

The pharmacodynamics and pharmacokinetics of rectal administration of artesunate in the initial treatment of moderately severe and severe malaria in South African adults in northern KwaZulu Natal.

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The pharmacodynamics and pharmacokinetics of rectal
administration of artesunate in the initial treatment of moderately
severe and severe malaria in South African adults in northern
KwaZulu Natal.

Minor Dissertation submitted to the University of Cape Town Faculty of Health Sciences towards
the degree of MMed (Clinical Pharmacology) – Part III.

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Declaration

I, Karen I Barnes, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise), and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Date: 10 November 2004

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Abstract

Problem statement:

The interventions which are shown to decrease malaria mortality, namely early effective parenteral antimalarial treatment and intensive care supportive management of malaria complications are not accessible to a considerable proportion of those living in malaria endemic areas, particularly in rural Africa.

Objective:

To evaluate the reliability of absorption, antimalarial efficacy and tolerability of a single rectal dose of artesunate in the initial management of non immune adults with moderately severe and severe *falciparum* malaria.

Methods:

Adequacy of absorption and antimalarial efficacy of a single rectal administration of artesunate (10 mg/kg) were assessed by rate of decrease in peripheral asexual parasitaemia, clinical response and area under the parasite time curve of artesunate and its active metabolite, dihydroartemisinin. To compare artesunate with standard parenteral therapy (quinine), one in five patients was randomly allocated to parenteral quinine. As soon as possible after 24 hours, both artesunate and quinine groups were treated with a single dose of sulfadoxine-pyrimethamine (1.25 mg/kg pyrimethamine) to complete therapy.

Results:

Among the adults with moderately severe malaria, parasitaemia at 12 hours was greater than 60% of baseline parasite density in 1/27 [4% (0.1-19%)] compared with 5/8 [63% (24-92%)] in the quinine treated group [RR 0.06 (0.01-0.44); $p < 0.001$]. This difference between treatments were greater at 24 hours. However, the proportional reduction in parasitaemia was not significant patients with severe malaria. Parasite density at 12 hours was greater than 60% of baseline in 2/5

(40%) in artesunate arm compared with 5/6 (83%) in the quinine arm; [RR 0.48 (0.15-1.49; p=0.19)]. Clinical response was equivalent with rectal artesunate and parenteral quinine both in patients with moderately severe malaria and those with severe malaria.

Artesunate and / or its active metabolite, dihydroartemisinin, were detectable in the plasma between 1 and 8 hours after administration in all 27 patients with moderately severe malaria and four of five patients with severe malaria. There was marked inter-individual variability of the pharmacokinetics of artesunate and dihydroartemisinin. Measurable drug exposure could not predict pharmacodynamic effect in terms of parasitological or clinical response to treatment.

Artesunate was generally well tolerated at 10 mg/kg administered rectally in the small cohort studied. The frequency of serious adverse events, and of adverse events overall, did not differ significantly between artesunate and control study groups. Rigorous evaluation of the central nervous system did not detect signals of abnormalities previously described in high dose animal studies.

Interpretation:

A single rectal dose of artesunate is expected to be a useful option for initial treatment of patients with moderately severe malaria unable to tolerate oral medication, particularly where parenteral treatment is not readily available. However, further studies of the adequacy of absorption of rectal artesunate in patients with severe *falciparum* malaria are needed.

Abbreviations

| | |
|------------------|---|
| AS | Artesunate |
| AUC | Area under the time curve |
| CI | 95% confidence intervals |
| CLAST | Last non-zero plasma drug concentration |
| C _{MAX} | Maximum plasma drug concentration |
| CRF | Case Record Form |
| DHA | Dihydroartemisinin |
| FCT | Fever Clearance Time |
| GLP | Good Laboratory Practice |
| HCT | Haematocrit |
| HPLC | High Performance Liquid Chromatography |
| ECD | Electrochemical Detection |
| IM | Intramuscular |
| IQR | Interquartile range |
| IV | Intravenous |
| MRT | Mean Residence Time |
| NPO | Non "per os" status; unable to tolerate oral medication |
| PCR | Polymerase Chain Reaction |
| PCT | Parasite Clearance Time |
| PD | Pharmacodynamic |
| PK | Pharmacokinetic |
| RBC | Red Blood Cell |
| SBP | Systolic Blood Pressure |
| SP | Sulfadoxine-Pyrimethamine |
| TLAST | Time of last non-zero plasma drug concentration |
| T _{MAX} | Time of maximum plasma drug concentration |
| WCC | White Cell Count |

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Abbreviations

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Appendix 1: Publications

Appendix 1.1: Barnes KI, Mwenechanya J, Tembo M, McIlleron H, Folb PI, Ribeiro I, Little F, Gomes M, Molyneux ME. Efficacy of rectal artesunate in the initial treatment of moderately severe malaria in African children and adults. *Lancet* 2004; **363** (9421):1598-605

Appendix 1.2: Barnes KI, Folb PI. The role of artemisinin-based combination therapy in malaria management. In "Reducing Malaria's Burden: Evidence of effectiveness for decision-makers. Global Health Council." Editors: Murphy C, Ringheim K, Woldehanna S, Volmink J. 2003. www.globalhealth.org

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Chapter 1:**Study aims and objectives****Study aim**

This thesis aimed to investigate the adequacy of absorption of a single administration of artesunate suppositories (10 mg/kg) over the initial 24 hours of management in non-immune adults with moderately severe or severe *Plasmodium falciparum* malaria. Such patients are unable to tolerate oral medication, and in many malaria transmission areas do not have early access to parenteral treatment. The hypothesis to be tested was that the early administration of rectal artesunate would provide adequate initial antimalarial cover, indicated by a rapid fall in the density of parasitaemia, and that this would be achieved without clinical deterioration and without serious adverse drug reactions. If this were to be confirmed, rectal artesunate might be recommended as a preliminary treatment to enable a patient unable to tolerate oral medication, to reach a health care facility where parenteral treatment could be safely administered.

Study subjects:

Adult patients presenting to Mosvold hospital or its satellite clinics in northern KwaZulu Natal with either:

- moderately severe *falciparum* malaria who were treated with either a single administration of rectal artesunate or repeated administration of intramuscular quinine, followed in both treatment arms by a single administration of sulfadoxine-pyrimethamine at 24 hours (or as soon as possible thereafter); or
- severe *falciparum* malaria who were treated with either a single administration of rectal artesunate PLUS intravenous quinine alone, followed in both treatment arms by a single administration of sulfadoxine-pyrimethamine at 24 hours (or as soon as possible thereafter).

Study objectives

1. To determine the adequacy of absorption of a single dose of rectal artesunate in terms of:
 - i) Rate of early parasite clearance from the peripheral blood defined by reduction in parasite density by 12 hours, by 24 hours and parasite clearance time.
 - ii) The clinical response to treatment defined by fever clearance time, time to tolerate oral medication, case fatality rate and, in patients with moderately severe malaria, progression to severe malaria.
 - iii) Plasma levels of artesunate and its metabolite dihydroartesunate as measures of the adequacy of absorption.

2. To compare a single dose of rectal artesunate with best available standard therapy, parenteral quinine, in terms of:
 - i. Rate of early parasite clearance from the peripheral blood defined by reduction in parasite density by 12 hours, by 24 hours and parasite clearance time.
 - ii. The clinical response to treatment defined by fever clearance time, time to tolerate oral medication, case fatality rate and, in patients with moderately severe malaria, progression to severe malaria.

Introduction

Almost all of the over one million malaria related deaths are attributable to *Plasmodium falciparum* (World Health Organisation 2000). The symptoms and signs of severe, life-threatening malaria can be identified by rural health workers and even by parents (Kidane and Morrow 2000). However, delay in receiving effective medication is an important contributor to the high malaria mortality rate. As a considerable proportion of patients with acute malaria are unable to tolerate oral therapy (due to vomiting or increased disease severity), parenteral treatment is often necessary. Unfortunately, few rural Africans have access to health facilities that are equipped to provide parenteral treatment. Malaria morbidity and mortality is further increasing as a result of the spread of drug resistance to the most commonly used antimalarials, chloroquine and sulfadoxine-pyrimethamine (Trape 1998). There is a clear need for an effective drug that can be administered simply at village or rural health centre level to those unable to tolerate oral medication, prior to transfer to a healthcare facility where parenteral treatment can be given. The development of a thermostable suppository formulation for rectal artesunate offers the opportunity of providing antimalarial cover to patients in the periphery, who are unable to tolerate oral medication, while they are being transferred to healthcare facilities able to provide parenteral treatment (Wilairatana et al 1997).

Plasmodium falciparum malaria can manifest as asymptomatic parasitaemia, through uncomplicated disease to severe life-threatening disease. In severe malaria, mortality is generally reported to range between 10-40%, with the higher rates usually seen in studies with more rigorously defined severe malaria (World Health Organisation 2000). Classification of patients as having severe malaria is not standardized, and can even include hyperpyrexia or weakness,

which clearly do not have the prognostic significance of, for example, cerebral malaria, acute renal failure or adult respiratory distress syndrome (World Health Organisation 2000). This has led to an intermediate classification of moderately severe malaria used in this study for those patients who are at increased risk of severe malaria without yet meeting rigorous criteria. Mortality rates from one form of moderately severe malaria (uncomplicated hyperparasitaemic malaria) was 3% (Luxemborger et al 1995), compared with under 1% in uncomplicated malaria (Korenromp et al 2003) and 10-40% in severe and complicated malaria (World Health Organisation 2000).

Conventional therapy for severe malaria, in addition to highest level of supportive care available, is parenteral quinine. This requires either repeated constant rate infusions or painful intramuscular injections, is associated with hyperinsulinaemic hypoglycaemia (White et al 1983) and has a narrow therapeutic index (White 1996).

Four randomised controlled trials have compared intramuscular artemether with parenteral quinine (Boele van Hensbroek et al 1996, Hien et al 1996, Murphy et al 1996, Taylor et al 1998). Artemether was consistently associated with a more rapid clearance of parasitaemia. Fever clearance times were more rapid with artemether in one study, and similar or slower in the other 3 studies. In the 2 largest trials, artemether was associated with a delayed recovery from coma, which is yet to be satisfactorily explained. This finding, which remains a cause for concern, was not evident in a meta-analysis of artemisinin derivatives for the treatment of severe malaria, which showed no difference in neurological sequelae. This meta-analysis (which included 2653 patients in 16 trials comparing artemisinin derivatives with quinine) found that the artemisinin drugs were associated with better survival (mortality odds ratio 0.61; 0.46 – 0.82, random effects model). This effect was however barely significant when only trials in which concealment of allocation was adequate (McIntosh and Olliaro 2003).

The early availability of modern intensive care, particularly haemofiltration rather than peritoneal dialysis in sepsis or malaria related acute renal failure, and positive pressure ventilation is expected to contribute to lowering mortality rates (White 2003). Unfortunately these resources are very seldom available to those at highest risk of malaria, emphasizing the need for early effective treatment to prevent at risk patients from progressing to severe malaria.

Artemisinin derivatives

Artemisinin, a sesquiterpene lactone peroxide, is the active antimalarial moiety isolated from the traditional Chinese medicinal herb *Artemisia annua*. *Artemisinin annua* has been used for many years by the Chinese as a traditional treatment for fever and malaria. Artemisinin and derivatives were tested extensively in China (Qinghaosu Antimalarial Research Group 1979). These are the most rapidly acting of all antimalarials (Hien 1993). Artemisinin and its derivatives are structurally distinct from other classes of antimalarial drugs and are thus not vulnerable to cross resistance and are effective even against re recrudescence multi-drug resistant *P falciparum* parasites (Price et al 1998).

The mechanism of action of artemisinin and its derivatives is not completely understood, although the dependence of this activity on an intact peroxide bridge is established. Recently Eckstein-Ludwig and colleagues have shown that the artemisinins work through irreversibly inhibiting a metabolic enzyme, the *Plasmodium falciparum* calcium dependent ATP-ase (PfATP6) which is situated throughout the parasite cytoplasm, through an iron-dependent mechanism which generates free radicals from artemisinin. This inhibition of PfATP6 results in a slowing of parasite growth (Eckstein-Ludwig U et al, 2003). This evidence contradicts previous theories that the specific antimalarial effect of artemisinins was due to its accumulation in the parasites food vacuole where it would interact with haem to release free radicals which would inhibit several key parasite components, eventually resulting in parasite death.

Two of the artemisinin derivatives have been evaluated more extensively, namely artesunate and artemether. Both the water-soluble artesunate and the lipid soluble artemether derivatives are rapidly converted to the major biologically active metabolite, dihydroartemisinin. Deoxy-metabolites are biologically inactive, as they do not have an intact peroxide bridge. All artemisinin derivatives bind modestly to plasma proteins. The pharmacokinetics of a single dose of artemisinin (500mg) in patients with liver cirrhosis were not different from results in healthy subjects, indicating that there is no significant first pass effect (Duc et al 1994, de Vries et al 1997). The areas under the time curve (AUCs) of artemisinin and artesunate have been shown to decrease after multiple oral dosing, which is considered a result of increased hepatocellular activity or an auto inductive effect (Ashton et al 1998, Khanh et al 1999). All metabolites undergo further glucoronidation and are excreted in the urine or faeces (although data on excretion is scanty). Although artemisinin and its derivatives are all rapidly eliminated, they have been shown to be equally effective whether given once, twice or three times daily (White 1997).

Knowledge of pharmacokinetics is incomplete owing to challenges with the development of a suitable assay. Rapid degradation of artemisinin derivatives occurs in plasma samples, stressing the need for immediate centrifugation and cold storage (Navaratnam et al 1995). Furthermore, dihydroartemisinin preferentially accumulates in parasitised erythrocytes (more than 300-fold greater concentration than the plasma concentration, compared with only 2-fold greater in uninfected erythrocytes *in vitro*) (Gu et al 1984). This accumulation is reversible and saturable. The artemisinin derivatives appear to have a wide therapeutic index although the exact therapeutic and toxic levels in humans remain to be defined. Thus the doses of artemisinin derivatives used have largely been derived empirically (Rowland 1995). Among adult patients with acute uncomplicated *falciparum* malaria in Thailand, no further reduction in parasite clearance times was found with use of single oral doses of artesunate higher than 2 mg/kg, implying that this reflects the average lower limit of the maximally effective dose (Angus 2002).

The artemisinin derivatives have the highest rates of parasite clearance of all the antimalarial drugs. This has been explained by their potency and the fact that their activity covers the broadest number of stages in the asexual intra-erythrocytic schizogonic part of the parasites' life cycle of known antimalarials (ter Kuile et al 1993). Artemisinin and its derivatives have the particular advantage in more severe *falciparum* malaria of acting principally on young parasites, preventing their development into more mature pathological stages, which adhere to the vascular endothelium (White 1994). In this way, sequestration of parasitised erythrocytes in the microvasculature of vital organs is reduced by artemisinins, but not by quinine (Udomsangpetch et al 1996).

Artemisinin and its derivatives have been administered as suppository formulations, and have been shown to consistently result in a rapid parasitological and clinical response, although absorption (while usually adequate) is more erratic following this route of administration when compared with intravenous administration (Cao et al 1997, Ha et al 1997).

When used as monotherapy artemisinin derivatives are associated with a high recrudescence rate, unless administered for 7 days. 28% - 40% recrudescence follows 3-day monotherapy, with 10% - 20% recrudescence after 5-day monotherapy (Borman et al 2002, Alin et al 1995, Hassan et al 1996). Recrudescence rates are higher in non-immune populations in Thailand and China (Bunnag et al 1991, Li et al 1994).

Artemisinin-based combination therapy

In addition to benefits in terms of delaying resistance and decreasing malaria transmission, artemisinin based combinations improve clinical cure rates. The principle of using combination therapy to delay the emergence and spread of resistance is established in the management of tuberculosis and HIV, and should apply equally well to malaria (White 1998). This has been most conclusively demonstrated with the combination of artesunate with mefloquine in an area in

western Thailand with low malaria transmission and strict control of access to these antimalarials (Nosten et al 2000). The benefits of artemisinin, when used in combination with a second antimalarial drug, have been established in the management of severe *falciparum* malaria, hyperparasitemia and in the management of uncomplicated malaria. Confirmation of clinical and parasitological efficacy has resulted in the widespread use of artemisinin based combination therapy, particularly in the treatment of multidrug resistant malaria in Thailand and Vietnam where these compounds are used in combination with mefloquine (Nosten et al 1994, Price et al 1998, Bunnag et al 1996). This has been followed more recently with artemisinin-based combination therapies being recommended as first line treatment of uncomplicated malaria in South America (western Peru) and in Africa (including KwaZulu Natal, Zambia, and Zanzibar) (Barnes and Baker 2001).

As the artemisinin derivatives are the most potent of all anti-malarial drugs and reduce the infecting parasite biomass by approximately 10 000 fold per asexual lifecycle, they would protect vulnerable anti-malarials (those with a prolonged terminal elimination half life, a shallow concentration-effect relationship and / or those in which a few base pair mutations confer a marked reduction in susceptibility) against the development of resistance (White 1998). Concomitant oral administration of artesunate does, however, result in significant changes to mefloquine kinetics in patients with uncomplicated malaria, although the clinical effects of this effect is not yet established and the efficacy of the combination has been sustained for over a decade (Karbwang et al 1994, Nosten et al 2000). Similar effects on the kinetics of sulfadoxine-pyrimethamine have not yet been reported.

It is of particular importance that artemisinin derivatives also reduce gametocyte carriage, and thus reduce the transmission of malaria, and particularly the transmission of resistant strains of *P falciparum* (Price 1996). Recrudescence infections are associated with increased gametocyte carriage rates, which provide a powerful selection pressure for the spread of resistance. With the

possible exception of artemisinin derivatives, resistance of *P falciparum* to all available alternatives has been frequently described.

The efficacy and safety of artesunate in combination with sulfadoxine / pyrimethamine (SP) has been evaluated in randomised controlled trials involving 2685 patients in sub Saharan Africa. Results from the first study published from the Gambia (von Seidlein et al 2000), where the 28-day cure rate with SP monotherapy was 93%, showed that cure rate and parasite clearance was significantly higher in patients who received 3 days of artesunate PLUS a stat dose of SP when compared to those who received SP monotherapy. Gametocyte carriage was 68% following SP monotherapy treatment in comparison to 21% following combination treatment ($p = 0.001$).

Safety of artemisinin derivatives

Artemisinin and its derivatives are generally considered safe and well tolerated. Most of the concerns regarding the safety of artemisinin derivatives derive from animal studies or mild to moderate adverse events that have been reported from clinical studies. The artemisinin drugs have been used extensively, especially in SE Asia, for the treatment of *P falciparum* malaria, with very little evidence of toxicity (Ribeiro and Olliaro 1998, Price et al 1999). However only a few studies have included young children, in whom malaria is most prevalent in Africa, and this apparent safety is undermined by the use of counterfeit drugs with sub-potent or no active ingredients (Newton et al 2001). Careful clinical neurological examination in studies designed to detect neurotoxicity has failed to detect deafness or permanent neurological abnormalities, and auditory evoked potentials were normal in all patients studied (Kissinger et al 2000, van Vugt et al 2000b). Concerns about potential neurotoxicity stem from reports of an unusual pattern of damage to the brainstem nuclei induced by high dose (>15mg/kg/day for 14 days) parenteral artemether and arteether in animal studies (Genovese et al 1998, Brewer et al 1994a, Brewer et al 1994b, Brewer et al 1998). Although prolonged coma time was observed in two of three randomised controlled trials comparing artemether with quinine in the treatment of cerebral malaria, a meta-analysis found no increased risk of prolonged coma in the artemisinin derivative

treated patients (McIntosh 2003). Autopsy of 28 fatal severe malaria cases exposed to either artemisinin derivatives or quinine found no evidence of neurotoxicity (White NJ, personal communication). There have been sporadic case reports of impaired balance, nystagmus, fine motor coordination, paraesthesia and seizures following treatment of severe *falciparum* malaria with an artemisinin derivative (Miller and Panosian 1997, Elias et al 1999). However, concomitant administration of mefloquine and malaria itself may have caused these neurological abnormalities (Davis et al 1997, White 2000). In animal studies, artesunate (water-soluble) has shown less neurotoxicity than artemether and arteether (fat-soluble) (World Health Organisation 2000).

Clinical studies have reported pruritis and mild skin rash in 1-4% of patients receiving artemisinin-based combination therapy, with the higher incidence observed with the concurrent administration of sulfadoxine-pyrimethamine (Bakshi et al 2000, Price et al 1999, Tjitra et al 2001, van Vugt et al 2000a). Two cases of severe allergic reactions to artesunate have been reported in Thailand, from which the authors have calculated a risk of 1 in 2833 (95% confidence intervals 1362-6944) (Leonardi et al 2001). Concomitant use of medication that can cause skin reactions (such as sulpha drugs), or HIV infection may increase the risk of such reactions.

Early animal studies have shown a possible risk of bone marrow suppression, particularly neutropenia and a decreased reticulocyte count. Some haematological changes (e.g. anaemia) may be attributable to malaria, or to rehydration of volume depleted patients. Although a systematic review reported neutropenia in 0.9% (52/5760) of patients (Ribeiro and Olliaro 1998), these results have not been supported by recent studies which may be limited by their small sample size and exclusion of patients at risk of neutropenia (Kshirsagar et al et al 2000, Bakshi et al 2000, LeFevre et al 2001, van Vugt et al 1999a, Price et al 1999). There have been no reports of agranulocytosis to date. HIV infection, malnutrition, amodiaquine and antifolate drugs may further contribute to haematological abnormalities, and their prevalence in malaria endemic countries may increase this risk.

There have been no reports of clinically significant hepatotoxicity. In patients exposed to artemisinin derivatives, there were no clinically important cardiac effects despite careful ECG monitoring (van Vugt et al 1999b, Kshirsagar et al 2000). Other studies have suggested small changes in the QTc interval, but these appear to be no different to other antimalarial drugs (and less cardiotoxic than quinine and halofantrine) (Bakshi et al 2000, LeFevre et al 2001, Price et al 1999).

Animal studies have shown foetal resorption and implantation loss, even with low doses of artemisinins administered early in gestation; artemisinins are also associated with significantly decreased birth weights (Wang 1989). A study in the Gambia described pregnancy outcome in 287 women exposed to artesunate plus SP in comparison to those not exposed; there was no difference in the proportion of congenital abnormalities, stillbirths, abortions or neonatal deaths (Deen 2001). In a study of pregnant women treated with artemisinins (artesunate in 528) during 539 malaria episodes, there was no evidence of adverse effects and birth outcomes did not differ significantly from community rates for abortion, stillbirth, or congenital abnormality (McGready 2001). Although these studies suggest that artemisinins may have a relatively good safety profile during pregnancy, only 80 of the women studied were exposed during the first trimester.

Artesunate

Although most studies in severe malaria have evaluated artemether or arteether, rather than artesunate (Hien et al 1992, Win et al 1992, Hien et al 1996, Boele van Hensbroek et al 1996, Murphy et al 1996, Taylor et al 1998, Pittler et al 1999, Phuong et al 1997, Newton et al 2003), intramuscular artemether may not be the most suitable formulation for patients with severe malaria. Firstly, artemether is an oil-based formulation that is converted slowly to DHA and may be inadequately and erratically absorbed following intramuscular administration in severely ill patients (Teja-Isavadharm et al 1996, World Health Organisation 2000). There is evidence that absorption is particularly impaired in children with acidosis (Murphy et al 1997). Delay in absorption is likely to be harmful in life threatening severe *falciparum* malaria. Artesunate can be

administered intravenously, and is more rapidly absorbed than artemether if administered intramuscularly. Clinical and parasitological response was shown in a small study to be similar in patients with severe falciparum malaria following intravenous or intramuscular administration of 2mg/kg artesunate followed by 1 mg/kg at 12 and 24 hours (Hien et al 1992). Secondly, in animal studies parenteral artemether and arteether were found to be more neurotoxic than artesunate, a water-soluble derivative (Nonprasert et al 1998). Lastly, artesunate is more potent than artemether: artesunate and its active metabolite DHA have *in vitro* antimalarial activities 2.9 and 4.0 greater than artemether, respectively (Brockman et al 2000), although active comparisons are complicated by the hydrolysis of artesunate in solution, particularly in an acid pH (Newton et al 2002). Recent case reports suggest that artesunate also has a diuretic effect, which may provide additional benefit in patients with acute renal failure and adult respiratory distress syndrome (Seguro et al 2002). Artesunate is thus theoretically preferable in severe malaria, although there has been no large-scale trial comparing intravenous artesunate with either quinine or artemether. A comparison of intravenous artesunate and quinine in 113 adults with severe malaria reported mortality of 12% with artesunate and 22% with quinine (p=0.22) (Newton et al 2003). Oral artesunate was found to be clinically and parasitologically superior to intravenous quinine, in patients with uncomplicated malaria with greater than 4% parasitaemia (Luxemborger et al 1995).

There are a number of small studies on the clinical efficacy of rectal artesunate in adults with severe *P falciparum* infections (Bhatt et al 1996, Eduardo et al 1996, Thwe et al 1996, Looareesuwan et al 1995, Looareesuwan et al 1996). In each of these regimens, approximately 4mg/kg rectal artesunate was administered repeatedly and was combined sequentially with a second oral antimalarial to prevent recrudescence of malaria. These data are summarized in Table 1 below. In both the studies comparing different dosage regimens of artesunate, increased total dose improved cure rate at 28 days. These studies generally used the WHO definition of severe malaria, which include hyperpyrexia and prostration alongside the more rigorous hyperparasitaemia, jaundice, cerebral impairment, renal impairment. The use in some of these studies of subjective clinical criteria of severity, particularly hyperpyrexia, which is not clearly

associated with a poor prognosis, prevents comparison of mortality rates between these studies and of these studies with studies of parenteral therapy.

| Reference | Country (sample size) | Artesunate regimen | 2 nd antimalarial regimen administered sequentially | 28-day ACPR | Mortality rate (%) |
|--------------------|-----------------------|---|---|-------------|--------------------|
| Awad 2003 | Sudan (n=100) | 200 mg 8hourly x 72 hours (1800mg). | Doxycycline 100mg 12 hourly x 4 days, OR Sulfadoxine 1500mg plus pyrimethamine 75mg single dose OR Mefloquine 15 mg/kg started at 84 h. | 99% | 1% |
| Bhatt 1996 | Kenya (n=22) | 200 mg at 0, 4, 8, 12, 18, 24, 48, 60 h (1600mg). | Mefloquine 1000mg administered as 2 doses 8h apart. | 86.4% | 13.6% |
| Eduardo 1996 | Ecuador (n=40) | 200 mg at 0, 8, 16, 24, 36, 48, 60 h. (1400mg) | Mefloquine 250 mg 8 hourly | 97.5% | 2.5% |
| Thwe 1996 | Myanmar (Buma) (n=18) | 200 mg at 0, 12, 24, 48, 72 (1000mg). | Mefloquine 750 mg plus 500mg 12 hours later. | 77.8% | 0% |
| Thwe 1996 | Myanmar (Buma) (n=13) | 200 mg at 0, 12, 24, 36, 48, 60 h (1200mg). | Mefloquine 750 mg plus 500mg 12 hours later. | 92.3% | 0% |
| Looaree-suwan 1996 | Thailand (n=31) | 200 mg at 0, 12, 24, 36, 48, 60 h (1200 mg). | Mefloquine 750 mg plus 500mg 12 hours later. | 89% | 0% |
| Looaree-suwan 1996 | Thailand (n=32) | 200 mg at 0, 4, 8, 12, 36, 48, 60 h (1400 mg). | Mefloquine 750 mg plus 500mg 12 hours later. | 96% | 0% |
| Looareesuwan 1995 | Thailand (n=30) | 200mg at 0, 4, 8, 12, 24, 36, 48, 60 h. (1600 mg) | Mefloquine 750 mg at 72 h, 500 mg at 84h. | 92% | 0% |

Table 1: Clinical trials of therapeutic efficacy and dosage regimen of repeated administration of rectal artesunate followed by mefloquine, doxycycline or sulfadoxine-pyrimethamine.

