. One indicated that the QT interval changed during the early phase of malaria and that those changes were independent of the type of antimalarial therapy given. The other identified no life-threatening cardiac arrythmias and detected no abnormality which could have resulted from the medications given (among them, artemisinin, artemether, artesunate).

3.5.3 Neurological assessment

The vast majority of patients did not have information on neurological evaluations. The exception occurred in patients with severe malaria with or without cerebral involvement.

Four patients experienced neuropsychiatric adverse events. One patient with history of epilepsy, seizure-free for 10 years, had a episode of tonic clonic seizures; one patient became restless, with insomnia and irritability; one developed frank psychosis and another, depression. All patients were also receiving mefloquine, a known cause of neuropsychiatric disturbances.

There was a single case report of ataxia and slurred speech following treatment with oral artesunate. There was no information on the use of prophylactic treatment and concomitant medications (particularly mefloquine), site of initial diagnosis, details of baseline clinical status, reasons for self-medication, reliability of patient's account, as well as, the worrisome lingering undefined malaria infection.

A retrospective analysis of prospectively retrieved neurological signs and symptoms was conducted in Myanmar on 283 patients who had received artemisinin-type compounds either alone or combined with either mefloquine or doxycycline for uncomplicated malaria during 1990-1996. Of them, 98 (35%) developed a total of 106 neurological episodes -mainly dizziness (88 episodes). Most of the symptoms occurred on the very first day of treatment. Artesunate- and to a lesser extend artemether-containing regimens (alone or in combination) appeared to produce significantly more neurological adverse events than artemisinin. Such difference disappears when considering the subgroup of patients on monotherapy, but then sample size decreases substantially. Artemether at 960 mg total dose appears to be associated to a higher risk

of neurological events than lower doses. The conclusions must be interpreted with caution since all analyses were post-hoc.

3.5.4 Neurologic sequelae and deaths

Fifty-seven (57) of 851 patients receiving an artemisinin derivative for the treatment of severe malaria in randomised comparative trials vs quinine suffered from neurological sequelae (on discharge from the hospital). Sixty-nine (69) of 836 patients receiving quinine suffered from neurological sequelae.

One hundred and forty seven (147) patients among the 930 on artemisinin-type compounds died, compared to 177 of 915 patients on quinine. There was no significant difference between the two groups.

3.6 Strength of Evidence and Applicability of Conclusions

Despite the obvious methodological deficiencies of compiling published and unpublished data from various studies, the current report is the most comprehensive attempt to identify all the available information on the safety of artemisinin-type compounds. These drugs have been widely believed to be the fastest and best tolerated of all antimalarials and there were clearly no surprises on this matter.

The safety data presented in this study, however, reflect the findings reported in individual trials and may not illustrate the overall incidence of adverse effects associated with the artemisinin derivatives in the population. Often information was inadequate on data collection, imputability of an observed event and criteria adopted for reporting of adverse events. To further complicate the discussion of safety, the adverse effects experienced by patients often mirrors the symptoms of malaria. Although these effects are attributed to artemisinin derivatives in some studies, one should also consider the expected incidence of disease-related effects when assessing tolerability in this setting.

There were remarkable few side effects reported with these compounds. The most commonly reported adverse events were gastrointestinal. Clinical laboratory evaluation showed a few cases of reduced reticulocyte counts, anaemia, neutropenia and elevated transaminases. All side effects were mild and transient. Electrocardiographic assessment revealed a very low incidence of bradycardia and QT prolongation, with the latter also seen in early phases of malaria.

Unfortunately, limited data were available on neurological assessments. Three patients had neuropsychiatric adverse events, all of which resolved spontaneously without any intervention. There was a single case report of ataxia and slurred speech in a patient receiving oral artesunate. Several aspects of this case, however, are vague, such as the use of prophylactic treatment and concomitant medications (particularly mefloquine), site of initial diagnosis, details of baseline clinical status, reasons for self-medication, reliability of patient's account, as well as, the worrisome lingering undefined malaria infection. Nonetheless, this report highlights the importance of continued surveillance and further studies to evaluate the neurotoxic potential of this class of compounds.

In summary, despite inevitable bias and methodological limitations, this review collated evidence corroborating the benign safety profile of the artemisinin derivatives.

Table 1:

Type of Compound	Route	Number of Patients
Artemether	Oral	863
	Im	2663
Artemisinin	Oral	1016
	Im	114
	Pr	1351
Artesunate	Oral	1742
	Im	194
	Iv	989
	Pr	208
Arteether	Im	50
Dihidroartemisinin	Po	51
Total		9241

Im: intramuscular Iv: intravenous Pr: per rectum

Patient consent form

Volunteer information

ARTESUNATE AND AMODIAQUINE: TOLERABILITY AND DRUG INTERACTION STUDY IN HEALTHY NORMAL VOLUNTEERS

Introduction- what this trial is about:

Malaria is an illness transmitted by mosquitoes. Millions of people in Africa die of malaria every year. The infectious organism is a parasite. The one that affects South Africa is usually Plasmodium falciparum. Most malaria can be treated, particularly if diagnosed early on. However, the falciparum strain is rapidly becoming resistant to drugs commonly used to treat it, such as chloroquine. New drug development will not be able to keep up with this rising rate of resistance.

Novel approaches are needed. One of these is to use two anti-malarials together. New drugs, to which Plasmodium Falciparum is still sensitive, can have their useful life prolonged by giving them with another anti-malarial.

This trial is to look at the combination of amodiaquine, an old anti-malarial with a similar structure to chloroquine, and artesunate, a new, rapidly acting anti-malarial. These drugs have not been used in combination before. This trial aims to establish whether the two drugs interact at a biological level when taken together, and whether a single dose of both drugs taken together is tolerable to a patient.

Duration of the trial:

Screening for the trial will happen in the 3 weeks preceding the study. Should you be accepted for the trial and decide to take part in it, you will be involved for 48 days from the time of taking the first study dose.

During these seven weeks you will spend 3 days (7am to 8pm) in a ward. These will be weekend days- days 0, 7 and 28. On these days you will be given the study medicines to take in the morning. Another 19 blood specimens are needed, on non-ward days. Blood samples will be taken between 7h00 and 8h30 on the required days.

Trial structure and procedures:

Screening:

Before you are accepted for the trial you will need to be screened to check whether it is safe for you to take the study drugs. This screen will involve a full medical history and clinical examination. Your heart will be monitored with an electrocardiograph (ECG). Some blood (15ml) will be taken to check for liver and kidney function and blood cell counts. Your blood and a sample of urine will also be screened for drugs of abuse. If you are a woman a pregnancy test will be sent.

You will not be accepted for the trial if:

- any of the results are abnormal
- you have been in a malarial area in the preceding 8 weeks
- you smoke (more than 5 a day) or drink heavily (more than 1 unit a day)
- you are pregnant
- you have any medical disorders or are taking any medications
- you do not agree to the trial regulations and to return for all the follow-up evaluations and blood specimens

If any abnormalities are found during the screening the trial doctor will explain these to you and refer you to the appropriate doctor as necessary.

You will be asked not to take any other medicines, drugs **OR ALCOHOL** for the duration of the trial.

Trial days:

See Trial Schedule table.

Once you have been accepted for the trial you will be asked to arrive at ward G13, Groote Schuur at 7am on day 0. **It is important that you do not eat or drink anything from midnight the night before.**

When you arrive you will be given a bed and a cannula (a thin plastic tube over a needle) will be inserted in your arm. This can be slightly painful at the time of insertion. Once the needle is removed and the plastic tube left in place it may feel uncomfortable, but should not cause pain. Blood can be taken through the cannula without any further pricks with a needle. If the cannula blocks it may be necessary to resite it or take the blood using a needle and syringe. You may end up with a bruise at the site of cannula insertion.

You will be asked to stay in your bed until after breakfast at 10am. No food or drink is allowed until breakfast. After breakfast you may drink water and after lunch (1pm) you may eat and drink fluids freely. Supper will be between 6 and 7pm. All these meals are standardised.

At 8am you will be given the first dose of the study drugs. On the first day everyone will take artesunate. In order to monitor the drug levels in the blood, samples (15 ml) will be taken through the cannula 12 times in the day. Artesunate has a short half-life and all the drug should be gone by 12 hours. The last specimen for the day will be taken at 8pm, after which you can leave.

Six days after you take the artesunate safety bloods (to check liver and kidney functions, as well as blood cell counts) will be taken.

Day seven is the second admission day. . **It is important that you do not eat or drink anything from midnight the night before.** This day is the same as day 0. The drugs taken will either be a dose of amodiaquine alone or a dose of amodiaquine with a dose of artesunate. Amodiaquine has a half-life of up to 3 weeks, so although the artesunate is gone by 12 hours we need to monitor the amodiaquine for longer.

After this round we need to take bloods the following morning and on days 2,3,4,5, 7 10, 14 and 20 after taking the drug. On the 20th day safety bloods will be taken too.
The third admission day follows. It is identical to the previous two. Again, **it is important that you do not eat or drink anything from midnight the night before.** You will receive the treatment (either amodiaquine alone or a dose of amodiaquine with a dose of artesunate) that you did not get the previous round. All the blood samples in the ward and for the 3 weeks afterwards are the same as above. The last specimen is on the 20th day after your last drug dose.

Your rights as a volunteer:

Your participation is completely voluntary. You can refuse to participate and withdraw at any time without stating a reason. The trial investigator retains the right to withdraw you from the study if it is considered important for your safety. If it is discovered you did not give an accurate history or did not follow the trial regulations you may also be withdrawn.

Risks of the trial:

Both of the trial drugs have the potential to cause side effects. Allergies or unexpected side effects may occur.

Amodiaquine:

The most common side effects experienced with amodiaquine are nausea, vomiting and itching of the skin. In higher doses it can cause darkening of the skin and nails, diarrhoea, loss of appetite and abdominal cramps. Weakness and irregular heartbeat have been reported.

It has two severe side effects, liver toxicity and suppression of the white cells in the bone marrow (agranulocytosis). Both of these are rare and have only been associated with long term administration of the drug (average 9 weeks). However, they can be fatal.

The safety of amodiaquine in pregnancy has not been established.

Artesunate:

Artesunate has very few clinically significant side effects. Animal studies have shown dose-related damage to the nervous system at high doses but this has not been seen in humans. A transient decrease in reticulocyte count (an early red blood cell) and white cells have been shown. Dizziness, itching, vomiting, diarrhoea, headache and skin rash have been reported.

Safety in pregnancy has not been fully established.

Ethical approval:

This trial will not go ahead until written ethical approval has been received from the University of Cape Town Research Ethics Committee, the Medicines Control Council of South Africa and the WHO Secretariat committee for research Involving Human Subjects (SCRIHS).

Financial and Insurance agreements:

You will be compensated for your participation in the trial on completion of the last sample. You will receive R100 for the screening, R625 for each of the three days in the ward and R50 for each blood sample thereafter. Total payment will therefore amount to R2875.00. Volunteers who are withdrawn from the study by the investigator will be compensated up to the point of withdrawal. Volunteers who withdraw themselves may not be compensated.

Insurance will be taken out by the manufacturers of the drugs to cover all participants and staff for the duration of the trial. The trial will not proceed until properly insured. You must notify the investigator immediately if you develop any complications or side effects.

Additional information:

For the duration of the trial you will be under the care of Dr Catherine Orrell. If at any time between your visits you feel you may be experiencing a side effect or have any questions, please contact her at 021-406.6353 or 021-404.9111 page 0507. Alternative number s: for Dr. K. Barnes 021- 406.6294 or Professor P. Folb 021-406.6286.

Additional information on the trial and study drugs is available if you are interested.

Confidentiality:

All information obtained during the course of this trial is confidential. Any publications will not identify you as a trial participant. All of the results of your blood and other tests are held in strict confidence.

Trial Schedule

Day	Action
Pre-trial screening	Examination, screening blood samples-, ECG, urine sample, pregnancy test for women.
Day prior to day 0	**NIL BY MOUTH OVERNIGHT**
Day O (Saturday) Artesunate dose	Admit to ward 7am to 8pm. Cannula, 12 x blood samples, ECG
Day 6 (Friday)	Safety blood samples- **NIL BY MOUTH OVERNIGHT**
Day 7 (Saturday) Amodiaquine or artesunate and amodiaquine	Admit to ward 7am to 8pm. Cannula, 12 x blood samples, ECG
Day 8 (Sunday)	Drug blood sample 8am
Day 9 (Monday)	Drug blood sample 8am
Day 10 (Tuesday)	Drug blood sample 8am
Day 11 (Wednesday)	Drug blood sample 8am
Day 12 (Thursday)	Drug blood sample 8am
Day 14 (Saturday)	Drug blood sample 8am
Day 17 (Tuesday)	Drug blood sample 8am
Day 21 (Saturday)	Drug blood sample 8am
Day 27 (Friday)	Drug and safety blood samples- **NIL BY MOUTH OVERNIGHT**
Day 28 (Saturday) Amodiaquine or artesunate and amodiaquine	Admit to ward 7am to 8pm. Cannula, 12 x blood samples, ECG
Day 29 (Sunday)	Drug blood sample 8am
Day 30 (Monday)	Drug blood sample 8am
Day 31 (Tuesday)	Drug blood sample 8am
Day 32 (Wednesday)	Drug blood sample 8am
Day 33 (Thursday)	Drug blood sample 8am
Day 35 (Saturday)	Drug blood sample 8am
Day 38 (Tuesday)	Drug blood sample 8am
Day 42 (Saturday)	Drug blood sample 8am
Day 48 (Friday)	Drug and safety blood samples-

-Safety and screening bloods involve two tubes: one for a full blood count, and one for liver and kidney functions.

Patient consent form

ARTESUNATE AND AMODIAQUINE: TOLERABILITY AND DRUG INTERACTION STUDY IN HEALTHY NORMAL VOLUNTEERS

Patient name (last and first- please print): _____

Date of birth (day/month/year): _____

Patient/trial number: _____

I confirm that I have been fully informed by Dr. _____ about the nature, schedule and risks of this clinical trial with artesunate and amodiaquine. I have also received, read and understood the patient information regarding the clinical trial.

I am aware that results of the trial will be published. I will in no way be identified in the publication.

I may withdraw from the trial at any stage.

I have had enough time and opportunity to ask questions. I am willing to participate, voluntarily, in this trial.

Patient's name: _____

Patient's signature: _____ Date: _____

I , Dr. _____ confirm that the above patient has been fully informed of the nature, conduct, schedule and risks of the above trial.

Investigator's signature: _____ Date: _____

Witness name (print): _____

Witness signature: _____ Date: _____

Appendix B: Randomization table

(as used for statistical analysis)

The three drug administrations were organised according to the following randomisation table.

Subject ID	Visit 2	Visit 4	Visit 14
A	1	3	2
B	1	2	3
C	1	3	2
D	1	2	3
E	1	3	2
F	1	2	3
J	1	3	2
K	1	2	3
L	1	2	3
M	1	3	2
N	1	2	3
P	1	3	2
Q	1	3	2

1 = Sodium Artesunate alone **Phase 1**

2 = Sodium Artesunate + Amodiaquine base } **Phase 2 and 3**

3 = Amodiaquine base alone

Appendix C: Normal laboratory ranges.

Full blood count:

Haemoglobin – women	11.6 – 15.6 g/dL
Haemoglobin – men	13.3 - 17.3 g/dL
Haematocrit – women	0.32 – 0.48 ratio
Haematocrit - men	0.37 – 0.53 ratio
Platelets	150 – 450 $x10^9$/L
White cell count	4.0 – 11.0 $x10^9$/L
Neutrophils	1.8 – 7.7 $x10^9$/L
Lymphocytes	1.0 – 4.0 $x10^9$/L

Chemistry:

Sodium	135 -145 mmol/L
Potassium	3.5 – 5.5 umol/L
Urea	1.7 – 6.7 umol/L
Creatinine	75 – 115 umol/L
Total bilirubin	1 – 17 umol/L
AST	1 – 25 units/L
ALT	1 – 25 units/L

Appendix D-1: Pharmacokinetic summary tables and graphs

Artesunate Summary Table: Artesunate administered (4 mg/kg) alone or in combination with Amodiaquine base (10 mg/kg).

Subject	Tmax (h)		Cmax (ng/ml)		Half-life (h)		AUC (0-last time point) (ng.h/ml)		AUC (0-inf) (ng.h/ml)	
	Art	Amo + Art	Art	Amo + Art	Art	Amo + Art	Art	Amo + Art	Art	Amo + Art
A	1.00	0.50	74.60	12.00	0.91	MD	66.15	28.50	94.64	MD
B	0.25	1.50	114.50	22.60	0.15	MD	39.28	27.80	40.44	MD
C	0.50	0.50	279.80	265.70	0.22	0.31	137.30	158.33	138.02	157.74
D	0.25	0.25	56.70	336.90	MD	0.52	17.18	259.06	MD	262.12
E	1.00	0.50	95.00	203.80	MD	0.34	65.88	111.74	MD	111.65
F	0.50	0.75	253.40	21.90	0.72	EXCL	152.85	54.98	169.24	162.66
J	1.00	1.50	75.90	50.60	MD	0.84	50.31	69.20	MD	77.79
K	0.75	1.00	388.00	182.40	0.16	0.52	158.08	171.98	157.83	184.42
L	0.50	1.50	557.00	105.20	0.44	0.53	233.76	96.08	235.31	99.32
M	0.25	0.50	332.60	283.00	0.75	0.81	264.59	324.74	265.60	332.83
N	0.50	0.50	389.40	96.50	0.45	0.55	271.11	79.13	272.95	82.58
P	0.75	0.50	252.70	174.50	0.28	0.26	222.54	93.31	221.39	94.77
Q	0.75	MD	143.20	MD	0.34	MD	87.26	MD	87.12	MD
Mean	0.62	0.79	231.8	146.3	0.44	0.52	135.9	122.9	168.3	156.6
SEM	0.08	1.09	42.99	32.19	0.08	0.07	24.76	26.40	25.19	26.63
95% CI	0.44-0.78	0.50-1.09	138.1-325.4	75.4-217.1	0.25-0.63	0.36-0.68	81.93-189.8	64.79-181.0	111.3-225.2	96.35-216.8
Paired t-test (two-tailed p-value)										
	0.25	nsd	0.12	nsd	0.08	nsd	0.62	nsd	0.25	nsd
Wilcoxon matched pairs signed rank (two-tailed p-value for normal approximation)										
	0.36	nsd	0.09	nsd	0.06	nsd	0.73	nsd	0.47	nsd

Abbreviations: Art = Sodium Artesunate Amo = Amodiaquine base Cmax = peak plasma concentration

AUC = Area under the concentration-time curve nsd = no significant difference sd = significant difference

MD = missing or incalculable data EXCL = excluded data (F Thalf >6 h)

Dihydroartemisinin (metabolite) Summary Table: Artesunate administered (4 mg/kg) alone or in combination with Amodiaquine base (10 mg/kg).

Subject	Tmax (h)		Cmax (ng/ml)		Half-life (h)		AUC (0-last time point) (ng.h/ml)		AUC (0-inf) (ng.h/ml)	
	Art	Amo + Art	Art	Amo + Art	Art	Amo + Art	Art	Amo + Art	Art	Amo + Art
A	0.75	1.50	971.00	458.20	1.16	1.73	1825.50	1351.13	1821.63	1370.41
B	0.50	0.75	592.10	296.30	1.54	2.80	943.21	916.61	1041.91	1057.98
C	0.75	0.50	1114.70	526.80	0.78	1.90	1326.75	778.64	1331.94	916.15
D	3.00	0.50	364.30	1072.90	1.05	0.85	1173.51	1543.81	1167.60	1546.46
E	1.50	0.75	443.50	456.80	0.88	0.99	588.11	684.33	591.28	689.43
F	0.75	4.00	1042.10	243.70	1.27	MD	1315.43	609.35	1339.55	MD
J	1.00	3.00	560.30	217.80	1.20	1.49	1066.26	758.25	1061.54	760.04
K	0.75	1.50	1336.50	508.10	1.05	2.25	1104.86	1250.56	1111.10	1281.03
L	0.50	1.50	1201.20	459.50	1.02	1.18	1677.69	708.66	1694.08	731.98
M	0.75	0.75	835.50	701.90	1.22	1.42	1320.01	1428.88	1317.20	1429.40
N	0.75	2.00	855.30	197.80	1.04	1.76	1523.51	607.63	1517.50	745.74
P	0.75	0.75	590.70	416.90	0.77	0.86	1264.93	749.08	1266.21	751.89
Q	1.00	3.00	1070.90	243.70	1.55	MD	1594.36	655.04	1597.17	MD
Mean	0.98	1.58	844.5	446.2	1.12	1.56	1286	926.3	1297	1026
SEM	0.18	0.31	85.83	66.41	0.07	0.18	91.23	94.14	89.37	97.91
95% CI	0.58-1.38	0.90-2.26	657.5-1031	301.5-590.9	0.97-1.27	1.16-1.97	1088-1485	721.2-1131	1102-1492	807.3-1244
Paired t-test (two-tailed p-value)										
	0.16	nsd	0.007	sd	0.008	sd	0.02	sd	0.09	nsd
Wilcoxon matched pairs signed rank (two-tailed p-value for normal approximation)										
	0.12	nsd	0.01	sd	0.01	sd	0.03	sd	0.15	nsd

Abbreviations:

Art = Sodium Artesunate
Amo = Amodiaquine base
Cmax = peak plasma concentration
AUC = Area under the concentration-time curve
nsd = no significant difference
sd = significant difference
MD = missing or incalculable data

Amodiaquine Summary Table: Amodiaquine base administered (10 mg/kg) alone or in combination with Artesunate (4 mg/kg).

Subject	Tmax (h)		Cmax (ng/ml)		Half-life (h)		AUC (0-last time point) (ng.h/ml)		AUC (0-inf) (ng.h/ml)	
	Amo	Amo + Art	Amo	Amo + Art	Amo	Amo + Art	Amo	Amo + Art	Amo	Amo + Art
A	3.00	3.00	38.814	27.132	4.591	4.205	287.227	199.129	292.959	199.582
B	4.00	1.50	19.455	11.744	4.499	MD	173.368	55.191	176.221	MD
C	1.50	1.00	9.298	14.038	1.60	4.967	20.377	65.327	24.131	89.947
D	3.00	1.50	28.236	39.54	2.575	5.006	114.321	217.136	125.872	223.933
E	3.00	2.00	26.25	22.175	MD	MD	62.747	31.121	MD	MD
F	0.75	4.00	29.95	13.20	1.628	MD	51.364	61.162	50.239	MD
J	3.00	2.00	27.58	24.57	13.804	2.873	252.824	113.667	547.658	123.821
K	1.50	3.00	46.822	18.281	10.822	2.811	248.954	140.939	340.447	99.898
L	2.00	2.00	30.049	24.676	4.445	3.91	219.352	158.082	222.37	156.31
M	4.00	1.00	37.684	36.642	3.70	4.762	299.094	163.493	293.332	205.156
N	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD
P	0.75	0.75	21.371	16.066	3.516	3.048	72.039	69.069	114.332	79.451
Q	2.00	0.75	44.57	26.771	2.669	2.923	251.368	100.39	244.056	102.618
Mean	2.38	1.88	30.01	22.90	4.90	3.83	171.10	114.60	221.10	142.30
SEM	0.33	0.29	3.10	2.57	1.17	0.31	29.29	17.56	44.95	18.45
95% CI	1.66-3.09	1.23-2.52	23.18-36.83	17.25-28.55	2.29-7.50	3.11-4.56	106.6-235.5	75.92-153.2	120.9-321.2	99.77-184.8
Paired t-test (two-tailed p-value)										
	0.33	nsd	0.04	sd	0.38	nsd	0.04	sd	0.09	sd
Wilcoxon matched pairs signed rank (two-tailed p-value for normal approximation)										
	0.36	nsd	0.03	sd	0.73	nsd	0.04	sd	0.10	nsd

Abbreviations:

Art = Sodium Artesunate

Amo = Amodiaquine base

Cmax = peak plasma concentration

AUC = Area under the concentration-time curve

nsd = no significant difference

sd = significant difference

MD = missing or incalculable data

D-Amodiaquine (metabolite) Summary Table: Amodiaquine base administered (10 mg/kg) alone or in combination with Artesunate (4 mg/kg).