Artesunate is a water-soluble, semi-synthetic, hemisuccinate derivative of artemisinin; this enhances its absorption (Baradell et al 1990). The detailed pharmacokinetic properties of artesunate and dihydroartemisinin has been studied using High Performance Liquid

Chromatography with Electro-chemical detection (HPLC-ECD) and bioassay methodology, although elucidation has been complicated by challenges in quantitative determination in biological fluids.

Artesunate has an elimination half-life ($t_{1/2}$) of 2-5 minutes, while DHA has a $t_{1/2}$ around 40-60 minutes (Bethell et al 1997, Batty et al 1998, Davis et al 2001, Newton et al 2000). The short $t_{1/2}$ of artesunate implies that its therapeutic efficacy is due primarily to the action of DHA. There was insufficient data to describe artesunate kinetics in the majority of studies. Two studies reported a Volume of distribution of artesunate of 0.08 and 0.14 L/kg (Batty et al 1998; Davis et al 2001); Batty et al reported an artesunate AUC of 2.98 $\mu\text{Mol.h/L}$.

The pharmacokinetics of dihydroartemisinin following artesunate oral or intravenous administration are summarized in Table 2. In patients with uncomplicated *falciparum* malaria, the relative bioavailability of dihydroartemisinin after oral administration of artesunate is 82% of that following intravenous artesunate, and the apparent oral bioavailability of combined artesunate-DHA using a sensitive bioassay was 61% in the acute phase, approximately double that found during convalescence (Batty et al 1998). Artesunate is absorbed with a significantly shorter median lag time and earlier median peak antimalarial activity than DHA (Newton et al 2002). This probably reflects the faster dissolution of the water-soluble artesunate tablet relative to the relatively insoluble DHA tablet. The clinical significance of these differences is not established.

Davis et al (2001) concluded that disease severity did not alter the pharmacokinetics of intravenous artesunate in their comparison of patients with either severe (n=12) or moderately severe malaria (n=8). In this small study a trend to a shorter parasite clearance time with higher DHA AUCs was found (Davis et al (2001)).

Table 2: Summary of pharmacokinetic parameters of the active metabolite dihydroartemisinin after initial administration of artesunate, and possibly influential study characteristics.

| | Newton 2000 | Newton 2000 | Batty 1998 | Batty 1998 | Bethell 1997 | Davis 2001 |
|--|---------------|---------------|---------------|---------------|--------------|--------------|
| Sample size | 19 | 19 | 26 | 24 | 10 | 12 |
| Age group | adults | adults | adults | Adults | children | adults |
| Disease Severity | uncomplicated | uncomplicated | uncomplicated | uncomplicated | moderate | severe |
| Study Site | Thailand | Thailand | Vietnam | Vietnam | Vietnam | Vietnam |
| Route | oral | IV | IV | Oral | oral | IV bolus |
| Assay | bioassay | bioassay | HPLC-ECD | HPLC-ECD | bioassay | HPLC-ECD |
| Artesunate Dose | 2mg/kg | 2mg/kg | 120mg | 100g | 3mg/kg | 120mg |
| Manufacturer | Guilin no 1 | Guilin no 2 | Guilin no 2 | Guilin no 2 | Guilin no 2 | Guilin no 2 |
| Pharmacokinetic parameters of Dihydroartemisinin | | | | | | |
| C _{max} | 1021ng/mL | 8240ng/mL | 9.31mcmol | 2.59mcmol | 664ng | 8.5mcmol/L |
| range / SD | 775-1267 | 2188-70763 | 7.06-10.87 | 1.84-3.94 | 179-1395 | -2.9 |
| T _{max} | 0.75hr | | 9.0min | 90min | 1.7hr | 10.4min |
| range / SD | 0.5-4 | | 7to12 | 60-135 | 0.8-2.6 | -7.5 |
| V _d | 1.33L/kg | 0.61L/kg | 0.76L/kg | | | 0.77L/kg |
| range / SD | 1.02-1.64 | 0.5-0.72 | 0.68-0.85 | | | -0.28 |
| t _{1/2} | 0.71hr | 0.73hr | 40.2 min | 39.3min | 1hr | 40min |
| range / SD | 0.55-0.87 | 0.62-0.83 | 37.1-43.4 | 31.6-47 | 0.8-1.4 | -24.8 |
| MRT | | 0.76h | 60.3min | 127.6min | | |
| range / SD | | 0.64-0.88 | 55.6-65 | 107.3-148 | | |
| AUC (L) | 1.738ng.h/mL | 3013ng.h/mL | 8.36mcmol.h/L | 4.53mcmol.h/L | | 7.3mcmol.h/L |
| range / SD | 1412-2064 | 2482-3544 | 7.6-9.12 | 3.79-7.16 | | -4.1 |
| Cl _f | 1.38L/kg/hr | | 0.75L/h/kg | | | 1.09 L/h/kg |
| range / SD | 1.03-1.73 | | 0.68-0.91 | | | -0.08 |

Virtually all artesunate and dihydroartemisinin was cleared within under 10 hours of artesunate administration (Bethell et al 1997; Ittarat et al 1998; Newton et al 2000). However, artesunate is equally effective whether administered once or twice daily (Bunnag et al 1991). This suggests that rapid parasite clearance can be achieved after a relatively brief exposure to artesunate and its active metabolite.

Artesunate can be administered parenterally, orally or rectally. Rectal artesunate is rapidly absorbed although there is considerable interindividual variation (Benakis et al 1996). It has been shown to be safe and highly effective in children and adults with uncomplicated *falciparum* malaria (Karunajeewa et al 2003, Sabchareon et al 1998). Two pharmacokinetic studies have suggested adequate absorption of 2mg/kg (range 0.86-2.55 mg/kg) rectal artesunate in 12

Gabonese children with uncomplicated malaria (Halpaap B et al 1998), and of 10 vs 20mg rectal artesunate in 34 Ghanaian children with moderately severe malaria(Krishna S et al, 2001).

The selection of a 10mg/kg dose for this study was based on findings in the study by Krishna et al (2001 in which rectal artesunate was more rapidly absorbed in the 10mg/kg than the 20 mg/kg group ($p=0.023$), and the median relative bioavailability of DHA was higher in the 10mg/kg than 20 mg/kg group (58% vs 23%; $p=0.018$) although there was wide inter-patient variation in the AUC (9-fold following 20mg/kg and 20-fold following 10mg/kg rectal artesunate). Although C_{max} was higher (5-fold) following IV than rectal artesunate, parasite clearance kinetics were comparable with 2.4 mg/kg IV artesunate for both 10mg/kg and 20 mg/kg rectal artesunate groups. Artesunate was eliminated significantly more rapidly and Volume of distribution significantly smaller following IV artesunate than rectal artesunate (Krishna S et al, 2001). In the only other published study on the pharmacokinetics of artesunate following rectal administration (in children with uncomplicated malaria), only the C_{Max} (0.09 $\mu\text{g/mL}$ for artesunate and 0.18 for DHA) and T_{max} (0.58 hr for artesunate and 1.13 for DHA) are reported (Halpaap et al 1998).

Summary of Literature review, and implications for thesis.

Although artesunate has theoretical advantages over both quinine and artemether for the management of more severe malaria, individual studies have only shown clinical equivalence despite more rapid parasite clearance.

There are no published studies of the reliability of absorption and therapeutic efficacy of a *single* dose of rectal artesunate in adult patients with moderately severe or severe malaria. Pilot pharmacokinetic studies have suggested adequate absorption of rectal artesunate in 24 Ghanaian children with moderately severe malaria. Evidence of the early therapeutic efficacy of repeated administration of rectal artesunate in severe malaria, and evidence that artemisinin derivatives are equally effective whether given once, twice or three times daily justify the evaluation of a single dose of rectal artesunate in patients with more severe malaria. However, since efficacy is not established, such a study should be conducted in a facility where careful monitoring and supportive care can be provided.

The dose of rectal artesunate was selected based on the pharmacokinetic-pharmacodynamic data made available to the WHO, demonstrating equivalent clinical and parasitological response of 10mg/kg of rectal artesunate to that of both 2.4 mg/kg intravenous and 20 mg/kg rectal artesunate, as was subsequently published by Krishna et al (2001).

Although the single dose of rectal artesunate evaluated in the present study is less likely to have toxic effects than a full treatment course, it is important to carefully monitor patients for adverse events, particularly those previously identified. Given that serious adverse drug reactions are likely to be uncommon (based on the low incidence in large clinical trials and meta-analyses), this study is not powered to draw conclusions regarding the safety of rectal artesunate. There is insufficient evidence of the safety of artemisinins in pregnancy, particularly in the first trimester, to justify inclusion of pregnant women in this study.

Current knowledge of the pharmacokinetics of artemisinins is incomplete. Rapid degradation of artemisinin derivatives occurs in plasma samples, and dihydroartemisinin preferentially accumulating in parasitised erythrocytes, suggesting that plasma levels should be interpreted with caution. The therapeutic range of artemisinin derivatives including artesunate is poorly defined, and pharmacokinetic parameters are highly variable, particularly following rectal administration. Despite this artemisinin derivatives consistently show more rapid reduction in peripheral parasite density when adequately absorbed than all other classes of antimalarials. Thus pharmacokinetic evidence of reliability of drug absorption should be considered of secondary importance to pharmacodynamic evidence of a rapid reduction in peripheral parasitaemia in this study.

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References

- Alin MH, Kihamia MC, Bjorkman A, Bwijo BA, Premji Z, Mtey GJ, Ashton M (1995). Efficacy of oral and intravenous artesunate in male Tanzanian adults with *Plasmodium falciparum* malaria and in vitro susceptibility to artemisinin, chloroquine and mefloquine. *Am J Trop Med Hyg*; **53**: 639-645.
- Angus BJ, Thaiaporn I, Chanthapadith K, Suputtamongkol Y, White NJ (2002). Oral artesunate dose response relationship in acute falciparum malaria. *Antimicrob Agents Chemother*; **46**(3): 778-82.
- Ashton M, Hai TN, Sy ND, Huong DX, Huong NV, Nieu NT, Cong LD (1998). Artemisinin pharmacokinetics is time dependent during repeated oral administration in healthy male adults. *Drug Metabolism and Disposition*; **26**(1): 25-27.
- Awad MI, Alkadru AMY, Behrens RH, Baraka OZ, Eltayeb IB (2003). Descriptive study of the efficacy and safety of artesunate suppository in combination with other antimalarials in the treatment of severe malaria in Sudan. *Am J Trop Med Hyg*; **68**(2): 153-8.
- Bakshi R, Hermeling-Fritz I, Gathmann I, Alteri E (2000). An integrated assessment of the clinical safety of artemether-lumefantrine: a new oral fixed-dose combination antimalarial drug. *Trans R Soc Trop Med Hyg*; **94**(4): 419-424.
- Baradell LB, Fitton A (1990). Artesunate, a review of its pharmacology and therapeutic efficacy in the treatment of malaria. *Drugs*; **50**: 714 - 741
- Barnes KI, Baker L (2001). New antimalarial treatment for KwaZulu Natal. *S Afr Med J*; **91**(5): 349-350.
- Batty KT, Thu LT, Davis TM, Ilett KF, Mai TX, Hung NC, Tien NP, Powell SM, Thien HV, Binh TQ, Kim NV (1998). A pharmacokinetic and pharmacodynamic study of intravenous vs. oral artesunate in uncomplicated falciparum malaria. *Brit J Clin Pharmacol*; **45**: 123-129.
- Benakis A, Paris M, Loutan L, Plessas CT, Plessas ST (1996). Pharmacokinetic study of a new pharmaceutical form of artesunate (Plasmotrim-200 Rectocaps) administered in healthy volunteers by rectal route. *Jpn J Trop Med Hyg*; **24**: 39-45.
- Bethell DB, Teja-Isavadharm P, Cao XT, Pham TT, Ta TT, Tran TN, Nguyen TT, Pham TP, Kyle D, Day NP, White NJ (1997). Pharmacokinetics of oral artesunate in children with moderately severe falciparum malaria. *Trans R Soc Trop Med Hyg*; **91**: 195-8.
- Bhatt K, Bhatt S, Omonge E, Otekol L, Andrial M (1996). Efficacy and tolerability of a sequential artesunate treatment of severe falciparum malaria. *Jpn J Trop Med Hyg*; **24**: 59-63.
- Boele van Hensbroek M, Onyiorah E, Jaffer S, Schneider G, Palmer A, Frenkel J, Enwere G, Forck S, Nusmeijer A, Bennett S, Greenwood B, Kwiatkowski D (1996). A trial of artemether or quinine in children with cerebral malaria. *N Eng J Med*, **335**: 69-75.
- Bormann S, Ayola AA, Missinou MA, Binder RK, Issifou S, Schindler A, Matsiegui PB, Kun JFJ, Krishna S, Lell B, Kremsner PG (2002). Short course artesunate treatment in uncomplicated *Plasmodium falciparum* malaria in Gabon. *Antimicrob Agents Chemother*; **47**(3): 901-4.
- Brewer T.G., Grate S.J., Peggins J.O., Weina P.J., Petras J.M., Levine B.S., Heiffer M.H., Schuster B.G (1994a). Fatal neurotoxicity of arteether and artemether. *Am J Trop Med Hyg*; **51**: 251-259.
- Brewer T.G., Peggins J.O., Grate S.J., Petras J.M., Levine B.S., Weina P.J., Swearngen J., Heiffer M.H., Schuster B.G (1994b). Neurotoxicity in animals due to arteether and artemether. *Trans R Soc Trop Med Hyg*; **88 Suppl 1**, S33-S36.

- Brewer T.G., Genovese R.F., Newman D.B., Li Q (1998). Factors relating to neurotoxicity of artemisinin antimalarial drugs "listening to arteether". *Med Trop (Mars)*; **58**, 22-27.
- Brockman A, Price RN, van Vugt M, Heppner DG, Walsh D, Sooklo P, Wimonwaltrawatee T, Looareesuwan L, White NJ, Nosten F (2000). *Plasmodium falciparum* drug susceptibility on the northwestern border of Thailand during five years of extensive artesunate-mefloquine use. *Trans R Soc Trop Med Hyg*; **94**: 537-544.
- Bunnag D, Viravan C, Looareesuwan S, Karbwang J, Harinasuta T (1991). Clinical Trial of artesunate and artemether on multi-drug resistant falciparum malaria in Thailand. A preliminary report. *Southeast Asian J Trop Med Public Health*; **22**: 380-385.
- Bunnag D, Viravan C, Looareesuwan S, Karbwang J, Harinasuta T (1991). Double blind randomised clinical trial of oral artesunate at once or twice daily dose in falciparum malaria. *Southeast Asian J Trop Med Public Health*; **22(4)**: 539-543.
- Bunnag D, Kanda T, Karbwang J, Thimasam K, Pungpak S, Harinasuta T (1996). Artemether or artesunate followed by mefloquine as a possible treatment for multidrug resistant falciparum malaria. *Trans Roy Soc Trop Med Hyg*; **90**: 415-417.
- Cao X, Bethell DB, Pham TP, Ta TTM, Tran TNT, Nguyen TTH, Pham TTT, Nguyen TTA, Day NPJ, White NJ (1997). Comparison of artemisinin suppositories, intramuscular artesunate and intravenous quinine for the treatment of severe childhood malaria. *Trans Roy Soc Trop Med Hyg*; **91**: 335-342.
- Davis TME, Edwards GO, McCarthy JS (1997). Artesunate and cerebellar dysfunction in falciparum malaria. *N Engl J Med*; **337**: 792.
- Davis TM, Phuong HL, Ilett KF, Hung NC, Batty KT, Phuong VDB, Powell SM, Thien HV, Binh TQ (2001). Pharmacokinetics and pharmacodynamics of intravenous artesunate in severe falciparum malaria. *Antimicrob Agents Chemother*; **45**: 181-6.
- De Vries PJ, Nguyen XK, Tran KD, Nguyen B, Nguyen TY, Dao DD, van Boxel CJ, Kager PA (1997). The pharmacokinetics of a single dose of artemisinin in subjects with liver cirrhosis. *Trop Med Int Health*; **2(10)**: 957-962.
- Deen JL, von Seidlein L, Pinder M, Welraven GE, Greenwood BM (2001). The safety of the combination of artesunate and pyrimethamine-sulfadoxine given during pregnancy. *Trans R Soc Trop Med Hyg*; **95(4)**:424-8
- Duc DD, de Vries PJ, Nguyen XK, Nguyen B, Kager PA, van Boxel CJ (1994). The pharmacokinetics of a single dose of artemisinin in healthy Vietnamese subjects. *Am J Trop Med Hyg*; **51(6)**: 785-790.
- Eckstein-Ludwig U, Webb RJ, Van Goethem ID, East JM, Lee AG, Kimura M, O'Neill PM, Bray PG, Ward SA, Krishna S. Artemisinins target the SERCA of Plasmodium falciparum. *Nature*. 2003 Aug 21; **424**(6951): 957-61.
- Eduardo A, Gomez L (1996). Efficacy of artesunate suppository followed by oral mefloquine in the treatment of severe malaria in endemic areas where resistance to chloroquine exists in Ecuador. *Jpn J Trop Med Hyg*; **24**: 17-24.
- Elias Z, Bonnet E, Marchou B, Massip P (1999). Neurotoxicity of artemisinins: possible counselling and treatment of side effects. *Clin Infect Dis*; **28**: 1330-1331.
- Genovese R.F., Newman D.B., Li Q., Peggins J.O., Brewer T.G (1998). Dose-dependent brainstem neuropathology following repeated arteether administration in rats. *Brain Res Bull* ; **45**: 199-202.
- Gu HM, Warhurst DC, Peters W (1984). Uptake of dihydroartemisinin derivatives by erythrocytes by erythrocytes infected with with Plasmodium falciparum in vitro. *Trans R Soc Trop Med Hyg*; **78(2)**: 265-270.

- Ha V, Nguyen NH, Tran TBH, Bui MC, Nguyen HP, Tran THC, Phan TQ, Arnold K, Tran TH (1997). Severe and complicated malaria treated with artemisinin, artesunate or artemether in Vietnam. *Trans Roy Soc Trop Med Hyg*; **91**: 465-467.
- Halpaap B, Ndjave M, Parish M, Benakis A, Kremsner PG (1998). Plasma levels of artesunate and dihydroartemisinin in children with *Plasmodium falciparum* malaria in Gabon after administration of 50mg artesunate suppositories. *Am J Trop Med Hyg*; **58**: 365-368.
- Hassan AM, Ashton M, Kihamia CM, Mtey GJ, Bjorkman A (1996). Multiple dose pharmacokinetics of oral artemisinin and comparison of its efficacy with that of oral artesunate in falciparum malaria patients. *Trans R Soc Trop Med Hyg*; **90**: 61-65.
- Hien TT, Phu NH, Mai NTH, Chau TTH, Trang TTM, Loc PP, Cuong BM, Dung NT, Vinh H, Waller D, White NJ (1992). An open randomised comparison of intravenous and intramuscular artesunate in severe falciparum malaria. *Trans R Soc Trop Med Hyg*; **86**: 584-585.
- Hien TT, White NJ (1993). Qionhaosu. *Lancet*; **341**: 603-608
- Hien TT, Day NP, Nguyen HP, Nguyen TH, Tran TH, Pham PL, Dinh VS, Ly VC, Walker D, Peto TE, White NJ (1996). A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. *New Eng J Med*; **335**: 76-83.
- Ittarat W, Looareesuwan S, Pootrakul P, Sumpunsirikul P, Vattanavibool P, Meshnick S (1998). Effects of α -Thalassemia on Pharmacokinetics of the Antimalarial Agent Artesunate. *Antimicrob Agents Chemother*; **42**: 2332-2335.
- Karbwang J, Na Bangchang K, Thanavibul A, Back DJ, Bunnag D, Harinasuta T (1994). Pharmacokinetics of mefloquine alone or in combination with artesunate. *Bull WHO*; **72(1)**: 83-87.
- Karunajeewa HA, Kemiki A, Alpers MP, Lorry K, Batty KT, Ilett KF, Davis TM (2003). Safety and therapeutic efficacy of artesunate suppositories for treatment of malaria in children in Papua New Guinea. *Pediatr Infect Dis J*; **22(3)**: 251-6.
- Khanh NX, de Vries PJ, Ha LD, van Boxtel CJ, Koopmans R, Kager PA (1999). Declining concentrations of dihydroartemisinin in plasma during 5-day oral treatment with artesunate for falciparum malaria. *Antimicrob Agents Chemother*; **43(3)**: 690-692.
- Kidane G, Morrow RH (2000). Teaching mothers to provide home treatment of malaria in Tigray, Ethiopia: a randomised trial. *Lancet*; **356**: 550 – 55.
- Kissinger E., Hien T.T., Hung N.T., Nam N.D., Tuyen N.L., Dinh V.B., Mann C., Phu N.H., Loc P.P., Simpson J.A., White N.J., & Farrar J.J. (2000). Clinical and neurophysiological study of the effects of multiple doses of artemisinin on brain-stem function in Vietnamese patients. *Am J Trop Med Hyg*; **63**: 48-55.
- Korenromp EL, Williams BG, Gouws E, Dye C, Snow RW (2003). Measurements of trends in childhood malaria mortality in Africa: an assessment of progress toward targets based on verbal autopsy. *Lancet Infect Dis*; **3(6)**: 349-358.
- Krishna S, Planche T, Agbenyega T, Woodrow C, Agranoff D, Bedu-Addo G et al. Bioavailability and clinical efficacy of intra-rectal artesunate in Ghanaian children with Moderate Malaria. *Antimicrob Agents Chemother* 2001; **45(2)**: 509-516.
- Kshirsagar NA, Gogtay NJ, Moorthy NS, et al (2000). A randomised, double blind, parallel group, comparative safety and efficacy trial of oral artemether-lumefantrine versus oral chloroquine in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in adults in India. *Am J Trop Med Hyg*; **63(3)**: 402-408.
- Kshirsagar NA, Gogtay NJ, Moorthy NS, et al (2000). A randomised, double blind, parallel group, comparative safety and efficacy trial of oral artemether-lumefantrine versus oral

- chloroquine in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in adults in India. *Am J Trop Med Hyg*; **63**(3): 402-408.
- Lefevre G, Looareesuwan S, Treeprasertsuk S, et al (2001). A clinical and pharmacokinetic trial of six doses of artemether-lumefantrine for multidrug-resistant plasmodium falciparum malaria in Thailand. *Am J Trop Med Hyg*; **64**(5): 247-256.
- Leonardi E, Gilvary G, White NJ, Nosten F (2001). Severe allergic reactions to oral artesunate: a report of two cases. *Trans R Soc Trop Med Hyg*; **95**(2): 182-183.
- Li GQ, Guo XB, Fu LC, Jian HX, Wang XH (1994). Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. *Trans R Soc Trop Med Hyg*; **84**(Suppl 1): S5-6.
- Looareesuwan S, Wilairatana P, Andrial M (1996). Artesunate suppository in the treatment of severe falciparum malaria in Thailand. *Jpn J Trop Med Hyg*; **24**: 25-32.
- Looareesuwan S, Wilairatana P, Vanijanonta S, Viravan C (1995). Efficacy and tolerability of a sequential, artesunate suppository plus mefloquine, treatment of severe falciparum malaria. *Ann Trop Med Parasitol*; **89**: 469-575.
- Luxemborger C, Thwai KL, Raimond SD, Chongsuphajaisiddhi T, White NJ (1995). Oral artesunate in the treatment of uncomplicated hyperparasitaemic *falciparum* malaria. *Am J Trop Med Hyg*; **53**: 522-525.
- McGready R, Cho T, Keo NK, Thwai KL, Villegas L, Looareesuwan S, White NJ, Nosten F (2001). Artemisinin antimalarials during pregnancy: a prospective treatment study of 539 episodes of multi-drug resistant *Plasmodium falciparum*. *Clin Infect Dis*; **33**(12): 2009-2016.
- McIntosh HM, Olliaro P (2003). Artemisinin derivatives for treating severe malaria. In *The Cochrane Library*, Issue 3: 1-25; Oxford: Update Software.
- Miller LG, Panosian CB (1997). Ataxia and slurred speech after artesunate treatment for falciparum malaria. *N Engl J Med*; **336**: 1328.
- Moyou-Somo R, Tietche F, Ondona M, Kouemeli LE, Ekoe T, Mbonda E, et al. (2001). Clinical trial of beta-artether versus quinine for the treatment of cerebral malaria in children in Yaounde, Cameroon. *Am J Trop Med Hyg*; **64**: 229-232.
- Murphy S, English M, Waruiru C, Mwangi I, Amokoye E, Krawley J, Newton C, Winstanley P, Peshu N, Marsh K (1996). An open randomised trial of artemether vs quinine in the treatment of cerebral malaria in African children. *Trans Roy Soc Trop Med Hyg*, **90**: 298-301.
- Murphy SA, Mberu E, Muhia D, English M, Crawley J, Wariuru C, Lowe B, Newton CRJ, Winstanley P, Marsh K, Watkins WM (1997). The disposition of intramuscular artemether in children with cerebral malaria: a preliminary study. *Trans Roy Soc Trop Med Hyg*; **91**: 331-334.
- Navaratnam V, Mansor SM, Chin LK, Mordi MN, Ashokan M, Nair NK (1995). Determination of artemether and dihydroartemisinin in blood plasma by high pressure liquid chromatography for application in clinical pharmacological studies. *J Chromatogr B*; **669**: 289-294.
- Newton P, Suputtamongkol Y, Teja-Isavadham P, Pukrittayakamee S, Navaratnam V, Bates I, White N, 2000. Antimalarial bioavailability and disposition of artesunate in acute falciparum malaria. *Antimicrob Agents Chemother*; **44**: 972-7.
- Newton P, Proux S, Green M (2001). Fake artesunate in southeast Asia. *Lancet*; **357**: 1948-50.
- Newton PN, van Vugt M, Teja-Isavadham, Siriyanonda D, Rasameesoraj M, Teerapong P, Ruangveerayuth R, Slight T, Nosten F, Suputtamongkol Y, Looareesuwan S, White NJ (2002). Comparison of oral artesunate and dihydroartemisinin antimalarial availabilities in acute falciparum malaria. *Antimicrob Agents Chemother*; **46**(4): 1125-1127.

- Newton PN, Angus BJ, Chierakul W, Dondorp A, Ruangveerayuth R, Silamut K, Teerapong P, Suputtamongkol Y, Looareesuwan S, White NJ (2003). Randomised comparison of artesunate and quinine in the treatment of severe malaria. *Clin Infect Dis*; **37**(1): 7-16.
- Nontprasert A, Nosten-Bertrand M, Pukrittayakamee S, Vanijjanonta S, Angus BJ, White NJ (1998). Assessment of the neurotoxicity of parenteral artemisinin derivatives in mice. *Am J Trop Med Hyg*; **59**: 519-522.
- Nosten F, Luxemborger C, ter Kuile FO, Woodrow C, Pa Eh J, Chongsuphajaisiddhi T, White NJ (1994). Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate mefloquine combination. *J Inf Dis*; **170**: 971-7.
- Nosten F, van Vugt M, Price R, Luxemborger C, Thway KL, Brockman A, McGready R, ter Kuile F, Looareesuwan S, White NJ (2000). Effects of artesunate – mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet*; **356**: 297-302.
- Phuong CXT, Bethell DB, Phuong PT, Mai TTT, Thuy TTN, Ha NTH, Thuy PTT, Anh NTT, Day NPJ, White NJ (1997). Comparison of artemisinin suppositories, intramuscular artesunate and intravenous quinine for the treatment of severe childhood malaria. *Trans R Soc Trop Med Hyg*; **91**: 335-342.
- Pittler MH, Ernst E (1999). Artemether for severe malaria. *Clin Infect Dis*; **28**: 597-601.
- Price RN, Nosten F, Luxemborger C, ter Kuile FO, Paiphun L, Chongsuphajaisiddhi T & White NJ (1996). Effects of artemisinin derivatives on malaria transmissibility. *Lancet*; **347**: 1654-8.
- Price R, van Vugt M, Nosten F, Luxemborger C, Brockman A, Phaipun L, Chongsuphajaisiddhi T, White NJ (1998). Artesunate versus artemether for the treatment of recrudescence multidrug-resistant falciparum malaria. *Am J Trop Med Hyg*; **59**(6): 883-888.
- Price R, van Vugt M, Phaipun L et al (1999). Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *Am J Trop Med Hyg*; **60**, 547-555.
- Qinghaosu Antimalarial Research Group (1979). Antimalarial studies on qinghaosu. *Chinese Medical Journal*, **92**: 811-816.
- Ribeiro I.R. & Olliaro P (1998). Safety of artemisinin and its derivatives. A systematic review of published and unpublished clinical trials. *Med Trop (Mars)*; **58**, 50-53.
- Rowland M, Thomas N (1995). Therapeutic regimens. In *Clinical Pharmacokinetics – concepts and applications* (3rd Ed.). Baltimore: Williams and Wilkins. 83-105.
- Sabchareon A, Attanath P, Chanthavanich P, Phanuaksook P, Prarinyanupharb V, Poonpanich Y, Mookmanee D, Teja-Isavadharm P, Heppner DG, Brewer TG, Chongsuphajaisiddhi T (1998). Comparative clinical trial of artesunate suppositories and oral artesunate in combination with mefloquine in the treatment of children with acute falciparum malaria. *Am J Trop Med Hyg*; **58**: 11-16.
- Seguro AC, Campos SB (2002). Diuretic effect of sodium artesunate in patients with malaria. *Am J Trop Med Hyg*, **67**(5): 473-4.
- Taylor TE, Wills BA, Courval JK, Molyneux ME (1998). Intramuscular artemether vs. intravenous quinine: an open randomised trial in Malawian children with cerebral malaria. *Trop Med Int Hth*; **3**:3-8.
- Teja-Isavadharm P, Nosten F, Kyle DE, Luxemborger C, ter Kuile F, Peggins JO, Brewer TG, White NJ (1996). Comparative bio-availability of oral, rectal and intramuscular artemether in healthy subjects: use of simultaneous measurement by high performance liquid chromatography and bioassay. *Br J Clin Pharmacol*; **42**: 599-604.
- ter Kuile F, White NJ, Holloway P, Pasvol G, Krishna S (1993). *Plasmodium falciparum*: in vitro studies of the pharmacodynamic properties of drugs used for the treatment of severe malaria. *Experimental Parasitology*; **76**: 85-95.

- Thuma PE, Bhat GJ, Mabeza GF, Osborne C, Biemba G, Shakankale GM, Peeters PAM, Oosterhuis B, Lugt CB, Gordeuk VR (2000). A randomised controlled trial of artemotil (barteether) in Zambian children with cerebral malaria. *Am J Trop Med Hyg*; **62**: 524-529.
- Thwe Y, Than M, Phay S, ZawOo A, YuSoe A (1996). Artesunate-mefloquine tablets (Plasmotrim Rectocaps, Mefloquine Lactab) in the treatment of severe malaria. *Jpn J Trop Med Hyg*; **24**: 25-32.
- Tjitra E, Suprianto S, Currie BJ, et al (2001). Therapy of uncomplicated falciparum malaria: a randomised trial comparing artesunate plus sulfadoxine-pyrimethamine versus sulfadoxine-pyrimethamine alone in Irian Jaya, Indonesia. *Am J Trop Med Hyg*; **65(4)**: 309-317.
- Trape JF, Pison G, Preziosi MP, Enel C, Desgrees du Lou A, Delaunay V, Samb B, Lagarde E, Molez JF, Simondon F (1998) Impact of chloroquine resistance on malaria mortality. *Comptes rendes de l'Academie des Sciences. Serie III, Sciences de la vie*; **321**: 689-697.
- Udomsangpetch R, Piptaporn B, Krishna S, Angus B, Pukrittayakamee S, Bates I, Supputamongkol Y, Kyle DE, White NJ (1996). Antimalarial drugs prevent cytoadherence and rosetting in falciparum malaria. *J Infect Dis*; **173**: 691-698.
- van Vugt M, Wilairatna P, Gemperli B, et al (1999a). Efficacy of six doses of artemether-lumefantrine in multidrug resistant falciparum malaria. *Am J Trop Med Hyg*; **60(6)**: 936-942.
- van Vugt M, Ezzet F, Nosten F, Gathmann I, Wilairatana P, Looareesuwan S, White NJ (1999b). No evidence of cardiotoxicity during antimalarial treatment with artemether-lumefantrine. *Am J Trop Med Hyg*; **61(6)**: 964-967.
- van Vugt M, Looareesuwan S, Wilaratana P, et al (2000a). Artemether lumefantrine for the treatment of multidrug resistant falciparum malaria. *Trans R Soc Trop Med Hyg*; **94**: 545-548.
- van Vugt M., Angus B., Price R., Mann C., Simpson J.A., Poletto C et al (2000b). A case-control auditory evaluation of patients treated with artemisinin derivatives for multidrug-resistant plasmodium falciparum malaria. *Am J Trop Med Hyg*; **62**: 65-69.
- von Seidlein L, Milligan P, Pinder M, Bojang K, Anyalebechi C, Gosling R, et al (2000). Efficacy of artesunate plus pyrimethamine – sulphadoxine for uncomplicated malaria in African children: a double-blind, randomised, controlled trial. *Lancet*; **355**: 352-357.
- Wang TY (1989). Follow up observation on the therapeutic effects and remote reactions of artemisinin (Qinghaosu) and artemether in treating malaria in pregnant women. *J Trad Chin Med*; **9**: 28-30.
- White NJ, Warrell DA, Chanthavanich P, Looareesuwan S, Warrell MJ, Krishna S, Williamson DH, Turner RC (1983). Severe hypoglycaemia and hyperinsulinaemia in falciparum malaria. *N Engl J Med*; **309**: 61-66.
- White NJ (1994). Clinical Pharmacokinetics and pharmacodynamics of artemisinin and derivatives. *Trans R Soc Trop Med Hyg*; **88 (Suppl 1)**: S41-S43.
- White NJ (1996). The treatment of malaria. *N Engl J Med*; **335**: 800-806.
- White NJ (1997). Assessment of the pharmacodynamic properties of the antimalarial drugs *in vivo*. *Antimicrob Agents Chemother*; **4**: 1413-1422.
- White NJ (1998). Preventing antimalarial drug resistance through combinations. *Drug Resistance Updates*; **1**:3-9
- White NJ (2000). Neurological dysfunction following malaria: disease or drug related? *Clin Infect Dis*; **30**: 836.

- White NJ (2003). The management of severe falciparum malaria. *Am J Resp Crit Care Med*; **167**: 673-674.
- Wilairatana P, Viriyavejakul P, Looreesuwan S, Chongsuphajsiddhi T (1997). Artesunate suppositories: an effective treatment for severe falciparum malaria in rural areas. *Ann Trop Med Parasit*; **91(7)**: 891-896.
- Win K, Than M, Thwe Y (1992). Comparison of combinations of parenteral artemisinin derivatives plus oral mefloquine with intravenous quinine plus oral tetracycline for treating cerebral malaria. *Bull WHO*; **70**: 777-782.
- World Health Organization (2000). Severe falciparum malaria. *Trans Roy Soc Trop Med Hyg*; **94 Supp1**: S1-90.

University of Cape Town

Overall study design and plan-description

This was a Phase III open-label randomised PK / PD comparison between rectal AS and parenteral quinine in southern African adults with moderately severe or severe malaria.

Patients were stratified into the moderately severe and severe malaria categories on the basis of their disease severity (see criteria below).

Moderately severe falciparum malaria:

Randomised to receive either:

artesunate suppository followed by sulphadoxine / pyrimethamine at 24 hours
(Group 1)

or

intramuscular quinine followed by sulphadoxine / pyrimethamine at 24 hours, if able to tolerate "per os" medication (Group 2)

Severe and complicated malaria

Randomised to receive either:

artesunate suppository PLUS intravenous quinine (Group 3)

or

intravenous quinine alone (Group 4) - i.e. best available treatment currently

Followed by oral quinine, once able to take oral medication, to complete at least 7 days of treatment, PLUS a stat dose of sulphadoxine / pyrimethamine.

The study was conducted at Mosvold District Hospital in northern KwaZulu Natal, South Africa, which serves a non-immune population resident in an area with low intensity (Entomological Inoculation Rate < 1), seasonal malarial transmission, which peaks between February and May. Only one small *in vivo* study had been conducted in the area, which demonstrated 18% resistance to sulphadoxine / pyrimethamine in 1997: (RI: 14.7%; RII: 0%, RIII: 2.9% (n = 34) ¹) which is has been treatment policy for uncomplicated malaria in KwaZulu Natal since 1998.

Discussion of study design

The randomised controlled design was chosen to allow estimates of relative efficacy and safety in comparison to the best available alternative, in the target population. Although intravenous (and often, intramuscular) quinine is not accessible in most rural malaria endemic areas where rectal artesunate is most needed, parenteral quinine is considered the comparator of choice in the hospital setting. Allocation ratios were chosen to maximise the collection of data on artesunate suppository efficacy and pharmacokinetics, while retaining a reference group receiving standard therapy of known efficacy and pharmacokinetics.

Rectal artesunate is intended for emergency use to provide antimalarial cover for the initial 24 hours to enable patients unable to tolerate oral medication to reach a health facility where safe parenteral treatment is available. As rectal artesunate is intended as an initial therapeutic intervention and not as curative treatment, and short courses of both artemisinins and quinine are associated with frequent recrudescence^{1,2}, all patients with

¹ Classification describes parasitological responses with increasing levels of resistance to a slowly eliminated drug; RI: Initially patients appear cured as parasite density decreases below detectable levels, whereafter parasitaemia reappears (with re-infection excluded by PCR); RII: parasite density decreases but fails to clear within 7 days; RIII: less than 75% reduction in parasite density by 72 hours.

moderately severe malaria received identical treatment with recommended first line treatment, sulfadoxine-pyrimethamine, after 24 hours or as soon as possible thereafter. In those with severe malaria, in addition to a stat dose of SP to complete treatment, 7 days of quinine therapy was completed to minimise the risk of treatment failure.

In an area with a similar intensity of malaria transmission to KwaZulu Natal, the risk of death in patients with moderately severe malaria (> 4% parasitaemia and no vital organ dysfunction) was 3%³, compared with 0.1% in all uncomplicated malaria⁴, and 18% in severe malaria⁵.

Sample size calculations

This study is a component of a multicentre study on which the WHO aims to recruit 300 patients. Secondary sample size calculations to establish adequacy of absorption in the local population were as follows:

For patients with moderately severe malaria: Assuming a parasite density at 24 hours of 5% that at baseline in patients treated with rectal artesunate and of 30% for patients treated with IM quinine, a sample size of 43 per treatment arm is needed to achieve significance at the 5% level with 80% power. 100 patients were to be recruited to ensure that at least 86 complete follow up.

For patients with severe malaria: Assuming a parasite density at 24 hours of 10% that at baseline in patients treated with rectal artesunate plus IV quinine and of 50% for patients treated with IV quinine alone, a sample size of 24 per treatment arm is needed to achieve significance at the 5% level with 80% power. 50 patients were to be recruited to ensure that at least 48 complete follow up.

Selection of study population

Inclusion criteria

For all groups:

- Age: 16 - 65 years
- *P. falciparum* malaria, clinically suspected, with a positive ICT® rapid diagnostic card test, confirmed with a positive *P. falciparum* thick smear.
- Patient or parent or guardian given informed consent.
- **PLUS** at least one of the following:

For groups 1-2 (moderately severe falciparum malaria):

the admitting physician considered oral therapy inappropriate, as patient (without any features of severe and complicated malaria - see below) is:

- Unable to eat or drink
- Repeatedly vomiting
- Dehydrated
- Prostrate (unable to stand / walk unaided)

For groups 3 - 4 (severe and complicated falciparum malaria):

- Respiratory distress (respiratory rate > 20 and HCO₃ < 15 mmol/L)
- Jaundice (detected clinically or a serum bilirubin > 50 µmol/L)
- Bleeding abnormality (spontaneous bleeding, disseminated intravascular coagulation)
- Shock (defined as a systolic blood pressure < 80 mmHg with signs of poor peripheral perfusion)
- Two or more generalised convulsions

- Decreased level of consciousness (failure to localise or make appropriate verbal response to a noxious stimulus, at least 30 minutes after a generalized convulsion)
- Serum Lactate level > 5 mmol/L
- *Plasmodium falciparum* asexual parasitaemia > 500 000 / μ l.
- Renal failure (urine output < 400ml in 24 hours, or a creatinine of more than 265 μ mol / l)
- Hypoglycaemia (defined as a whole blood glucose concentration < 2.2 mmol/L);

Exclusion criteria

- Diarrhoea (defined as > 2 liquid stools in the previous 24hours)
- A history of having received an effective anti-malarial in the previous 24 hours
- Pregnant (using Beta-HCG card test in all female patients) or breast feeding women
- Diseases of the rectum, previous rectal surgery.
- A history of hypersensitivity to artemisinin derivatives or quinine (NOTE: patients with a history of hypersensitivity reactions to sulphadoxine / pyrimethamine or any "sulpha" drugs were to be included in the study, and to receive 7 days of doxycycline instead of sulphadoxine / pyrimethamine).

Criteria for withdrawal from treatment or assessment

Groups 1 & 2

Patients in Groups 1 & 2 who failed to respond or deteriorated in the first 24 hours (indicated by developing any of the above features of severe and complicated malaria during the 24 hour period of observation after rectal therapy, or if the parasite density at 12 and 24 hours is 60% or more of the admission density), were to be rescued immediately with intravenous quinine. Sampling continued according to the initially allocated schedule in these patients.

Groups 1 - 4

Patients judged by the investigator to require transfer to a referral hospital, for intensive care (e.g. requiring ventilation or haemodialysis - indicated with metabolic acidosis, hyperkalaemia and fluid overload or urea > 50 mmol/L or patients who remain anuric after adequate rehydration)).

Discontinuation of study arm

After eleven patients with severe malaria had been evaluated, consensus was reached between investigators and sponsors, that the study arm evaluating severe and complicated malaria would be discontinued. This decision was based on the realisation that the referral systems in place in KwaZulu Natal (both for transfer and intensive care) were unable to meet the standard of care specified in the protocol, as was demonstrated by a review of the management of two patients who died following referral.

Treatments administered

Patients received either rectal AS (Plasmotrim®, 50 or 200mg Rectocaps®; Batch nos. 24398 and 23742 respectively, Mepha AB, Basel, Switzerland) or Parenteral Quinine (Adco-Quinine dihydrochloride®, Adcock Ingram Generics Ltd., South Africa) which was followed up with oral sulfadoxine – pyrimethamine (Fansidar®, Roche Products (Pty) Ltd.) and, in patients with severe malaria, oral quinine (Lennon Quinine Sulphate tablets®, Phamacare Ltd, South Africa).

Patients were included in four treatment groups:

- Group 1: 10 mg/kg artesunate suppository at 0 hours followed by 3 tablets sulphadoxine / pyrimethamine (or 10 mg/kg intramuscular quinine if unable to tolerate oral medication) at 24 hours
- Group 2: 10 mg/kg intramuscular quinine at 0, 4, 12 and 20 hours followed by 3 tablets sulphadoxine / pyrimethamine (or 10 mg/kg intramuscular quinine if unable to tolerate oral medication) at 24 hours
- Group 3: 10 mg/kg artesunate suppository PLUS 20 mg/kg loading dose of intravenous quinine and then 10 mg/kg intravenous quinine 8 hourly until able to take oral medication; then 10 mg/kg oral quinine 8 hourly to complete 7 days of treatment, PLUS a stat dose of 3 tablets of sulfadoxine / pyrimethamine.
- Group 4: 20 mg/kg loading dose of intravenous quinine and then 10 mg/kg intravenous quinine 8 hourly until able to take oral medication; then 10 mg/kg oral quinine 8 hourly to complete 7 days of treatment, PLUS a stat dose of 3 tablets of sulfadoxine / pyrimethamine.

Suppositories were administered whole, without the use of lubricant, and the actual dose given was the nearest approximation to the recommended dose of 10 mg/kg. Intramuscular doses of quinine were diluted in normal saline to a concentration of 60

mg/ml, with half the dose administered in each anterior thigh. Intravenous quinine was administered as an infusion over 4 hours in 5% dextrose saline (5-10 ml/kg depending on the patient's fluid balance). Patients with persistent parasitaemia were to receive curative therapy with 750mg mefloquine (Lariam®, Roche products (Pty) Ltd., South Africa) orally.

Method of assigning patients to treatment groups.

Patients were allocated to the different treatment arms according to a textbook randomisation list, in a ratio of 1 control: 4 artesunate in each arm. These were prepared for 100 moderately severe patients and 50 severe patients. These were not prepared in blocks. Treatment category for each patient was provided in a sealed envelope, labelled by disease severity, which was opened at the time of enrolment to the study. The exact timing of each patient's dose was documented; however the relationship of dosing to meals was not specified. All supportive measures required in the management of malaria were authorised. IV fluids were administered to maintain circulating intravascular volume. Fever was treated with paracetamol and seizures were managed with parenteral diazepam and phenytoin. Severe anaemia was corrected with transfusion of HIV and Hepatitis B screened blood. Concomitant infections and diseases were treated appropriately.

Patients with severe and complicated malaria were to receive the best management available at a rural district hospital level, following WHO guidelines⁶. All concomitant treatment was documented. Patients with moderately severe malaria were to remain in hospital until they were clinically well, able to tolerate oral medication and were aparasitaemic and afebrile for at least 24 hours. Patients with severe malaria were required to remain in hospital until they had met the above criteria and had completed 7 days of oral quinine

Prior to discharge, all treatment was administered by study nurses, who documented time of administration. Nursing staff monitored patients for expulsion of suppositories at 5, 15, 30, 60 minutes, and then hourly for 6 hours after insertion. If the suppository was expelled in < 10 minutes, it was reinserted; if expelled between 10 and 60 minutes a new suppository was inserted. Time of re-insertion was documented. At discharge patients were motivated to adhere to follow up appointments. At follow up, patients were questioned regarding compliance with outpatient medication.

Patients who failed initial treatment were administered:

- Parenteral Quinine (Adco-Quinine dihydrochloride®, Adcock Ingram Generics Ltd., South Africa) for severe malaria, or
- Oral quinine (Lennon Quinine Sulphate tablets®, Pharmacare Ltd, South Africa), for moderately severe malaria persisting despite treatment with rectal artesunate, or
- Mefloquine (Lariam®, Roche Products (Pty) Ltd.) for recrudescence of *P. falciparum* parasitaemia

Efficacy and safety variables

Pre-treatment evaluation

Clinical assessment

During screening, a detailed history and physical examination was performed, with emphasis on assessing the severity of the malaria, the neurological status, including assessment of extra-ocular movements, cerebellar signs (including heel to toe walking, nystagmus) and fine motor coordination (including placing small tablets in a narrow container and drawing a line between 2 crosses 10 cm apart). Glasgow and Blantyre Coma Scale scores (GCS) were monitored in all patients.

Parasitologic diagnosis

Initial diagnosis was made using the ICT® rapid diagnostic antigen test and confirmed on thick and thin blood films stained with reverse Field's stain and expressed per 1000 red blood cells and 200 white blood cells, respectively. Slides were read by the laboratory technicians in the study team, and quality assurance of parasite density conducted thereafter by a single haematologist at the University of Cape Town Department of Haematology.

Baseline laboratory tests

Haemoglobin (Hb), Haematocrit (Hct), Mean Cell Volume (MCV), White Cell Count (WCC) and platelets were estimated using a Coulter counter®. Whole blood lactate was measured on an automated lactate analyser (Accusport®, Boeringer Mannheim, South Africa). Blood gas analysis was performed using the IL 1640 BGE® analyser (Ilex, South Africa). Liver function tests and glucose tests were determined using the Vision® Analyser (Abbotts, South Africa). Normal ranges are defined in Appendix 3. These instruments were calibrated daily, before analysis of the first sample, with glucose and lactate standards provided by the manufacturer. In addition, after each 5 samples were analysed, an automatic re-calibration occurred with the use of internal standards. Pregnancy was excluded in all female patients with HCG Combo®, Abbotts, South Africa.

On-treatment evaluation

Patients were under care of study nurses and physicians. Vital signs including pulse, blood pressure (and Central Venous Pressure if required), respiratory rate, temperature and coma score were recorded at 0, 1 and 2 hours then 2 hourly until 24 hours, 4-hourly until 48 hours, 6-hourly thereafter until 96 hours, and then daily until discharge. Parasitaemia was monitored every 6h until clearance of parasites; Full Blood Count (FBC), blood gas, blood glucose and lactate levels were monitored every 6h until 24 hours. Time to eat, drink, stand and walk unaided were monitored.

Drug-free follow-up

Patients were asked to attend follow-up 7, 14 and 42 days after entry into the study. A full history and physical examination, including neurological assessment, as well as, peripheral smear and FBC were carried out. Patients were encouraged to attend earlier if they developed fever or other symptoms. If patients did not attend the follow-up appointment, a member of the study team visited the patient's homestead to conduct the assessment. If the patient was absent, a history regarding the health of the patient was taken from family members.

Efficacy definitions

Parasitological response:

Standard definitions of parasitological response were used, including time to reduction of parasite count by 50% and 90% of baseline (PC50 and PC90) and parasite clearance time (PCT). The latter was defined as the time to the first of at least 2 successive blood smears with <1parasite/200 white cell nuclei. The fractional reduction of parasite count at 24 hours (absolute parasite count at 24 hours / absolute parasite count at baseline

expressed as a percentage) was felt to be the best indicator of response to the study medications in view of the administration of standard antimalarial treatment to both arms at 24 hours.

Clinical response:

Fever clearance time (FCT) was defined as the time elapsed before the temperature remained below 37.5°C for 2 subsequent 4-hourly readings. Time to eat, drink, stand and walk unaided were defined as the time elapsed from study entry until the patient was first able to perform these activities without help. Time to return to *per os* status was defined as the time taken for a patient to return to being able to eat, drink, and walk. Patients in the moderately severe category were classified as having progressed to severe and complicated malaria if they developed any of the WHO criteria of severe and complicated malaria⁷, and / or if their parasite density 12 hours after inclusion, remained above 60% of baseline. Coma recovery time is defined as the number of hours taken for the Blantyre Coma Score to return to 5 after being less than 5.

Safety evaluation

Safety was assessed by means of adverse event monitoring. Adverse event data was volunteered, with no specific checklist followed. An adverse event was defined as any symptom, sign of deterioration in laboratory value that developed or increased in severity after the administration of study treatment. The adverse event form in the CRF included rating of severity and likelihood of causal association.

Drug concentration measurements

A fine gauge (20 or 22G) IV cannula (Introcan®, Braun Omnimed, South Africa) was inserted into a peripheral vein for sampling purposes. An admission sample of plasma (from 10ml whole blood) was obtained before study drug administration. At 1h, 2 h, 4 h, 6 h and 8 h after study drug administration, sampling (from 10ml whole blood) for AS and

DHA was performed in patients randomised to Groups 1 and 3. Whole blood was collected into appropriately labelled (with subject number and assay time) heparinised tubes and the plasma separated within 5 minutes of collection by centrifugation at 12,000g for 3 min. Samples were stored and transported at -70°C in liquid nitrogen.

Concentrations of artesunate (AS) and its active metabolite dihydroartemisinin (DHA) in plasma were determined by a specific and sensitive high-performance liquid chromatographic method with electrochemical detector operating in the reductive mode⁸. The assays were conducted according to Good Laboratory Practice (GLP) procedures and requirements at the Centre for Drug Research, University Sains Malaysia, Penang, Malaysia and duplicate samples were analysed by the less sensitive but reliable HPLC with post column derivitisation and ultraviolet detection⁹ in the Division of Clinical Pharmacology of the University of Cape Town with the intention of developing UCT's laboratory capacity in this regard.

Data quality assurance

The author conducted training sessions at the study site. Data were source-verified by the Study Monitors, with double data entry. Data auditing was performed in October 1998, and again by the author prior to the start of analysis.

Statistical methods planned in the protocol and determination of sample size

This study was one site in a multicentre PK/PD study. The number of patients in each treatment group was expected to be sufficient to estimate the fractional reduction of parasitaemia, the main objective of the study. It was initially intended that a total of 100 patients would be recruited into the study. Unfortunately, only 46 patients met the inclusion criteria during the study period. Descriptive statistics were used for demographic and baseline characteristics. Normally distributed data were compared with parametric tests (t-test, one-way ANOVA) and non-normally distributed data using the Mann Whiney U and Kruskal-Wallis tests. Correlation of non-normally distributed data was analysed using the Spearman Correlation. Changes over time in vital signs were compared with the Wilks lambda test. Kaplan Meier Survival Curves were compared using the Log Rank test. Logistic regression was used to explore the influence of explanatory variables on the fractional reduction in parasitaemia over time. AUC of AS and DHA were calculated using WinNonLin 3.3 (Pharsight®). All results were assessed at a significance level of $p=0.05$ with 95% confidence intervals. Analyses were conducted using SPSS 8.0® and Stata 7.0®.