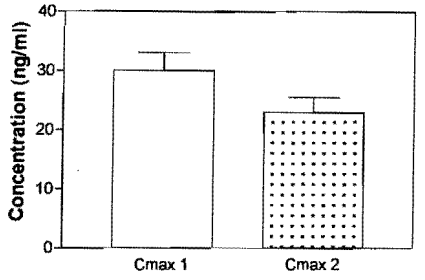
Subject	Tmax (h)		Cmax (ng/ml)		Half-life (h)		AUC (0-last time point) (ng.h/ml)		AUC (0-inf) (ng.h/ml)	
	Amo	Amo + Art	Amo	Amo + Art	Amo	Amo + Art	Amo	Amo + Art	Amo	Amo + Art
A	3.00	3.00	324.65	327.14	219.19	266.86	12526.89	29497.83	14590.04	42443.15
B	4.00	4.00	227.18	267.10	165.21	29.46	11194.30	3571.432	13330.31	4092.886
C	3.00	1.00	155.86	178.04	106.98	168.99	7284.21	7550.05	8001.973	8565.872
D	3.00	1.50	223.03	458.70	579.79	144.11	10927.82	8349.786	19765.85	9270.824
E	3.00	2.00	324.91	657.72	207.71	190.84	10650.79	16126.56	12456.83	17921.34
F	6.00	4.00	163.51	201.32	121.23	99.49	14678.67	8289.724	15698.35	9563.785
J	3.00	2.00	300.42	266.61	282.10	141.01	9962.464	8457.925	12284.74	9433.391
K	8.00	3.00	256.67	260.64	427.68	133.24	19254.30	14365.29	26327.09	15192.44
L	2.00	2.00	329.40	261.29	203.23	79.28	11408.59	5858.69	13496.02	6432.519
M	4.00	1.50	371.48	493.38	129.71	184.93	10761.65	14049.74	12223.65	15214.01
N	MD	MD	MD	MD	MD	MD	MD	MD	MD	MD
P	1.50	1.50	271.27	112.40	245.03	96.96	16352.65	7967.574	21295.58	8787.827
Q	3.00	1.50	332.00	158.02	216.57	92.25	10865.24	3774.60	14107.30	4420.213
Mean	3.63	2.25	273.40	303.50	242.00	135.60	12160	10650	15300	12610
SEM	0.51	0.29	20.05	45.76	39.44	18.06	920.80	2066.00	1421.00	2978.00
95% CI	2.50-4.75	1.61-2.89	229.2-317.5	202.8-404.2	155.2-328.8	95.87-175.4	10129-14182	6108-15202	12170-18426	6056-19167
Paired t-test (two-tailed p-value)										
	0.007	sd	0.49	nsd	0.03	sd	0.49	nsd	0.43	nsd
Wilcoxon matched pairs signed rank (two-tailed p-value for normal approximation)										
	0.007	sd	0.52	nsd	0.03	sd	0.23	nsd	0.15	nsd

Abbreviations: Art = Sodium Artesunate; Amo = Amodiaquine base; Cmax = peak plasma concentration

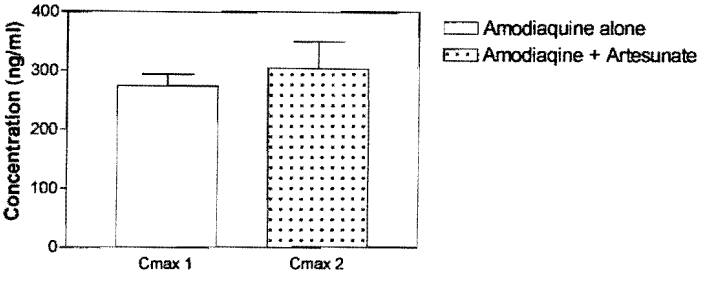
AUC = Area under the concentration-time curve; nsd = no significant difference; sd = significant difference

MD = missing or incalculable data

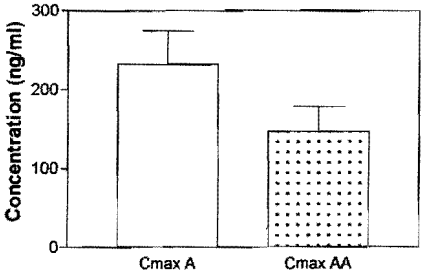
Amodiaquine Summary



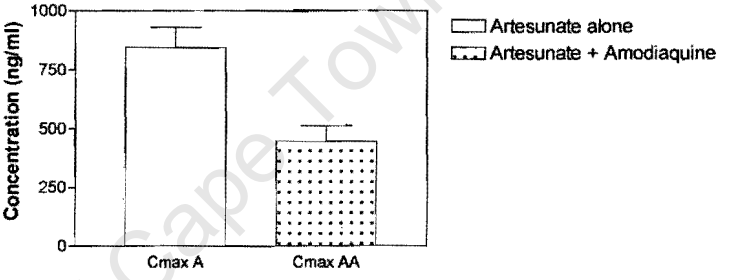
D-Amodiaquine Summary



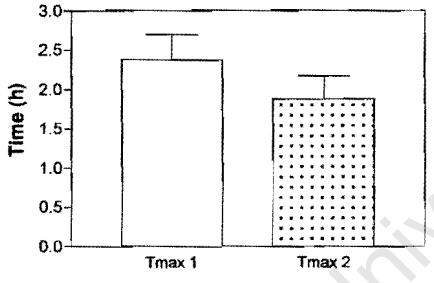
Artesunate Summary



Dihydroartesunate Summary



Amodiaquine Summary



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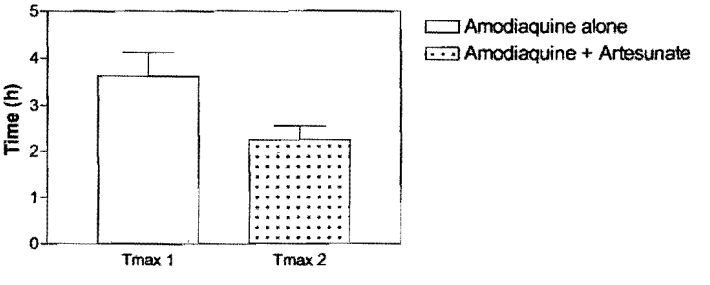
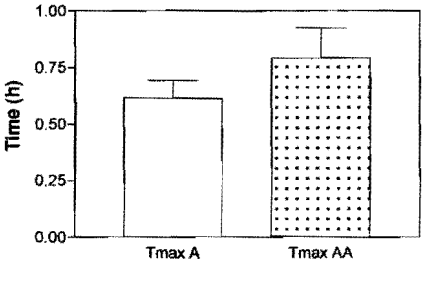


Artesunate Summary



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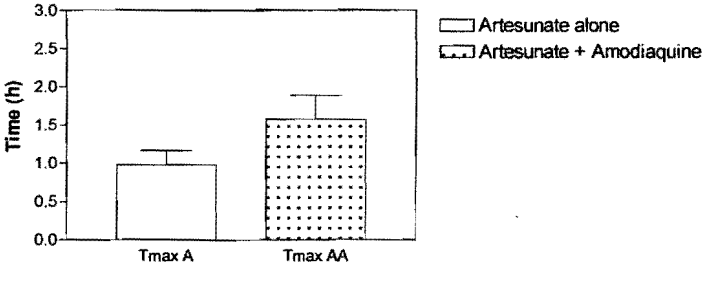

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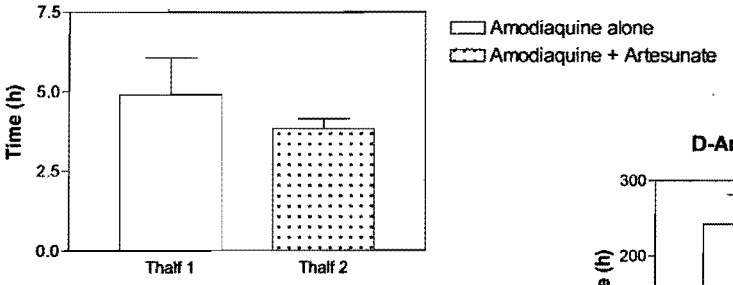

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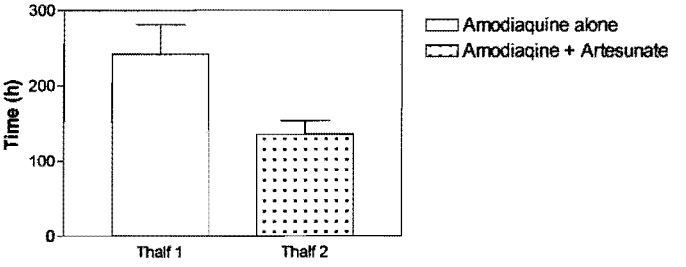
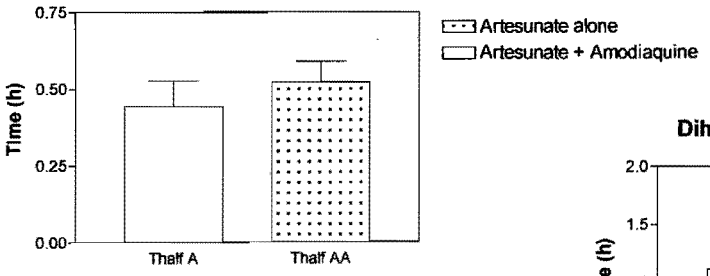


Artesunate Summary



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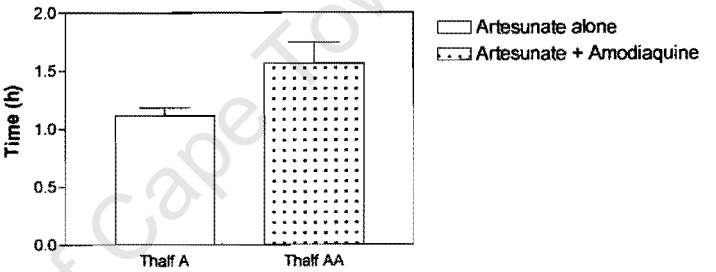

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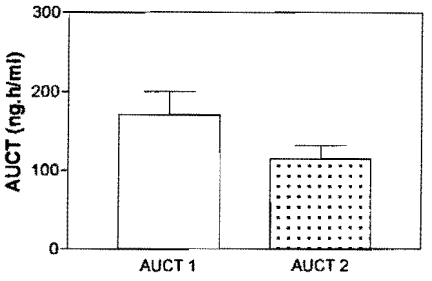
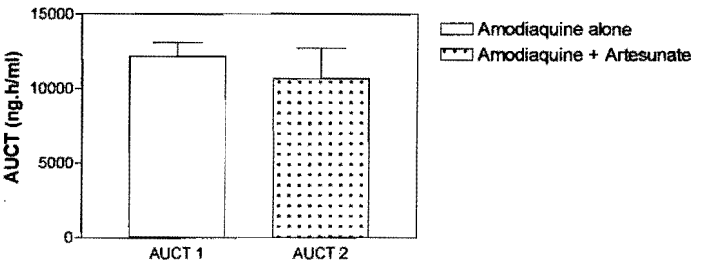
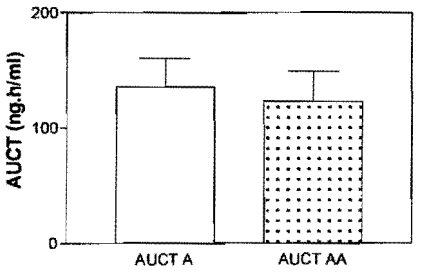


D-Amodiaquine Summary



Artesunate Summary



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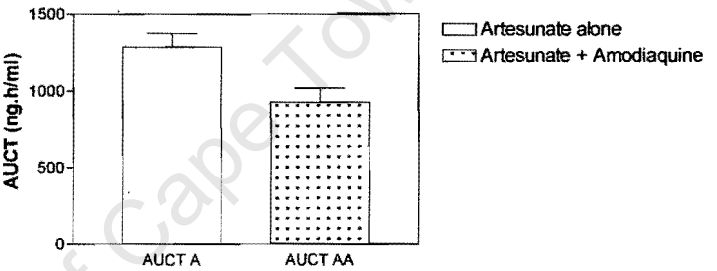
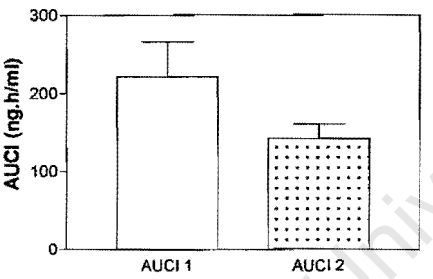


Amodiaquine Summary



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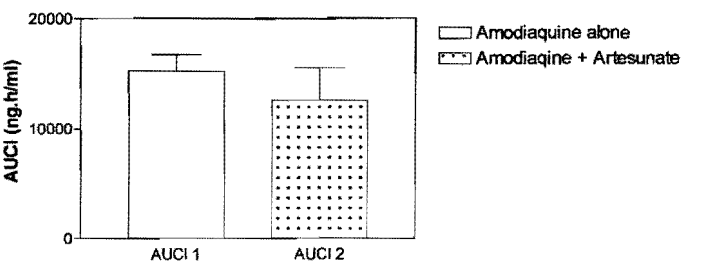

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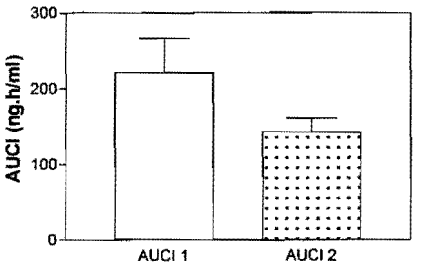
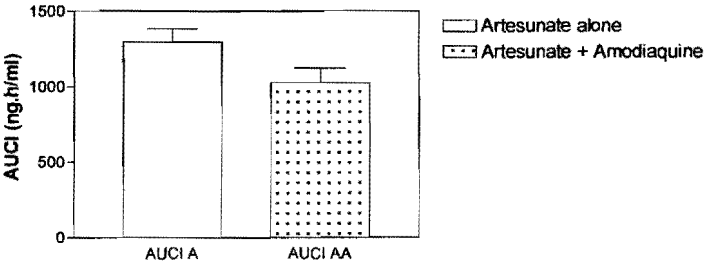


Dihydroartesunate Summary



Appendix D-2: All individual PK data

Artesunate:

Subject	Time	Artesunate only		Artesunate + Amodiaquine		Subject	Time	Artesunate only		Artesunate + Amodiaquine	
	(hrs)	*Art (ng/ml)*	*Dihydro (ng/ml)*	*Art (ng/ml)*	*Dihydro (ng/ml)*		(hrs)	*Art (ng/ml)*	*Dihydro (ng/ml)*	*Art (ng/ml)*	*Dihydro (ng/ml)*
A	0	0	0	0	0	E	0	0	0	0	0
A	0.25	0	0	0	0	E	0.25	0	0	0	0
A	0.5	12	125.2	12	17.9	E	0.5	62.7	73.2	203.8	400.9
A	0.75	12	971	12	126.5	E	0.75	34.3	159.7	115.2	456.8
A	1	74.6	919	12	86.6	E	1	95	306.9	45.3	219.8
A	2	12	739.2	12	458.2	E	2	12	443.5	12	161.2
A	3	34.9	449.5	MD	MD	E	3	0	147.8	12	148.5
A	4	0	214.4	MD	MD	E	4	0	40.2	0	98.6
A	6	0	78.6	0	167.8	E	6	0	12	0	48.6
A	8	0	65.9	0	50.2	E	8	0	12	0	12
A	12	0	12	0	39.1	E	12	0	0	0	0
A	24	0	0	0	0	E	24	0	0	0	0
B	0	0	0	0	0	F	0	0	0	0	0
B	0.25	114.5	85.2	0	0	F	0.25	24.2	12	0	0
B	0.5	30.6	592.1	0	0	F	0.5	253.4	798.6	0	0
B	0.75	12	432.9	12	296.3	F	0.75	51.5	1042.1	21.9	41.9
B	1	0	328.3	12	207.7	F	1	79.2	726	12	39.2
B	2	0	179.8	22.6	137.8	F	2	36.3	320.1	12	88
B	3	0	81.2	12	177.8	F	3	30.3	224.4	MD	MD
B	4	0	271	0	142.5	F	4	0	115.5	12	104.5
B	6	0	80.5	0	217.8	F	6	0	52.1	12	243.7
B	8	0	0	0	46.6	F	8	0	29	0	0
B	12	0	0	0	0	F	12	0	0	0	0
B	24	0	0	0	0	F	24	0	0	0	0
C	0	0	0	0	0	J	0	0	0	0	0
C	0.25	95	12	77.9	14.6	J	0.25	0	51.4	0	0
C	0.5	279.8	841.5	265.7	526.8	J	0.5	12	60.6	28.6	57.3
C	0.75	116.8	1114.7	123.8	480.2	J	0.75	12	96.3	12	74.6
C	1	22.4	635.6	70.6	467.5	J	1	75.9	560.3	34	107.2
C	2	12	405.9	12	42.3	J	2	31.7	339.2	50.6	197.8
C	3	0	272.6	12	164.5	J	3	0	35.1	12	212.5
C	4	0	126.7	0	84.6	J	4	0	258	12	217.8
C	6	0	41.5	0	79.2	J	6	0	162.3	0	77.2
C	8	0	0	0	0	J	8	0	27.7	0	28.6
C	12	0	0	0	0	J	12	0	17.1	0	12
C	24	0	0	0	0	J	24	0	0	0	0

Subject	Time	Artesunate only		Artesunate + Amodiaquine		Subject	Time	Artesunate only		Artesunate + Amodiaquine	
	(hrs)	*Art (ng/ml)*	*Dihydro (ng/ml)*	*Art (ng/ml)*	*Dihydro (ng/ml)*		(hrs)	*Art (ng/ml)*	*Dihydro (ng/ml)*	*Art (ng/ml)*	*Dihydro (ng/ml)*
D	0	0	0	0	0	K	0	0	0	0	0
D	0.25	56.7	0	336.9	246.4	K	0.25	0	0	0	0
D	0.5	12	196.6	309.7	1072.9	K	0.5	153.1	95.7	65	62.6
D	0.75	0	153.1	179.1	969	K	0.75	388	1336.5	63.7	178.5
D	1	0	207.9	23.3	679.3	K	1	44.8	650.7	182.4	463.5
D	2	0	142.5	45.8	432.9	K	2	12	445.5	69.9	508.1
D	3	0	223.7	12	305.2	K	3	0	169.6	48.6	421.5
D	4	0	364.3	12	163.8	K	4	0	76.5	0	169.8
D	6	0	167.6	0	55.9	K	6	0	34.9	0	84.6
D	8	0	58.7	0	12	K	8	0	12	0	37.9
D	12	0	12	0	0	K	12	0	0	0	24.6
D	24	0	0	0	0	K	24	0	0	0	0
L	0	0	0	0	0	Q	0	0	0	-	0
L	0.25	175.6	97	0	0	Q	0.25	0	0	-	0
L	0.5	557	1201.2	12	54.12	Q	0.5	58.7	82.5	-	47.3
L	0.75	87.1	1005.2	23.3	50.6	Q	0.75	143.2	1058.9	-	161.9
L	1	36.9	742.5	24.6	62.6	Q	1	58.1	1070.9	Spoilt	183.1
L	2	12	403.9	105.2	459.5	Q	2	12	546.1	Data	100.6
L	3	12	442.2	17.9	256.4	Q	3	12	344.3	-	MD
L	4	0	124	12	104.5	Q	4	0	179.1	-	243.7
L	6	0	64	0	43.9	Q	6	0	71.9	-	85.9
L	8	0	34.9	0	33.3	Q	8	0	26.6	-	0
L	12	0	0	0	0	Q	12	0	12	-	0
L	24	0	0	0	0	Q	24	0	0	-	0
M	0	0	0	0	0						
M	0.25	332.6	598.6	227.1	160.5						
M	0.5	139.2	633.6	283	698.6						
M	0.75	90.4	835.5	279	701.9						
M	1	69.3	756.3	192.5	617.4						
M	2	12	37.2	42.6	416.2						
M	3	75.2	285.7	29.3	246.4						
M	4	17.8	146.5	12	147.2						
M	6	11.9	53.4	0	97.2						
M	8	0	12	0	42.6						
M	12	0	12	0	12						
M	24	0	0	0	0						

N	0	0	0	0	0
N	0.25	0	0	39.9	71.9
N	0.5	389.4	475.2	96.5	137.2
N	0.75	237.6	855.3	68.6	165.2
N	1	166.3	793.3	16.6	41.2
N	2	62	593.3	25.3	161.8
N	3	12	376.8	12	197.8
N	4	12	147.2	0	134.5
N	6	0	71.3	0	89.9
N	8	0	12	0	0
N	12	0	12	0	0
N	24	0	0	0	0
P	0	0	0	0	0
P	0.25	62	45.5	12	26.4
P	0.5	212.5	281.1	174.5	243
P	0.75	252.7	590.7	93.9	416.9
P	1	MD	MD	45.9	279
P	2	29.7	585.6	12	209.8
P	3	12	437.5	0	142.5
P	4	0	151.8	0	131.2
P	6	0	12	0	70.6
P	8	0	12	0	12
P	12	0	0	0	0
P	24	0	0	0	0

Amodiaquine:

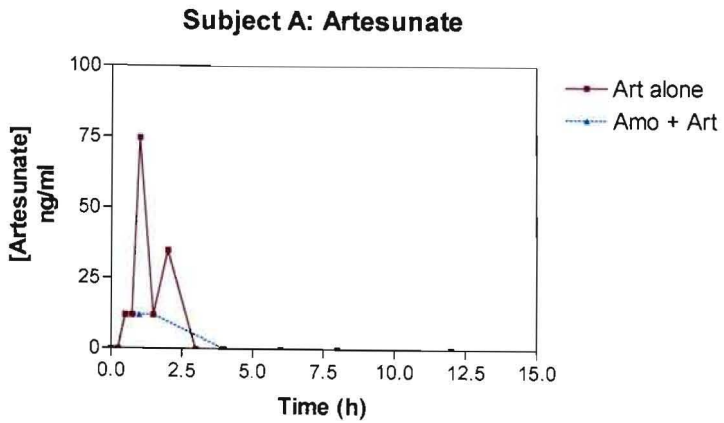
Subject	Time (hrs)	Amodiaquine only		Amodiaquine + Artesunate		Subject	Time (hrs)	Amodiaquine only		Amodiaquine + Artesunate	
		Amo (ng/ml)	*D-Amo (ng/ml)*	*Amo (ng/ml)*	*D-Amo (ng/ml)*			*Amo (ng/ml)*	*D-Amo (ng/ml)*	*Amo (ng/ml)*	*D-Amo (ng/ml)*
A	0	0	0	0	0	C	0	0	0	0	0
A	0.25	0	0	0	-	C	0.25	0	0	0	0
A	0.5	0	0	7.2	5.11	C	0.5	0	0	0	0
A	0.75	0	0	6.62	4.86	C	0.75	0	12.09	11.99	76.28
A	1	31.02	10.21	-	-	C	1	5.23	15.52	14.04	178.04
A	1.5	15.35	33.79	15.07	61.07	C	1.5	9.3	100.7	9.82	173.26
A	2	37.99	107.1	17.8	127.78	C	2	5.99	142.17	9.18	163.11
A	3	38.81	324.65	27.13	327.14	C	3	4.64	155.86	10.28	137.57
A	4	22.2	256.79	24.41	317.94	C	4	-	106.33	7.78	125.51
A	6	23.13	237.67	14.06	198.05	C	6	0	104.52	-	115.75
A	8	15.81	181.75	8.19	152.66	C	8	0	111.77	-	77.88
A	12	9.2	168.64	6.85	180.63	C	12	0	79.45	0	77.32
A	24	0	94.05	0	105.46	C	24	0	45.65	0	39.68
A	48		54.39		68.7	C	48		38.65		25.49
A	72		35.01		71.33	C	72		22.23		16.86
A	96		29.44		74.67	C	96		22.72		19.56
A	120		27.64		36.9	C	120		18.92		15.55
A	168		16.7		81.84	C	168		11.98		15.74
A	240		14.62		53.81	C	240		13.95		10.39
A	336		9.22		50.21	C	336		4.65		8.07
A	480		6.52		33.62	C	480		-		4.17
B	0	0	0	0	0	D	0	0	0	0	0
B	0.25	0	-	0	0	D	0.25	0	0	13.89	0
B	0.5	0	-	0	0	D	0.5	0	0	30.45	343.47
B	0.75	-	-	9.32	-	D	0.75	24.13	10.81	23.47	170.22
B	1	11.67	-	5.84	4.77	D	1	6.19	9.83	24.26	410.4
B	1.5	10.95	15.45	11.74	10.62	D	1.5	8.501	22.92	39.54	458.7
B	2	12.01	71.9	9.33	50.29	D	2	6.62	39.01	28.05	334.29
B	3	17.27	185.32	10.35	151.07	D	3	28.24	223.03	19.14	245.87
B	4	19.46	227.18	10.98	267.1	D	4	16.63	91.47	20.76	151.18
B	6	15.09	201.59	14.06	131.13	D	6	-	111.42	12.64	134.04
B	8	9.99	179.01	0	137.28	D	8	6.74	90.08	10.08	131.55
B	12	5.81	131.74	0	69.88	D	12	0	96.51	5.56	96.77
B	24	0	74.55	0	40.7	D	24	0	53.91	0	66.12
B	48		44.23		28.19	D	48		43.31		34.5
B	72		34.27		-	D	72		35.49		25.47
B	96		27.12		0	D	96		36.67		15.84
B	120		22.32		0	D	120		29.8		16.88
B	168		18.25		0	D	168		16.52		11.67
B	240		7.37		0	D	240		14.08		5.72
B	336		13.04		0	D	336		12.56		4.67
B	480		8.96		0	D	480		10.57		4.43

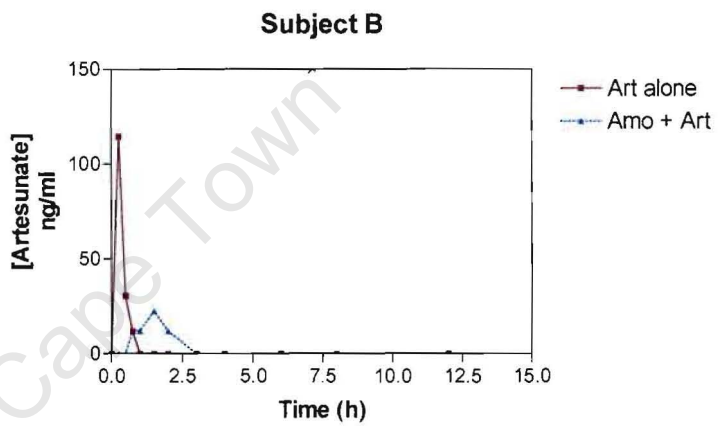
Subject	Time	Amodiaquine only		Amodiaquine + Artesunate		Subject	Time	Amodiaquine only		Amodiaquine + Artesunate	
	(hrs)	*Amo (ng/ml)*	*D-Amo (ng/ml)*	*Amo (ng/ml)*	*D-Amo (ng/ml)*		(hrs)	*Amo (ng/ml)*	*D-Amo (ng/ml)*	*Amo (ng/ml)*	*D-Amo (ng/ml)*
E	0	0	0	0	0	J	0	0	0	0	0
E	0.25	0	0	0	0	J	0.25	11.82	0	0	0
E	0.5	0	0	0	0	J	0.5	19.35	0	5.86	0
E	0.75	0	0	9.35	185.24	J	0.75	18.78	5.54	14.47	-
E	1	0	66.03	12.99	550.83	J	1	18.58	12.33	11.21	3.86
E	1.5	14.033	220.81	14.56	399.22	J	1.5	21.04	41.48	21.86	69.34
E	2	17.34	280.16	22.17	657.72	J	2	23.22	120.2	24.57	266.61
E	3	26.25	324.91	0	224.4	J	3	27.58	300.42	16.48	253.67
E	4	10.98	218.29	0	288.44	J	4	20.52	261.02	15.38	241.92
E	6	0	179.53	0	220.99	J	6	21.22	200.72	8.87	209.76
E	8	0	178.96	0	223.57	J	8	25.01	171.05	-	163.72
E	12	0	149.38	0	171.58	J	12	14.81	151.1	0	161.68
E	24	0	66.96	0	122.61	J	24	-	74.64	0	77.97
E	48		56.8		78	J	48		37.3		31.52
E	72		39.31		50.86	J	72		31.38		24.71
E	96		20.48		47.95	J	96		21.59		22.97
E	120		18.03		29.01	J	120		13.73		13.79
E	168		13.93		24.22	J	168		12.88		11.09
E	240		9.92		15.6	J	240		9.87		-
E	336		6.72		10.92	J	336		8.45		4.8
E	480		6.03		6.52	J	480		5.71		-
F	0	0	0	0	0	K	0	0	0	0	0
F	0.25	10.53	0	0	0	K	0.25	0	0	0	0
F	0.5	22.53	6.89	0	0	K	0.5	14.29	12.3	2.69	0
F	0.75	29.95	65.33	0	0	K	0.75	11.9	33.62	12.33	4.98
F	1	10.03	112.42	0	0	K	1	7.93	57.87	9.4	12.3
F	1.5	7.6	109.53	0	0	K	1.5	46.82	89.39	7.72	62.54
F	2	-	122.72	7.11	4.02	K	2	17.96	154.07	15.76	222.44
F	3	7.46	126.55	5.7	34.58	K	3	18.28	182.57	18.28	260.64
F	4	-	132.45	13.2	201.31	K	4	15.99	179.59	11.13	219.7
F	6	-	163.51	7.58	156.95	K	6	18.66	238.31	8.3	176.81
F	8	0	95.23	-	129	K	8	-	256.67	-	135.25
F	12	-	126.46	0	99.39	K	12	9.52	219.71	-	141.29
F	24	0	84.89	0	65.91	K	24	0	174.73	0	141.44
F	48				39.73	K	48		97.09		75.041
F	72		37.31		22.92	K	72		83.98		34.79
F	96		31.32		17.32	K	96		62.51		37.41
F	120		25.59		21.67	K	120		41.9		13.85
F	168		34.87		13.21	K	168		18.79		24.54
F	240		26.49		4.951	K	240		17.2		15.5
F	336		18.53		6.01	K	336		14.23		16.83
F	480		5.83		8.88	K	480		11.46		4.3

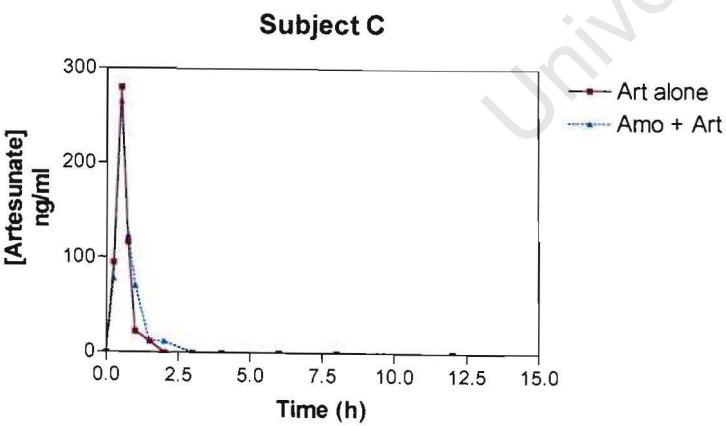
Subject	Time	Amodiaquine only		Amodiaquine + Artesunate		Subject	Time	Amodiaquine only		Amodiaquine + Artesunate	
	(hrs)	*Amo (ng/ml)*	*D-Amo (ng/ml)*	*Amo (ng/ml)*	*D-Amo (ng/ml)*		(hrs)	*Amo (ng/ml)*	*D-Amo (ng/ml)*	*Amo (ng/ml)*	*D-Amo (ng/ml)*
L	0	0	0	0	0	P	0	0	0	0	0
L	0.25	0	0	0	0	P	0.25	8.37	4.63	0	0
L	0.5	12.07	5.17	0	0	P	0.5	15.31	5.46	0	0
L	0.75	10.52	7.29	0	0	P	0.75	21.37	97.71	16.07	14.9
L	1	21.51	21	11.82	0	P	1	19.46	213.73	15.87	67
L	1.5	29.54	179.58	11.8	24.2	P	1.5	18.48	271.27	9	112.4
L	2	30.05	329.4	24.68	261.29	P	2	17.58	267.56	7.28	102.14
L	3	23.51	272.44	23.36	240.78	P	3	15.48	195.28	6.93	103.51
L	4	25.6	230.79	17.92	154.45	P	4	10.38	169.88	10.75	107.61
L	6	11.89	163.06	9.91	139.75	P	6	0	124.22	6.54	108.88
L	8	8.03	134.02	6.55	113.66	P	8	0	145.87	4.33	76.72
L	12	7.32	149.01	4.94	74.35	P	12	0	103.74	0	88.08
L	24	0	58.7	0	53.9	P	24	0	73.88	0	54.56
L	48		50.39		32.52	P	48		52.89		36.06
L	72		22.56		20.09	P	72		51.71		27.98
L	96		21.14		13	P	96		46.63		22.08
L	120		22		11.28	P	120		31.56		17.29
L	168		20.18		7.99	P	168		31.61		17.32
L	240		16.43		-	P	240		30.78		7.2
L	336		10.93		5.02	P	336		19.66		5.43
L	480		7.12		-	P	480		13.98		5.86
M	0	0	0	0	0	Q	0	0	0	0	0
M	0.25	0	0	12.15	0	Q	0.25	5.93	0	11.7	0
M	0.5	0	0	30.58	17.68	Q	0.5	7.1	0	24.2	4.86
M	0.75	5.22	0	36.56	131.45	Q	0.75	22.53	-	26.77	44.02
M	1	17.88	0	36.64	416.19	Q	1	33.13	6.82	20.02	101.47
M	1.5	28.98	10.75	28.51	493.38	Q	1.5	32.1	39.8	18.39	158.02
M	2	15.84	47.31	28.21	479.96	Q	2	44.57	69.31	14.2	129.1
M	3	30.05	293.93	17.93	261.59	Q	3	41.26	332	11.08	111.04
M	4	37.68	371.48	16.33	261.54	Q	4	19.06	185.77	10.29	87.48
M	6	26.78	213.27	13.21	251.49	Q	6	29.73	146.14	-	65.18
M	8	18.34	203.08	8.55	213.07	Q	8	8.01	118.85	-	59.53
M	12	8.69	146.31	0	128.78	Q	12	3.4	86.52	0	52.72
M	24	0	92.21	0	101.76	Q	24	0	49.69	0	33.56
M	48		57.46		83.13	Q	48		25.04		19.12
M	72		33.21		55.35	Q	72		28.46		15.08
M	96		23.66		36.48	Q	96		23.48		13.2
M	120		23.19		29.86	Q	120		17.36		8.58
M	168		19.1		14.33	Q	168		15.78		5.84
M	240		10.76		11.61	Q	240		22.52		4.85
M	336		7.81		9.74	Q	336		15.72		-
M	480		-		4.36	Q	480		10.38		-

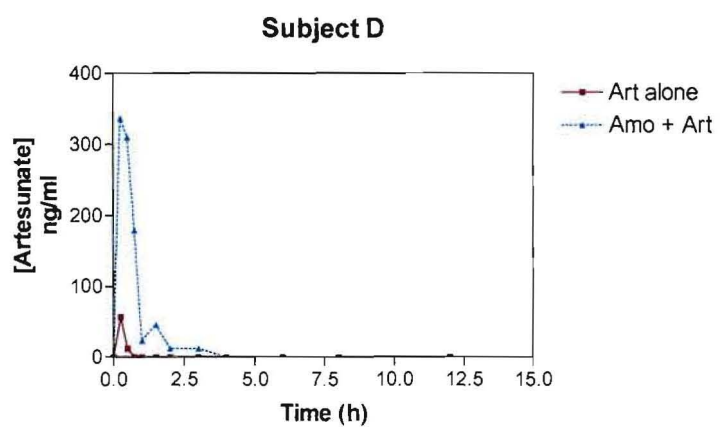
Appendix D-3: Artesunate, DHAS, amodiaquine and D-amodiaquine individual PK graphs.