Ethical Issues

Ethical clearance was obtained from the Research and Ethics Committee of the University of Cape Town, the Ethics Committee of the South African Medicines Control Council and the WHO Secretariat Committee on Research Involving Human Subjects (SCRIHS).

The study was conducted in accordance with the principles laid down in the WHO Health Assembly of 1975 on Ethics in Human Experimentation and the Declaration of Helsinki. Patients were informed of the purpose of the trial and potential risks and benefits of the investigational drug. Patients were considered eligible for enrolment only if witnessed signed informed consent was given during screening (see English consent form, Appendix 2). Information was provided verbally to patients in their native language with subsequent witnessed signature of the consent form. Written informed consent form was available in both English and Zulu.

References

- ¹Bunnag D. Double blind randomised clinical trial of two different regimens of oral artesunate in falciparum malaria. Viravan, C., Looareesuwan, S., Karbwang, J., and Harinasuta, T. *Southeast Asian J Trop Med Public Health* 1991; 22(4): 534 – 538.
- ² Ringwald P, Louis FJ, Bickii J, Ringwald C, Daban A, Ravinet L. Efficacy and tolerance in adults of a short (3 days) course of quinine for uncomplicated falciparum malaria. *Ann Soc Belg Med Trop* 1995 ;75(2):141-3.
- ³ Luxemburger C, Nosten F, Shotar, Raimond D, Chongsuphajaisiddhi T, White NJ. Oral artesunate in the treatment of uncomplicated hyperparasitemic falciparum malaria. *Am J Trop Med Hyg* 1995; **53**: 522-525
- ⁴ Luxemburger C, Kyaw Ley Thew, White NJ, Webster HK, Kyle DE, Maelankiri L, Chongsuphajaisiddhi T, Nosten F. The epidemiology of malaria in a Karen population on the western border of Thailand. *Trans R Soc Trop Med Hyg* 1996; 90: 105-111.
- ⁵ Luxemburger C, Ricci F, Nosten F, Raimond D, Saw Bathet, White NJ. The epidemiology of severe malaria in an area of low transmission in Thailand. *Trans R Soc Trop Med Hyg* 1997; **91**: 256-262.
- ⁶ Warrell DA, Molyneux ME and Beales PF, Severe and Complicated Malaria, Second Edition, *Transactions of the Royal Society of Tropical Medicine and Hygiene*, Vol 84, Supp 2.
- ⁷ World Health Organization. Severe falciparum malaria. *Trans R Soc Trop Med Hyg* 2000; 94 Suppl 1: S1/36.
- ⁸ Navaratman V, Mordi MN, Mansor SM. Simultaneous determination of artesunic acid and dihydroartemisinin in blood plasma by high performance liquid chromatography for application in clinical pharmacological studies. *J Chromatography B* 1997; 692: 157-162
- ⁹ Batty KT, Thu LTA, Davis TME, et al. A pharmacokinetic and pharmacodynamic study of intravenous vs oral artesunate in uncomplicated falciparum malaria. *Br J Clin Pharmacol* 1998; 45: 123-129.

Chapter 4:**Therapeutic efficacy****Study patients**

Between 21 March and 6 June 1998, 47 patients were admitted to the study. Three initial patients in the study were “dummy” patients who were treated with parenteral quinine (IM or IV, depending on disease severity) and monitored as if they were enrolled in the study. These patients were not included in the analysis as they were not randomised.

No accurate record was kept of patients screened as many patients were excluded from the study (e.g. as a result of prior treatment, age < 16 years, pregnancy or uncomplicated malaria) by those who would refer patients into the study, without completing screening forms. Three patients were formally screened (as they were expected to meet inclusion and exclusion criteria), but two were found to be ineligible (prior use of antimalarials (n=1), pregnant (n=1), and one had severe malaria (after study arm for severe malaria was discontinued). One patient (M98) was randomised but then admitted recent prior use of an antimalarial, so was withdrawn before administration of study drug. This patient was thus not included in the analysis.

Defining features of disease severity

Patients frequently (67%) had more than one defining feature of moderately severe malaria. Inability to walk was the most frequent (91%) defining feature among patients with moderately severe disease, while clinical jaundice was the most frequent (73%) defining feature among patients with severe malaria. No patients presented with hypoglycaemia or shock. Three patients presented with a decreased level of

consciousness (2 in Group 3 (GCS = 12 and 14) and 1 in Group 4 (GCS = 8). Hepatomegaly was found in 11 patients and splenomegaly in 10 patients.

History of presenting complaint and prior treatment

On average, patients had been symptomatic for 3 days at the time of presentation (Table 4.1). Patients who had been recorded as symptomatic for greater than 5 days on the screening form (which did not stipulate number of days beyond this point) were assumed to have been symptomatic for 6 days. All patients had a history of fever. One quarter of patients had taken prior antimalarial treatment, and a similar proportion had taken "traditional" medication, as specified in Table 4.1. The more prevalent prior antimalarial use in the artesunate groups did not reach significance among those with moderately severe malaria ($p=0.154$, Fischer's exact test), nor among those with severe malaria ($p=0.18$, Fischer's exact test). Over one half of patients had taken an antipyretic, and prior antipyretic use was significantly more common in Group 1 compared with Group 2 ($p = 0.032$, Fischer's exact test). Rectal administration of commercial shoe polish was the most frequent (67%) "traditional" medication.

Protocol deviations

Protocol deviations occurred in four patients. In three patients (M62, M64 and M85) intravenous quinine rescue therapy was indicated due to a fractional parasitaemia greater than 60% at 12 hours. One patient was not given rescue therapy as the 12 hour result was obtained at the same time as that of 24 hours, which showed a parasitaemia of less than 60% of baseline. A second patient received rescue therapy late, and the third was rescued with oral rather than IV quinine, as this patient was clinically considered too well to receive IV quinine. The last protocol violation was a patient (M50) who had received oral SP, in error, at time 0 instead of at 24 hours.

Table 4.1: Duration of symptoms and prior treatment by treatment group

| | ALL (N=46) | GROUP 1 (n=27) | GROUP 2 (n=8) | GROUP 3 (n = 5) | GROUP 4 (n = 6) |
|--|---------------|-------------------|------------------|--------------------|--------------------|
| Duration of symptoms [median days] (range) | 3 (1 – 6) | 3 (1 – 6) | 2.5 (1– 5) | 4 (2 – 6) | 2.5 (1 - 6) |
| Prior anti-malarial, n (%) | | | | | |
| None | 35 (76%) | 19 (70%) | 8 (100%) | 2 (40%) | 6 (100%) |
| Antimalarial unknown | 7 (15%) | 4 (15%) | 0 (0%) | 3 (60%) | 0 (0%) |
| SP plus primaquine | 3 (7%) | 3 (11%) | 0 (0%) | 0 (0%) | 0 (0%) |
| SP plus unknown | 1 (2%) | 1 (4%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Prior anti-pyretic n (%) | | | | | |
| Paracetamol | 20 (43%) | 16 (59%) | 2 (25%) | 2 (40%) | 0 (0%) |
| Aspirin | 5 (11%) | 3 (11%) | 0 (0%) | 0 (0%) | 2 (33%) |
| Paracetamol / "compral" | 1 (2%) | 1 (4%) | 0 (0%) | 0 (0%) | 0 (0%) |
| None | 20 (43%) | 7 (26%) | 6 (75%) | 3 (60%) | 4 (67%) |
| Other treatment, n (%) | | | | | |
| None | 34 (74%) | 21 (8%) | 6 (75%) | 3 (60%) | 4 (67%) |
| Shoe polish PR | 7 (15%) | 4 (15%) | 1 (12.5%) | 2 (40%) | 0 (0%) |
| Shoe polish PR / herbal | 1 (2%) | 1 (4%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Herbal emetic tea | 1 (2%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (17%) |
| Toothpaste PR | 1 (2%) | 1 (4%) | 1 (12.5%) | 0 (0%) | 0 (0%) |
| Liquid soap enema | 1 (2%) | 1 (4%) | 0 (0%) | 0 (0%) | 0 (0%) |
| Oral purgative | 1 (2%) | 0 (0%) | 0 (0%) | 0 (0%) | 1 (17%) |

Measurements of treatment compliance

In-patient treatment was fully supervised by the responsible team of nurses and clinicians. Plasma drug levels were obtained from all patients enrolled. In one patient with severe malaria (S26) neither artesunate nor its active metabolite were detected at any time point (0-8 hours). Pharmacokinetic results are presented in Chapter 5. Patients were provided with information to encourage them to remain compliant with follow up treatment after discharge, and were questioned regarding compliance at follow up visits.

Efficacy evaluation*Baseline Characteristics*

There was a slight male predominance (25:21), and patients were aged between 16 and 60 years. Table 4.2 summarise patient baseline characteristics by group. Treatment groups 1 and 2 were comparable for these baseline features ($p > 0.10$), with the exception of GGT, which was higher in group 2 ($p = 0.028$, Mann Whitney U). Treatment groups 3 and 4 were comparable for most baseline parameters, although total bilirubin was significantly lower in group 3 ($p = 0.047$, Mann Whitney U).

Table 4.2: Selected baseline clinical and laboratory parameters by treatment group.

| Variable | GROUP 1 (n=27) | GROUP 2 (n=8) | GROUP 3 (n = 5) | GROUP 4 (n = 6) |
|---|--------------------------------|---------------------------------|-------------------------------|----------------------------------|
| Male: Female | 16:11 | 2:6 | 3:2 | 4:2 |
| Age (years) (mean, CI) | 32 (27-38) | 28 (19 - 37) | 45 (30 - 59) | 30 (18-41) |
| Oral Temp (°C) (mean, CI) | 38.7 (38.2-39.2) | 38.5 (37.4– 39.5) | 38.0 (35.4 – 40.5) | 39.2 (36.5 – 41.9) |
| Weight (kg) (mean, CI) | 62 (58 – 66) | 62 (47 – 76) | 65 (53 – 76) | 65 (54 – 76) |
| Pulse (/min) (mean, CI) | 89 (85 - 92) | 93 (85 - 102) | 84 (76 - 91) | 80 (67 – 92) |
| Resp. rate (/min) (mean, CI) | 24 (22 – 26) | 24 (19 - 29) | 21 (19 – 22) | 22 (16 – 27) |
| Systolic BP (mmHg) (mean, CI) | 106 (100 - 112) | 109 (99 – 118) | 100 (82 - 122) | 105 (96 - 114) |
| Parasitaemia (/ μ l) Median, IQR | 56 480 (26 536- 126 000) | 58 340 (45 110 - 118 230) | 23 000 (5580 – 177 593) | 113 793 (49 683 – 459 600) |
| Albumin (G/L) (mean, CI) | 37 (35 – 39) | 37 (33 – 42) | 33 (29 - 37) | 36 (28 - 44) |
| GGT (IU/L) (median, range) | 19 (3 – 429) | 35* (25 – 104) | 51 (23 – 59) | 88 (11 – 300) |
| C-reactive protein (ng/L) | 99 (77 – 121) | 97 (49 – 144) | 173* (153 – 192) | 129 (98 – 161) |
| Total Bilirubin (μ mol/l) (median, range) | 19 (9 – 64) | 22 (7 - 43) | 27 (15 - 63) | 65* (37 - 136) |

| | | | | |
|------------------------------|---------------|---------------|---------------|---------------|
| Platelets ($\times 10^9$) | 78 | 38.5 | 56 | 93 |
| (mean, CI) | (29 - 187) | (27 - 223) | (23 - 110) | (20 - 104) |
| Glucose (mmol/L) | 7.2 | 7.7 | 9.6 | 6.7 |
| (mean, CI) | (5.5 - 12.3) | (5.2 - 10.6) | (5.9 - 10.3) | (4.1 - 8.1) |
| Lactate (mmol/L) | 2.5 | 2.2 | 4.6 | 3.4 |
| (mean, CI) | (2.3 - 2.8) | (1.5 - 2.9) | (2.1 - 7.2) | (1.8 - 5.0) |
| Haematocrit (g/dl) | 39.1 | 33.9 | 28.1 | 34.2 |
| Mean, CI | (36.2 - 41.9) | (30.2 - 37.6) | (14.1 - 42.0) | (24.8 - 43.5) |
| Base deficit | 0.72 | 2.17 | -4.8 | -2.6 |
| (mean, CI) | (-0.4 - 1.9) | (0.6 - 4.9) | (-11.6 - 2.0) | (-4.9 - -0.4) |
| Urea : Creatinine ratio | 0.04 | 0.04 | 0.04 | 0.05 |
| (median, range) | (0.03 - 0.08) | (0.02 - 0.05) | (0.04 - 0.05) | (0.03 - 0.06) |
| Creatinine $\mu\text{mol/l}$ | 140 | 131 | 194 | 146 |
| (mean, CI) | (83 - 234) | (94 - 169) | (141 - 446) | (111 - 489) |

* Statistically significantly different from comparator group of same disease severity.

Data sets analysed

There were two data sets analysed, namely an Intention to treat analysis of all patients who received study medication, and an efficacy analysis data set where patients who received additional antimalarial medication, either in error or as "rescue medication" as a result of inadequate clinical or parasitological response, were excluded.

Intention To Treat Analysis

All patients (46) receiving at least one dose of study medication were included in the intention to treat analysis.

Parasitological outcome

i) **Primary endpoint: Fractional reduction in Parasitaemia at 24 hours**

All patients had a decrease in parasitaemia over 24 hours. The median proportion or fraction of baseline parasitaemia at 24 hours was 0.6% in Group 1 (range 0 – 41%) and 16.6% in Group 2 (range 2 - 75%). The fractional reduction in parasitaemia was significantly greater in Group 1 than in group 2 (Mann-Whitney $p = 0.002$), although this difference was not significant between groups 3 and 4 ($p=0.273$) This reduction reflects the effect of a single rectal 10 mg / kg dose of artesunate in group 1 compared with intramuscular quinine administered at 0, 4, 12 and 20 hours in group 2. Logistic regression analysis of the Fractional Reduction in parasitaemia at 24 hours (Table 4.3) showed that this was decreased with prior rectal treatment ($p = 0.003$), a decreased baseline Blantyre coma score ($p = 0.036$), and a decreased baseline serum albumin level ($p = 0.018$). Other parameters that approached significance are "treatment group" (Both artesunate treatment groups 1 and 3 vs. both control groups 2 and 4 $p = 0.08$) and prior antimalarial treatment ($p = 0.093$).

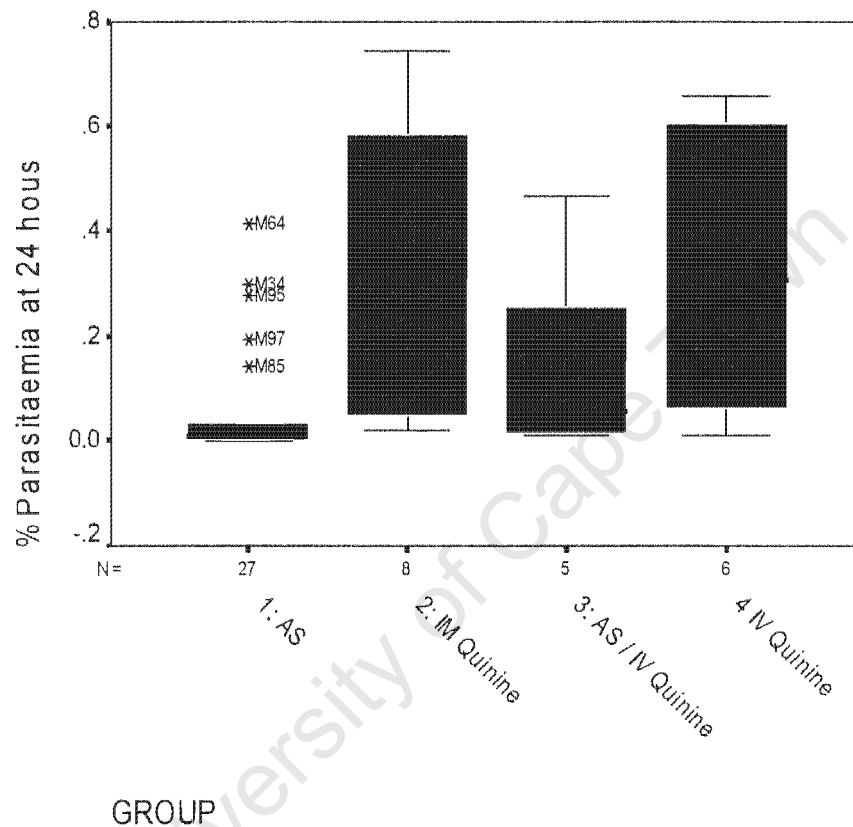
Table 4.3: Logistic regression analysis of the Fractional Reduction in parasitaemia at 24 hours.

| | Beta-Coefficient | Std. Err. | p-value | 95% CI |
|---------------------|------------------|-----------|---------|---------------|
| Treatment group | 11.48 | 6.55 | 0.08 | -1.36 - 24.33 |
| Prior rectal Rx | 1.65 | 0.55 | 0.003 | 0.56 - 2.73 |
| BCS-baseline | -2.74 | 1.31 | 0.036 | -5.31 - -0.17 |
| Albumin-baseline | -0.11 | 0.04 | 0.018 | -0.20 - -0.02 |
| Prior antmalaria Rx | -1.97 | 1.18 | 0.093 | -4.28 - 0.33 |

NOTE: A beta-coefficient of 0 is equivalent to an Odds Ratio of 1.

Figure 4.1:

Boxplots of Intention to Treat analysis of the percentage of baseline parasitaemia at 24 hours by treatment group



Descriptive and non-parametric statistics

Tables 4.4a and 4.4b summarise the descriptive and non-parametric statistics of parasite density related efficacy variables. Parasite clearance times have been right censored where patients left the study (owing to transfer to referral hospital or death) prior to parasite clearance.

Table 4.4a: Intention to Treat analysis of the Parasite density over time by Treatment Group (Median, interquartile range) for Group 1 and Group 2 (Moderately severe malaria)

| | PR AS N = 27 | IM Quinine N = 8 | Significance (p) (Mann Whitney U) |
|---------------------------|------------------------------|------------------------------|--------------------------------------|
| Baseline parasitaemia | 56 480 (26 536 – 126 000) | 58 340 (45 110 – 118 230) | 0.666 |
| Parasitaemia (12hours) | 5 560 (1 295 – 25 840) | 35 160 (22 720 – 80 400) | 0.025* |
| 12 hour % parasitaemia | 12.8% (3.2 – 33.8%) | 64.7% (35.5 – 85.5%) | 0.002* |
| Parasitaemia (24 hours) | 400 (120 – 1 000) | 17 320 (4 980 – 37 403) | 0.004* |
| 24 hour % parasitaemia | 1% (0 – 3%) | 27.5% (5 – 58.5%) | <0.001* |
| PCT (median, IQR) | 48h (36 - 54) | 60h (35 - 86) | 0.0791 (Log Rank Test) |
| Censored PCT observations | 1 | 0 | - |

* Statistically significantly different from comparator group

Table 4.4b: Intention to Treat analysis of the Parasite density over time by Treatment Group (Median, interquartile range) for Group 3 and Group 4 (Severe Malaria)

| | Group 3 N = 5 | Group 4 N = 6) | Significance (p value) (Mann Whitney U) |
|---------------------------|-----------------------------|-------------------------------|--|
| Baseline parasitaemia | 23 000 (5 580 – 177 593) | 113 793 (49 682 – 459 600) | 0.14 |
| Parasitaemia (12hours) | 5714 (2 260 – 136 000) | 215 580 (29 682 – 443 928) | 0.10 |
| 12 hour % parasitaemia | 52.9% (26.3 – 93.2%) | 101.3% (56.9 – 180%) | 0.10 |
| Parasitaemia (24 hours) | 1 640 (361 – 17 318) | 26 074 (7 620 – 115 315) | 0.068 |
| 24 hour % parasitaemia | 5.5% (1.1 – 36.0%) | 30.7% (0.5 – 62%) | 0.273 |
| PCT (Median, IQR) | 48h (42 - 69) | 69h (51 - 89) | 0.0901 (Log Rank Test) |
| Censored PCT Observations | 1 | 4 | - |

Proportion and number of patients with 50%, 90% reduction and clearance of parasitaemia at 12 and 24hours (Table 4.5)

≥50% reduction of absolute parasite count

At 12 and 24 hours the proportion of patients with greater than 50% reduction of absolute parasite count from baseline was comparable between Groups 1 and 2 and between groups 3 and 4.

$\geq 90\%$ reduction of absolute parasite count

At 12 hours, 11 of 27 patients had greater than 90% reduction of absolute parasite count from baseline in group 1, which is a significantly greater proportion (Fischer's Exact Test, $p = 0.037$) than that of group 2 where none of the 8 patients had achieved this. At 24 hours, 22 of 27 patients in Group 1 had greater than 90% reduction of parasite count from baseline, which again reached a significance of $p = 0.027$ (Fischer's Exact Test) when compared with group 2. The differences between groups 3 and 4 did not reach significance.

Parasite clearance at 12 and 24 hours

The proportion of patients achieving parasite clearance at 12 hours did not differ significantly between Group 1 vs 2 and Group 3 vs 4. However, at 24 hours the proportion achieving parasite clearance was significantly higher in Group 1 (Fisher's exact test, $p = 0.037$). This difference was not significant between Groups 3 and 4 at 24 hours.

Parasite Clearance Time

Parasite clearance time was defined as the time to the first of two negative thick smears. The PCT by treatment group were summarised using Kaplan Meier Survival Curves, and compared with the Log Rank Test. The PCT was significantly faster ($p = 0.0084$) following rectal artesunate (Group 1) than IM quinine (Group 2) (Figure 4) although this did not achieve significance between Groups 3 and 4 ($p = 0.0901$).

Figure 4.2: Kaplan Meier Survival Curves of parasitaemia in patients with moderately severe malaria by treatment group (Intention to Treat analysis).

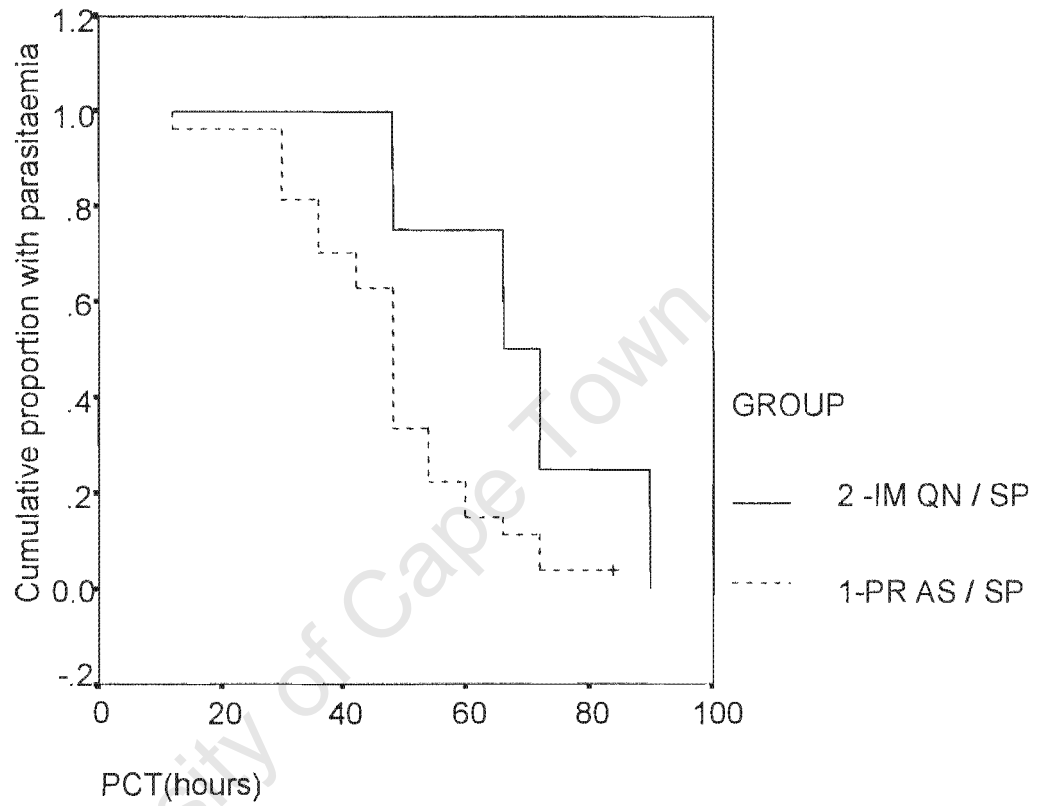


Table 4.5: Intention to Treat analysis of the Percentage (and number) of patients with 50%, 90% reduction and clearance of parasitaemia from baseline at 12 and 24 hours

Table 4.5a: Groups 1 and 2 – Moderately severe malaria

| Number of patients | GROUP 1 N = 27 | GROUP 2 N = 8 | SIGNIFICANCE (FISHER'S EXACT) |
|-----------------------|-------------------|------------------|----------------------------------|
| ≥50% reduction at 12h | 96% (26) | 38% (3) | 0.117 |
| ≥90% reduction at 12h | 41% (11) | 0 | 0.037* |
| Cleared at 12h | 4% (1) | 0 | 1.0 |
| ≥50% reduction at 24h | 100% (27) | 63% (5) | 0.433 |
| ≥90% reduction at 24h | 81% (22) | 38% (3) | 0.027* |
| Cleared at 24h | 11% (3) | 0 | 0.037* |

* Statistically significantly different from comparator group

Table 4.5b: Groups 3 and 4 – Severe malaria

| Number of patients | GROUP 3 N = 5 | GROUP 4 N = 6 | SIGNIFICANCE (FISHER'S EXACT) |
|-----------------------|------------------|------------------|----------------------------------|
| ≥50% reduction at 12h | 40% (2) | 17% (1) | 0.545 |
| ≥90% reduction at 12h | 0 | 0 | - |
| Cleared at 12h | 0 | 0 | - |
| ≥50% reduction at 24h | 100% (5) | 50% (3) | 0.182 |
| ≥90% reduction at 24h | 60% (3) | 33% (2) | 0.567 |
| Cleared at 24h | 0 | 0 | - |

Fever clearance times (Table 4.6)

Defervescence occurred with the four treatment regimens. There was no significant difference in reduction in fever in 24 hours (delta T24) between groups 1 and 2 (t test $p=0.38$), nor between groups 3 and 4 (t test $p = 0.767$). Mean body temperature at 24 hours did not differ significantly between Group 1 and Group 2 (t test $p = 0.575$), nor between Group 3 and Group 4 (t test $p = 0.575$). Moreover, there was no difference in the number of patients with fever $> 37.5^{\circ}\text{C}$ at 24 hours between groups 1 and 2 (Fisher's exact test $p = 0.41$), nor between groups 3 and 4 (Fisher's exact test $p = 1.0$). Temperature at 24 hours was not recorded for 1 patient (M50) in Group 1. Fever clearance time was significantly more rapid in Group 2 compared to Group 1 (Log Rank test $p = 0.0394$), and comparable between group 3 and 4 (Log Rank test $p = 0.3042$). Two patients (M12 – Group 1 and S24 – Group 3) were excluded from the analysis of FCT as they were apyrexial on admission.

Table 4.6: Intention to Treat analysis of the effect of treatment on fever related parameters (reported as Mean, 95% Confidence Intervals unless specified)

| | Delta T24 | T @ 24h | $>37.5^{\circ}\text{C}$ @24hours | FCT (hrs) Median, IQR | Right censored |
|-------------------|--------------------------|-----------------------|-------------------------------------|--------------------------|-------------------|
| Group 1 N = 27 | -1.55 (-2.05 – -1.05) | 37.2 (36.9 – 37.5) | 44% (8) | 36 (22 – 66) | 6 |
| Group 2 N = 8 | -1.09 (-2.30 – 0.12) | 37.4 (36.7 – 38.1) | 50% (4) | 23* (14.5 – 30) | 1 |
| Group 3 N = 5 | -1.12 (-2.47 – 0.23) | 37.0 (36.2 – 37.8) | 20% (1) | 54 (13.5 – 86) | 0 |
| Group 4 N = 6 | -1.43 (-3.59 – 0.73) | 37.4 (36.0 – 38.7) | 33% (2) | 44 (6.25 – 79.5) | 4 |

* Statistically significantly different from comparator group

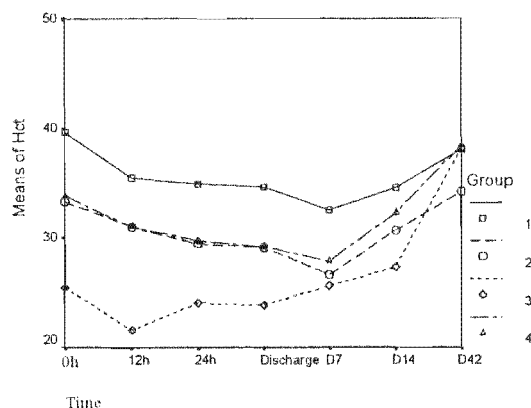
Efficacy: laboratory evaluations

Changes in laboratory parameters are also described in Chapter 6: Drug Safety.

The effects of treatment over time on HCT, WCC, platelets, glucose, lactate, pH, HCO₃, Base deficit, Sodium, Potassium, Urea, Creatinine, Bilirubin and Calcium were tested (at baseline, 12 hours, 24 hours, discharge and on days 7, 14 and 42). These generally did not change significantly over time, and changes generally reflected a normalisation of values.

A random effects regression model showed no significant ($p > 0.10$) effects of time or treatment on haematocrit overall, in patients with moderately severe, or severe, malaria. There was no interaction between treatment and time. However, there was a significant decrease in haematocrit in patients with moderately severe malaria ($p = 0.006$) but not severe malaria ($p = 0.218$) over the initial 24 hours. Treatment groups did not differ significantly, although the decrease tended to be more rapid in patients with severe malaria treated with rectal artesunate and IV quinine (Group 3) compared with those treated with IV quinine alone ($p = 0.070$).

Figure 4.3: Intention to Treat analysis of the Mean haematocrit at 0, 24 hours, discharge, day 7, 14 and 42) by treatment group (Group 1: AS / SP; Group 2 IM Quinine / SP; Group 3: AS / IV Q



The decrease in serum lactate over time was not significant, in patients with either moderately severe or severe malaria. As expected, serum lactate was significantly higher in patients with severe malaria ($p=0.002$). One patient (M96, Group 1) whose lactate was recorded as 5.9 mmol/L at 24 hours, recovered uneventfully without rescue medication.

One patient (M53 Group 1) developed mild jaundice clinically at 24 hours, which although not confirmed on laboratory evaluation resulted in the patient being withdrawn and treated with quinine. This clinical jaundice had resolved by day 3.

Platelets increased significantly over time in all patients with moderately severe malaria ($p = 0.001$), as would be expected in patients receiving effective antimalarial treatment, but not in patients with severe malaria ($p=0.678$). This effect did not differ between treatment groups ($p=0.39$ in moderately severe and 0.948 in severe malaria).

Vital signs

Pulse, SBP, and Respiratory Rate were monitored over time. Across all groups there was a statistically significant overall decrease in Pulse Rate (Wilks Lambda $p = 0.01$), and Respiratory Rate (Wilks Lambda $p = 0.008$) over time. There was a significant overall increase in Systolic Blood Pressure (Wilks Lambda $p = 0.004$), over time. The differences between Groups 1 and 2 and between Groups 3 and 4 did not reach significance at any time point. As expected with effective treatment, these showed a normalisation of values.

Case Fatality Rates

No deaths occurred in Groups 1 and 2. One patient (S24) died in Group 3, and 2 patients (S31 and S23) died in Group 4. Details of each death are provided in Chapter 6. The difference in the Case Fatality Rates between Group 3 (20%) and Group 4 (33%); was not significant (Fisher's exact test $p = 1.0$). All 3 deaths were considered consistent with death from severe and complicated malaria.

Progression to severe and complicated malaria

No patients in Groups 1 and 2 developed WHO criteria¹ for the diagnosis of severe and complicated malaria, although one patient (M53 Group 1) developed mild jaundice diagnosed clinically at 24 hours, which was not confirmed on laboratory evaluation of total bilirubin. This jaundice had resolved by day 3. Another patient (M96, Group 1) whose lactate was recorded as 5.9 mmol/L at 24 hours, recovered uneventfully without rescue medication.

Time to return to per os status

Once patients were able to eat, drink and walk, they were considered "per os". The differences in time to per os status between Groups 1 and 2 (Log Rank test $p = 0.5521$), and between groups 3 and 4 (Log Rank test $p = 0.4248$) were not significant. All patients with moderately severe malaria received oral administration of SP at 24 hours. Time to return to per os status was right censored in 4 patients (1 in group 3, 3 in Group 4), who died or were transferred to a referral hospital for intensive care without returning to a "per os" status.

¹ World Health Organization(2000). Severe *falciparum* malaria. Trans R Soc Trop Med Hyg; 94 (Suppl 1)

Final treatment outcome

No patients were found to be *P. falciparum* smear positive between day 7 and day 42, although this cannot be excluded in patients lost to follow up. Three patients were lost to follow up (M37, M85 and M97); 2 of which were in Group 1 and 1 in Group 2. PCR samples were missing on 1 of 3 follow up visits in 3 patients (M19, M54, M64); these patients were smear and PCR negative on both of the other follow up visits.

Seven patients had positive PCR results, despite negative smears, suggesting very low density parasitaemia or gametocytaemia. PCR was used to differentiate recrudescence from re-infection. PCR suggested recrudescence in 5 patients (2/25 in Group 1, 2/7 in Group 2 and 1/4 in Group 3), and non-recrudescence in 2 further patients (1/25 in Group 1, 1/4 in Group 4). In one patient (M45) no PCR amplification was obtained at 3 of 3 loci, which precluded interpretation. Since all patients had received at least 2 antimalarial drugs, and SP was the only drug administered in curative therapeutic dosages, final treatment outcome is most likely to reflect resistance to SP.

Two patients (M17 and S46) presented to the primary health care clinic on day 10 and day 30 respectively, and were treated with sulfadoxine / pyrimethamine after testing ICT positive. Since smears and PCR before and after re-treatment were negative, these might be considered false positive ICT tests. One patient (M47) received rescue therapy with mefloquine on d7, in addition to AS and SP in error, following identification of gametocytes; this patient was found by PCR on d7, 14 and 42 to have a recrudescence infection.

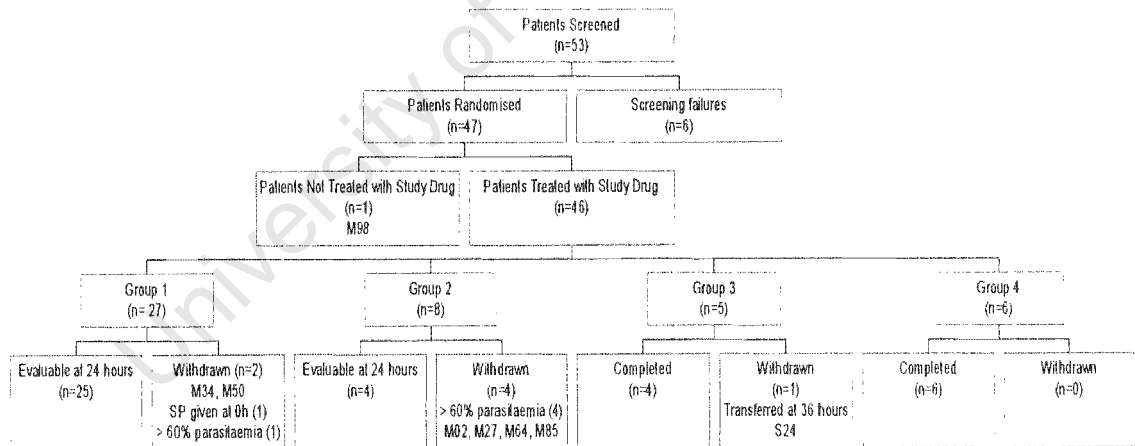
Table 4.7: Intention to Treat analysis of the Smear and PCR results of patients with evidence of persistent parasitaemia, summary of treatment administered and interpretation of final treatment outcome (+ positive, - negative, NK not known)

| Code | Group | Smear d7 | PCRd7 | Smear-d+4 | PCR d+4 | Smear-d42 | PCR d42 | Directly observed treatment (number of doses administered) | Interpretation |
|------|-------|----------|-------|-----------|---------|-----------|---------|--|---|
| M02 | 2 | - | + | - | - | - | - | IM Quinine (2); IV Quinine (2); PO Quinine (8); PO SP (1) | PCR Recrudescence d7 |
| M17 | 1 | NK | - | - | - | - | - | PR Artesunate (1); PO SP (2) | SP retreatment at clinic as rapid test pos. |
| M27 | 2 | - | + | - | + | - | - | IM Quinine (2); IV Quinine (2); PO SP (1) | PCR Recrudescence day 7, 14 |
| M34 | 1 | - | + | - | - | - | - | PR Artesunate (1); IV Quinine (1); PO Quinine (11); PO SP (1) | PCR Non recrudescence day 7 |
| M45 | 1 | - | + | - | - | - | - | PR Artesunate (1); PO SP (1) | PCR positive. No amplification. |
| M47 | 1 | - | + | - | + | - | - | PR Artesunate (1); PO SP (1); PO Mefloquine (1) d7 | PCR Recrudescence day 7 and d14. |
| M49 | 1 | - | + | - | + | - | + | PR Artesunate (1); PO SP (1) | PCR Recrudescence day 7, 14, 42 |
| S04 | 3 | - | + | - | + | - | - | PR Artesunate (1); IV Quinine (2); PO Quinine (10) PO SP (1) | PCR Recrudescence d7, 14 |
| S30 | 4 | - | + | - | - | - | - | IV Quinine (9); PO Quinine (5); PO SP (1) | PCR Non recrudescence d7 |
| S46 | 4 | NK | NK | - | - | - | - | IV Quinine (5); Cont. at referral hospital. SP (1) | Received SP on d30 – ICT pos. |

Efficacy Data Set

Of the 46 initially evaluable patients, 1 patient was withdrawn between 0-12hours (M50, Group 1) as a result of early (0 hours) administration of sulfadoxine / pyrimethamine in error. A further 5 patients were withdrawn between 12 and 24 hours as a result of administration of quinine rescue therapy for parasite density greater than 60% of baseline at 12 hours (Group 1: M34; Group 2: M02, M27, M62 and M64). Between 24 and 36 hours a further patient was withdrawn due to the development of mild jaundice clinically (not confirmed by laboratory bilirubin levels) and consequent treatment with additional quinine. (All 46 patients receiving study treatment were included in the safety analysis.)

Figure 4.4: Patient disposition (efficacy data set)



Parasitological outcome

Primary endpoint: Fractional reduction in Parasitaemia at 24 hours

All patients had a decrease in parasitaemia over 24 hours. The median proportion or fraction of baseline parasitaemia at 24 hours was 0.6% in Group 1 (range 0 – 28%) and 31% in Group 2 (range 3 - 60%). The fractional reduction in parasitaemia was significantly greater in Group 1 than in group 2 (Mann-Whitney $p = 0.007$). This reduction reflects the effect of a single rectal 10 mg / kg dose of artesunate in group 1 compared with intramuscular quinine administered at 0, 4, 12 and 20 hours in group 2. This difference in fractional reduction in parasitaemia at 24 hours was not significant between groups 3 (median 5.5%) and 4 (median 30.7%) - Mann Whitney $p = 0.273$.

Descriptive and non parametric statistics

Table 4.8 summarises the descriptive and non-parametric statistics of parasite density related efficacy variables.

Table 4.8: Per protocol analysis of Parasite density over time by Treatment Group
(Median, interquartile range)

Table 4.8a: Group 1 and Group 2 – Moderately severe malaria

| | Group 1 | Group 2 | Significance (p value) (Mann Whitney U) |
|----------------------------|------------------------------|------------------------------|---|
| Baseline parasitaemia | 56 480 (26 536 - 126 000) | 58 340 (40 564 – 129 480) | 0.666 |
| Parasitaemia (12hours) | 4 829 (1 171 – 22 960) | 35 160 (22 080 – 100 200) | 0.017* |
| 12 hour % parasitaemia | 11.9% (3 - 33%) | 64.7% (32 – 91%) | 0.001* |
| Parasitaemia (24 hours) | 320 (76 - 990) | 19 160 (803 – 72 231) | 0.076 |
| 24 hour % parasitaemia | 0.6% (0.1 – 2%) | 31% (4-59%) | 0.007* |
| PCT | 48h 36 – 54h | 60 34.5 – 85.5 | 0.2032 (Log Rank Test) |
| Censored obs. PCT | 1 | 0 | - |

* Statistically significantly different from comparator group

Table 4.8b: Group 3 and Group 4 (Severe Disease)

| | Group 3 N = 5 | Group 4 N = 6) | Significance (Mann Whitney U) |
|----------------------------|-----------------------------|-------------------------------|----------------------------------|
| Baseline parasitaemia | 23 000 (5 580 – 177 593) | 113 793 (49 682 – 459 600) | 0.14 |
| Parasitaemia (12hours) | 5714 (2 260 – 136 000) | 215 580 (29 682 – 443 928) | 0.10 |
| 12 hour % parasitaemia | 53% (26 – 93%) | 101% (57 – 180%) | 0.10 |
| Parasitaemia (24 hours) | 1640 (362 – 17 317) | 26 074 (7 620 – 115 315) | 0.068 |
| 24 hour % parasitaemia | 5.5% (1 – 36%) | 30.7% (5 – 62%) | 0.273 |
| PCT (hours) | 48 (42 - 69) | 69 (51 – 88.5) | 0.0901 (Log Rank Test) |
| Censored obs. PCT | 1 | 4 | - |

Proportion and number of patients with 50%, 90% reduction and clearance of parasitaemia at 12 and 24hours

≥50% reduction of absolute parasite count (Table 4.9)

At 12 and 24 hours the proportion of patients with greater than 50% reduction of absolute parasite count from baseline was significantly a greater proportion (Fisher's exact $p = 0.001$ and $p = 0.015$ respectively) in Group 1 compared with Group 2. The differences between groups 3 and 4 were not significant ($p=0.54$ at 12 hours and $p=0.18$ at 24 hours)

≥90% reduction of absolute parasite count (Table 4.9)

At 12 hours, 11 of 26 patients had greater than 90% reduction of absolute parasite count from baseline in group 1, which is a significantly greater proportion (Fischer's Exact Test, $p = 0.034$) than that of group 2 where none of the 8 patients had achieved 90% parasite reduction. At 24 hours, 22 of 25 patients in Group 1 had greater than 90% reduction of parasite count from baseline, which was comparable with this reduction in 50% of those in Group 2 ($p = 0.127$ Fischer's Exact Test). The differences between groups 3 and 4 were not significant ($p = 0.57$ at 24 hours).

Parasite Clearance Time

The mean parasite clearance time overall was 55 hours (with 6 censored observations). The PCT by treatment group were summarised using Kaplan Meier Survival Curves, and compared with the Log Rank Test. The difference in PCT between Groups 1 and 2 ($p = 0.2032$) and between Groups 3 and 4 ($p = 0.0901$) did not achieve significance.

Table 4.9: Per protocol analysis of the Percentage (and number) of patients with 50%, 90% reduction and clearance of parasitaemia from baseline at 12 and 24 hours

9a: Groups 1 and 2

| | PR AS (N=26) | IM QUININE (N=8) | SIGNIFICANCE (FISHER'S EXACT) |
|-----------------------|-----------------|---------------------|-------------------------------------|
| ≥50% reduction at 12h | 96% (25)* | 38% (3) | 0.001* |
| ≥90% reduction at 12h | 42% (11)* | 0 | 0.034* |
| Cleared at 12h | 8% (2) | 0 | 1.0 |
| ≥50% reduction at 24h | 100% (25)* | 50% (2) | 0.015* |
| ≥90% reduction at 24h | 81% (22) | 50% (2) | 0.127 |
| Cleared at 24h | 44% (11) | 0 | 0.268 |

* Statistically significantly different from comparator group

Table 4.9b: Groups 3 and 4

| Number of patients | PR AS / IV QUININE (N=5) | IV QUININE (N=6) | SIGNIFICANCE (Fisher's Exact) |
|-----------------------|-----------------------------|---------------------|----------------------------------|
| ≥50% reduction at 12h | 40% (2) | 17% (1) | 0.545 |
| ≥90% reduction at 12h | 0 | 0 | - |
| Cleared at 12h | 0 | 0 | - |
| ≥50% reduction at 24h | 100% (5) | 50% (3) | 0.182 |
| ≥90% reduction at 24h | 60% (3) | 34% (2) | 0.567 |
| Cleared at 24h | 0 | 0 | - |

Fever Clearance (Table 4.10)

Defervescence occurred with the four treatment regimens. There was no significant difference in reduction in fever by 24 hours (delta T24) between groups 1 and 2, nor between groups 3 and 4. There was no difference in the number of patients with fever > 37.5°C at 24 between groups 1 and 2 (Fisher's exact $p = 0.568$), nor between groups 3 and 4 (Fisher's exact $p = 1.0$). Fever clearance time was significantly more rapid in Group 2 compared to Group 1 (Log Rank test $p = 0.0367$), and comparable between group 3 and 4 (Log Rank test $p = 0.3042$). Two patients (M12 – Group 1 and S24 – Group 3) were excluded from the analysis of FCT as they were afebrile on admission. Fever clearance times were classified as right censored in patients who were discharged without defervescence.

Table 4.10: Per protocol analysis of the Effect of treatment on fever related parameters

| | Change in temperature: between 0 and 24hours | Temperature (Celcius) @ 24hours | >37.5°C @24h | FCT (hrs) (median, IQR) | Right censored |
|--------------------------|--|---------------------------------|--------------|-------------------------|----------------|
| PR AS | -1.6 (-2.1 – -1.0) | 37.2 (36.9 – 37.5) | 28% (7) | 46 (34 – 58) | 5 |
| IM Quinine | -0.8 (-1.9 – 0.4) | 37.3 (35.8 – 38.8) | 50% (2) | 23* (14 - 31) | 0 |
| PR AS / IV Quinine | -1.1 (-2.5 – 0.2) | 37.0 (36.2 – 37.8) | 20% (1) | 51 (14 – 88) | 0 |
| IV Quinine | -1.4 (-3.6 – 0.7) | 37.4 (36.0 – 38.7) | 33% (2) | 84 (44 – 124) | 4 |

Case Fatality Rates

No deaths occurred in Groups 1 and 2. One patient (S24) died in Group 3, and 2 patients (S31 and S23) died in Group 4. Details of each death are provided in Chapter 6. The difference in Case Fatality Rates between Group 3 (20%) and Group 4 (33%) was not significant (Fisher's exact test $p = 1.0$).

Progression to severe and complicated malaria

No patients in Groups 1 and 2 developed WHO criteria for the diagnosis of severe and complicated malaria, although one patient (M53 Group 1) developed mild jaundice clinically at 24 hours, which was not confirmed on laboratory evaluation. This jaundice had resolved by day 3. Another patient (M96, Group 1) whose lactate was recorded as 5.9 at 24 hours, recovered uneventfully without rescue medication.

Time to return to per os status

Once patients were able to eat, drink and walk, they were considered "per os". Time to return to per os status was right censored in 4 patients (1 in group 3, 3 in Group 4), who died or were transferred to a referral hospital without returning to "per os" status. The differences between Groups 1 and 2 (Log Rank test $p = 0.3647$), and between groups 3 and 4 (Log Rank test $p = 0.4248$) were not significant.

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Chapter 5: Pharmacokinetics of artesunate and dihydroartemisinin

Extent of Exposure

All patients received the closest possible dose to 10mg/kg AS rectally, using 200 and 500 mg suppositories. The mean artesunate dose administered in patients with moderately severe malaria (Group 1) was 9.95 mg/kg (95% CI: 9.86-10.05) and in severe malaria (Group 3) was 10.11 (95% CI: 9.63-10.58).

Pharmacokinetic parameters

Pharmacokinetic parameters were determined for artesunate (AS) and its active metabolite dihydroartemisinin (DHA), as well as for the sum of both artesunate and dihydroartemisinin. As AS is rapidly and completely hydrolysed to DHA (probably by plasma and/or tissue esterases¹), AS is sometimes considered a "prodrug" for DHA. Since artemisinins are tightly membrane bound, a validated methodology for evaluating drug levels at the site of action (within the RBC) has not been established and our results reflect plasma levels.

Samples were analysed for AS and DHA by HPLC equipped with an electro-chemical detector², generally considered the reference technique for pharmacokinetic studies as this has greater sensitivity and specificity than the HPLC-UV detection or bioassay methods³. The reliability and reproducibility of this assay is reported in Appendix 4. All samples were analysed according to GLP (ISO9000 accredited) procedures. Levels below the detectable limit in this study (4.0 ng/ 0.5mL) were assumed to be zero for the determination of pharmacokinetic parameters. The minimum level of quantification of this

assay was 10ng/mL. There were 90/192 AS levels and 134/192 DHA levels above the detectable limit following treatment. Nine AS levels were classified as missing as the "peak spoilt". All AS and DHA levels prior to exposure were zero. Levels reported as "out of range" were included in this analysis as these made up a substantial proportion of the results (7/90 AS concentrations and 23/148 DHA concentrations above the level of detection). In the majority of patients AS and DHA were detectable 1 hour after administration. In four patients only DHA was detected. In one patient with severe malaria, neither AS or DHA were detected at any time point (0-8 hours). AS and DHA plasma concentrations did not appear to decline with time in one and six patients, respectively.

In children, both artesunate and dihydroartemisinin were cleared before 8 hours⁴. As this study in children was the only rectal artesunate PK data available when our study was designed, we assumed that these kinetics would also be seen in adults. Unfortunately, AS and DHA plasma concentrations declined more slowly over time in our non immune adults when compared with children with moderately severe malaria. Consequently our sampling time was inappropriately limited to 8 hours. Elimination half life and $AUC_{0-\infty}$ could not be calculated for AS or DHA due to insufficient data points on the elimination curve.

AUC_{0-8} was calculated using a non-compartmental model in WinNonLin4.0 in this study as the limited data precluded the use of a compartmental model. A one compartment model with first order appearance and elimination kinetics including a lag time was observed to best fit the dihydroartemisinin data in previous studies with more complete data sets. These studies have observed that it is not possible to perform compartmental modelling on AS data^{5,6,7}.

Table 5.1 and Figures 5.1a-c summarise the observed plasma levels for patients with moderately severe (Group 1) and severe malaria (Group 3).

Table 5.1: Observed plasma levels of artesunate and dihydroartemisinin, separately and combined, for patients with moderately severe (Group 1) and severe malaria (Group 3).

| Time | Moderately Severe (n=27) | | | Severe Malaria (n=5) | | |
|------------------------------------|-----------------------------|----------------|------------------|-------------------------|--------------|------------------|
| | Median | (IQR) | N (out of range) | Median | (IQR) | N (out of range) |
| Artesunate | | | | | | |
| 0 | 0 | (0-0) | 27 (0) | 0 | (0-0) | 5 (0) |
| 1 | 88.8 | (0-195.2) | 27 (2) | 0 | (0-107.9) | 5 (0) |
| 2 | 148.3 | (0-265.5) | 24 (2) | 0 | (0-62.1) | 4 (0) |
| 4 | 161.8 | (16.7-297.7) | 24 (2) | 0 | (0-72.7) | 5 (0) |
| 6 | 68.7 | (0-211.7) | 26 (0) | 0 | (0-68.8) | 5 (0) |
| 8 | 0 | (0-157.1) | 26 (1) | 0 | (0-0) | 5 (0) |
| Dihydroartemisinin | | | | | | |
| 0 | 0 | (0-0) | 27 (0) | 0 | (0-0) | 5 (0) |
| 1 | 176.8 | (20.8-435.6) | 27 (3) | 34.8 | (0-97.1) | 5 (1) |
| 2 | 390.2 | (87.5-598.6) | 27 (5) | 63.4 | (29.0-104.0) | 5 (0) |
| 4 | 466.6 | (194.0-889.6) | 27 (5) | 109.6 | (22.5-142.5) | 5 (0) |
| 6 | 343.0 | (87.5-1039.0) | 27 (4) | 70.4 | (0-231.0) | 5 (0) |
| 8 | 86.6 | (0-724.4) | 27 (5) | 0 | (0-142.4) | 5 (0) |
| Artesunate plus dihydroartemisinin | | | | | | |
| 0 | 0 | (0-0) | 0 | 0 | (0-0) | 0 |
| 1 | 232.1 | (49.4-629.7) | 27 (3) | 34.8 | (0-205.0) | 5 (1) |
| 2 | 457.0 | (108.6-806.7) | 27 (5) | 63.4 | (29.0-145.4) | 5 (0) |
| 4 | 579.8 | (213.0-1213.6) | 27 (5) | 115.8 | (22.5-212.1) | 5 (0) |
| 6 | 425.4 | (87.5-1170.2) | 27 (4) | 70.4 | (0-299.8) | 5 (0) |
| 8 | 86.6 | (0-787.2) | 27 (5) | 0 | (0-142.4) | 5 (0) |

Figures 5.1 summarise the observed plasma levels of artesunate and dihydroartemisinin, separately and combined, in moderately severe (Group 1) and severe malaria (Group 3).