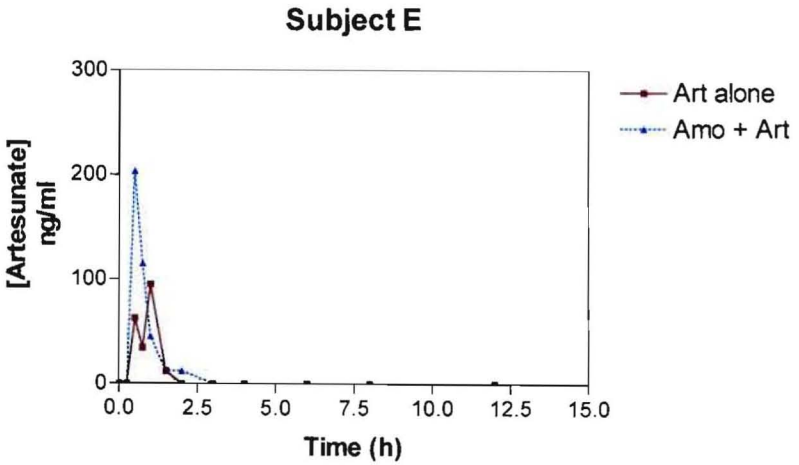
Artesunate profiles

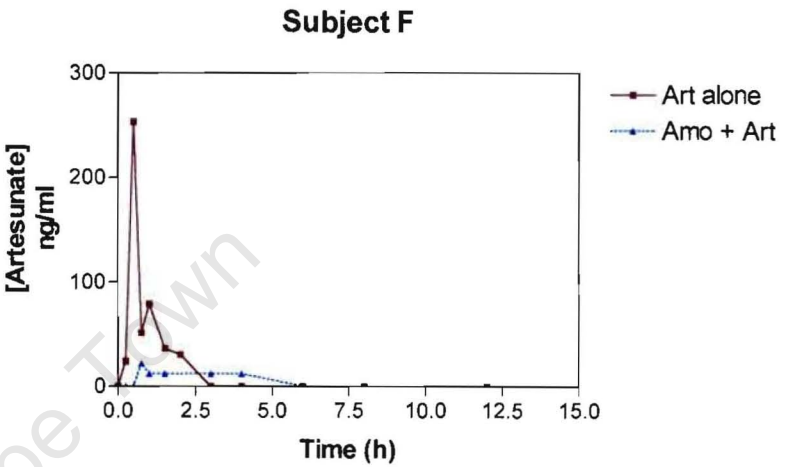


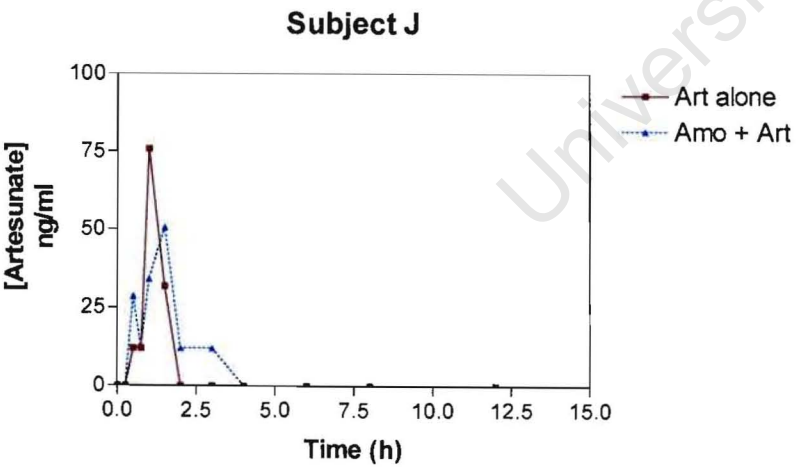


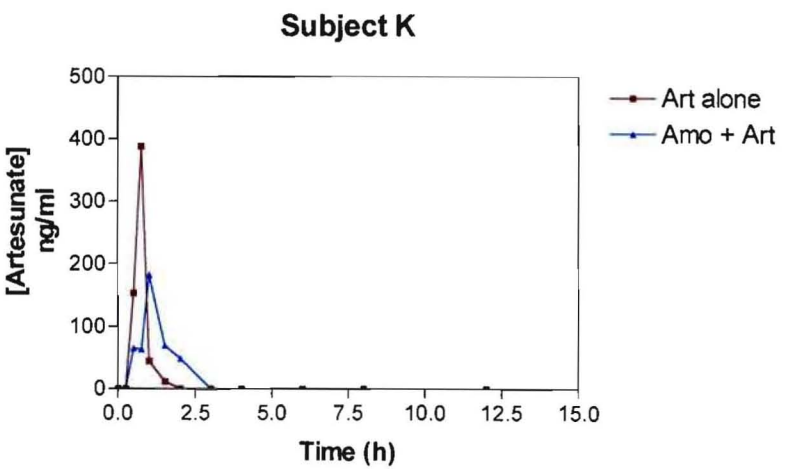


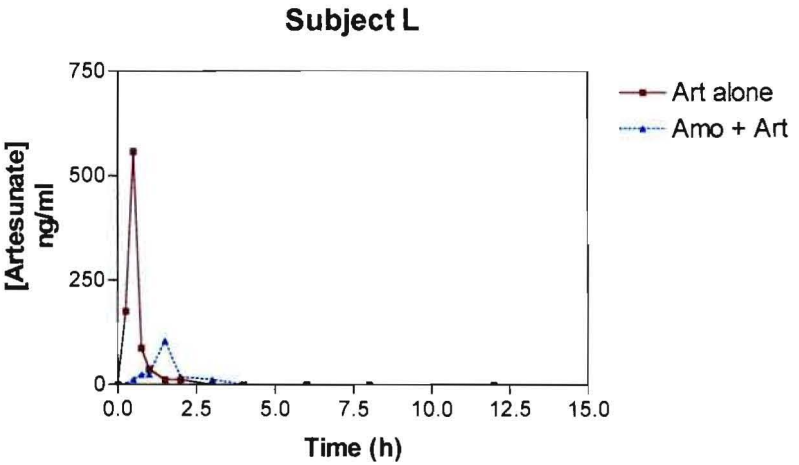


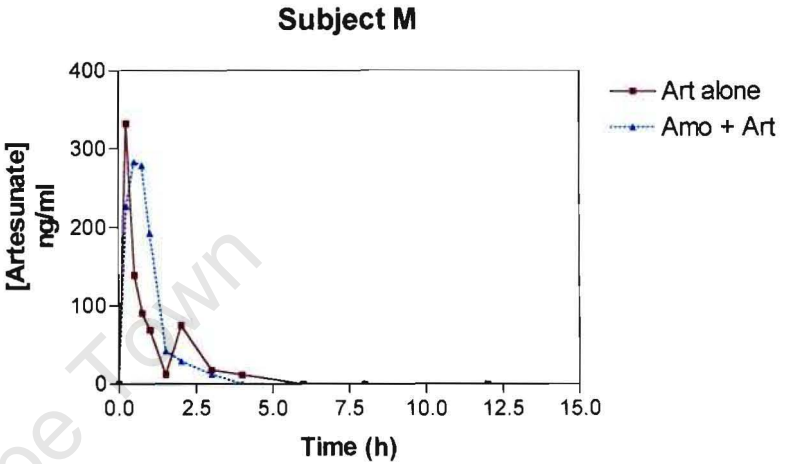


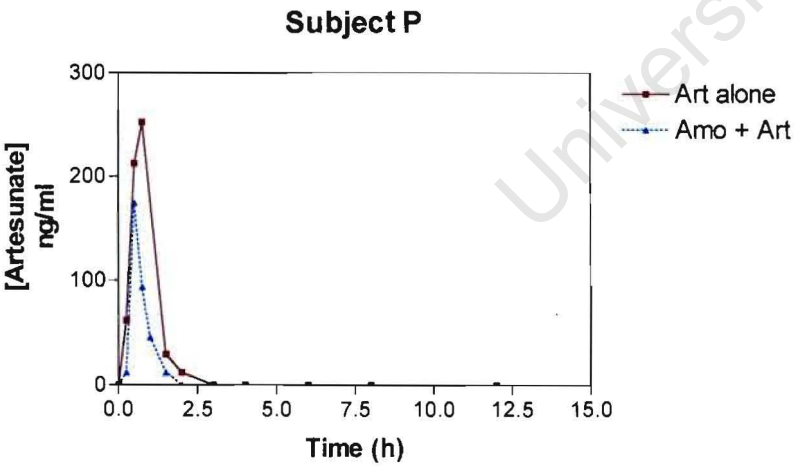


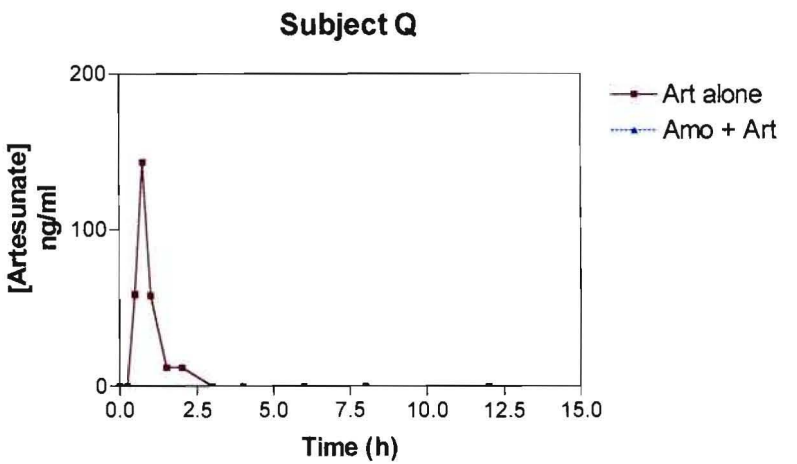


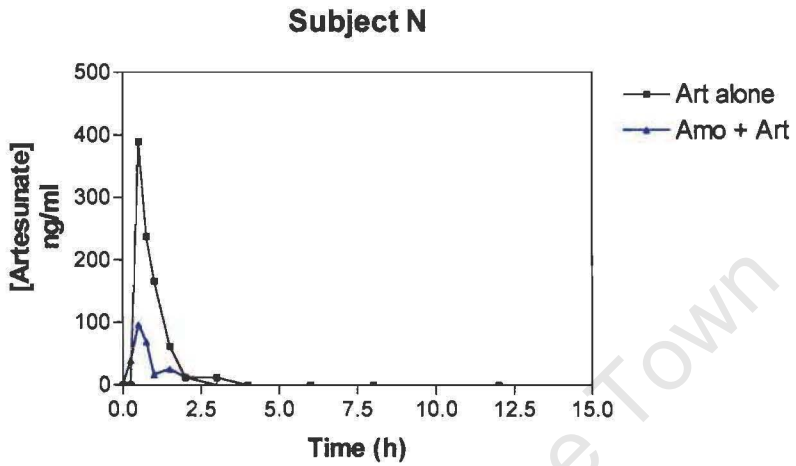




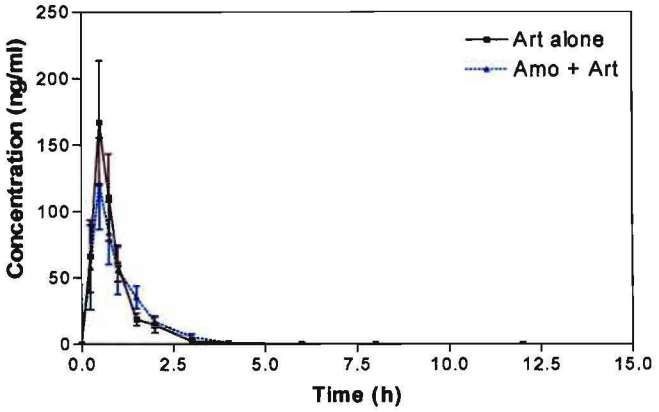




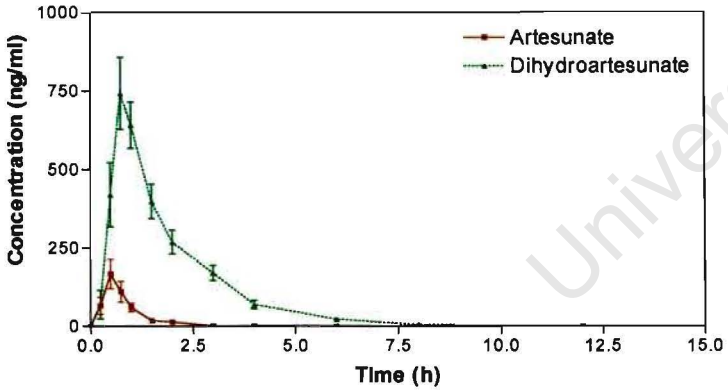




Mean Artesunate Plot

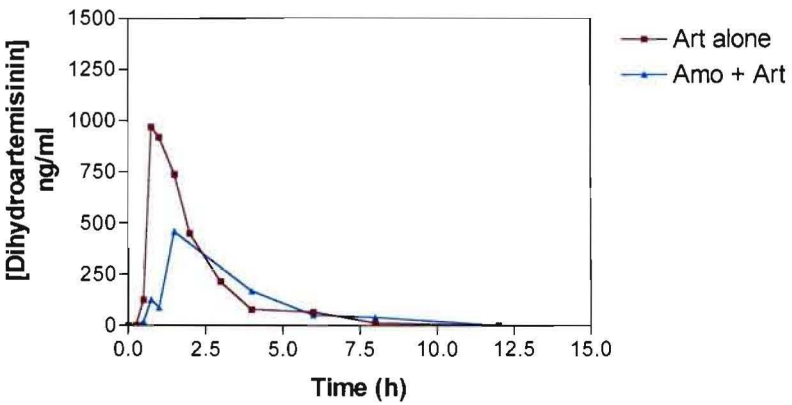


Artesunate + Dihydroartesunate
Artesunate administered alone

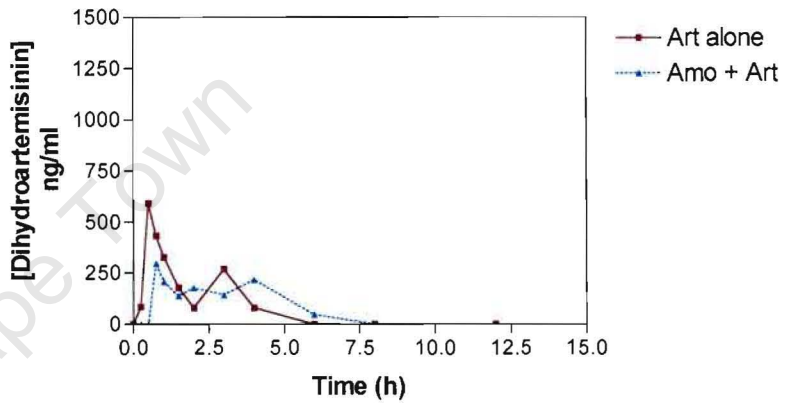


Dihydroartemisinin profiles

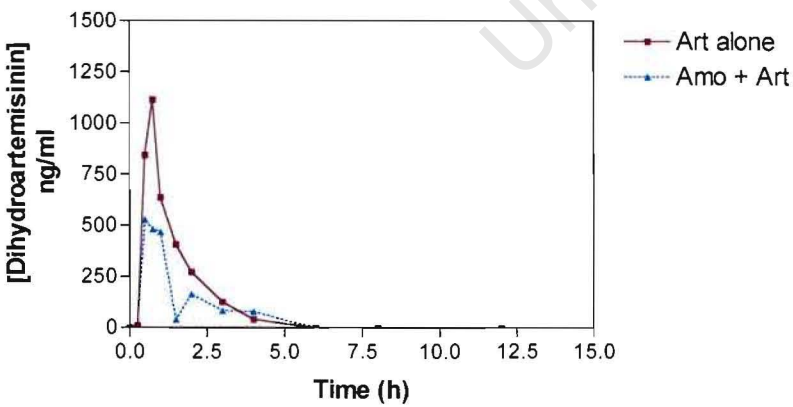
Subject A: Dihydroartemisinin



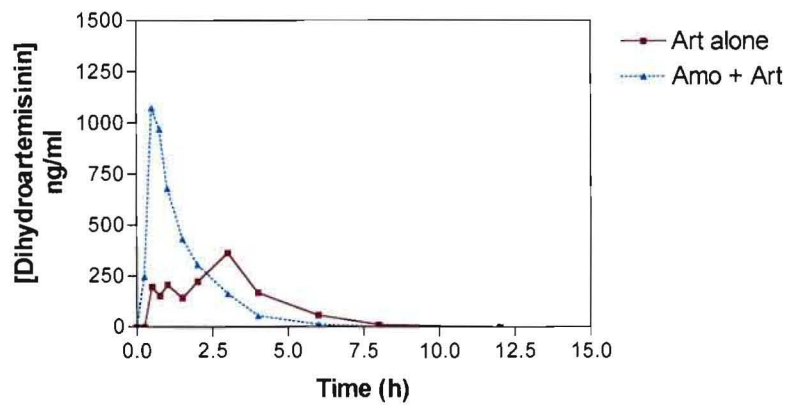
Subject B: Dihydroartemisinin



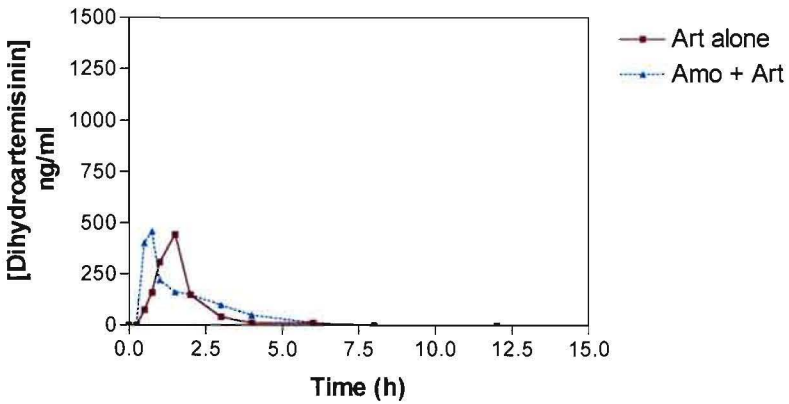
Subject C: Dihydroartemisinin



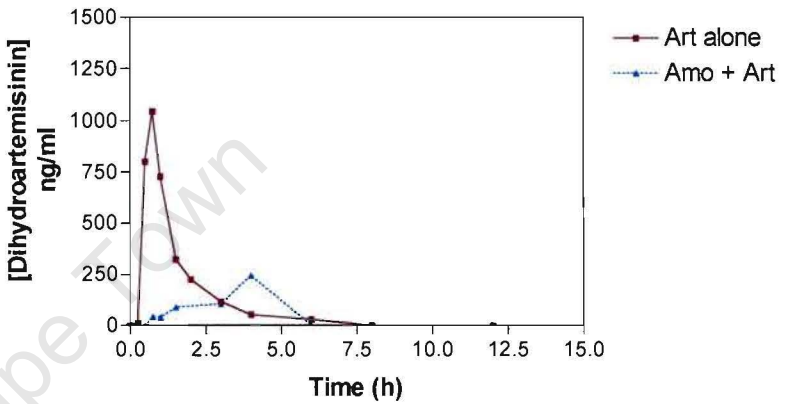
Subject D: Dihydroartemisinin



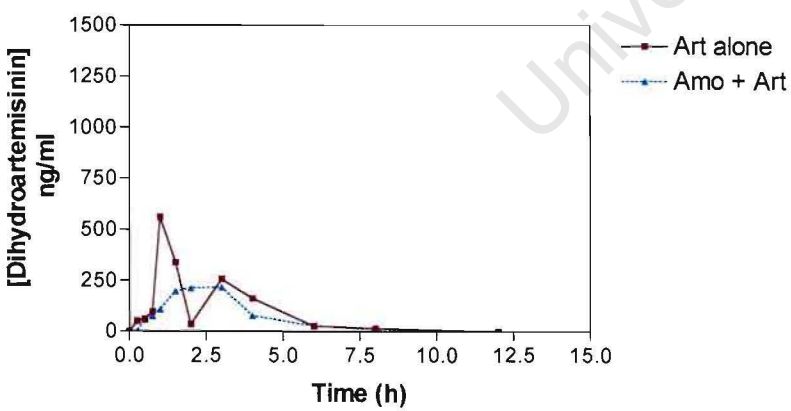
Subject E: Dihydroartemisinin



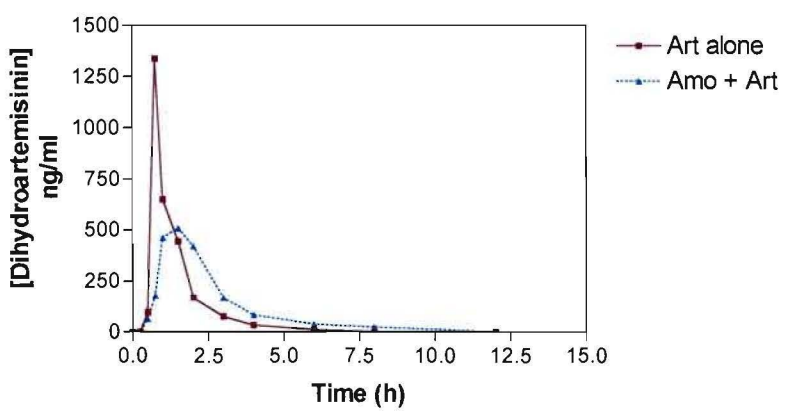
Subject F: Dihydroartemisinin



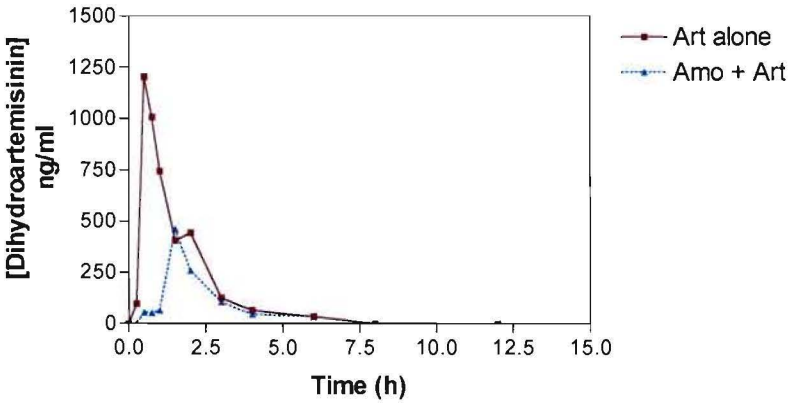
Subject J: Dihydroartemisinin



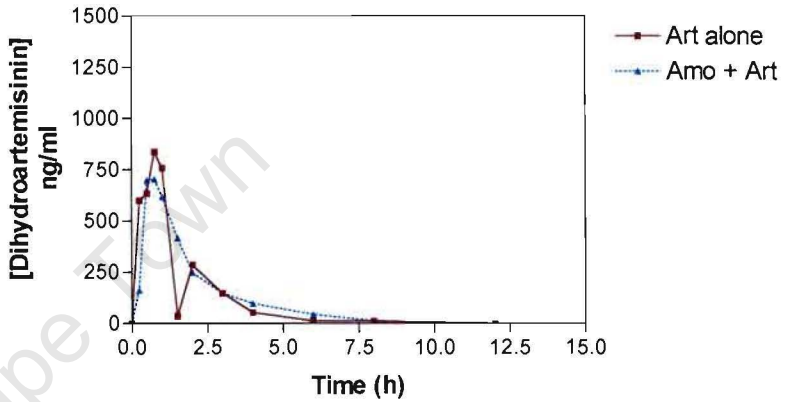
Subject K: Dihydroartemisinin



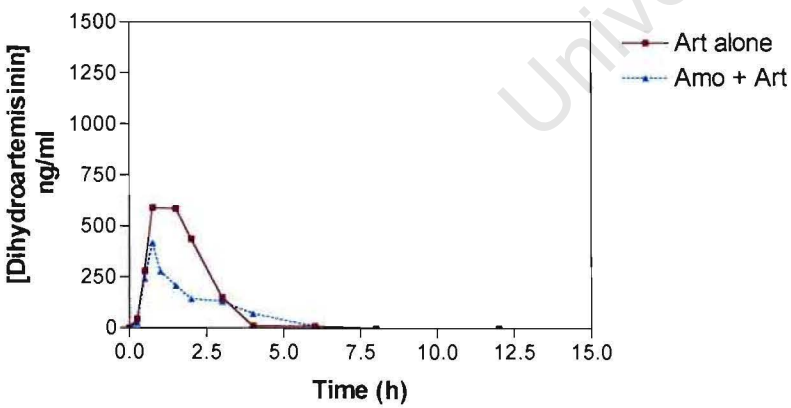
Subject L: Dihydroartemisinin



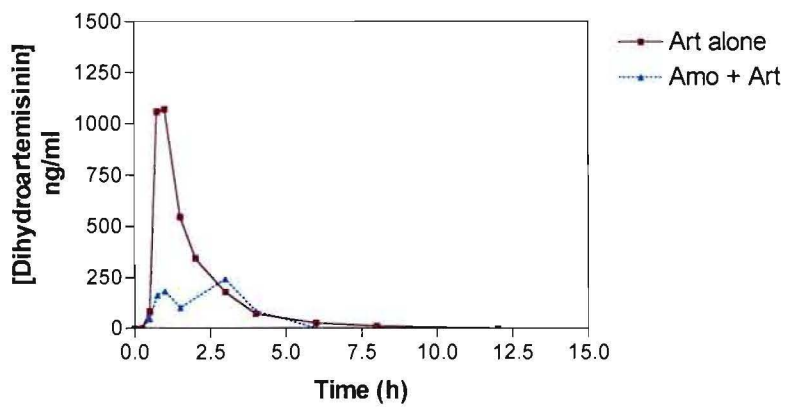
Subject M: Dihydroartemisinin

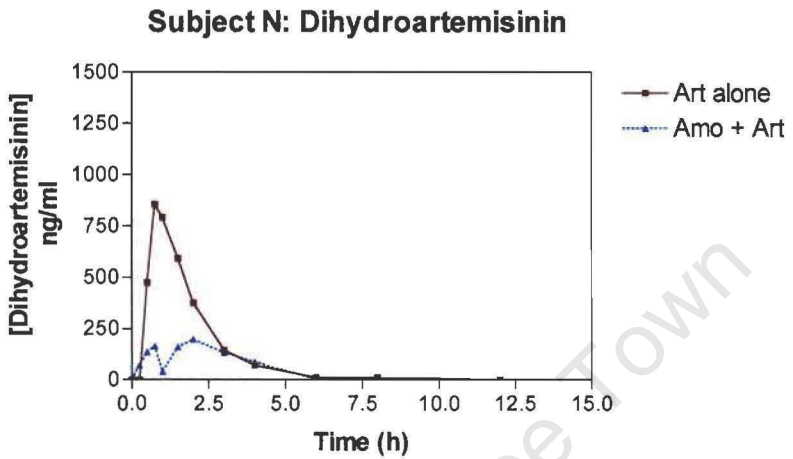


Subject P: Dihydroartemisinin

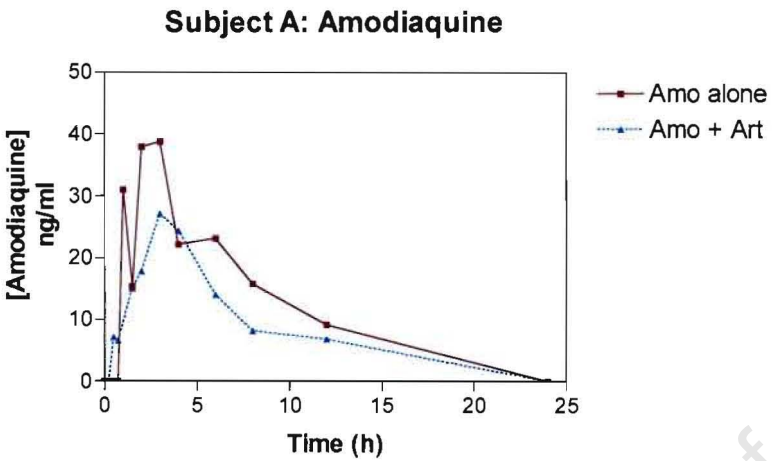


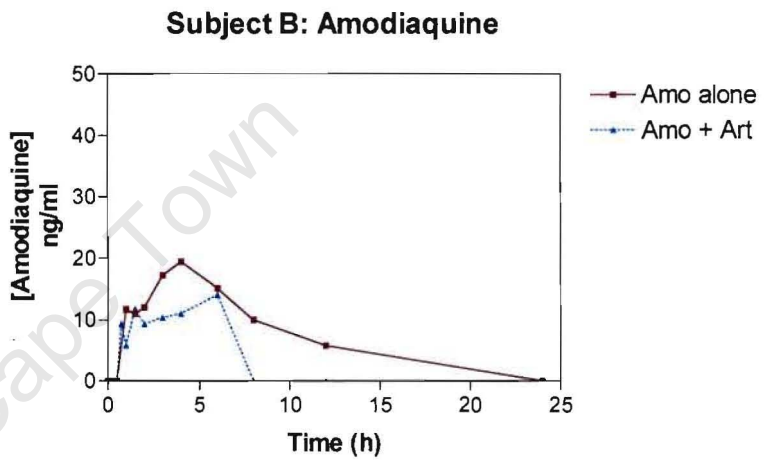
Subject Q: Dihydroartemisinin

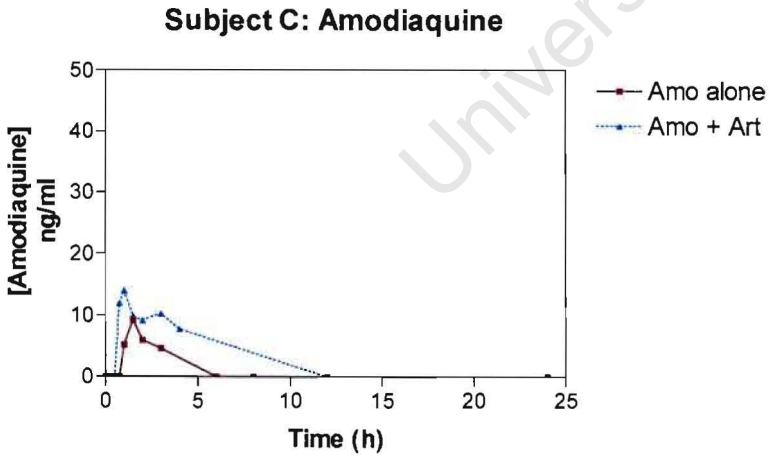


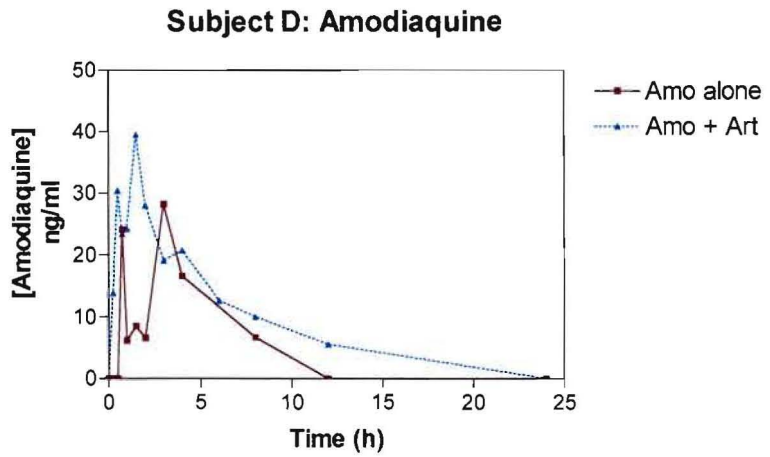


Amodiaquine profiles

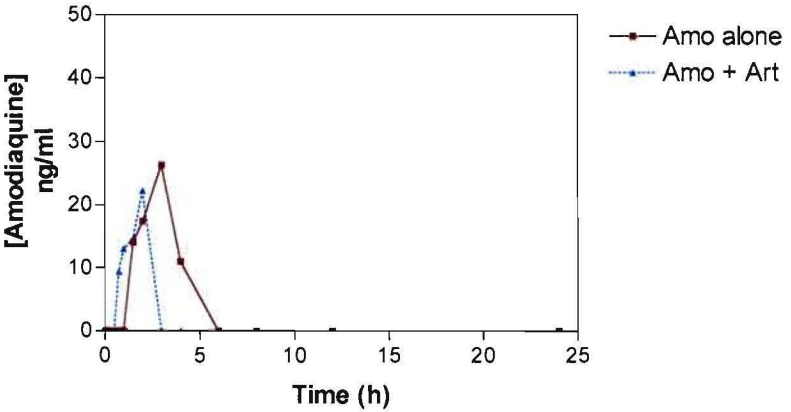