Figure 5.1a: Plasma concentrations of artesunate over time

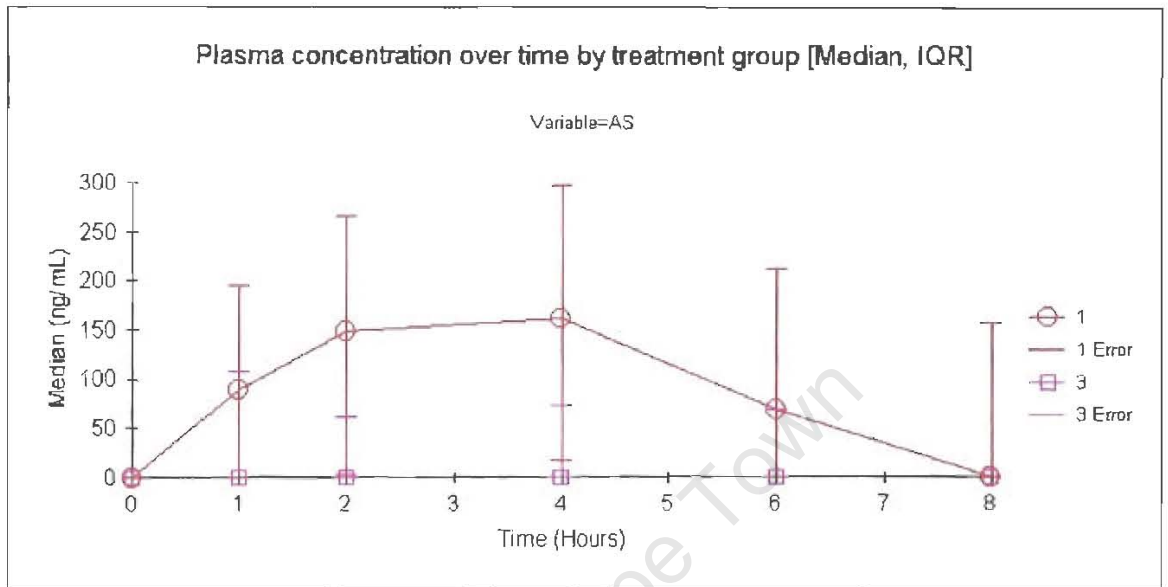


Figure 5.1b: Plasma concentrations of dihydroartemisinin over time

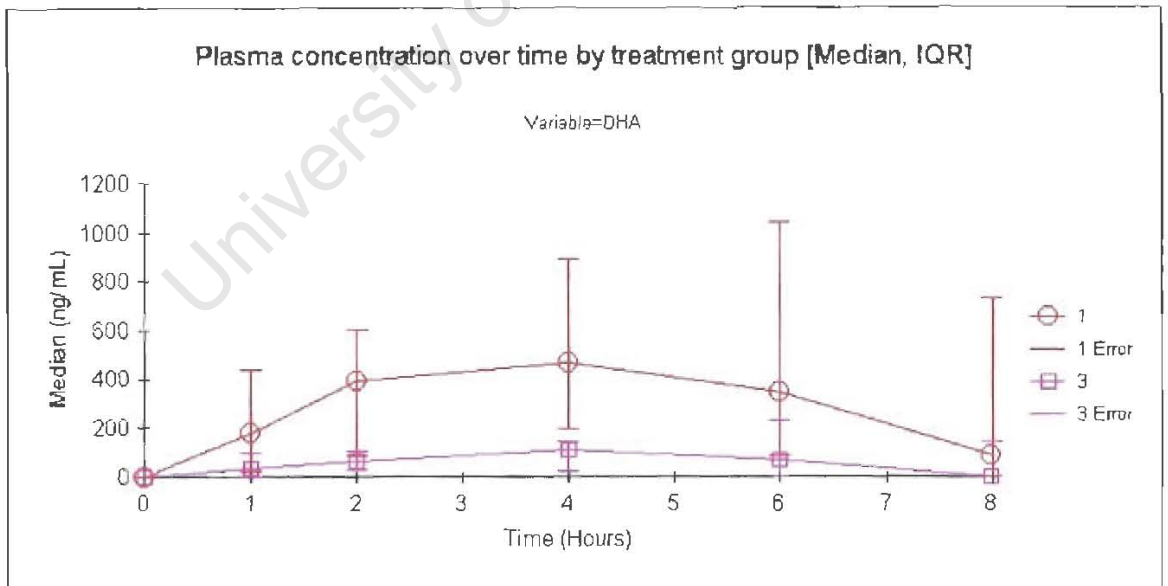
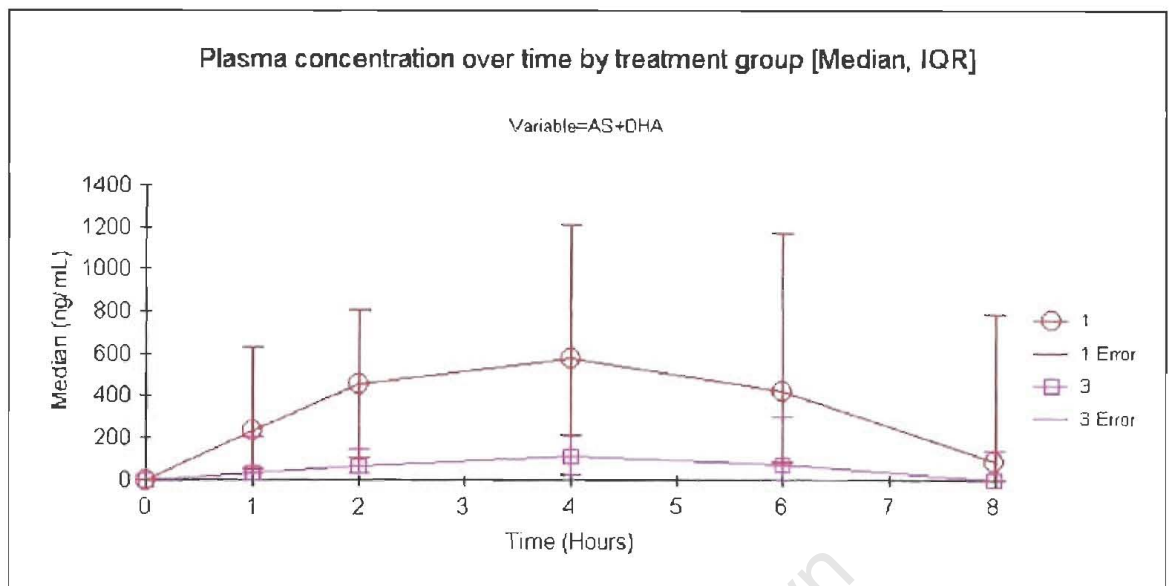


Figure 5.1b: Plasma concentrations of artesunate plus dihydroartemisinin over time



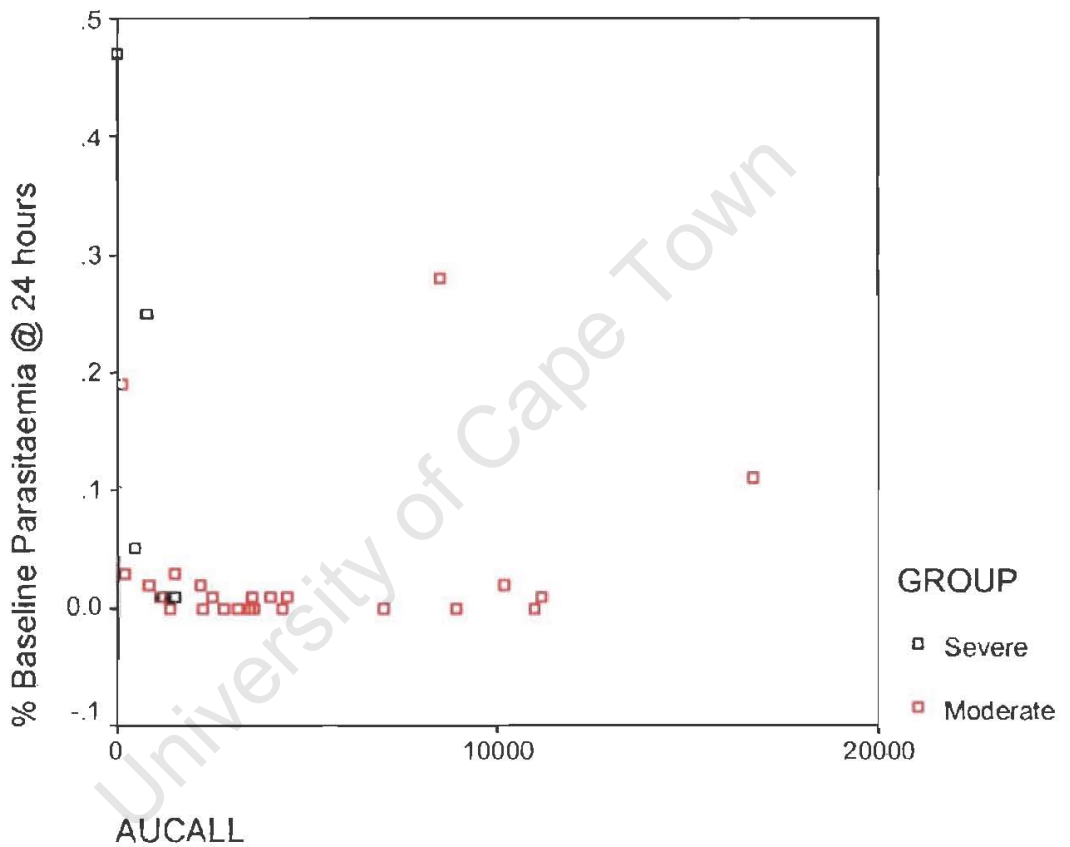
Marked inter-individual variability was noted in this study (100% coefficient of variation for both AUC and Cmax). The Cmax ($p=0.008$) and AUC (Figures 5.1a-c) of both AS and of DHA, separately and combined, were significantly higher in patients with moderately severe malaria when compared with severe malaria (Table 5.2). This significance of the difference for combined AS plus DHA was preserved whether AUC all ($p=0.006$) or AUC last ($p=0.008$) were compared.

Table 5.2: Pharmacokinetic parameters of Artesunate plus DHA by disease severity [median and interquartile range (IQR)]

| | Moderately Severe (Group 1) | | | Severe (Group 3) | | | p-value (Mann-Whitney U) |
|---------------------|-----------------------------|--------|-----------------|------------------|--------|----------------|--------------------------|
| | N | Median | (IQR) | N | Median | (IQR) | |
| C _{MAX} | 27 | 773.4 | (429.0-1792.0) | 5 | 196.6 | (42.6-378.7) | 0.008 |
| T _{LAST} | 27 | 8 | (6-8) | 5 | 6 | (2-8) | 0.241 |
| C _{LAST} | 27 | 105.6 | (53.2-787.2) | 5 | 118.0 | (42.6-182.2) | 0.511 |
| AUC _{LAST} | 27 | 3398.3 | (1422.1-8479.1) | 5 | 593.9 | (218.9-1271.7) | 0.008 |
| AUC _{ALL} | 27 | 3457.1 | (1509.6-8479.1) | 5 | 758.7 | (218.9-1330.7) | 0.006 |
| MRT _{LAST} | 27 | 3.7 | (3.1-4.9) | 4 | 4.0 | (2.5-5.5) | 0.977 |

The negative correlation between fractional reduction in parasitaemia at 24 hours and AUC all (Spearman correlation $p=0.13$), AUC last (Spearman correlation $p=0.133$) or Cmax (Spearman correlation $p=0.18$), did not reach significance (Figure 5.2).

Figure 5.2: Scatter graph of AUCall of AS plus DHA with Percentage of baseline parasitaemia at 24 hours, in patients with moderately severe and severe malaria.



References

- ¹ Lee and Hufford. Metabolism of antimalarial sesquiterpene lactones. *Pharmacol Ther* 1990; 48(3): 345-355.
- ² Navaratnam V, Mordi MN, Mansor SM. Simultaneous determination of artesunic acid and dihydroartemisinin in blood plasma by high-performance liquid chromatography for application in clinical pharmacological studies. *J Chromatogr B Biomed Sci Appl* 1997; 692(1): 157-162.
- ³ Khanh NX, de Viries PJ, Ha LD, van Boxtel CJ, Koopmans R, Kager PA. Declining concentrations of Dihydroartemisinin in Plasma during 5-day oral treatment with artesunate for falciparum malaria. *Antimicrob Agents Chemother* 1999; 43(3): 690-692.
- ⁴ Halpaap et al. Plasma levels of artesunate and dihydroartemisinin in children with Plasmodium falciparum malaria in Gabon after administration of 50mg artesunate suppositories. *Am J Trop Med Hyg* 1998; 58: 365-368.
- ⁵ Newton PN et al. Antimalarial bioavailability and disposition of artesunate in acute falciparum malaria. *Antimicrob Agents Chemother* 2000; 44: 972-7.
- ⁶ Batty KT et al. A pharmacokinetic and pharmacodynamic study of intravenous vs. oral artesunate in uncomplicated falciparum malaria. *Br J Clin Pharmacol* 1998; 50: 173-182.
- ⁷ Krishna S et al. Bioavailability and preliminary clinical efficacy of intrarectal artesunate in Ghanain children with moderate malaria. *Antimicrob Agents Chemother* 2001; 45(2): 509-516.

CHAPTER 6:**Tolerability***Extent of exposure*

Thirty two patients received AS (27 moderately severe and 5 severe malaria) according to randomisation, while fourteen received quinine (8 moderately severe and 6 severe malaria). Demographics of these patients are described under Chapter 4: Therapeutic efficacy. Patients received the closest possible dose to 10mg/kg AS rectally, using 200 and 500 mg suppositories. The mean dose in Group 1 was 9.95 mg/kg (CI: 9.86-10.05) and in Group 3 was 10.11 (CI: 9.63-10.58). Quinine was administered strictly according to body weight, with 20 mg/kg administered initially as a loading dose, followed by 10 mg/kg at 4 hours, 12 hours and then 8 hourly until able to tolerate oral medication.

Definitions

Adverse event (AE): any untoward medical occurrence (symptom, sign or special investigation) in a subject administered pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

Adverse drug reaction (ADR): all noxious and unintended responses to a medicinal product (a causal relationship is at least a reasonable possibility, i.e. cannot be ruled out) related to any dose.

Serious adverse event (SAE): An adverse event that is fatal or life-threatening, results in persistent or significant disability/incapacity, requires inpatient hospitalisation or prolongation of existing hospitalisation, is a congenital anomaly/birth defect, or is an important medical event as detailed below:

Life-threatening: Immediate risk of death from the adverse event as it occurred, in the view of the investigator/reporter. It does not refer to an adverse event that, had it occurred in a more severe form, might have caused death.

Disability: A substantial disruption of a person's ability to conduct normal life functions.

Important medical event: Any adverse event that may not be immediately life-threatening or result in death or hospitalisation but required medical intervention in order to prevent a serious outcome. The medical intervention can occur in any setting (e.g, emergency room, home, etc).

Frequency of Adverse Events

Sixteen patients (59%) with moderately severe malaria in Group 1 experienced one or more adverse events, compared with seven patients (87.5%) in Group 2; this difference in frequency was not significant (Fisher's Exact Test $p = 0.145$).

Adverse events were grouped according to the WHO Adverse Reaction Dictionary Preferred terms¹, as summarised in Table 6.1. Gastro-intestinal, followed by respiratory adverse events were the most frequent event in both Groups 1 and 2. Three patients in Group 2 developed reactions at the site of IM quinine injection. None of the adverse events in the patients with moderately severe malaria were assessed as being "serious".

A total of 7 adverse events were described in 5 patients in Group 3 and 21 events in 6 patients in Group 4. Gastro-intestinal adverse events were the most frequent in both

¹ World health Organisation Collaborating Centre for International Drug Monitoring, Uppsala, Sweden (1995). WHO Adverse Reaction Dictionary.

Groups 3 and 4. The frequency of serious events was not significantly different between Group 3 and Group 4 (Fisher's Exact test $p = 1.0$).

Table 6.1: Frequency of Adverse Event Preferred Terms by Treatment Group

Table 6.1a: Patients with MODERATELY SEVERE malaria

| | Code | PR Artesunate / SP N=27 | | IM Quinine / SP N = 8 | | Total |
|---------------------------|------|----------------------------|-----|--------------------------|-----|-------|
| | | N | % | n | % | |
| None | 0 | 11 | 41% | 1 | 13% | 12 |
| Application site reaction | 0058 | 0 | 0% | 3 | 38% | 3 |
| Thrombophlebitis | 0467 | 1 | 4% | 0 | 0% | 1 |
| Dysdiadochokinesis | 0097 | 0 | 0% | 1 | 13% | 1 |
| Dizziness | 0101 | 1 | 4% | 0 | 0% | 1 |
| Headache | 0109 | 1 | 4% | 0 | 0% | 1 |
| Nystagmus | 0131 | 1 | 4% | 1 | 13% | 2 |
| Vertigo | 0158 | 1 | 4% | 0 | 0% | 1 |
| Tinnitus | 0264 | 3 | 11% | 2 | 25% | 5 |
| Hearing impaired | 1368 | 1 | 4% | 0 | 0% | 1 |
| Reflexes abnormal | 1451 | 1 | 4% | 0 | 0% | 1 |
| Concentration impaired | 1127 | 0 | 0% | 1 | 13% | 1 |
| Diarrhoea | 0205 | 0 | 0% | 1 | 13% | 1 |
| GIT disorder | 1262 | 0 | 0% | 1 | 13% | 1 |
| Vomiting | 0228 | 5 | 19% | 0 | 0% | 5 |
| Abdominal pain | 0268 | 3 | 11% | 2 | 25% | 5 |
| Dyspepsia | 0279 | 1 | 4% | 0 | 0% | 1 |
| Nausea | 0308 | 2 | 7% | 0 | 0% | 2 |
| Jaundice | 0356 | 1 | 4% | 0 | 0% | 1 |
| Hepatomegaly | 0354 | 0 | 0% | 1 | 13% | 1 |
| Cough | 0513 | 1 | 4% | 2 | 25% | 3 |
| Sore throat | 0523 | 1 | 4% | 0 | 0% | 1 |
| Pneumonia | 0528 | 1 | 4% | 0 | 0% | 1 |
| Rhinitis | 0539 | 4 | 15% | 0 | 0% | 4 |
| URTI | 0543 | 1 | 4% | 1 | 13% | 2 |
| Ejection systolic murmur | 1471 | 1 | 4% | 0 | 0% | 1 |

| | | | | | | |
|--------------------------------|------|----|----|----|----|----|
| Increased lactate | 0364 | 1 | 4% | 0 | 0% | 1 |
| Creatinine clearance decreased | 0598 | 1 | 4% | 0 | 0% | 1 |
| Vaginitis | 0669 | 1 | 4% | 0 | 0% | 1 |
| Pelvic Inflammation | 0964 | 1 | 4% | 0 | 0% | 1 |
| Herpes simplex | 0867 | 1 | 4% | 0 | 0% | 1 |
| Total | | 35 | - | 16 | - | 51 |

Table 6.1b: Patients with SEVERE AND COMPLICATED malaria

| | Code | PR Artesunate / IV Quinine / SP | | IV Quinine / SP | | Total |
|-------------------------|------|------------------------------------|-----|-----------------|-----|-------|
| | | N | % | n | % | |
| None | 0 | 0 | 0% | 0 | 0% | 0 |
| Dysdiadochokinesis | 0097 | 1 | 20% | 0 | 0% | 1 |
| Tinnitus | 0264 | 0 | 0% | 2 | 33% | 2 |
| Hearing impaired | 1368 | 0 | 0% | 1 | 17% | 1 |
| Convulsions | 0410 | 0 | 0% | 1 | 17% | 1 |
| Diarrhoea | 0205 | 2 | 40% | 1 | 17% | 3 |
| Vomiting | 0228 | 0 | 0% | 5 | 83% | 5 |
| Abdominal pain | 0268 | 1 | 20% | 3 | 50% | 4 |
| Nausea | 0308 | 1 | 20% | 0 | 0% | 1 |
| Cough | 0513 | 0 | 0% | 1 | 17% | 1 |
| Pneumonia* | 0528 | 0 | 0% | 1 | 17% | 1 |
| URTI | 0543 | 0 | 0% | 1 | 17% | 1 |
| Bronchitis | 0805 | 0 | 0% | 1 | 17% | 1 |
| Cystitis | 0599 | 0 | 0% | 1 | 17% | 1 |
| Renal function abnormal | 0619 | 1 | 20% | 0 | 0% | 1 |
| Vaginitis | 0669 | 0 | 0% | 1 | 17% | 1 |
| Total | | 6 | | 20 | | 26 |

* in addition to pneumonias described under deaths below.

Deaths and other serious adverse events

There were no deaths or serious adverse events among the study patients with moderately severe malaria. Three patients (1 in Group 3, 2 in Group 4), all whom presented with severe and complicated malaria, died while on study. Mosvold hospital is a rural district hospital without facilities for intensive care, ventilation or dialysis. During the planning of this study it was agreed by the staff of both Mosvold and the referral hospitals that ambulance services and referral systems were adequate for transferring patients requiring these facilities. An international review panel however decided to stop the severe arm of this study on May 18 1998 following substantial problems experienced in these referral systems, which may have contributed to the death of both patients transferred to a referral hospital for ICU management. These problems are clearly not specific to this study but reflect the challenges of treating patients with severe malaria in this region. The panel agreed that all deaths appeared to be attributable to malaria.

One death occurred among 5 patients in Group 3, the study patients with severe malaria who were treated with both rectal artesunate and intravenous quinine. This patient (S24) was a 52 year-old woman who presented to hospital with severe malaria 8 days after the onset of symptoms. She had apparently been treated at a nearby clinic with oral SP two days prior to admission, despite already being prostrated and confused. On admission she was found to be prostrate, confused (GCS 14/15), and had hepatosplenomegaly and a lobar pneumonia. Within 24 hours her level of consciousness deteriorated (GCS 9/15) and she developed metabolic acidosis and renal failure, despite treatment per protocol with intravenous quinine, a single rectal dose of artesunate and cefuroxime (750 mg 8 hourly). Her parasite density decreased by 95% within 24 hours. She was withdrawn from the study 25 hours after admission so that she could be referred to the provincial academic hospital intensive care unit for haemodialysis and ventilation. She appeared to have remained stable throughout transfer (6.5 hours). However, there was a further 8-

hour delay before she was admitted to the medical ward (not ICU as arranged) and a further 2-hour delay before administration of quinine was documented (total 19 hour quinine dosage interval). Haemodialysis was refused as she was haemodynamically unstable by the time of referral for haemodialysis. After her second cardio-respiratory arrest she was finally referred to the ICU (despite telephonic ICU acceptance of referral), where she arrested again soon after arrival and this resuscitation was unsuccessful. She died 61 hours after inclusion in the study, and 36 hours after withdrawal from the study for referral to the provincial academic hospital. Post mortem examination showed that her lungs were congested and voluminous with black oedema fluid; her kidneys, heart, liver and brain were very pale; her spleen was very dark red black and diffuent. No signs of meningitis or ATN. On the basis of these findings the pathologist confirmed that this death appeared to be attributable to severe malaria.

Two deaths occurred among six patients in Group 4, the study patients with severe malaria who were treated with intravenous quinine only. The one death in this group occurred in a 29-year old man who was referred for dialysis 24 hours after study inclusion, after consultation with the ICU physician at the regional referral hospital. Unfortunately he was returned 11 hours later still anuric with signs of fluid overload, a right sided pneumonia and a GCS of 4/15. ICU admission and peritoneal dialysis had been refused on the assumption of his poor prognosis based on his HIV positive status (indicated by a single rapid diagnostic card test done at the referral hospital) and decreased level of consciousness. He died 6 days after study enrolment despite per protocol treatment of his severe malaria, careful management of fluid balance (including central venous pressure monitoring) and appropriate treatment of his pneumonia (initially cefotaxime, then adding gentamycin and cloxacillin and later rifampicin, isoniazid, pyrazinamide and ethambutol). Relatives refused permission for autopsy or lung biopsy. The other patient in Group 4 who died (S31) was a 49 year old man who presented with cerebral malaria (GCS 8/15). Although his level of consciousness improved, he died on

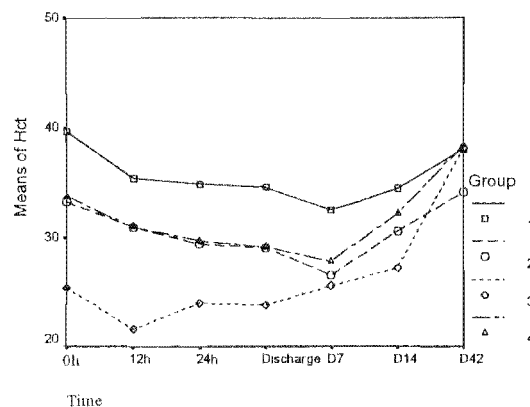
day 6 after developing ARDS despite careful management of fluid balance (including central venous pressure monitoring) and appropriate treatment of a possible pneumonia (initially cefotaxime, then adding gentamycin and cloxacillin).

Clinical and laboratory evaluations

The effects of treatment over time on HCT, WCC, platelets, glucose, lactate, pH, HCO₃, Base deficit, Sodium, Potassium, Urea, Creatinine, Bilirubin and Calcium were tested (at baseline, 12 hours, 24 hours, discharge and on days 7, 14 and 42). These generally did not change significantly over time.

A random effects regression model showed no significant ($p > 0.10$) effects of time or treatment on haematocrit overall, in patients with moderately severe, or severe, malaria. There was no interaction between treatment and time. However, there was a significant decrease in haematocrit in patients with moderately severe malaria ($p = 0.006$) but not severe malaria ($p = 0.218$) over the initial 24 hours. Treatment groups did not differ significantly, although the decrease tended to be more rapid in patients with severe malaria treated with rectal artesunate and IV quinine (Group 3) compared with those treated with IV quinine alone ($p = 0.070$).

Figure 6.1: Mean haematocrit at 0, 24 hours, discharge, day 7, 14 and 42) by treatment group (Group 1: AS / SP; Group 2 IM Quinine / SP; Group 3: AS / IV Quinine; Group 4: IV Quinine)



The decrease in serum lactate over time was not significant, in patients with either moderately severe or severe malaria. As expected, serum lactate was significantly higher in patients with severe malaria ($p=0.002$). One patient (M96, Group 1) whose lactate was recorded as 5.9 mmol/L at 24 hours, recovered uneventfully without rescue medication.

One patient (M53 Group 1) developed mild jaundice clinically at 24 hours, which although not confirmed on laboratory evaluation, resulted in the patient being withdrawn and treated with quinine. This clinical jaundice had resolved by day 3.

Platelets increased significantly over time in all patients with moderately severe malaria ($p = 0.001$), as would be expected in patients receiving effective antimalarial treatment, but not in patients with severe malaria ($p=0.678$). This effect did not differ between treatment groups ($p=0.39$ in moderately severe and 0.948 in severe malaria).

Vital signs

Pulse, SBP, and Respiratory Rate were monitored over time. Across all groups there was a statistically significant overall decrease in Pulse Rate (Wilks Lambda $p = 0.01$), and Respiratory Rate (Wilks Lambda $p = 0.008$) over time. There was a significant overall increase in Systolic Blood Pressure (Wilks Lambda $p = 0.004$), over time. The differences between Groups 1 and 2 and between Groups 3 and 4 did not reach significance at any time point. As expected with effective treatment, these showed a normalisation of values.

Neurological Evaluation

Patients were assessed for neurological abnormalities (as defined in Chapter 3: Methods) by treatment group at baseline, discharge and day 7, 14 and 42. There was no association between the frequency of any neurological abnormality and treatment group.

Changes in clinical state following treatment may potentially bias the rigour with which the neurological evaluation could be conducted at admission. Dysdiadochokinesis was only included in the neurological evaluation conducted at admission, and (in error) not systematically at further time points. This precludes comparison of frequency of this adverse event.

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Chapter 7:**Discussion of findings and limitations of study**

The clinical and parasitological responses observed in non-immune adults following a single dose of rectal artesunate support its use as an early intervention to provide initial antimalarial therapy in patients with potentially life threatening malaria, particularly for those who do not have ready access to effective parenteral treatment. These findings suggest that such treatment would provide useful therapy to cover the period before the patient could reach a health care facility equipped to provide parenteral treatment.

This and previous studies¹⁵ indicate that in patients treated with parenteral quinine for *P falciparum* malaria, the density of peripheral parasitaemia seldom falls below 60% of baseline by 12 hours, or below 10% of baseline by 24 hours. In the present study, parasitaemia fell below these levels in 26 of 27 adults with moderately severe malaria and 3 of 5 patients with severe malaria treated with artesunate. This rate of reduction in the density of parasitaemia is characteristic of the action of artemisinin derivatives, and suggests that artesunate was responsible for the antiparasitic effect observed. In 25 of 27 adults this rate of reduction occurred in the absence of any antimalarial treatment administered during the study other than artesunate. It appears unlikely that the more prevalent prior use of antimalarial treatment in the artesunate groups might have contributed substantially to the observed differences between treatment groups. The differences in prior antimalarial use did not achieve significance with Fischers exact test ($p=0.15$) nor logistic regression regression analysis ($p=0.09$). Primaquine is considered effective monotherapy for *Plasmodium falciparum*¹, and chloroquine resistance was prevalent (47% in 1997) at the study site (Brian Sharp, unpublished report). Although sulfadoxine-pyrimethamine resistance was rapidly increasing from 18% observed in 1997 (Brian Sharp, unpublished report) to 88% in 2000², prior exposure to SP may have

enhanced the efficacy of SP by increasing drug concentrations given its long elimination half-life.

The sampling schedule which precluded the calculation of elimination half-life and of $AUC_{0-\infty}$ is a major limitation of this study. Inclusion of this data in a multi-centre population pharmacokinetic evaluation will facilitate further exploration of the pharmacokinetics of rectally administered artesunate. In all 27 adults with moderately severe malaria, and in four of five adults with severe malaria, at least one blood sample collected within the 8 hours after treatment contained detectable levels of artesunate or its active metabolite, dihydroartemisinin.

Of 27 patients with moderately severe malaria, 26 had parasitological evidence of adequate absorption of artesunate, while only three of five patients with severe malaria demonstrated the rate of reduction of parasitaemia associated with artemisinins. Measurable drug exposure could not predict parasitological or clinical response to treatment. There was no significant association between fractional reduction in parasitaemia at 24 hours and AUC ($p=0.13$), or C_{max} ($p=0.18$) in this small cohort. Marked inter-individual variability was noted (100% coefficient of variation for both AUC and C_{max}), which was not explained by inter-individual differences in disease severity alone. Studies to date have generally achieved plasma concentrations in excess of those required for maximum antimalarial activity, thus precluding the description of this concentration-effect relationship^{3,4,5}. This may suggest that therapeutic levels of these potent artemisinin derivatives approach the minimum detectable level of the HPLC artesunate and dihydroartemisinin assay. Additional explanations for the lack of correlation between pharmacokinetic parameters and parasitological effect could be that a rapid exposure to artesunate and/or dihydroartemisinin is sufficient for a pharmacological effect or that other biologically active metabolites exist which have yet to

be identified. The clinical relevance of plasma levels of these agents, whose site of action is intra-erythrocytic, remains to be confirmed once a whole blood assay is developed.

This study aimed to provide evidence in support of the use of artesunate suppositories for the initial treatment of patients with potentially life-threatening malaria, in rural settings where injections cannot be safely administered. Because the use of artesunate suppositories had not yet been established in this context, we studied only patients presenting to a reasonably well-equipped dedicated ward in a rural district hospital where close observations could be made. Malaria diagnosis was confirmed by microscopy in our study and this may not be feasible in many of the communities requiring access to rectal artesunate. Thus the outcomes observed in this study may be associated with the monitoring, nursing and supportive management provided to patients with confirmed malaria and may not be generalisable to facilities where these are not available.

Many individuals with life-threatening malaria for whom rectal artesunate has been developed suffer from severe malaria, including coma, severe acidosis, severe anaemia and impairments of pulmonary and renal function. It is therefore of concern that the CMAX and AUC of both AS and of DHA, separately or combined, were significantly lower in patients with severe malaria when compared with those with moderately severe malaria. Although findings in such a small sample should be interpreted with caution, our results may suggest that the rectal absorption of AS is reduced or delayed by increased disease severity. These differences may (in part, at least) be accounted for by:

- decreased gastro-intestinal perfusion with increased disease severity, thus decreasing or delaying the absorption of rectal artesunate;
- a possible drug interaction with intravenous quinine, although no evidence of such an interaction was found; or
- an increased concentration of artesunate and dihydroartemisinin within the parasitized red blood cells in patients with more severe malaria since DHA is

preferentially accumulated in parasitized red blood cells⁶. Parasite density generally increases in patients with more severe disease. This may result in a decrease in plasma but not RBC levels of AS and/or DHA, although an equilibrium between plasma and RBC levels might have been expected. However, there was no correlation between AUC or C_{max} with baseline density of peripheral parasitaemia. Peripheral parasite density, however, may not reflect total parasite load since parasitised red blood cells are more frequently sequestered in severe disease.

The discontinuation of the severe arm of this study following unexpected but substantial concerns regarding the public sector referral system in northern KwaZulu Natal, resulting in limiting this sample to only eleven patients, is a major limitation of this study. The challenges of managing severe malaria at this rural district hospital, and even at the referral, hospital emphasise the critical importance of preventing the progression of moderately severe malaria. Studies to confirm the adequacy of absorption and parasitological and clinical efficacy of rectal artesunate in patients with severe malaria are needed.

Fever clearance times have been shown to be shorter in artemisinin-treated than quinine-treated patients in most studies. However, in patients with moderately severe malaria in this study fever clearance was significantly slower in the artesunate than the quinine group. Rapid fever clearance could be a danger if patients believe that further treatment is not needed, as single-dose treatment with any artemisinin is insufficient to cure malaria. Deployment of rectal artesunate for severe malaria in peripheral units should be accompanied by adequate explanation to patients or guardians that the initial therapy on its own is not sufficient, even if the patient appears to get better. They must understand that further effective curative treatment at a referral centre is necessary. In our study, PCR detected parasitaemia in 5/40 (12.8%) patients, but these can not be classified as

treatment failures (recrudescences) as no parasites were detected microscopically to differentiate asexual parasitaemia from gametocytaemia. This rate is lower than the 18% parasitological SP failure rate reported for this study site in 1997 (Brian Sharp, unpublished report).

In this study, the final treatment outcome depended on the efficacy of SP, as this was the only treatment given at a curative dose. It was possible to follow up the majority (91%) of patients for 42 days and to use PCR to differentiate recrudescence from re-infection. Treatment failed in 8% of patients in the artesunate group compared with 29% in the quinine group ($p>0.10$). Initial artesunate achieved the desired effect of improving the patient's clinical condition and reducing parasitaemia. The appropriate follow-on treatment to effect a cure is dependent upon local patterns of *P. falciparum* drug resistance.

Concerns that the use of rectal artesunate for life-threatening disease would increase the development or spread of artemisinin-resistant *P. falciparum* are unlikely to be justified. By ensuring this rapid reduction of parasite biomass, administration of artesunate suppositories is likely to reduce the probability of the presence of a parasite resistant to SP (or alternative curative therapies) at 24 hours⁷.

If this emergency treatment is deployed in peripheral areas, staff, patients and parents must be informed of the need to observe for expulsion of suppositories: two of 27 adults (7%) in this expelled suppositories, usually within the first fifteen minutes. Reinserted suppositories were usually retained.

Patients and their relatives in South Africa, readily accepted the use of rectal therapy in this study. The unexpected prevalence of prior use of rectal home treatment (using shoe

polish and toothpaste) indicates the acceptability of the rectal route of administration in this community.

Artesunate was generally well tolerated at 10 mg/kg administered rectally in the small cohort studied. The frequency of serious adverse events, and of adverse events overall did not differ significantly between artesunate and control study groups. Artemisinin drugs have been used extensively, especially in SE Asia, for the treatment of *P falciparum* malaria, with very little evidence of toxicity⁸. Careful clinical neurological examination in this and previous studies has failed to detect any signs of neurotoxicity, which is consistent with other findings in human studies of artesunate⁹. Concerns about potential neurotoxicity stem from reports of an unusual pattern of damage to the brainstem nuclei induced by high dose parenteral artemether and arteether in animal studies, and a prolonged coma time observed when artemether was compared with quinine in the treatment of cerebral malaria^{10,11}. Although these findings remain a cause for concern, a meta-analysis found no increased risk of prolonged coma in the artemisinin derivative treated groups¹². The one or two doses of the water soluble artesunate administered rectally for initial treatment of moderately severe disease are even less likely to have toxic effects, and no such effects were observed in the present study.

While artemisinin derivatives have been widely used in Southeast Asia, this is not yet the case in Africa where they have a potentially important role, both in combination therapies¹³ and as prompt treatment of potentially life threatening malaria. Our results suggest that at least in non-immune adults with malaria of moderate severity, and possibly in those with severe malaria, artesunate given by suppository is absorbed to a beneficial extent in the majority of individuals. Artesunate suppositories can be safely administered in minimally equipped health care facilities, and even homes, and result in a more rapid reduction in parasitaemia than is seen with parenteral quinine. Provided that early administration of rectal artesunate does not deter patients from reaching a health

care facility able to provide parenteral treatment and appropriate supportive management, this intervention has the potential to reduce malaria related morbidity and mortality in those rural areas in Africa which bear the heaviest malaria burden.

References

- ¹ Baird JK, Wiady I, Sutanihardja A et al. Short report: therapeutic efficacy of chloroquine combined with primaquine against *Plasmodium falciparum* in northeastern Indonesia. *Am J Trop Med Hyg* 2002; **66(6)**: 659-660.
- ² Bredenkamp BLF, Sharp BL, Durrheim DN, Barnes KI. Failure of Sulfadoxine - pyrimethamine in treating *Plasmodium falciparum* malaria in KwaZulu Natal province. *S Afr Med J* 2001; **91**: 970 – 972.
- ³ Newton PN et al. Antimalarial bioavailability and disposition of artesunate in acute *falciparum* malaria. *Antimicrob Agents Chemother* 2000; **44**: 972-7.
- ⁴ Batty KT et al. A pharmacokinetic and pharmacodynamic study of intravenous vs. oral artesunate in uncomplicated *falciparum* malaria. *Br J Clin Pharmacol* 1998; **50**: 173-182.
- ⁵ Bethell DB et al. Pharmacokinetics of oral artesunate in children with moderately severe *falciparum* malaria. *Trans R Soc Trop Med Hyg* 1997; **91**: 195-198.
- ⁶ GU HM et al. Uptake of dihydroartemisinin derivatives by erythrocytes infected with *Plasmodium falciparum* *in vitro*. *Trans R Soc Trop Med Hyg* 1984; **78(2)**: 265-270.
- ⁷ White NJ. Preventing antimalarial drug resistance through combinations. *Drug Res Updates* 1998; **1**:3-9.
- ⁸ Ribeiro, I.R, Olliaro, P. Safety of artemisinin and its derivatives. A review of published and unpublished clinical trials. *Médecine Tropicale* 1998; **58** : Suppl 1: 50 - 53
- ⁹ van Vugt M et al. A case control auditory evaluation of patients treated with artemisinin derivatives for multi-drug resistant *Plasmodium falciparum* malaria. . *Am J Trop Med Hyg* 2000; **62(1)**: 65-69.
- ¹⁰ Brewer TG et al. Neurotoxicity in animals due to arteether and artemether. *Trans R Soc Trop Med Hyg* 1994 Jun; **88 Suppl 1**: 33-6.
- ¹¹ Tran TH et al. A controlled trial of artemether or quinine in Vietnamese adults with severe *falciparum* malaria. *N Eng J Med* 1996; **335(2)**: 76-83.
- ¹² McIntosh HM, Olliaro P. Artemisinin derivatives for treating severe malaria. *The Cochrane Library* 2000; **2**: 1-25.
- ¹³ von Seidlein L et al. Efficacy of artesunate plus pyrimethamine – sulfadoxine for uncomplicated malaria in Gambian children: a double blind, randomized, controlled trial. *Lancet* 2000; **355**: 352-57.

Chapter 8:**Conclusions and implications for future research**

The findings of this study suggest that a single rectal dose of artesunate is expected to be a useful option for initial treatment of patients with moderately severe malaria who are unable to tolerate oral medication, particularly where parenteral treatment is not readily available. The benefits of rapidly reducing parasite density and maintaining clinical stability while patients are being transferred to a facility where they have access to parenteral treatment and supportive management, need to be confirmed within the normal context of use. Patients with malaria who do not have access to parenteral treatment are unlikely to have access to the definitive diagnosis, monitoring or supportive management received by patients in this study.

Although rectal artesunate appeared well tolerated in this study, ongoing pharmacovigilance is required, particularly regarding potential embryo-, haematological- and neuro-toxicity. The effect of increased disease severity, and co-morbidity (particularly with HIV/AIDS, malnutrition and tuberculosis) on the frequency and nature of adverse reactions needs to be determined. Given the prevalence of the use of concomitant treatment (both western and traditional medication), and the necessity to complete treatment with another effective antimalarial, it is important to evaluate *in vivo*, as well as *in vitro*, the potential for drug interactions with commonly used antimalarials (particularly quinine, as this is currently the treatment most widely used in severe malaria, and those used in artemisinin-based combination therapies) as well as for interactions with other essential drugs which have a similar adverse effect profile.

The limited data obtained from only five patients with severe *falciparum* malaria in this study raise concerns as to whether rectal artesunate is adequately absorbed in this group. As the target population for whom rectal artesunate has been developed includes those with severe malaria, it is essential that studies are conducted to establish the pharmacokinetic, parasitological and clinical evidence of benefit of a single administration of a rectal artesunate in this population.

Appendix 1:

Publications

Appendix 1.1

Barnes KI, Mwenechanya J, Tembo M, McIleron H, Folb PI, Ribeiro I, Little F, Gomes M, Molyneux ME. Efficacy of rectal artesunate in the initial treatment of moderately severe malaria in African children and adults. *Lancet* 2004; **363**: 1598-1605.

Appendix 1.2

Barnes KI, Folb PI. The role of artemisinin-based combination therapy in malaria management. In "Reducing Malaria's Burden: Evidence of effectiveness for decision-makers. Global Health Council." Editors: Murphy C, Ringheim K, Woldehanna S, Volminck J. 2003. www.globalhealth.org

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Efficacy of rectal artesunate compared with parenteral quinine in initial treatment of moderately severe malaria in African children and adults: a randomised study

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Summary

Background Many patients with malaria of increasing severity cannot take medicines orally, and delay in injectable treatment can be fatal. We aimed to assess the reliability of absorption, antimalarial efficacy, and tolerability of a single rectal dose of artesunate in the initial management of moderately severe falciparum malaria.

Methods 109 children and 35 adults were randomly assigned to rectal artesunate (single dose of about 10 mg/kg) or parenteral quinine treatment (10 mg/kg at 0, 4, and 12 h). The primary endpoint was the proportion of patients with peripheral asexual parasitaemia of less than 60% of that at baseline after 12 h. Secondary endpoints were clinical response and concentrations of drug in plasma. Analysis was by intention-to-treat.

Findings All artesunate-treated patients had pharmacodynamic or pharmacokinetic evidence of adequate drug absorption. 80 (92%) of 87 artesunate-treated children had a 12 h parasite density lower than 60% of baseline, compared with three of 22 (14%) receiving quinine (relative risk 0.09 [95% CI 0.04–0.19]; $p < 0.0001$). In adults, parasitaemia at 12 h was lower than 60% of baseline in 26 (96%) of 27 receiving artesunate, compared with three (38%) of eight receiving quinine (relative risk 0.06 [0.01–0.44]; $p = 0.0009$). These differences were greater at 24 h. Clinical response was equivalent with rectal artesunate and parenteral quinine.

Interpretation A single rectal dose of artesunate is associated with rapid reduction in parasite density in adults and children with moderately severe malaria, within the initial 24 h of treatment. This option is useful for initiation of treatment in patients unable to take oral medication, particularly where parenteral treatment is unavailable.

Lancet 2004; **363**: 1598–605

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Introduction

Plasmodium falciparum malaria causes hundreds of thousands of deaths annually, especially in children in sub-Saharan Africa. One of the most important reasons for the high mortality is delay in receiving effective medication. Rural health workers and even parents can be taught to identify the symptoms and signs of severe, life-threatening malaria.¹ However, many patients with malaria of increasing severity are not able to take medicines orally. Many of those living in malaria-endemic areas do not have ready access to health facilities that can provide parenteral treatment. For patients unable to take oral medication, delay in access to prompt injectable therapy can be fatal. Progression from being unable to take oral treatment to severe malaria can happen within hours.² Once the opportunity for early therapeutic intervention is lost and organ failure has developed, the prognosis worsens substantially and survival depends largely on the health-care facility's resources to manage malaria complications.³ Most patients in malaria-endemic areas do not have access to intensive-care facilities. Rectal artesunate makes for a rapidly acting and effective drug for patients with acute malaria who are unable to take oral medication where parenteral antimalarial treatment is not available.

WHO (UNDP, World Bank, and WHO Special Program for Tropical Diseases Research [WHO TDR]) selected artesunate for investigation as a candidate for emergency rectal administration for patients unable to take oral medication, to provide therapeutic cover for the initial 24 h after presentation. Artesunate was chosen on the basis of its rapid antimalarial activity,^{4,5} favourable bioavailability,⁶ and reassuring safety⁷ and efficacy⁸ profiles compared with other available treatments. Despite isolated case reports of failed supervised treatment,^{9,10} no evidence exists of stable parasite resistance developing during lengthy clinical use with artemisinins.¹¹ A thermostable suppository formulation of artesunate was chosen since unrefrigerated storage and easy administration are essential where facilities are rudimentary.

In our study, the absorption and initial therapeutic (but not curative) efficacy of single administration of suppositories in a dose of 10 mg/kg rectal artesunate were recorded during 1997 and 1998 in Malawian children presenting to the Queen Elizabeth Central Hospital, Blantyre, and in a smaller number of South African adults presenting to Mosvold District Hospital, KwaZulu Natal, all with moderately severe malaria. Because intensity of malaria transmission is low in South Africa, these adults are unlikely to have acquired any protective immunity. A few patients received standard treatment with repeated parenteral administration of quinine from the time of admission. The study was confined to patients regarded as having moderately severe malaria, in the absence of organ complications indicative of severe malaria, to establish

proof of principle in patients not at immediate risk of dying from the disease. Since artemisinin derivatives are the most rapidly acting antimalarials known,³ the rate of early parasite clearance from peripheral blood within the first 24 h after initiation of treatment is a sensitive indicator of drug absorption. Clinical response to treatment, and concentrations of artesunate and its metabolite dihydroartemisinin in plasma, were additional outcome measures. In both study groups, treatment was completed with the administration of sulfadoxine-pyrimethamine according to prevailing national policy, and the continued efficacy of this definitive treatment was also monitored.

The study aimed to determine whether early administration of rectal artesunate would provide beneficial initial antimalarial cover, indicated by a rapid fall in the density of parasitaemia and clinical improvement without serious adverse reactions. If this were confirmed, rectal artesunate might be considered suitable as initial treatment for patients who cannot be treated immediately either orally or parenterally, to cover the period until they are able to reach a health facility for definitive treatment and, if needed, further supportive management.

Methods

Participants

In Malawi, children aged 1–10 years were eligible for enrolment if they had consent of their parent or guardian, an illness suggestive of malaria, *P. falciparum* parasitaemia of 20 000–500 000 ring forms per μL in the blood, and features of moderately severe malaria judged by one or more of the following: inability to sit or stand unsupported; inability to eat, drink, or breastfeed; a Blantyre coma score¹² of 3 or 4 out of 5; and repeated vomiting. Exclusion criteria included any manifestation of more severe malaria—namely, acidosis, severe respiratory distress, deep coma, shock, jaundice, spontaneous bleeding, convulsions, or severe anaemia (packed cell volume <18%). Children who were enrolled were admitted to the national hospital in Blantyre. *P. falciparum* transmission in this area is year-round with increased intensity in the rainy season (November to May).

In South Africa, adults aged 16–65 years were enrolled if they gave written informed consent, had clinical features of malaria with a *P. falciparum* parasitaemia of <500 000 ring forms per μL in the peripheral blood, and were classified as having moderately severe malaria on the basis of one or more of the following: inability to eat or drink, dehydration, inability to stand or walk unaided or repeated vomiting, in the absence of WHO criteria of severe malaria,¹¹ or a serum lactate concentration greater than 5 mmol/L.¹³ Adult patients were admitted to Mosvold Hospital, a rural district hospital in northern KwaZulu Natal, where malaria transmission is low (annual entomological inoculation rate <1) and seasonal, with peaks between March and May.

Adults and children with diarrhoea (defined as three or more liquid stools in the previous 24 h) or who received an antimalarial drug in the previous 24 h, and women who were pregnant or breastfeeding were excluded.

Study design and procedures

During screening, a detailed history was taken and an examination done, with emphasis on assessing severity of malaria and neurological status. Neurological examination included Glasgow or Blantyre coma score rating, or both, examination of cranial nerves, and assessment of cerebellar and motor function. Parasitological diagnosis

was made on thick and thin blood films stained with Field's stain and reversed Field's stain, respectively. Parasite density was estimated by counting asexual parasites against 200 white cells on the thick film or 500 red cells on the thin film, and the white cell or red cell count was used to calculate the number of parasites per μL of blood. A sample was judged to be parasite-negative if the thick film revealed no asexual parasites in the fields in which 200 white cells had been counted. Blood samples were taken at baseline to measure full blood count (Onyx, Coulter Electronics, New York, USA), lactate (Accusport, Boehringer Mannheim, South Africa, or Chiron Diagnostics, Malawi), blood gases (Ilex Laboratories 1640 Blood Gases and Electrolytes, Ilex, South Africa, or ABL330, Radiometer, Copenhagen, for Malawi), glucose (Boehringer Mannheim Accutest strips read in an Accutest Alpha reflectance meter), and electrolytes. In adults, liver enzymes were measured (Vision, Abbotts, South Africa) and pregnancy was excluded (human chorionic gonadotrophin Combo, Abbotts, South Africa) in all adult women.

Artesunate suppositories were administered in a single dose as close as possible to 10 mg/kg, without lubricant, in a combination of 50 mg and 200 mg suppositories (Plasmotrim Rectocaps, Mepha AB, Basel, Switzerland). Children received a maximum of three, and adults a maximum of five, suppositories. Each patient was closely monitored for 5 h to detect expulsion of suppositories. Any suppository expelled intact was reinserted. If a disrupted suppository was expelled a new one of the same dosage was inserted.

To compare rectal artesunate with standard parenteral quinine therapy, sealed envelopes were used to allocate one in five patients to receive parenteral quinine (Adco-Quinine dihydrochloride, Adcock Ingram Generics, South Africa), using randomisation tables in blocks of size five. In all adults and in 17 children this was given as intramuscular injection, 20 mg/kg loading dose followed by 10 mg/kg diluted in normal saline to a concentration of 60 mg/mL, with half the dose given in each anterior thigh at 0, 4, and 12 h, then every 12 h until the patient was able to take oral treatment. In five children who required intravenous fluids, quinine was given in the same dosage and schedule infused intravenously over 2 h in half-strength Darrow's solution with 5% dextrose. The allocation ratio between quinine and artesunate was selected to obtain the most data on artesunate suppository efficacy and pharmacokinetics, while retaining a reference group receiving standard treatment of known efficacy and pharmacokinetics.¹⁵ A sealed prerandomised envelope which assigned a treatment was opened once the decision to enrol the patient had been made. Since the efficacy of a single dose of artesunate had not previously been established over 24 h in moderately severe malaria, the studies were not masked.

Both artesunate-treated and quinine-treated groups were given oral sulfadoxine-pyrimethamine (Fansidar, Roche Products, South Africa), at a dose of pyrimethamine 1.25 mg/kg, as consolidation therapy 24 h after initiation of treatment, or as soon thereafter as they were able to take fluids by mouth. Therapeutic efficacy of sulfadoxine-pyrimethamine monotherapy *in vivo*¹⁶ was 86% (14 day adequate clinical response) in Blantyre¹⁷ in 1998 and 82% (28 day adequate clinical and parasitological response) in KwaZulu Natal in 1997 (Sharp B, personal communication). Those unable to drink at 24 h were given intramuscular quinine until able to take sulfadoxine-pyrimethamine by mouth. Patients were discharged from hospital after parasite clearance

once clinically recovered and were asked to attend follow-up visits on days 7, 14, and 28 after the start of treatment; adults were also followed up on day 42.

The efficacy and adequacy of drug absorption was assessed in three ways: rate of reduction of peripheral parasitaemia, clinical response, and concentrations of artesunate and its active metabolite, dihydroartemisinin, in plasma.