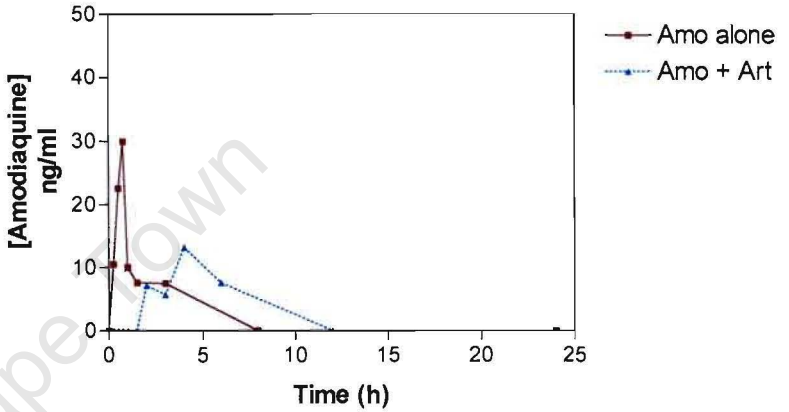




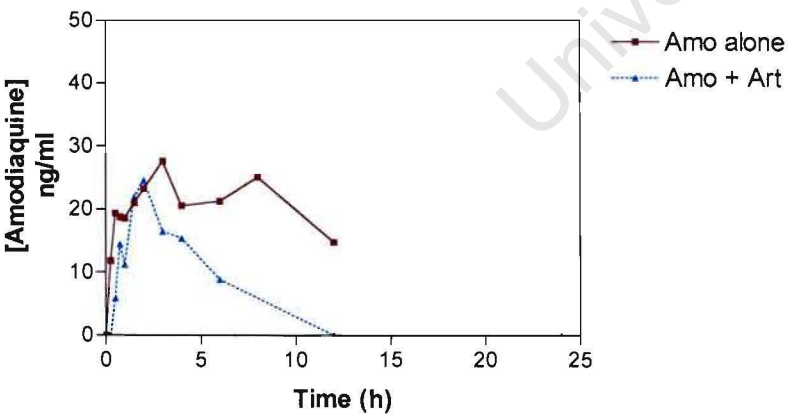
Subject E: Amodiaquine



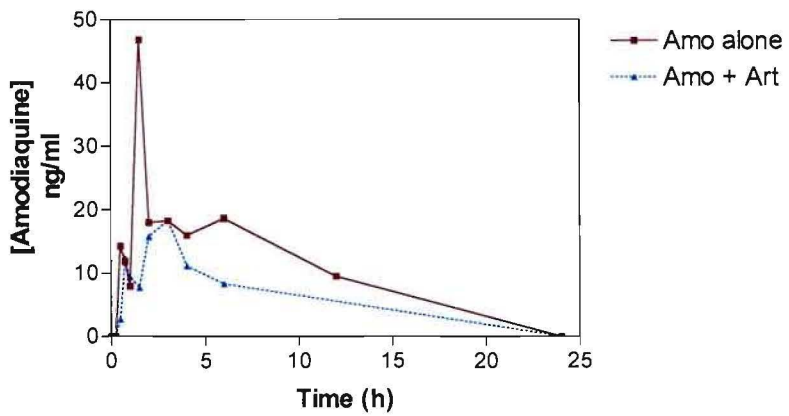
Subject F: Amodiaquine



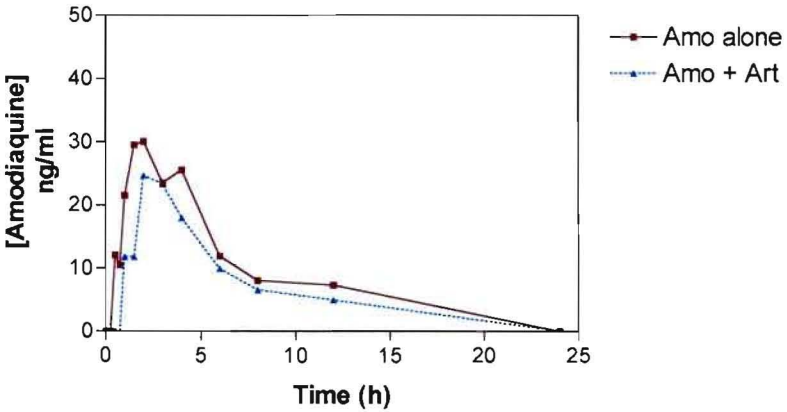
Subject J: Amodiaquine



Subject K: Amodiaquine



Subject L: Amodiaquine



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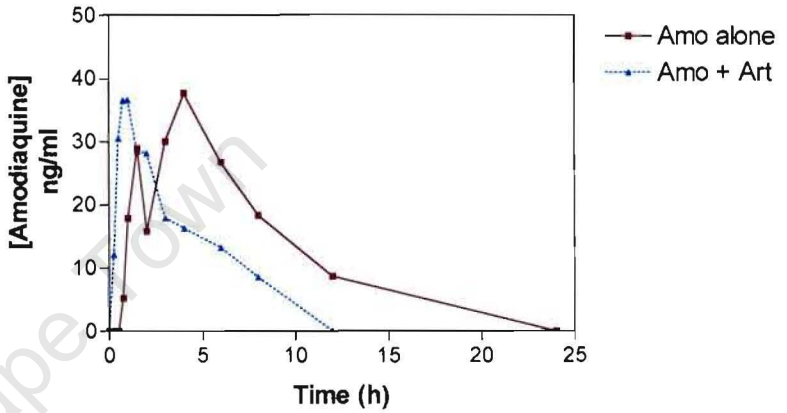

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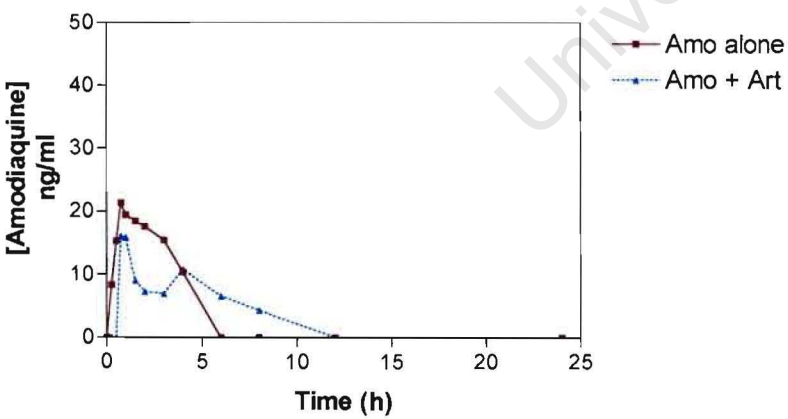

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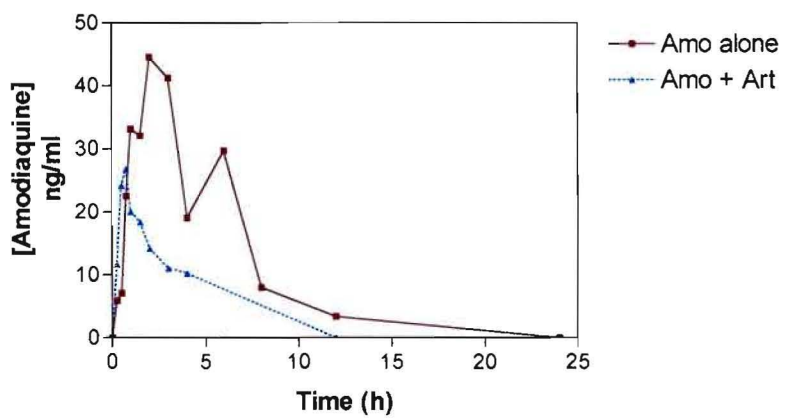
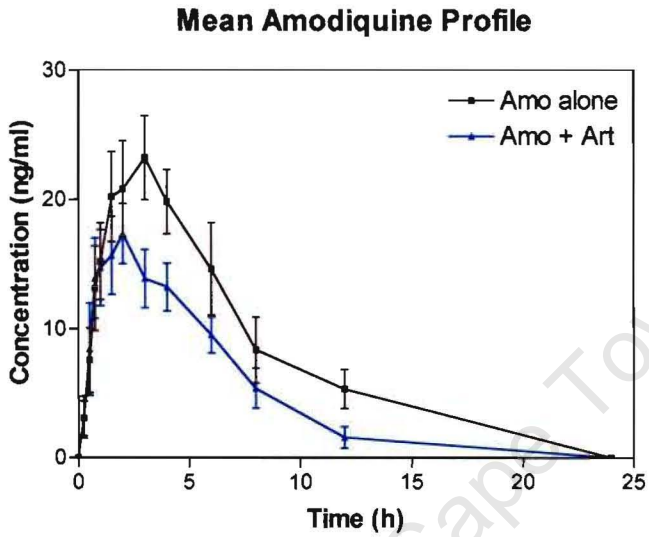
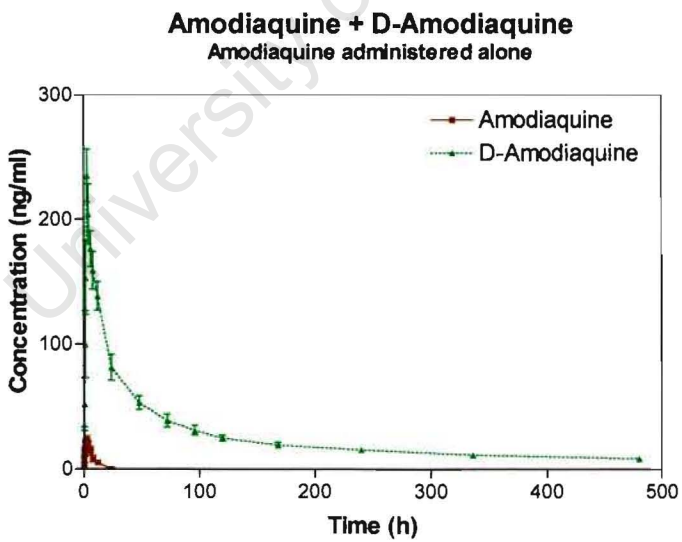
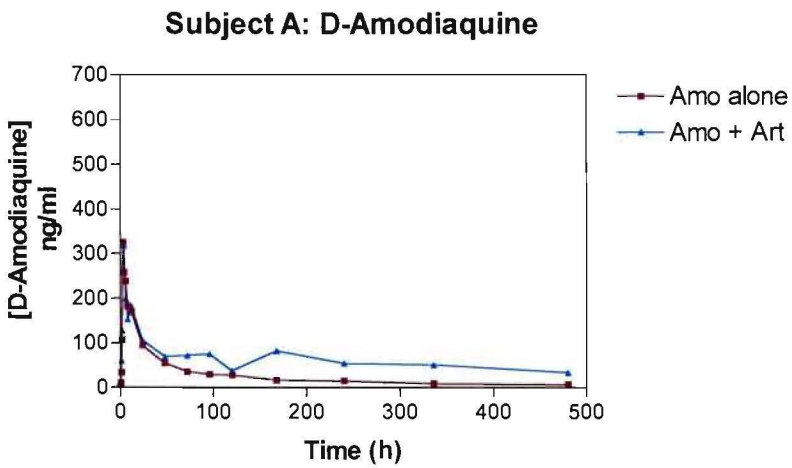
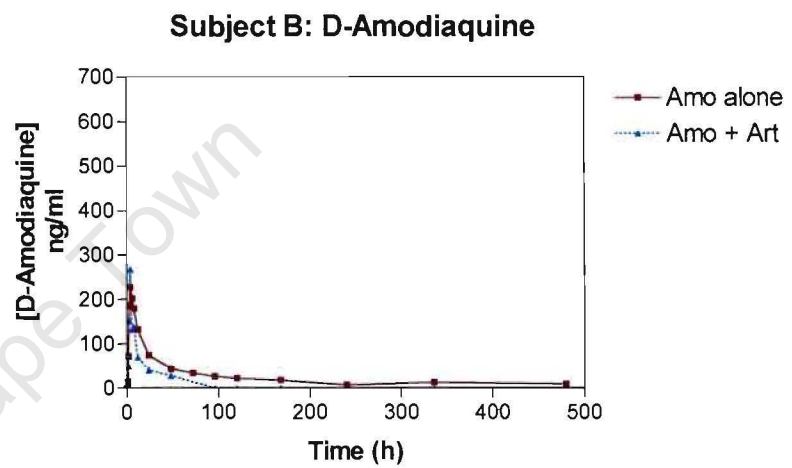


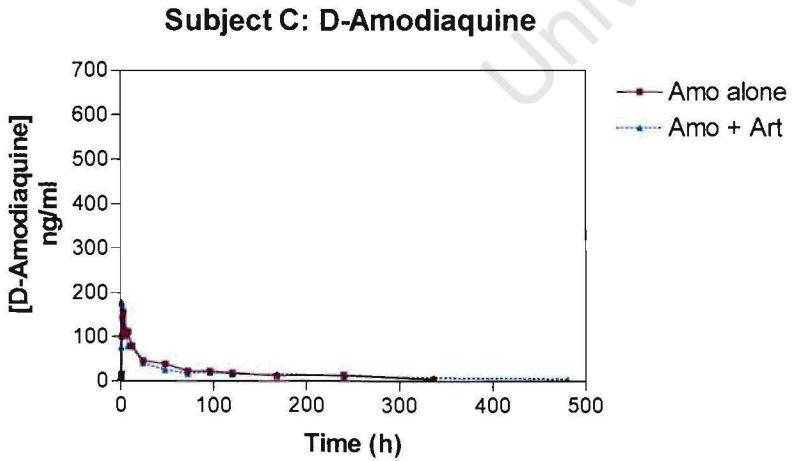


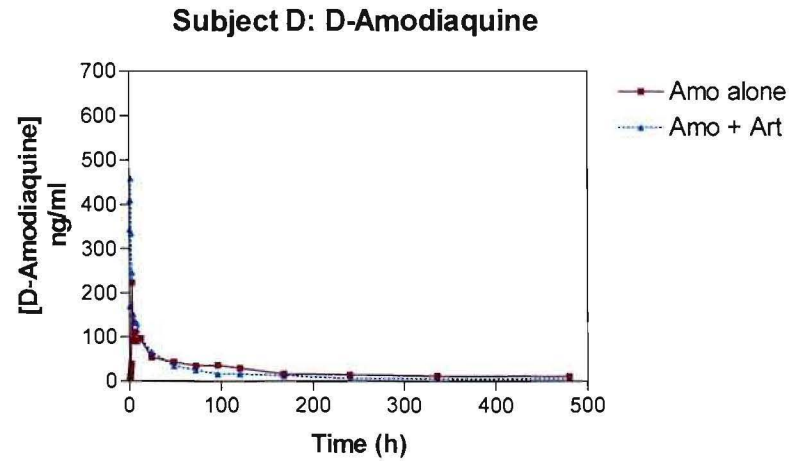


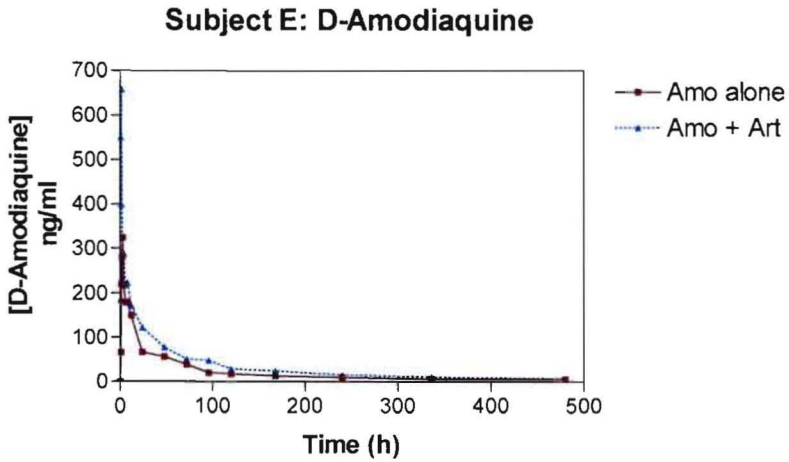
D-amodiaquine profiles

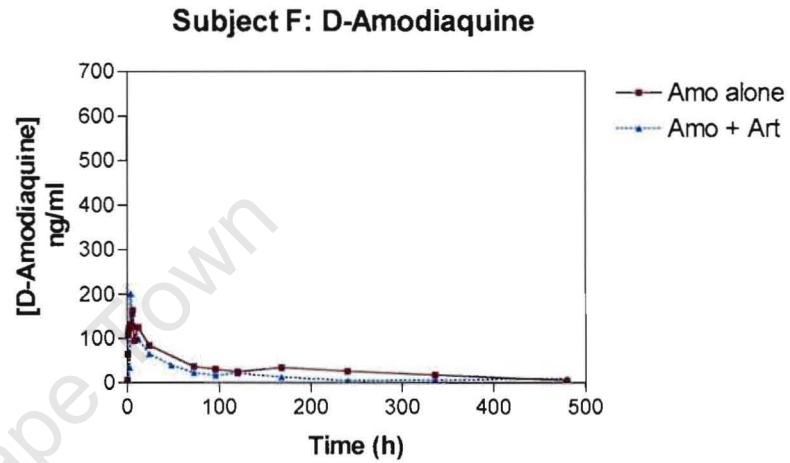


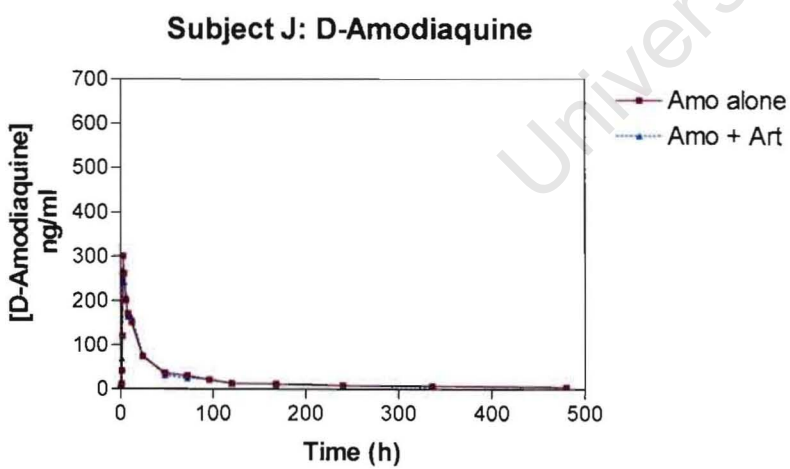


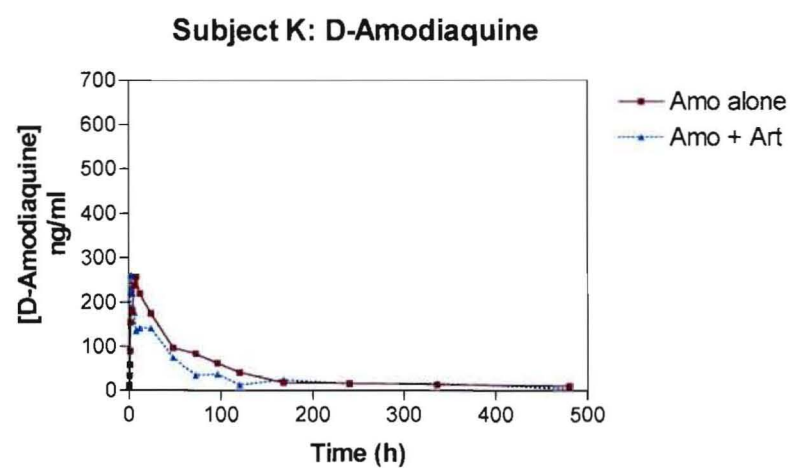


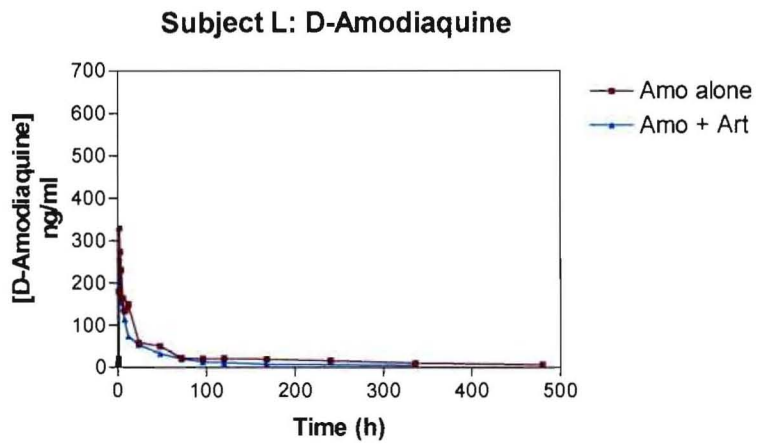


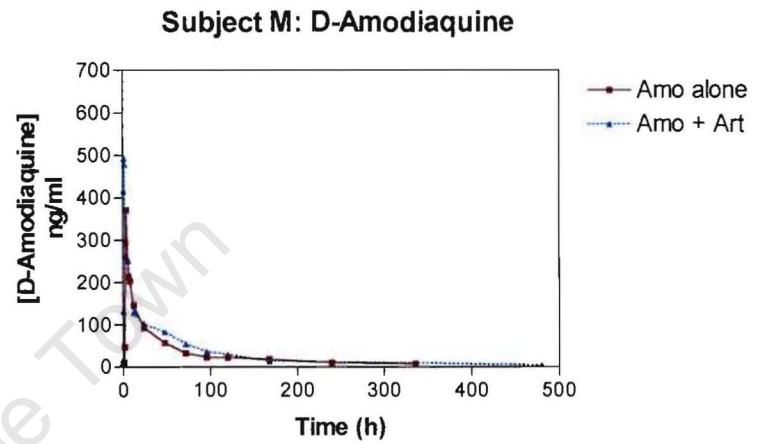


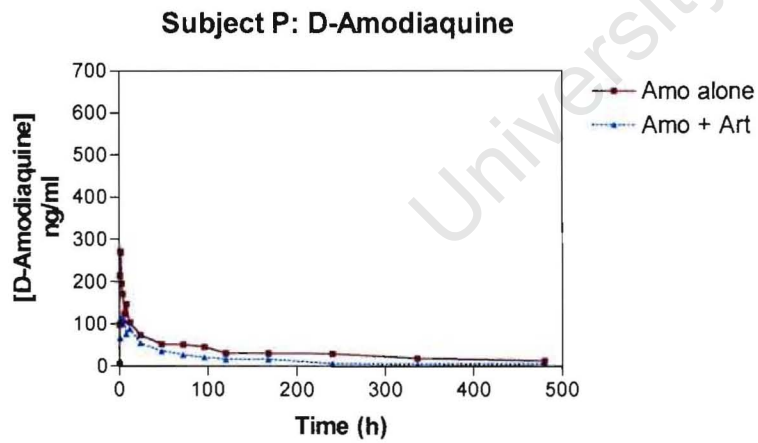


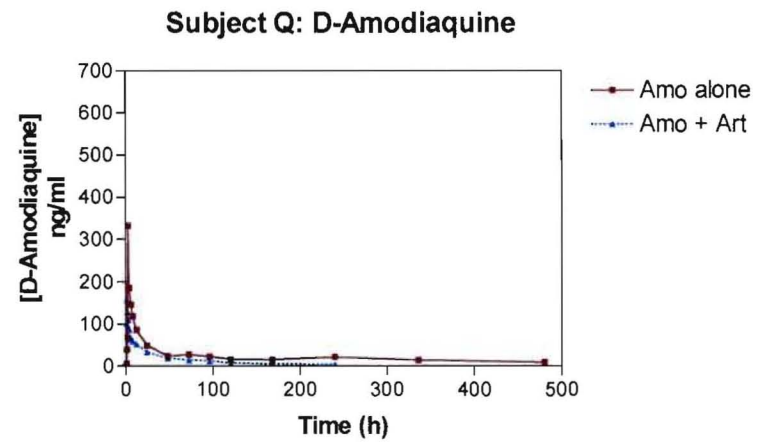












Appendix E-1: HPLC methodology for AS and DHAS

A high performance liquid chromatographic (HPLC) method for the determination of artesunate and dyhydroartemisinin in human plasma

References:

Batty , K.T. et al (1996) J. Chromatography 677 345 - 350

Navaratnam V. et al (1997) J. Chromatography 692 157 - 162

1. PURPOSE

To describe a High Performance Liquid Chromatographic (HPLC) method for the determination of artesunate and dyhydroartemisinin in human plasma to be performed at the Department of Pharmacology, University of Cape Town.

2. APPLICABILITY

All projects in the HPLC laboratory dealing with artesunate and dyhydroartemisinin in human plasma.

3. RESPONSIBILITY

Applicable to the persons responsible for the performance of assays on artesunate and dyhydroartemisinin in human plasma.

4. PROCEDURE

4.1 Description of assay components

Matrix	:	human plasma
Anticoagulant	:	heparin
Volume required	:	500 ul
Extraction method	:	solid phase
Concentration range	:	20 - 1000 ng/ml
Detection mode	:	Postcolumn decomposition UV 290 nm
Quantitation by	:	peak area
Regression	:	linear

4.2 INSTRUMENTATION

NAME	SPECIFICATIONS MODEL/BRAND	COMPANY
Solvent pump	600 Multisolvent Delivery System	Waters
Autosampler	WISP 712	Waters
Detector	484 Tunable Abs. Detector	Waters
System Controller	600 E	Waters
Integrator	Peaksimple	
Analytical Column	Symmetry C8 5 um 15 cm x 0,46 cm	Waters
Guard column	Pelliguard LC 8 2,5 cm x 0,46 cm	Supelco Packed in house
Centrifugal Vaccuum Concentrator	RC 10.10	Jouan
Low temperature trap	RCT 90	Jouan
Vaccuum extractor		Analytichem Int.

4.3 CHEMICALS

NAME CAT NUMBER	SPECIFICATIONS	COMPANY	
Water	Type 1 Rgt grade	Millipore	In house
Acetic acid	99,8%	Merck	Art 63
Acetonitrile	99,9%	BDH	152516Q
Trifluoroacetic acid	99%	Merck	808260
Potassium hydroxide	98%	Merck	4873
Phenyl extraction column 1210 2025	1 ml/70 mg packing	Varian/Anateck	

4.4 PREPARATION OF STOCK SOLUTIONS

4.4.1. Artesunate stock: Solution A (1 mg/ml)

a. On an analytical balance accurately weigh approximately1 mg of artesunate into an eppendorf tube.

b. Add the appropriate amount of acetonitrile to produce a final concentration of 1 mg/ml. Vortex well.

c. Remove 100 ul of stock and make up to 1 ml with mobile phase 1 to give a working standard of 100 ug/ml

d. Take 10 ul of working standard and make up to 1 ml with drug free plasma to give an upper reference standard of 1000ng/ml

4.4.2. Dyhydroartemisinin stock: Solution A (1 mg/ml)

a. On an analytical balance accurately weigh approximately 1 mg of DHA into an eppendorf tube.

b. Add the appropriate amount of acetonitrile to produce a final concentration of 1 mg/ml. Vortex well.

c. Remove 100 ul of stock and make up to 1 ml with mobile phase 1 to give a working standard of 100 ug/ml

d. Take 10 ul of working standard and make up to 1 ml with drug free plasma to give an upper reference standard of 1000ng/ml

4.5 PREPARATION OF REAGENTS

4.5.1 1,2 M Potassium hydroxide (Expiry 1 month)

Weigh 67 g of KOH and dissolve in 1 litre of MilliQ water.

4.5.2 Mobile Phase 1: 45 % acetonitrile in 0,05M acetic acid

4.5.3 Mobile Phase 2: 1,2 M KOH in 90% methanol

4.6 PREPARATION OF CALIBRATION STANDARDS IN PLASMA BY SERIAL DILUTION

a. Obtain 200 ml of human plasma collected and screened for interference at the appropriate retention times. Allow the plasma to thaw at room temperature.

b. Pipette 0,5 mls of plasma into each of 5 eppendorf tubes. Remove 0,5 mls from the upper reference standard (URS) and add it to a vial containing 0,5 mls of plasma. Vortex well.

c. Remove 0,5 mls from this tube and place in another tube containing 0,5 mls of plasma. Vortex well.

d. Continue the serial dilution in this manner until all the vials have been spiked.. Discard the 0,5mls removed from the final vial.

e. Process immediately or store at -80 degrees until used.

4.7 ANALYTICAL PROCEDURES

4.7.1. SAMPLE PREPARATION

a. Thaw samples and transfer 0,5 ml to a mini centrifuge tube

b. Add 0,5 ml water and 10 ul 5 N HCl. Vortex well.

c. Centrifuge, 10 000g for ten minutes

4.7.2. METHOD OF EXTRACTION

a. Wash the bondelut extraction cartridges twice with 1 ml of acetonitrile and then twice with 1 ml of water. Ensure that the water is completely pulled through the column.

b. Apply 950 ul of prepared sample to the column. Pull through to waste.

c. Wash with 2 x 1ml of water. Pull through till completely dry.

d. Elute into glass kimble tube as follows using 3 x 0,5 mls of acetonitrile

e. dry under vaccuum in centrifugal concentrator.

f. Add 100 ul HPLC mobile phase, vortex and place at 4 C for 18 hours.

4.7.3. HPLC Conditions

Analytical column : Symmetry C 8, 5 um, 15 cm x 4,6 mm.

Mobile Phase (1) : 45% acetonitrile in 0,05M acetic acid, pH 6,0

Mobile phase (2)	: 1,2 M KOH in 90% methanol
Flow rate	: 0,7 mls per minute (mobile phase 1) : 0,3 mls per minute(mobile phase 2)
Injection volume	: 50 ul
Reaction coil	: 1 ml, Waters
Column temperature	: 25 degrees centigrade
Reaction coil temperature	: 69 degrees centigrade
Autosampler temperature	: 25 degrees centigrade

The reaction coil and the Waters reagent delivery module which delivers the mobile phase (2) are linked to the postcolumn outflow via a T-piece

4.7.4. UV detector conditions

Wavelength : 290 nm

4.7.5. Retention times

Artesunate : 6,9 minutes

Dihydroartemisinin : 8,8 minutes

Run time : 13 minutes

4.8 LOWER LIMIT OF DETECTION

At present the reliable lower limit of detection using 0,5 mls of plasma as sample is 20 - 25 ng/ml. If 1 ml of plasma is used as sample, the lower limit of detection is 10 - 15 ng/ml.

Appendix E-2: HPLC methodology for AQ and des-ethyl AQ

A High Performance Liquid Chromatographic (HPLC)-Mass Spectrometric Method for the Determination of amodiaquine and desethylamodiaquine in Human Plasma

NB: All procedures must be carried out under yellow light conditions. All HPLC vials must be amber.

1. PURPOSE

To describe a High Performance Liquid Chromatographic (HPLC) method for the determination of amodiaquine and desethylamodiaquine in human plasma to be performed at the Division of Pharmacology, Department of Medicine, University of Cape Town.

2. APPLICABILITY

This procedure applies to all projects in the HPLC laboratory dealing with amodiaquine and desethylamodiaquine in human plasma.

3. RESPONSIBILITY

This procedure applies to all the persons responsible for performing assays for amodiaquine and desethylamodiaquine in human plasma.

4. PROCEDURE

4.1 Description of assay components

Matrix	human plasma (heparin added as an anticoagulant)
Sample Volume required	50 µl
Extraction method	protein precipitation
Concentration range	amodiaquine: 5 - 100 ng/ml Desethylamodiaquine: 5 – 400 ng/ml
Detection mod	Mass Spectrometer
Quantitation	Peak area
Regression	linear

4.2 Instrumentation Specifications

Instrument	Model/Brand	Supplier
HPLC	Agilent	Hewlet Packard
Detector	Mass Spectrometer	Hewlet Packard
Analytical Column	Xterra C18 50 x 4.6 mm	Waters
Guard Column	Pelliguard LC18 2.5 cm x 0.46 cm	Supelco Packed in house

4.4 Chemical Specifications

Chemical	Specifications	Supplier	Catalogue Number
Water	Type 1, Reagent Grade	Millipore	In house purification
Methanol	99.8%	BDH	101586B
Acetonitrile	99.9%	BDH	152516Q
Ammonia	98.0	Merck	001116.500

4.5 Preparation of Stock Solutions

4.5.1

4.5.2 Amodiaquine/Desamo: Standard Solution A (1 mg/ml)

a. On an analytical balance weigh approximately 1mg of amodiaquine or metabolite into a clean polypropylene microcentrifuge tube.
b. Add acetonitrile to produce a final concentration of 1 mg/ml. Vortex well.
c. Store at minus 80 degrees centigrade.

Amodiaquine/ Desamo stock: Standard Solution B (0,01 mg/ml)

a. Accurately pipette 10 ul of Standard Solution A into a polypropylene tube.
b. Add 990 ul acetonitrile
c. Store at minus 80 degrees centigrade

Mobile Phase preparation

4.6.2 Mobile Phase : 75% acetonitrile: pH 10.6 with ammonia

4.7 Preparation of calibration standards in plasma

a. Obtain 200 ml drug-free human plasma, which has been screened for interference at the appropriate retention times. Allow the plasma to thaw at room temperature.
b. Pipette 25 mls of plasma into clean 25 ml volumetric flasks

c. Remove the appropriate volume of plasma equivalent to the volume of standard to be added and then add an equal volume of the appropriate standard
d. Mix the contents of the volumetric flask by repeated inversion for 30 seconds.
e. Remove 0.5 ml aliquots of each standard into a clean eppendorf tube.
f. Store at minus 80 degrees C in the dark until used.

4.8 Analytical Procedures

4.8.1 Method of Sample preparation

Accurately pipette 200 ul of sample or standard into a clean eppendorf vial. Add 400 ul of acetonitrile. Vortex for 1 minute. Centrifuge for 3 minutes at 3000 g. Transfer supernatant to an HPLC insert.

4.8.2 HPLC Resolution Conditions

Analytical column	Waters Xterra C18.
Mobile Phase	75% acetonitrile : pH 10.4 with ammonia
Flow rate	0.1 ml per minute
Injection volume	5 µl
Column temperature	25 °C (Room temperature)
Autosampler temperature	25 °C (Room temperature)

4.8.3 *Detection*

Amodiaquine was detected at a Mass of 356 and desethylamodiaquine at a Mass of 328

4.8.6 Calibration

A series of calibration standards is run with every series of assays as were quality control samples. The standard curve is prepared with an ascending concentration of each analyte. For each calibration standard, the peak area is determined. A linear regression describing the calibration curve is then calculated.

Appendix F: Hepatitis case report

Orrell C, Taylor W, Olliaro P. (2001). Acute asymptomatic hepatitis in a healthy normal volunteer exposed to 2 oral doses of amodiaquine and artesunate. Trans R Soc Trop Med Hygiene. 95(5): 517-8. September 2002.

Multidrug resistant *Plasmodium falciparum* is a global problem (Wernsdorfer & Payne, 1991). The use of antimalarial drug combinations with independent mechanisms of action is a rational approach to delay the onset the of drug resistance and safeguard existing drugs (White & Olliaro, 1996). Current field research is assessing oral artesunate combined with standard antimalarials e.g. sulfadoxine/pyrimethamine, amodiaquine, for treating acute uncomplicated falciparum malaria. Artesunate has a short half life and produces a rapid and substantial reduction of malaria parasites; those that remain are then killed by the combinant drug acting over a longer time period. To complement the field studies, an interactive pharmacokinetic study of artesunate and amodiaquine was conducted in normal human volunteers at the University of Cape Town. We report the occurrence of asymptomatic hepatitis in a woman during this study.

This was a single dose three phase cross over study conducted in 15 volunteers, 10 male and 5 female, of age range 19 to 42 years. All volunteers received one dose of oral artesunate (4mg/kg) on day 0, followed, on day 7, by either one dose of oral amodiaquine (10 mg/kg) alone or combined with a single dose of artesunate (4mg/kg). Subjects were given the alternate regimen on day 28. Blood for routine haematology and biochemistry was taken on days 0, 6, 27 and 48.

Our subject was a healthy 20 years old, South African female of African – Caucasian descent, with no previous significant illnesses. During the study she did not take any prescribed or over the counter drugs, including paracetamol, and had no reported drug allergies. She did not drink alcohol and had no risk factors for hepatitis B. Her family history was unremarkable. Clinically, there were no abnormal physical signs and her baseline blood tests were all normal. She received artesunate (day 0), amodiaquine (day 7), followed by the combination (day 28). Six days after taking artesunate alone, her AST and total bilirubin were normal but she had a slight increase in ALT of 31 IU/L (normal 1-25 IU/L) that was not considered clinically significant (figure 1). On day 27, all blood tests were normal. On day 48, her

transaminases were elevated, rising to peak values (AST 236 IU/L, ALT 483 IU/L) on day 69, decreasing thereafter to normal values over 45 days (day 124). LDH and γ GT were also elevated. She remained asymptomatic and afebrile throughout her illness. She did not develop any physical signs of either liver dysfunction or of the possible aetiology of her hepatitis; in particular no rash, lymphadenopathy, splenomegaly, enlarged tonsils. Pertinent investigations were non-contributory and included a normal urea, creatinine, CPK, total white cell, neutrophil, lymphocyte and eosinophil counts, and prothrombin time. There were no atypical lymphocytes. Serologies for hepatitis A, B & C, and autoantibodies (ANF, anti mitochondrial & smooth muscle antibodies) were negative. A liver ultrasound on day 72 was normal. A liver biopsy was postponed after the first improved transaminase results.

This normal, healthy female volunteer developed an asymptomatic rise in liver enzymes following the sequential administration of artesunate and amodiaquine which we believe was most probably caused by the amodiaquine alone. Other causes of asymptomatic hepatitis e.g. EBV, CMV, toxoplasmosis were considered unlikely in the absence of other physical signs, the lack of lymphocytosis, her immune competent state (Mandell *et al.*, 1995). Furthermore toxoplasmosis is rare in Cape Town (personal communication).

Amodiaquine-induced hepatitis during malaria prophylaxis is well described and may be associated with neutropaenia (Larrey *et al.*, 1986; Neftel *et al.* 1986; Woodtli *et al.*, 1986; Charmot *et al.*, 1987; Bernuau *et al.*, 1988; Raymond *et al.*, 1989). These side effects are thought to be immune mediated but the pathogenesis remains unclear (Clarke *et al.*, 1991). Hepatitis has occurred from as early as 3 weeks (exposure to 3 weekly doses) to as late as 10 months of prophylaxis (Charmot *et al.*, 1987). Clinically, reported cases have ranged from a mild, transient elevation of liver enzymes with few symptoms (Larrey *et al.*, 1986; Charmot *et al.*, 1987), to fulminant hepatitis with either slow recovery of function (Larrey *et al.*, 1986; Neftel *et al.* 1986), or death (Neftel *et al.* 1986; Bernuau *et al.*, 1988; Raymond *et al.*, 1989).

Data on hepatic toxicity and amodiaquine use in malaria endemic areas are few. Amodiaquine appears to be safer as treatment rather than as prophylaxis.

However, we are unable to identify studies examining delayed onset hepatitis following treatment, or longitudinal studies of repeated treatments (Olliaro *et al.*, 1996). Artesunate has been widely used in south east Asia and China and is well tolerated - there have been no reported cases of hepatitis (Price *et al.*, 1999; Ribeiro & Olliaro, 1998). Although we believe the amodiaquine alone was the probable cause of the hepatitis in our subject, we cannot exclude the possibility of an interaction between artesunate and amodiaquine. Close to two hundred patients have received this combination in studies ongoing at the present time. Toxicity data from the field are awaited.

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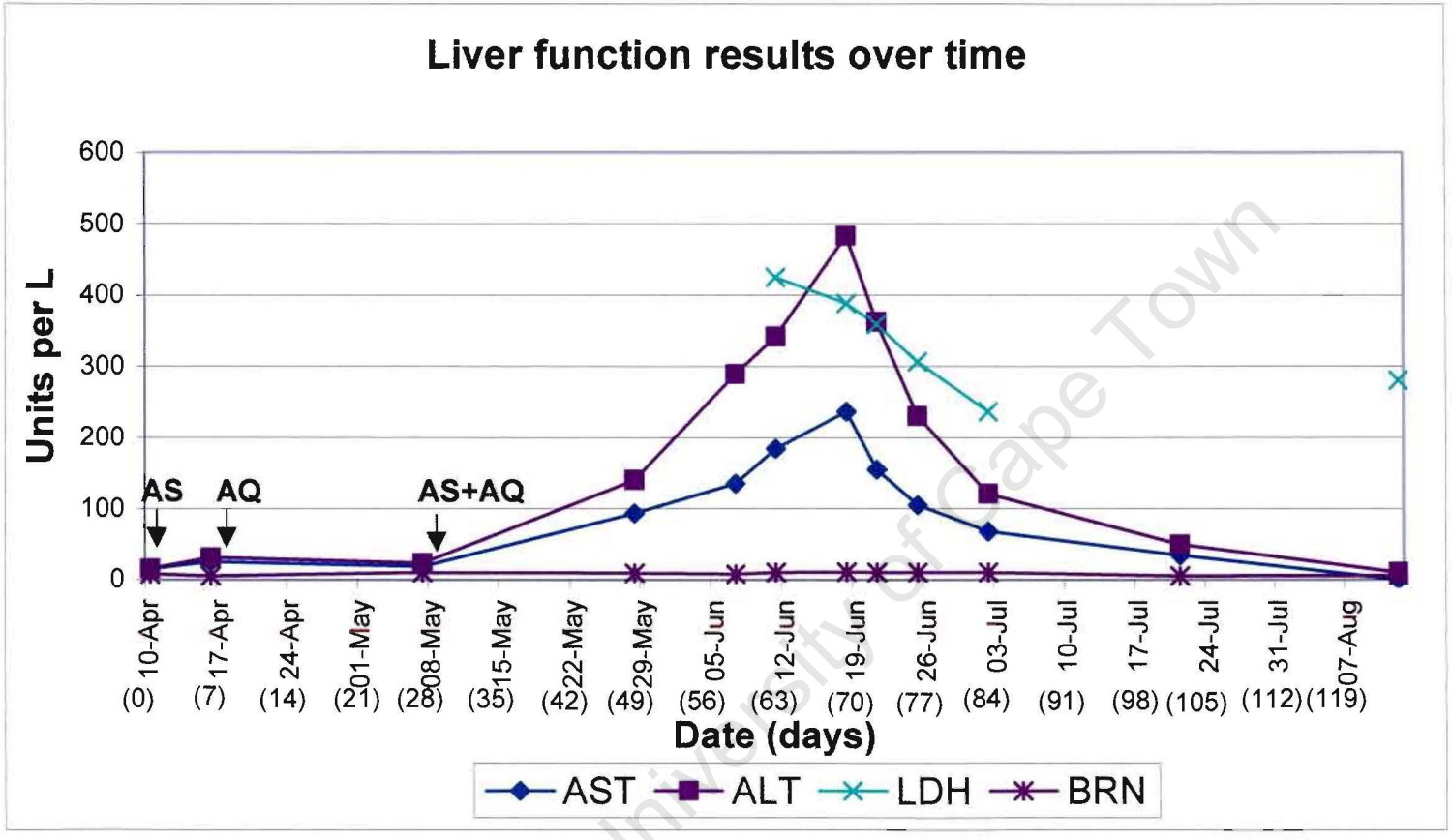
& The centre for Infectious Diseases, Royal Free and University College Medical School, London, UK.

Olliaro, P. UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), WHO, Geneva, Switzerland

Figure 1. Liver enzyme profiles of a healthy female volunteer participating in an interactive cross over pharmacokinetic study of oral artesunate and oral amodiaquine. (Normal ranges: AST 7 – 25 units/L, ALT 1 – 25 units/L, total bilirubin 1 – 17umol/L, LDH 175– 350 units/L, GGT 0 – 40 units/L).



AS = dose of artesunate taken AQ = dose of amodiaquine taken AS+AQ = dose of both drugs taken

Appendix G-1: Haematology mean values

SUMMARY OF HAEMATOLOGY MEASUREMENTS - TREATMENT MEANS AND CONFIDENCE INTERVALS

Haemoglobin (g/dL)

Treatment	Mean	Std. Err.	[95% Conf.	Interval]
Baseline	14.0538	.4074	[13.1662	14.9415]
Sodium Artesunate	13.7769	.3732	[12.9639	14.5900]
Combination	13.9000	.3686	[13.0968	14.7032]
Amadiaquine	14.2154	.3993	[13.3453	15.0855]

Haematocrit

Treatment	Mean	Std. Err.	[95% Conf.	Interval]
Baseline	.4331	.0117	[.4075	.4586]
Sodium Artesunate	.4234	.0107	[.4006	.4471]
Combination	.4123	.0109	[.3886	.4360]
Amadiaquine	.4223	.0098	[.4011	.4436]

Platelet count ($x10^{-9}/L$)

Treatment	Mean	Std. Err.	[95% Conf.	Interval]
Baseline	235.1538	11.6789	[209.7078	260.5999]
Sodium Artesunate	223.3077	9.9049	[201.7267	244.8887]
Combination	244.5385	11.3203	[219.8737	269.2032]
Amadiaquine	230.6154	11.4922	[205.576	255.6548]

White cell count ($x10^{-9}/L$)

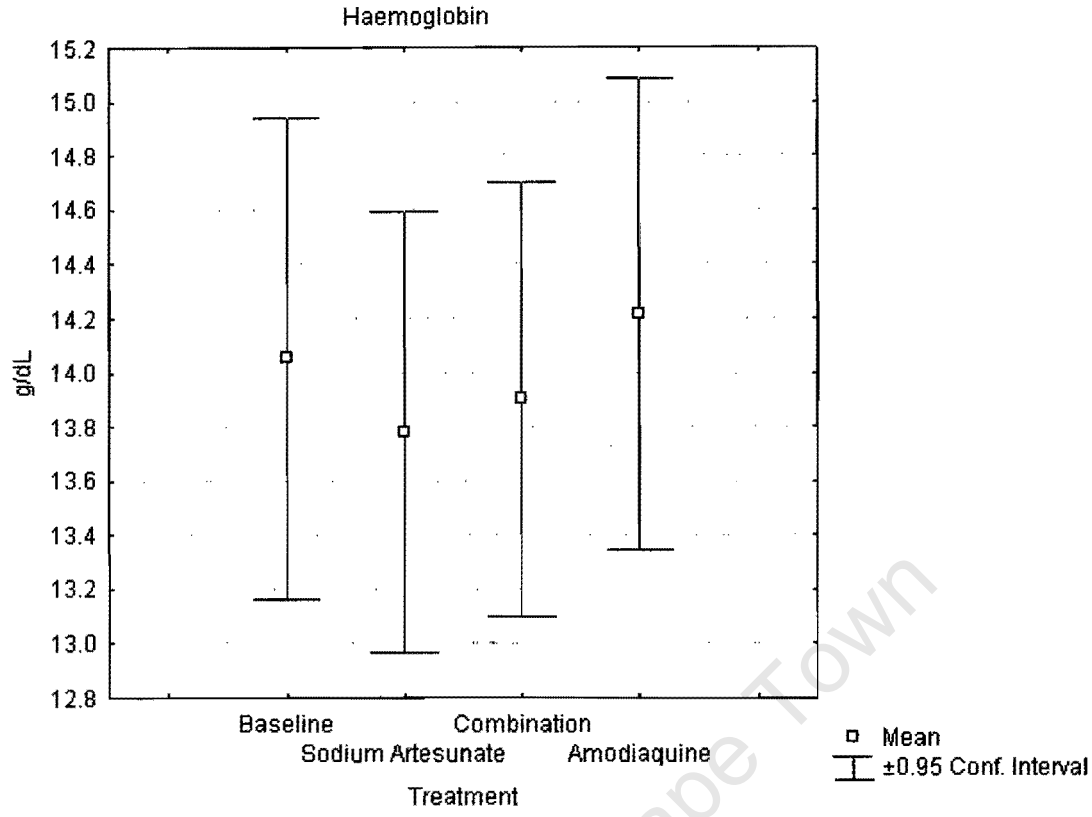
Treatment	Mean	Std. Err.	[95% Conf.	Interval]
Baseline	7.0923	.4779	[6.0510	8.1336]
Sodium Artesunate	6.1538	.4174	[5.2445	7.0632]
Combination	5.6923	.4736	[4.6604	6.7242]
Amadiaquine	5.3923	.3259	[4.6822	6.1024]

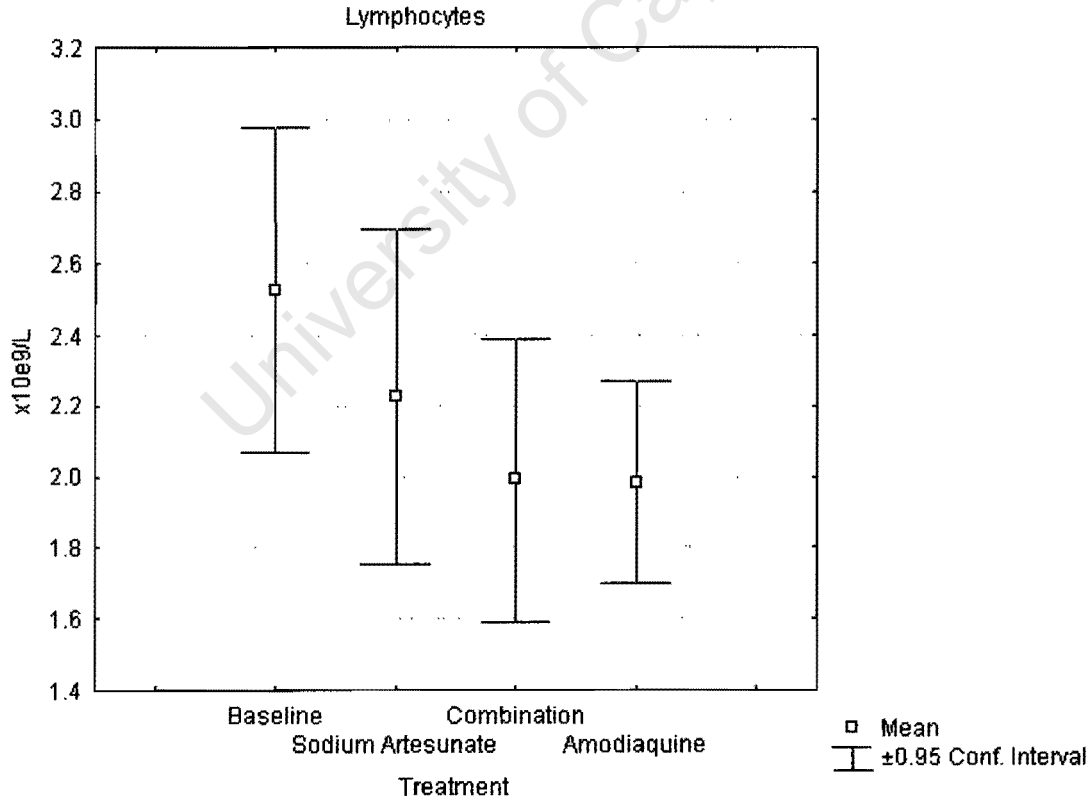
Neutrophils ($x10^{-9}/L$)

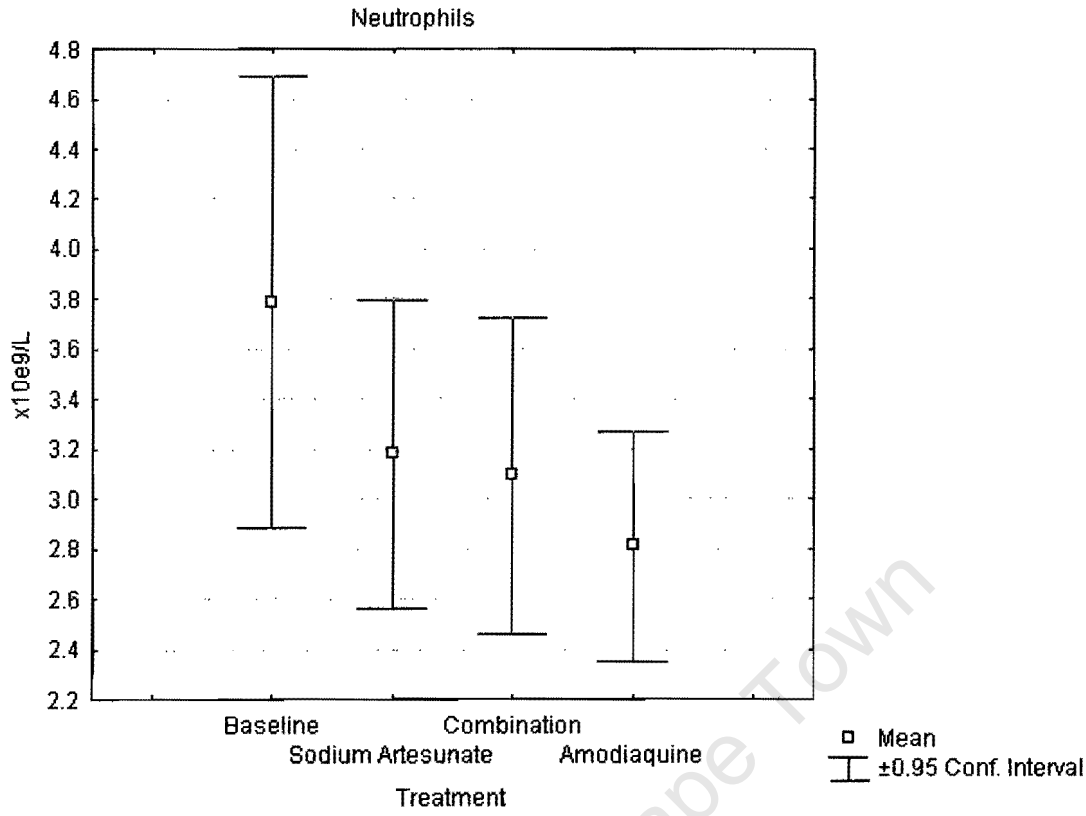
Treatment	Mean	Std. Err.	[95% Conf.	Interval]
Baseline	3.7869	.4140	[2.8848	4.6890]
Sodium Artesunate	3.1785	.2813	[2.5656	3.7913]
Combination	3.0923	.2888	[2.4630	3.7217]
Amadiaquine	2.8100	.2123	[2.3475	3.2725]

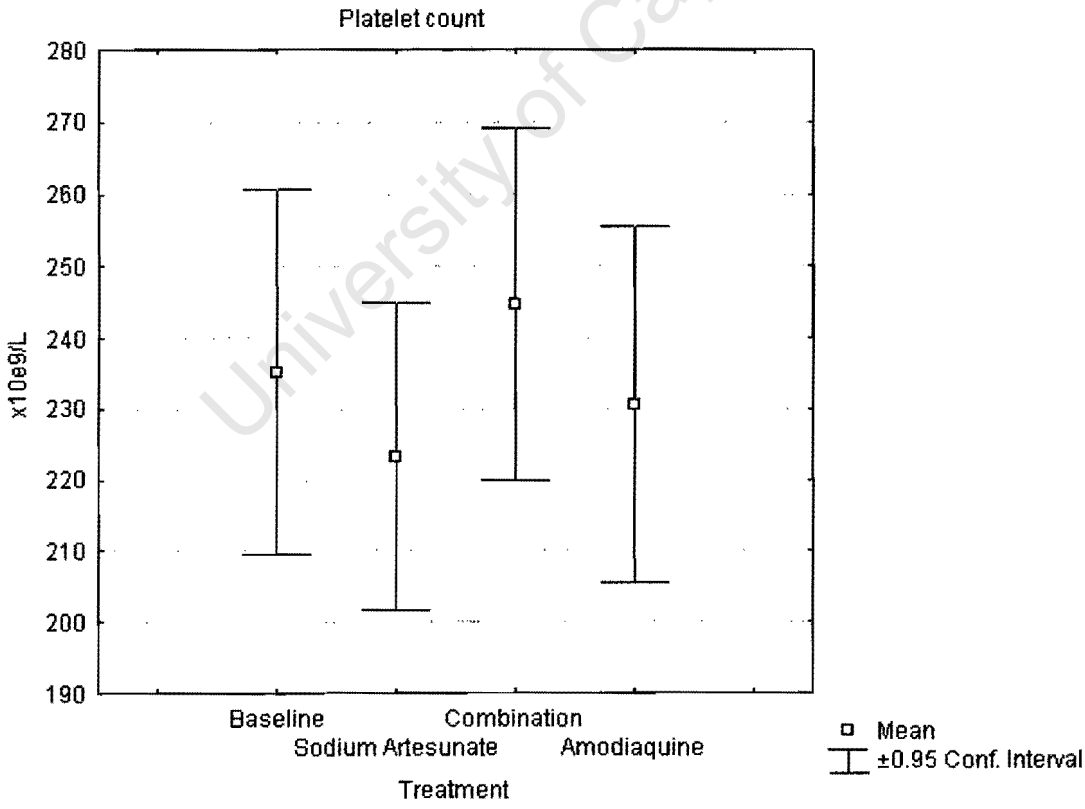
Lymphocytes ($x10^{-9}/L$)

Treatment	Mean	Std. Err.	[95% Conf.	Interval]
Baseline	2.5254	.2087	[2.0706	2.9802]
Sodium Artesunate	2.2246	.2162	[1.7535	2.6958]
Combination	1.9908	.1829	[1.5922	2.3894]
Amadiaquine	1.9846	.1321	[1.6968	2.2724]







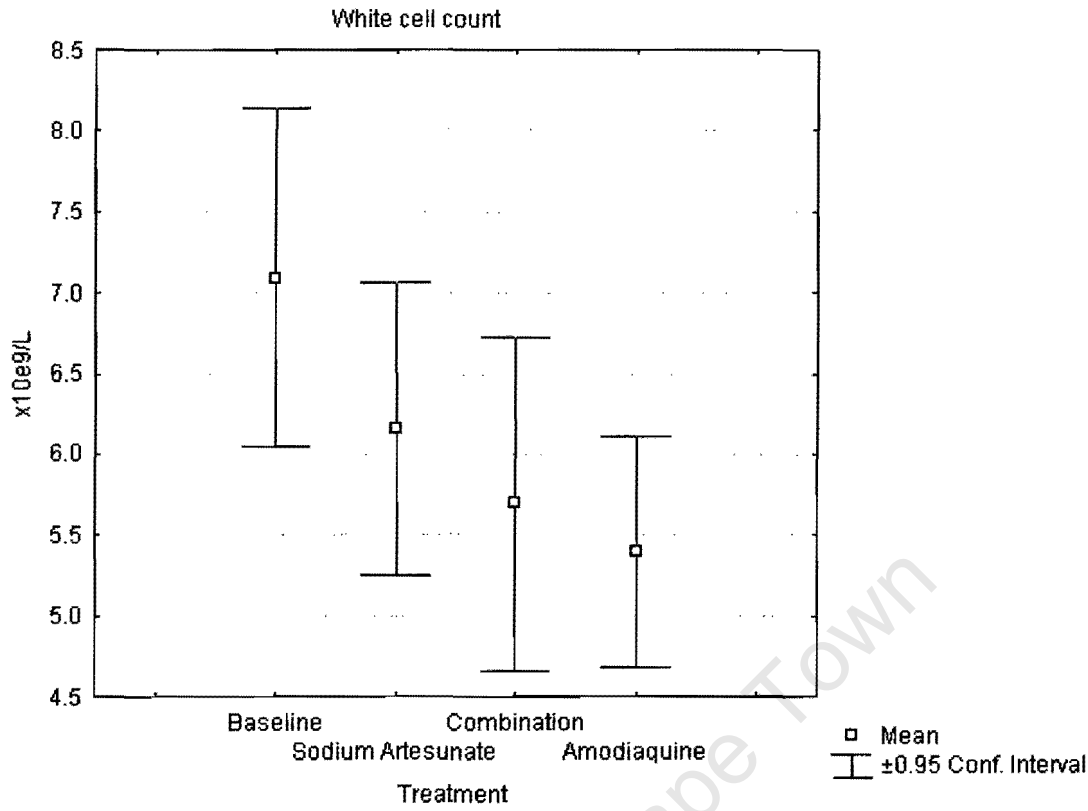


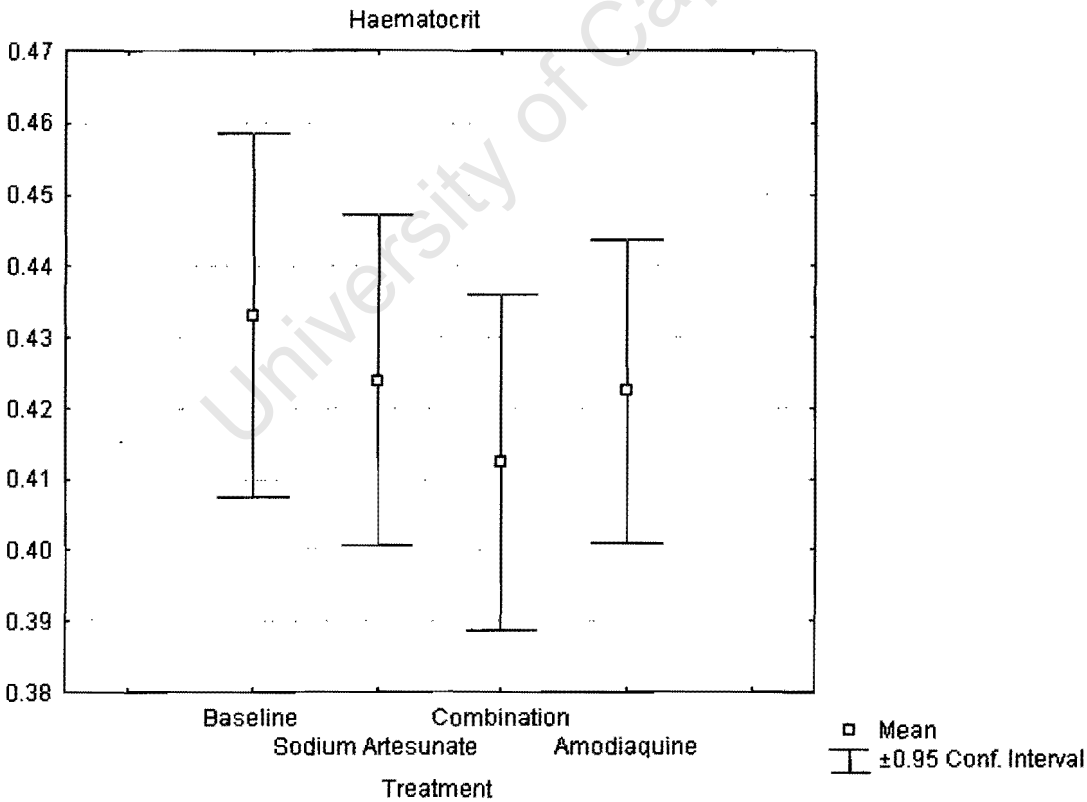
Demographic variables:

Subject	race	age (years)	gender	weight (kg)	height (cm)
A	c	20	f	69	169
B	c	42	m	70	166
C	w	23	m	82	185
D	w	22	m	63	173
E	b	23	f	68	158
F	w	34	m	73	180
G	w	23	m	61	171
H	b	22	f	59	159
J	a	19	f	50	161
K	b	24	f	54	157
L	c	22	m	74	181
M	c	21	m	97	189
N	a	23	m	68	176
P	w	24	m	64	179
Q	b	24	m	58	159
Mean:		24.4		67.33	170.87
Std deviation:		5.90		11.61	10.61

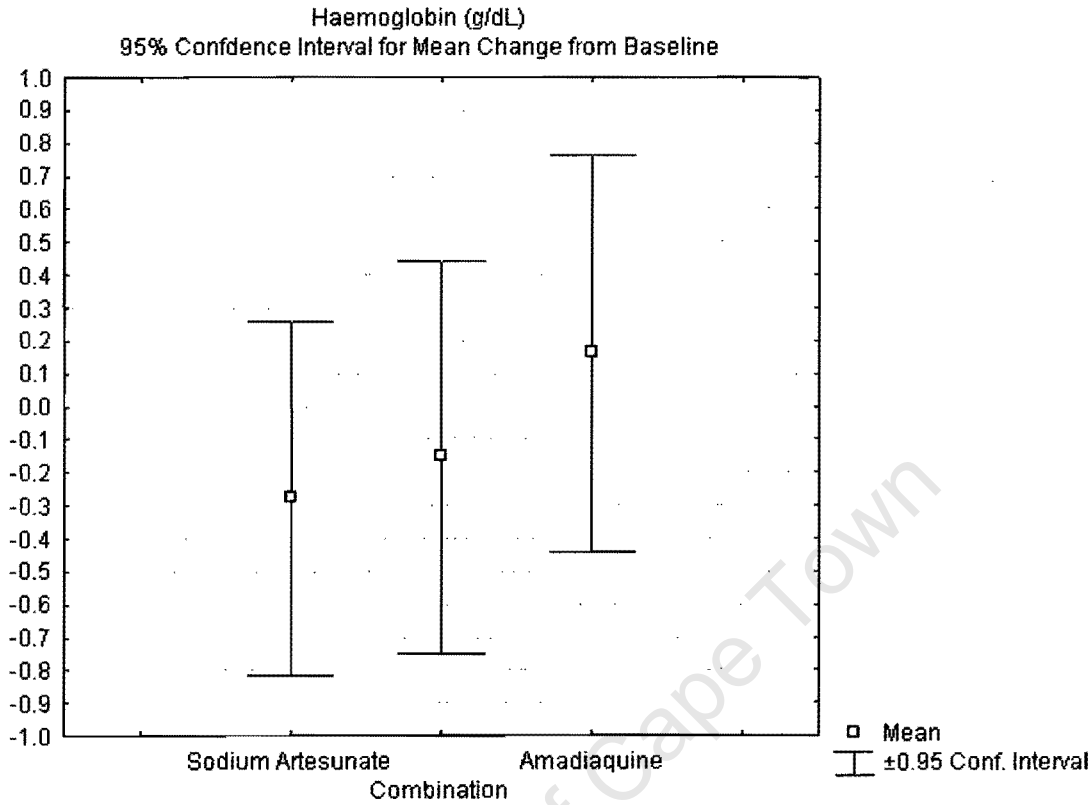
b=black
c=coloured
w=white

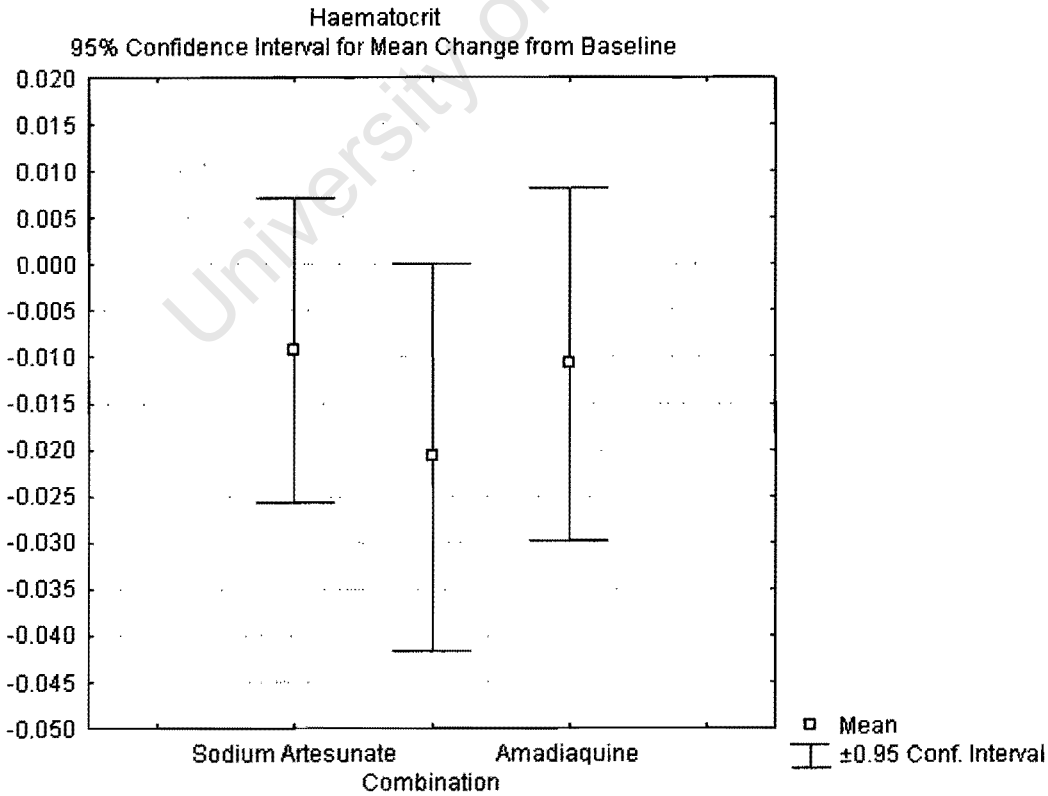
f=female
m=male

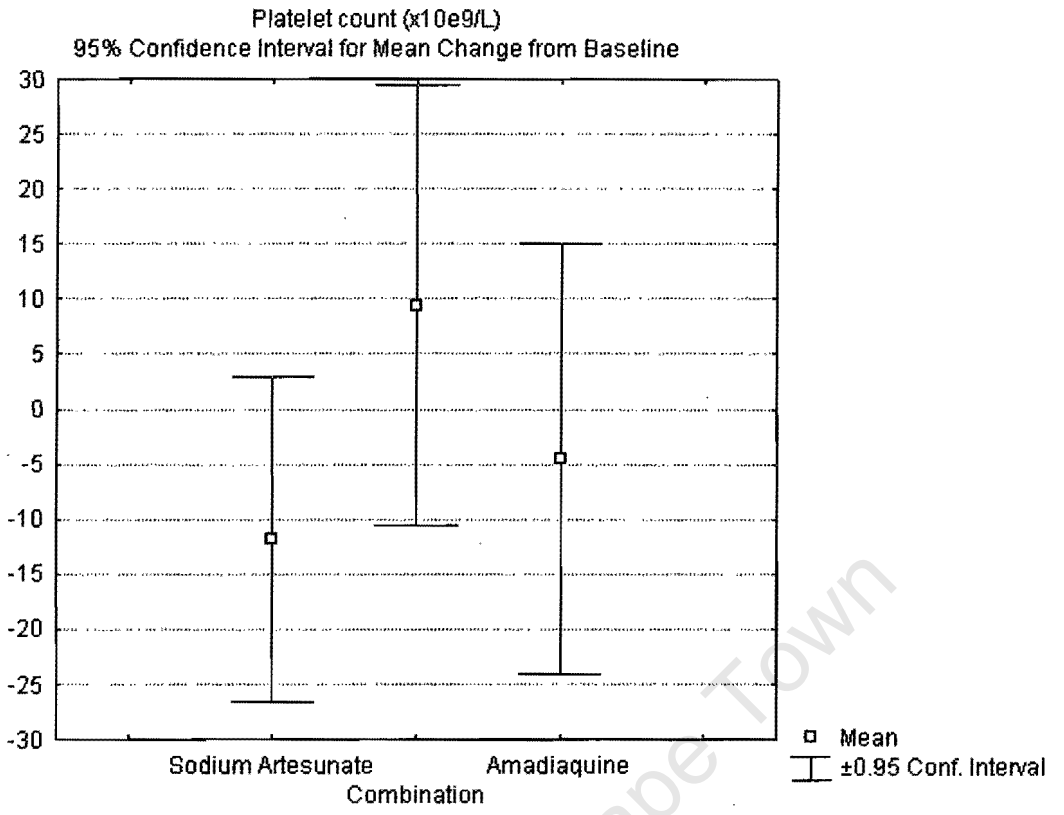


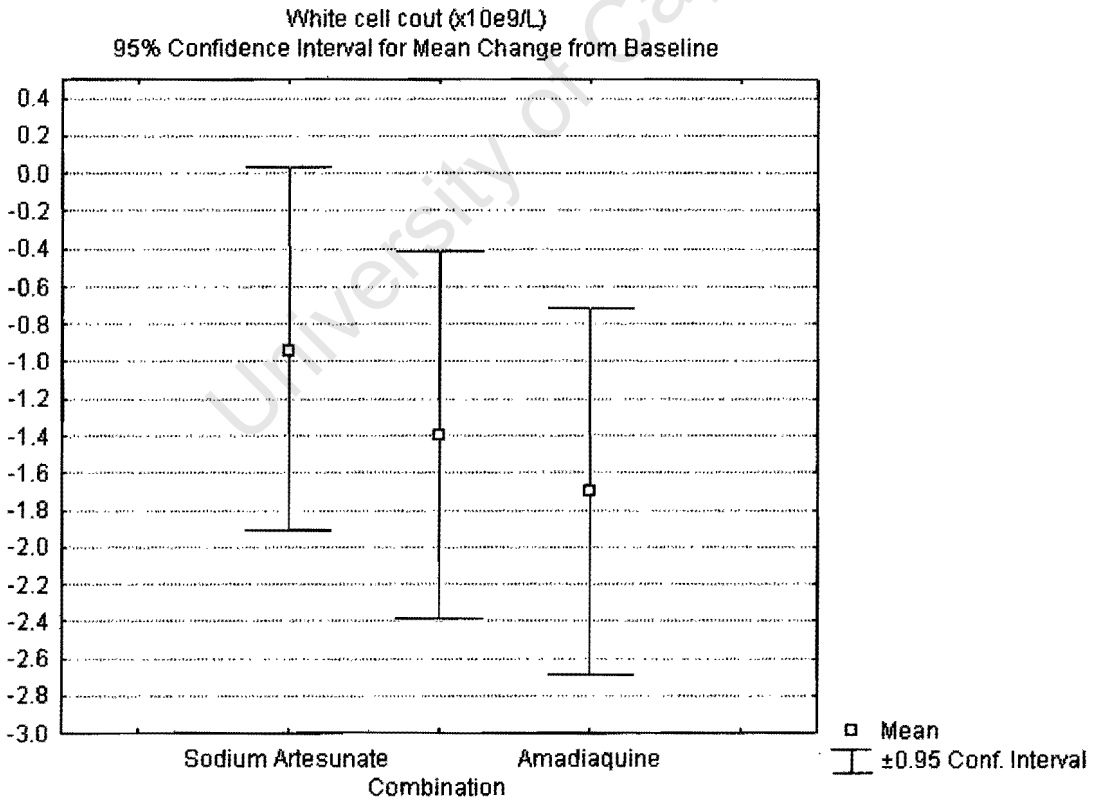


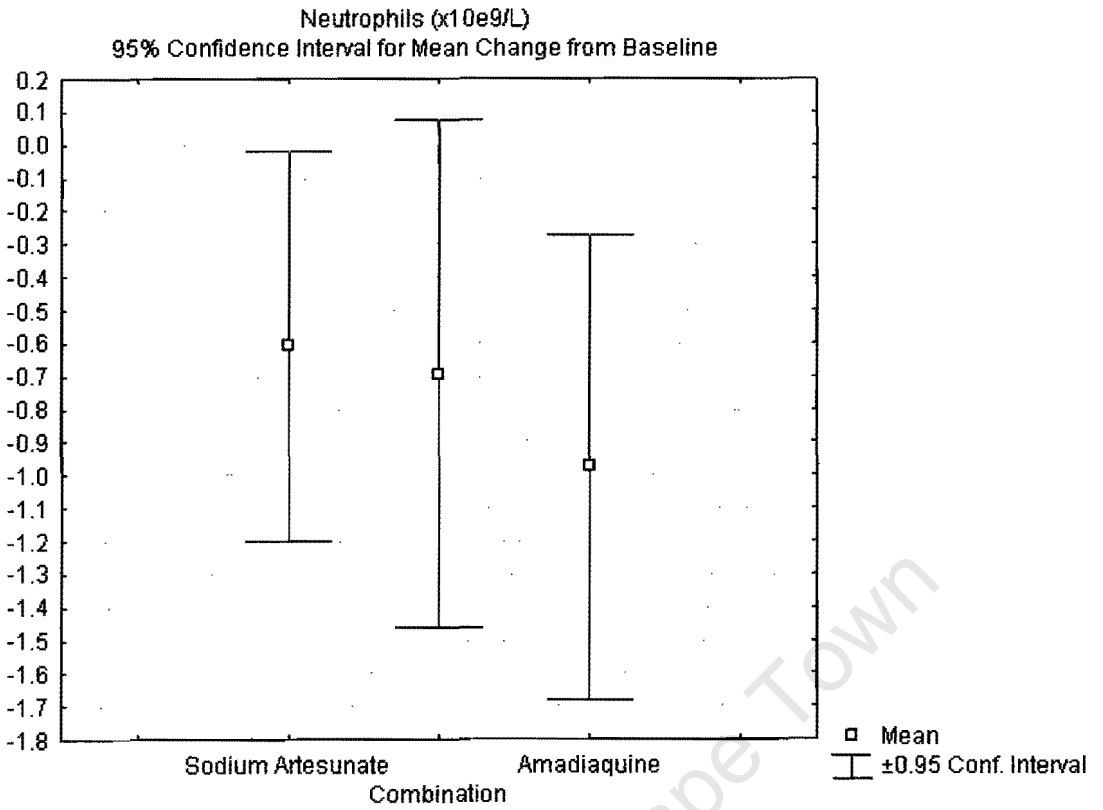
Appendix G-2: Change in haematological parameters

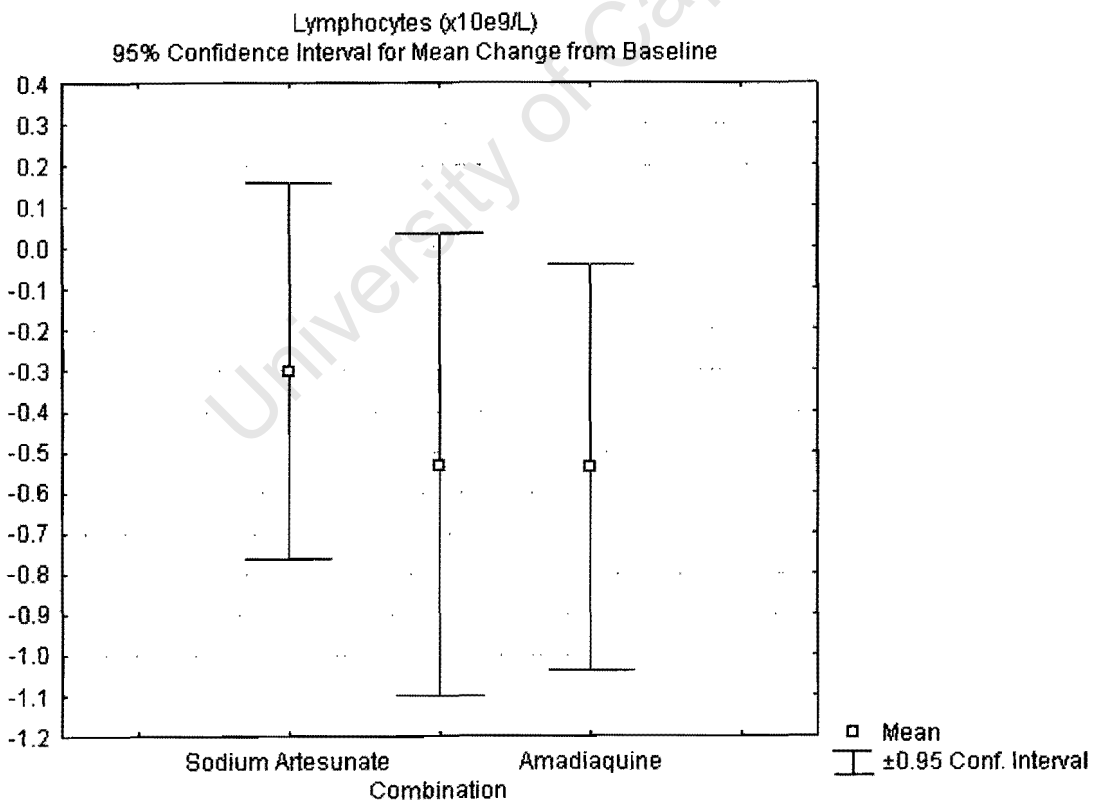












Appendix G-3: Individual haematological parameters.

White cell count (x10e9/L)

Subject	Code	AS in phase 2 or 3	Sex	baseline	day 6 safety phase 1 - AS only	day 27 safety phase 2 - AQ +/- AS	day 48 safety phase 3 - AQ +/- AS	
BL-01	Q	3	m	9.6	7.8	7.4	9.3	
TM-02	F	2	m	4.9	5.3	5.8	4.8	
BT-03	L	2	m	6.5	7.5	5.4	7.0	
TKM-07	K	2	f	5.2	5.2	**3.2**	4.3	
TTM-08	E	3	f	5.7	4.2	**3.2**	**3.4**	
CB-09	P	3	m	10.6	7.3	5.8	5.8	
GL-11	D	2	m	5.2	5.6	4.7	5.7	
NS-12	B	2	m	6.3	4.6	5.5	5.6	
SB-14	N	2	m	7.7	6.3	6.2	6.1	
DS-15	C	3	m	7.8	4.6	4.3	5.5	
AAS-16	J	3	f	7.3	9.4	6.3	8.6	
MA-17	M	3	m	8.2	6.4	4.6	4.9	
AS-18	A	3	f	7.2	5.8	5.0	5.7	
MEAN				**7.09**	**6.15**	**5.18**	**5.90**	
Withdrawn:								
SV-10	G	3	m	5.6	5.5	5.4	withdrew day 7- allergic reaction to AQ	
CL19	H	2	f	9.1	6.5	4.3	withdrew at day 28 - began fluoxetine	
Screen failures:								
RM-04			m	**2.9**			screen failure - neutropaenia	
MM-05			f	5.2			screen failure - chose not to commence	
US-06			f	5.9			screen failure - chose not to commence	
RW-13			m	**3.4**			screen failure - neutropaenia	

Neutrophils (x10e9/L)

Subject Code		AS in phase 2 or 3	Sex	baseline	day 6 safety phase 1 - AS only	day 27 safety phase 2 - AQ +/- AS	day 48 safety phase 3 - AQ +/- AS	
BL-01	Q	3	m	7.3	4.87	4.17	4.88	
TM-02	F	2	m	2.31	2.65	3.85	2.53	
BT-03	L	2	m	3.72	4.54	3.05	3.9	
TKM-07	K	2	f	2.33	2.51	1.37	1.8	
TTM-08	E	3	f	2.33	2.01	1.7	1.89	
CB-09	P	3	m	6.35	4.69	2.97	3.19	
GL-11	D	2	m	3.37	2.98	2.68	3.56	
NS-12	B	2	m	3.21	2.15	2.84	2.82	
SB-14	N	2	m	4.07	2.8	3.05	3.05	
DS-15	C	3	m	4.03	2.31	2.14	2.85	
AAS-16	J	3	f	3.44	4.05	2.82	4.84	
MA-17	M	3	m	2.99	2.48	2.08	2.03	
AS-18	A	3	f	3.78	3.28	2.99	3.68	hepatitis day 42
MEAN				**3.79**	**3.18**	**2.75**	**3.16**	
Withdrawn:								
SV-10	G	3	m	3.07	2.61	2.62		withdrew day 7- allergic reaction to AQ
CL19	H	2	f	5.15	3.12	3.35		withdrew at day 28 - began fluoxetine
Screen failures:								
RM-04			m	1.26				screen failure - neutropaenia
MM-05			f	2.83				screen failure - chose not to commence
US-06			f	3.56				screen failure - chose not to commence
RW-13			m	1.53				screen failure - neutropaenia

Lymphocytes (x10e9/L)

Subject	Code	AS in phase 2 or 3	Sex	baseline	day 6 safety phase 1 - AS only	day 27 safety phase 2 - AQ +/- AS	day 48 safety phase 3 - AQ +/- AS	
BL-01	Q	3	m	1.50	2.06	2.37	3.48	
TM-02	F	2	m	1.91	1.96	1.48	1.65	
BT-03	L	2	m	2.08	2.22	1.75	2.40	
TKM-07	K	2	f	2.26	2.04	1.50	2.02	
TTM-08	E	3	f	2.70	1.53	1.14	1.10	
CB-09	P	3	m	3.04	1.89	2.14	1.87	
GL-11	D	2	m	1.36	1.84	1.53	1.65	
NS-12	B	2	m	2.50	1.82	2.05	2.26	
SB-14	N	2	m	2.79	2.69	2.50	2.34	
DS-15	C	3	m	2.78	1.63	1.55	1.95	
AAS-16	J	3	f	3.16	4.32	2.78	2.81	
MA-17	M	3	m	4.23	3.23	2.14	2.42	
AS-18	A	3	f	2.52	1.69	1.36	1.44	hepatitis day 4
MEAN				**2.53**	**2.22**	**1.87**	**2.11**	
Withdrawn:								
SV-10	G	3	m	1.82	2.16	2.19	withdrew day 7- allergic reaction to AQ	
CL19	H	2	f	3.03	2.63	2.88	withdrew at day 28 - began fluoxetine	
Screen failures:								
RM-04			m	1.32			screen failure - neutropaenia	
MM-05			f	1.88			screen failure - chose not to commence	
US-06			f	1.74			screen failure - chose not to commence	
RW-13			m	1.34			screen failure - neutropaenia	

Haemoglobin (g/dL)

Subject	Code	AS in phase 2 or 3	Sex	Initial Hb	day 6 safety phase 1 - AS only	day 27 safety phase 2 - AQ +/- AS	day 48 safety phase 3 - AQ +/- AS	
BL-01	Q	3	m	14.2	14.9	15.5	15.9	
TM-02	F	2	m	15.4	15.2	14.5	15.6	
BT-03	L	2	m	15.5	14.4	14.6	15.7	
TKM-07	K	2	f	**11.4**	11.9	12.0	12.5	
TTM-08	E	3	f	13.6	13.0	12.7	13.2	
CB-09	P	3	m	15.0	14.2	15.0	14.4	
GL-11	D	2	m	16.0	15.9	14.7	14.8	
NS-12	B	2	m	14.8	14.5	15.0	15.9	
SB-14	N	2	m	11.7	13.6	13.3	13.8	
DS-15	C	3	m	14.1	13.6	14.1	14.4	
AAS-16	J	3	f	12.5	11.6	11.7	**11.2**	
MA-17	M	3	m	15.1	14.4	15.0	14.8	
AS-18	A	3	f	13.4	11.9	12.5	12.7	hepatitis day 4
MEAN				**14.05**	**13.78**	**13.89**	**14.22**	

Withdrawn:

SV-10	G	3	m	14.0	14.1	14.1	withdrew day 7- allergic reaction to AQ
CL19	H	2	f	**11.3**	**10.7**	**10.4**	withdrew at day 28 - began fluoxetine

Screen failures:

RM-04	m	14.7		screen failure - neutropaenia
MM-05	f	13.6		screen failure - chose not to commence
US-06	f	11.8		screen failure - chose not to commence
RW-13	m	13.4		screen failure - neutropaenia

Haematocrit

Subject	Code	AS in phase 2 or 3	Sex	baseline	day 6 safety phase 1 - AS only	day 27 safety phase 2 - AQ +/- AS	day 48 safety phase 3 - AQ +/- AS	
BL-01	Q	3	m	0.43	0.46	0.47	0.47	
TM-02	F	2	m	0.49	0.47	0.45	0.45	
BT-03	L	2	m	0.49	0.43	0.43	0.45	
TKM-07	K	2	f	0.35	0.37	0.37	0.36	
TTM-08	E	3	f	0.43	0.41	0.39	0.38	
CB-09	P	3	m	0.43	0.43	0.44	0.44	
GL-11	D	2	m	0.49	0.49	0.44	0.43	
NS-12	B	2	m	0.43	0.41	0.42	0.44	
SB-14	N	2	m	0.39	0.43	0.41	0.43	
DS-15	C	3	m	0.44	0.42	0.43	0.42	
AAS-16	J	3	f	0.4	0.37	0.37	0.33	
MA-17	M	3	m	0.46	0.45	0.45	0.43	
AS-18	A	3	f	0.4	0.37	0.38	0.37	hepatitis day 4
MEAN				**0.43**	**0.42**	**0.42**	**0.42**	
Withdrawn:								
SV-10	G	3	m	0.44	0.44	0.43		withdrew day 7- allergic reaction to AQ
CL19	H	2	f	0.37	0.36	0.34		withdrew at day 28 - began fluoxetine
Screen failures:								
RM-04			m	0.45				screen failure - neutropaenia
MM-05			f	0.45				screen failure - chose not to commence
US-06			f	0.38				screen failure - chose not to commence
RW-13			m	0.42				screen failure - neutropaenia

Platelet count (x10e9/L)

Subject	Code	AS in phase 2 or 3	Sex	baseline	day 6 safety phase 1 - AS only	day 27 safety phase 2 - AQ +/- AS	day 48 safety phase 3 - AQ +/- AS	
BL-01	Q	3	m	240	203	226	266	
TM-02	F	2	m	221	207	308	209	
BT-03	L	2	m	254	241	237	257	
TKM-07	K	2	f	273	291	297	338	
TTM-08	E	3	f	197	229	193	238	
CB-09	P	3	m	323	277	275	295	
GL-11	D	2	m	185	194	192	220	
NS-12	B	2	m	206	198	190	218	
SB-14	N	2	m	200	200	200	220	
DS-15	C	3	m	193	194	186	233	
AAS-16	J	3	f	292	274	252	279	
MA-17	M	3	m	252	200	206	227	
AS-18	A	3	f	221	195	198	217	hepatitis day 4
MEAN				**235.15**	**223.31**	**227.69**	**247.46**	

Withdrawn:

SV-10	G	3	m	210	231	224	withdrew day 7- allergic reaction to AQ
CL19	H	2	f	295	288		withdrew at day 28 - began fluoxetine

Screen failures:

RM-04	m	184	screen failure - neutropaenia
MM-05	f	279	screen failure - chose not to commence
US-06	f	275	screen failure - chose not to commence
RW-13	m	203	screen failure - neutropaenia

Appendix H

Dosing variables:

Subject	weight (kg)	Calculated AQ dose (10mg.kg)	AQ dose given (mg)	Actual AQ mg/kg	Calculated AS dose (4mg/kg)	AS dose given (mg)	Actual AS mg/kg
A	69	690	800	11.59	46	300	4.35
B	70	700	800	11.43	46	300	4.29
C	82	820	800	9.76	39	350	4.27
D	63	630	600	9.52	38	300	4.76
E	68	680	800	11.76	47	300	4.41
F	73	730	800	10.96	44	300	4.11
G	61	610	600	9.84	39	250	4.10
H	59	590	600	10.17	41	250	4.24
J	50	500	600	12.00	48	200	4.00
K	54	540	600	11.11	44	250	4.63
L	74	740	800	10.81	43	300	4.05
M	97	970	1000	10.31	41	400	4.12
N	68	680	800	11.76	47	300	4.41
P	64	640	600	9.38	38	250	3.91
Q	58	580	600	10.34	41	250	4.31
Mean:	67.33			10.72			4.26
Std deviation:	11.61			0.88			0.23

Demographic variables:

Subject	race	age (years)	gender	weight (kg)	height (cm)
A	c	20	f	69	169
B	c	42	m	70	166
C	w	23	m	82	185
D	w	22	m	63	173
E	b	23	f	68	158
F	w	34	m	73	180
G	w	23	m	61	171
H	b	22	f	59	159
J	a	19	f	50	161
K	b	24	f	54	157
L	c	22	m	74	181
M	c	21	m	97	189
N	a	23	m	68	176
P	w	24	m	64	179
Q	b	24	m	58	159
Mean:		24.4		67.33	170.87
Std deviation:		5.90		11.61	10.61

b=black
c=coloured
w=white

f=female
m=male