Parasitaemia was measured every 6 h until two consecutive smears were negative, and reduction in parasite density in the first 24 h served as an indicator of drug effect (and, by inference, of drug absorption). In patients with uncomplicated hyperparasitaemic falciparum malaria, peripheral parasites exposed to artemisinins are reduced to less than 60% of baseline within 12 h, and to below 10% within 24 h in 95% of cases.¹⁸ On this basis, the primary indicator of adequate artesunate activity was set at a fall in peripheral parasitaemia to 60% or less of baseline density at 12 h, without subsequent increase above 60% of baseline. Reduction of parasitaemia to less than 10% of baseline at 24 h was a confirmatory measure of efficacy. Although quinine is the current standard treatment recommended for more severe malaria, it does not have a consistent effect on peripheral parasite density in the first 12–24 h of treatment.¹²

Patients were monitored closely for clinical response in a dedicated ward. Vital signs and coma score were recorded at 0, 1, and 2 h, then every 2 h until 24 h, every 4 h until 48 h, every 6 h until 96 h, and then daily until discharge. Full blood count, blood gases, and blood glucose and lactate concentrations were monitored every 6 h for 24 h. Paracetamol was given to patients with temperatures of 38°C or greater. Fluids were given orally or, if indicated, by intravenous infusion. Seizures, hypoglycaemia, and intercurrent infection were treated according to standard protocols. Ability to eat, drink, and walk unaided, and detailed neurological assessment were recorded daily.

Blood samples were taken before treatment and twice (in children) or five times (in adults) during the first 12 h after treatment. Concentrations of both artesunate and dihydroartemisinin in plasma were measured by a validated high performance liquid chromatographic method with electrochemical detector operating in the reductive mode by the Centre for Drug Research, University Sains Malaysia, Malaysia.¹⁹

The study in children was approved by the National Health Sciences Research Committee of the Ministry of Health and Population, Malawi, and the adult study by the Research and Ethics Committee of the University of Cape Town. Both were approved by the WHO Secretariat Committee on Research Involving Human Subjects. The studies were done in accordance with the principles laid down in the World Health Assembly of 1975 on Ethics in Human Experimentation and in the Helsinki Declaration. Witnessed written consent was obtained from literate patients, a guardian, or both. Verbal consent was normally signed and dated by a witness. For 25 children a witness only signed to reaffirm consent during a follow-up visit rather than on the day of enrolment; in three children verbal consent was obtained without a witness's signature.

Any patient treated with artesunate who subsequently developed any of the exclusion criteria was also treated with parenteral quinine. Those in whom parasitaemia did not fall below 60% of the baseline density by 12 h were given parenteral quinine, unless their condition was improving clinically.

All patients were asked to attend follow-up at 7, 14, and 28 days (and for adults again at 42 days) after entry into the study. A full history and physical examination (including detailed neurological examination), full blood count, and peripheral malaria smear were taken at each visit. Reappearance of asexual parasitaemia was treated in adults with mefloquine (1500 mg total dose), whereas children were given halofantrine (10 mg/kg every 6 h three times) on day 7 and either halofantrine or sulfadoxine-pyrimethamine on days 14–28. PCR was used to differentiate recrudescence from reinfection in adults.²⁰

Parasite densities were counted by two independent microscopists. The study sites were visited regularly by experienced clinical study monitors. Data were source-verified by the investigators and clinical study monitors, double-checked at the investigation sites, and double entered.

Statistical analysis

Analyses are presented separately for the Malawian children and for the smaller study of adults in South Africa. Analyses were done with EpiInfo version 3.01, SPSS version 8.0, and Stata versions 5.0 and 6.0. Categorical data were analysed with Fisher's exact test, normally distributed data with parametric tests, and non-normally distributed data with the Mann-Whitney *U* test. Parasite and fever clearance times were compared by survival analysis and the log rank tests for the equality of survivor functions, censoring patients at the time of withdrawal, or loss to follow-up. Both intention-to-treat analysis and analysis of patients who received only rectal artesunate or parenteral quinine in the initial 24 h were done. All tests were two-tailed with a 5% level of significance.

Role of the funding source

WHO TDR conceptualised and provided financial support for these studies and provided clinical monitors for both study sites. WHO TDR had no role in data collection or the decision to publish these studies. IR and MG, who are employed by WHO TDR, contributed to study design, interpretation of data, and preparation of the manuscript.

Results

Malawian children

109 children were recruited to the study (figure 1). Nine were younger than 24 months. The demographic and clinical characteristics of the children on presentation were similar in the two groups (table 1). The mean duration of illness before admission was 2.4 days. In the month before enrolment, 15 (17%) of 87 children in the artesunate group and three (14%) of 22 in the quinine group had received an antimalarial drug (in most cases sulfadoxine-pyrimethamine).

Suppositories were retained, and no stool passed in the hour after insertion, in 70 (80%) of 87 children. 17 patients expelled suppositories, 12 within 15 min of insertion, four within an hour, and one at 80 min, most commonly with passing a stool. Staff succeeded in reinserting the same or new suppositories in all except one (1%), in whom attempts to reinsert suppositories were abandoned after they had been expelled five times; this child was treated with intramuscular quinine.

Ten children receiving artesunate were given additional parenteral quinine according to protocol, six within the first 12 h and four between 12 and 24 h. The reasons for giving quinine to children allocated to the artesunate group were: repeated expulsion of suppositories (one, see

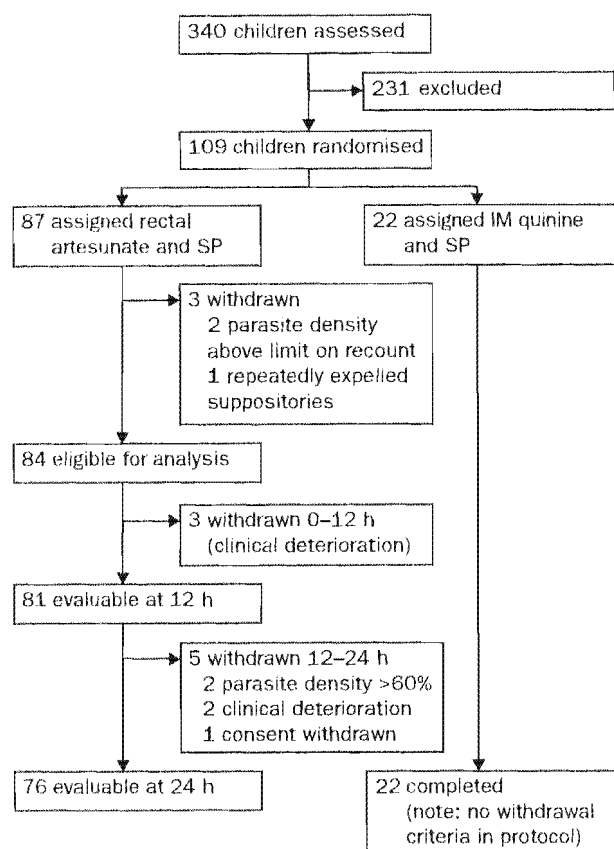


Figure 1: Trial profile for children in Malawi

IM=intramuscular, SP=sulfadoxine-pyrimethamine. One patient with parasite density >60% was given quinine because of clinical deterioration before 12 h.

previous paragraph), excessively high baseline parasite density on recount (two), parasite density at 12 h exceeding 60% of baseline count in the absence of clinical improvement (two), and clinical deterioration (five). Of the five children with clinical deterioration, three had convulsions (two within the first 2 h and one at 24 h), one developed deepening coma progressing to a Blantyre score of 2 out of 5 at 5 h, and one progressed to severe anaemia at 12 h. One child in the artesunate-treated group was withdrawn from the study by his mother at 22 h. In accordance with the protocol, patients in the artesunate group received a single dose of artesunate, whereas those in the quinine group received a minimum of three doses of quinine in the first 24 h (with total doses over 72 h ranging between three and seven, median four). Curative treatment was completed with oral sulfadoxine-pyrimethamine in both study groups at 24 h, or as soon thereafter as the patient was able to take drugs by mouth.

All infections were confirmed microscopically as *P. falciparum* and there were no mixed plasmodial infections. The median fractional reduction of parasitaemia at 24 h was 99.9% for artesunate and 41% for quinine ($p < 0.0001$, table 2). Of the 87 children who received artesunate, 80 (92%) had a 12 h parasite density below 60% of baseline, compared with three (14%) of 22 in the quinine group (relative risk 0.09 [95% CI 0.04–0.19], $p < 0.0001$). Of the 87 children in the artesunate group, of whom ten had received quinine by 24 h, 82 were available for evaluation of parasitaemia at 24 h (in four cases the 24 h slide was missing or spoilt and one child was withdrawn by his mother at 22 h). Of the 82 evaluable patients who had received artesunate, 79 (96%) had a 24 h parasite count less than 10% of baseline, compared with 3 (14%) out of 22 in the quinine group ($p < 0.0001$). For both the 12 h and 24 h analyses, the differences are similar when the artesunate-treated patients who were parasitological successes but received additional quinine as a result of clinical deterioration are removed from the analysis or are considered as failures. For the four in the artesunate group whose 24 h slides were missing, the 18 h parasitaemia was less than 1% of baseline in three, and was 19% of baseline in the remaining one, whose 30 h parasite density was 0.3% of baseline.

Clinical progress was similar in the two treatment groups. All children made a full recovery. Three of 87 in the artesunate group and one of 22 in the quinine group had a convulsion at some point after start of treatment. One artesunate recipient and one quinine recipient became severely anaemic and required blood transfusion. Convulsions and severe anaemia are features of malaria. 24 h after start of treatment, 77 (89%) of 87 artesunate-treated and 21 (95%) of 22 quinine-treated patients were able to drink ($p = 0.3$), and all were able to drink by 48 h. The median fever clearance time was 20 h (IQR 14–30) in the artesunate group and 44 h (30–58) in the quinine group ($p < 0.0001$). In the artesunate group the mean packed cell volume was 30.5% on admission and 26.0% on the day of discharge from hospital, with an average fractional fall of 14%; the data for the quinine group were 28.9% and 24.1%, with an average fall of 16% ($p = 0.32$).

Children were reviewed 7, 14, and 28 days after admission. 15, 20, and 28 patients in the artesunate group and three, five, and ten patients in the quinine group did not attend follow-up on days 7, 14, and 28, respectively. Time to reappearance of parasites was earlier in patients treated with a single dose of rectal artesunate than in those administered three to seven doses of parenteral quinine (log rank $p = 0.035$). Reappearance of parasitaemia among those followed up on day 7 was significantly more common in patients treated with artesunate than in those with quinine (14 of 71 vs 0 of 19, $p = 0.035$), but not for those followed up on days 14 ($p = 0.5$) and 28 ($p = 0.7$). Of

| | Children | | Adults | |
|--|--------------------------|-------------------------|--------------------------|-------------------------|
| | Rectal artesunate (n=87) | Quinine IM/IV (n=22) | Rectal artesunate (n=27) | Quinine IM (n=8) |
| Male | 54 | 13 | 16 | 2 |
| Female | 33 | 9 | 11 | 6 |
| Median (range) age | 48.2 months (16–120) | 45.7 months (18–121) | 29.0 years (16–58) | 24.5 years (17–45) |
| Mean (SD) weight (kg) | 14.2 (3.7) | 13.4 (3.6) | 62 (10.4) | 62 (20.5) |
| Mean (SD) respiratory rate (breaths/minute) | 45 (14.3) | 46 (13.0) | 24 (5.2) | 24 (7.1) |
| Mean (SD) temperature (°C)* | 38.7 (1.0) | 39.1 (1.1) | 38.7 (1.3) | 38.5 (1.5) |
| Median (range) parasitaemia (ring forms per μ L) | 185 977 (10 995–848 732) | 230 738 (4 293–701 784) | 54 800 (520–246 000) | 101 173 (7 000–190 000) |
| Mean (SD) plasma lactate (mmol/L) | 2.77 (2.1) | 3.02 (0.9) | 2.5 (0.7) | 2.2 (1.0) |
| Mean (SD) packed cell volume (%) | 30.4 (5.8) | 28.9 (3.6) | 39.1 (7.4) | 33.9 (5.2) |

IM—intramuscular, IV—intravenous. *Rectal in children, oral in adults.

Table 1: Selected baseline clinical and laboratory parameters by allotted treatment group

| | Children | | | Adults | | |
|--|---------------------------|---------------------------|---------|--------------------------|-------------------------|--------|
| | Rectal artesunate (n=87) | IM/IV quinine (n=22) | p | Rectal artesunate (n=27) | IM quinine (n=8) | p |
| Parasitaemia (ring forms per μL blood) | | | | | | |
| Baseline | 185 977 (112 411–336 000) | 230 738 (170 078–325 029) | 0.69 | 56 480 (26 536–126 000) | 58 340 (45 110–118 230) | 0.666 |
| 12 h | 50 596 (18 771–107 698) | 210 335 (170 078–325 029) | <0.0001 | 5560 (1 295–25 840) | 35 160 (22 720–80 400) | 0.025 |
| % baseline | 26.7% (13.8–42.7%) | 82.0% (62.5–102%) | <0.0001 | 12.8% (3.2–33.8%) | 64.7% (35.5–85.5%) | 0.002 |
| 24 h | 222 (37–625) | 111 223 (24 590–168 143) | <0.0001 | 400 (120–1 000) | 17 320 (4 980–37 403) | 0.004 |
| % baseline | 0.1% (0.02–0.5%) | 59.2% (15–75%) | <0.0001 | 1% (0–3%) | 27.5% (5–58.5%) | 0.0004 |
| PCT (h) | | | | | | |
| Mean (95% CI) | 36 (30–42) | 45 (42–48) | 0.0003* | 49 (43–55.7) | 63 (45.4–80.6) | 0.10* |
| Censored observations | 1 | 1 | | 1 | 0 | |

Data are median (IQR) unless specified otherwise. IM=intramuscular, IV=intravenous, PCT=parasite clearance time. *Unpaired t test.

Table 2: Parasite density over time by allotted treatment group

those patients who completed 28 days of follow-up, 39 (67%) of 58 treated with rectal artesunate and five (42%) of 12 treated with parenteral quinine had had a positive parasite smear by day 28 (relative risk 1.61 [95% CI 0.81–3.23], $p=0.11$). Recrudescence was not differentiated from reinfection in these children. The 44 patients with recurring parasitaemia were either asymptomatic or had features of uncomplicated malaria—namely, history of fever (29), vomiting (six), decreased appetite (seven), cough (four), and abdominal pain (one). None was associated with severe or moderately severe illness. All patients had a normal Blantyre coma score (5) and tolerated treatment with an oral antimalarial. No deaths occurred in this group.

South African adults

Of 53 patients screened, 36 met the criteria for the study. Of these, 28 were assigned to receive rectal artesunate and eight to receive parenteral quinine (figure 2). One patient was not given the assigned artesunate because he admitted to recent use of an antimalarial on requestioning. The two groups were similar in most clinical and laboratory variables (table 1), in duration of symptoms (median 3 days range 1–6), and in previous treatment of the current illness. Seven patients (20%) had taken antimalarial medication more than 24 h before

presentation, whereas eight (23%) reported use of so-called traditional home remedies, of which rectal administration of commercial shoe polish (in six of eight) was most common. The other traditional treatments were rectal toothpaste and liquid soap enema. Two adults expelled artesunate suppositories (at 5 min and 100 min); the suppository was successfully reinserted or replaced in both.

One patient in the artesunate group received oral sulfadoxine-pyrimethamine, in error, at 0 h instead of 24 h. Two in the artesunate group were given additional parenteral quinine according to protocol: one patient had a parasite density exceeding 60% of baseline at 12 h, and the other developed mild clinical jaundice at 24 h. Four in the quinine group had a parasite density exceeding 60% of baseline at 12 h. Mefloquine was given to one in the artesunate group on day 7 for persistent parasitaemia.

In adults the results were similar to those in children (table 2)—ie, significantly faster parasite clearance in those who received a single dose of rectal artesunate than in those given at least three doses of parenteral quinine. At 12 h, parasitaemia was less than 60% of baseline in 26 (96%) of 27 patients allocated to artesunate and in three (38%) of eight patients allocated to quinine (relative risk 0.06 [95% CI 0.01–0.44], $p=0.0009$). At 24 h, parasitaemia was less than 10% of baseline in 22 (81%) of 27 of patients allocated to artesunate, compared with three (38%) of eight in the quinine-treated group (relative risk 2.17 [0.87–5.41], $p=0.027$). The median fractional reduction of parasitaemia at 24 h was 99% and 72% in patients treated with artesunate and quinine, respectively ($p=0.007$). Of the 27 artesunate-treated patients, two had received quinine by 24 h (one at 12 h and the other at 24 h). Results are similar when the artesunate-treated patients who received additional quinine are removed from the analysis or are regarded as having treatment failure.

Clinical improvement was similar in the two treatment groups. All patients with moderately severe malaria tolerated oral administration of sulfadoxine-pyrimethamine at 24 h, and subsequently made a full clinical recovery. None developed severe malaria according to WHO criteria. One patient (artesunate group) was regarded as having progressed to severe malaria on the basis of mild clinical jaundice at 24 h (although

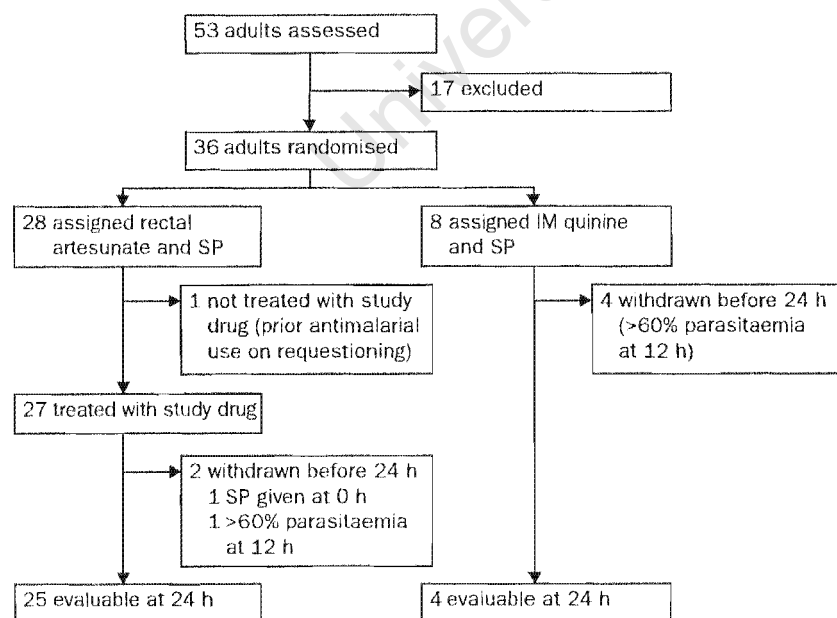


Figure 2: Trial profile for adults in South Africa
IM=intramuscular, SP=sulfadoxine-pyrimethamine.

bilirubin was not measured) and was treated with parenteral quinine. The jaundice had resolved by the following day. There were no deaths. Median fever clearance time was more rapid in the quinine group (23 h) than in the artesunate group (36 h), although this difference was not significant ($p=0.116$).

32 (91%) completed 42 days of follow-up. Three (two of 27 in the artesunate group and one of eight in the quinine group) were lost to follow-up, but were reported by family members to be well. One in the artesunate group was parasitaemic but asymptomatic on days 7, 14, and 42 (a recrudescence infection shown by PCR) despite treatment with therapeutic doses of both sulfadoxine-pyrimethamine and mefloquine. No other patients were parasitaemic as shown by microscopy between days 7 and 42. Five had *P. falciparum*-positive PCR results,²¹ suggesting low density asexual parasitaemia or gametocytaemia. PCR suggested recrudescence in four (two of 25 in artesunate group, two of seven in quinine group, relative risk 0.28 [95% CI 0.05-1.65], $p=0.20$) and reinfection in one (in artesunate group).

Tolerability

A single dose of artesunate suppositories at a dose of 10 mg/kg was well tolerated in both children and adults. There was no significant difference in frequency of adverse events (defined as any new symptom, or worsening of any existing symptom, sign, or abnormal laboratory value) between treatment groups. Other than local reactions at the site of intramuscular quinine injection in three adult patients, the few adverse events that happened could have been attributable to falciparum malaria or to pre-existing disease. Careful clinical assessment of the central nervous system detected no signs of adverse neurological events after administration of artesunate, although one patient of eight in the adult quinine group developed transient dysdiadochokinesis.

Pharmacokinetics

Artesunate, its active metabolite dihydroartemisinin, or both, were detected in the plasma of all 27 adults and in 84 of 87 children. The minimum detectable concentration for artesunate and dihydroartemisinin was 4.0 µg/L. Concentrations of any drug in plasma did not correlate with parasitological or clinical outcome, and striking interindividual variation was noted (data not shown).

Discussion

We have shown that rectal artesunate, given in a single dose of 10 mg/kg, has rapid antimalarial efficacy within 24 h of administration in moderately severe falciparum malaria in children and adults. All patients had either pharmacodynamic or pharmacokinetic evidence of absorption of the drug. Clearance of asexual parasites from the peripheral blood was consistently faster with rectal artesunate than parenteral quinine, as is expected when an artemisinin is absorbed adequately. The results were highly significant not only in the large study of children, but also in the smaller study in adults, which was powered to detect only large effects. The clinical and parasitological responses show that rectal artesunate provides effective initial management of acute malaria in patients who cannot take medication by mouth, particularly when parenteral treatment is not available.

A faster decrease in peripheral parasitaemia does not necessarily ensure improved clinical outcome. In this study, the clinical success rate for rectal artesunate was similar to that for parenteral quinine. This is consistent with other studies comparing intramuscular artemether

with intravenous quinine in severe malaria, where artemether showed more rapid decrease in parasitaemia but equivalent clinical outcomes.²²

Fever clearance times are shorter in artemisinin-treated patients than in quinine-treated patients.²³ Rapid fever clearance is an important clinical benefit, but it does not reflect cure and might create a false sense of security since combination with a second effective antimalarial agent, or a prolonged treatment regimen for 5-7 days, is required to achieve cure.¹⁷ Health-care providers need to ensure that patients or caregivers understand the need for further curative treatment at a referral centre.

For the purposes of this study, the detection of artesunate or dihydroartemisinin in the plasma was taken as a secondary indicator of drug absorption, as the therapeutic drug level of either is not yet clearly defined. Despite the consistent pharmacodynamic effect of rectal artesunate, reflected in parasite clearance, we found marked interindividual variability in pharmacokinetics. This is consistent with the variability described for the artemisinins.⁴ There was no correlation between pharmacokinetic measurements and parasite clearance rates. The relevance of pharmacokinetic data from unbound plasma concentrations of artesunate and dihydroartemisinin needs to be confirmed because these drugs accumulate selectively in infected erythrocytes.²⁴

A single administration of rectal artesunate alone is not intended to cure malaria. Although repeated administration of parenteral quinine and artemisinin derivatives are proven effective treatments for severe *P. falciparum* malaria once a patient is admitted to hospital,^{25,26} short-course treatment with either is associated with recrudescence unless followed by full course of effective consolidation treatment.^{29,30} Rates of recrudescence indicate both drug exposure to the initial treatment (to which no resistance is proven in Africa) and the efficacy of the follow-up treatment, which is highly dependent on the prevalent patterns of drug resistance. Widespread resistance now precludes the use of sulfadoxine-pyrimethamine for completion of treatment in much of east and southern Africa.³¹

In our study of children in Malawi, the reappearance of parasites occurred significantly earlier with artesunate than with quinine. Reappearance of parasites at 7 days suggests that a single dose of artesunate followed up with a single treatment with sulfadoxine-pyrimethamine at 24 h did not eradicate the infection. These findings emphasise that treatment with a single dose of rectal artesunate should be completed as soon as possible with an effective antimalarial. Sulfadoxine-pyrimethamine was first-line treatment for uncomplicated malaria in both study sites. Despite reported therapeutic efficacy of sulfadoxine-pyrimethamine monotherapy in uncomplicated malaria before our studies, parasitological failure rates had increased to 76.7% by 1999 in the Malawi site and to 88% by 2000 in KwaZulu Natal.^{31,32} The earlier reappearance of parasites in artesunate-treated patients might reflect the fact that they received a single dose of medication (at the time of admission), whereas patients on quinine received a total of three to seven doses (over 24-72 h). Late parasitaemia could be due either to failed treatment or to reinfection. Our study in children was not designed to distinguish between recrudescence and reinfection, but rather to establish the benefit of a single dose of rectal artesunate over the initial 24 h. The addition of 3 days of artesunate to standard antimalarial treatments has been shown in most studies, but not all, to substantially reduce treatment failure, recrudescence, and gametocyte carriage.³¹

Careful clinical neurological examination in our studies detected no signs of neurotoxicity, which is consistent with other human studies of the artemisinins,^{7,33-35} although few data are available in young children, and neurological examinations are less reliable in patients under 5 years of age.^{33,36} Concerns about potential neurotoxicity stem from an unusual pattern of damage to the brainstem nuclei induced by high-dose parenteral artemether and artemotil reported in animal studies.³⁷⁻⁴⁰ The single dose of the water-soluble artesunate in our study is less likely to have toxic effects than a full treatment course.

Our study was confined to patients with moderately severe malaria, presenting to well-equipped units where close observations could be made. Many patients with potentially life-threatening malaria have more severe disease than those in this study, with deeper levels of coma, severe acidosis, severe anaemia, and impairment of pulmonary and renal function. Although several small studies confirm the therapeutic efficacy of repeated administration of rectal artesunate (followed by mefloquine, doxycycline, or sulfadoxine-pyrimethamine) in severe malaria in adults,^{8,41-45} further investigations are needed to confirm this benefit in children, and the therapeutic benefit of initial management with a single dose of rectal artesunate in patients with severe malaria. Studies are being done to investigate the early administration of rectal artesunate in the planned context of remote rural communities in Africa and Asia.

Although artemisinin derivatives have been widely used in southeast Asia, this is not yet the case in Africa where they have a potentially important role, both in combination therapies and as prompt treatment of potentially life-threatening malaria. Our results provide evidence that in children and adults with malaria of moderate severity, artesunate given by suppository is effective in most individuals and is well tolerated. Artesunate suppositories can be given safely by personnel with little training, even in the home. Staff, patients, and parents should be informed of the need to watch for the expulsion of suppositories, and of the need to ensure follow-up with an effective curative treatment. Patients and their relatives readily accepted the use of rectal treatment in this study. Provided early administration of rectal artesunate does not deter patients from reaching a health-care facility that can provide further effective antimalarial treatment and appropriate supportive management, prereferral rectal artesunate has the potential to reduce malaria related morbidity and mortality. This treatment is of greatest relevance to communities in rural areas of malaria-endemic countries, which commonly bear the heaviest malaria burden and for whom parenteral treatment is often not immediately available.

Contributors

Within the WHO TDR programme to develop clinical applications for artemisinin drugs, M Gomes convened the meetings of the WHO TDR Artesunate Task Force to discuss rectal artesunate studies in general, chaired by P J Folb. The study in children was coordinated and supervised by M Molyneux, and K Barnes adapted the study design, coordination, supervision, and analysis of the study in adults. K Barnes, J Mwenechanya, M Tembo, H McMiller, and M Molyneux provided patient care and acquired data. I Ribeiro contributed to the analyses of the study in children. F Little supervised statistical analysis of the study. All authors made substantial contributions to the study and to the writing of the manuscript. K Barnes and M Molyneux took responsibility for the integrity of the work as a whole, from inception to published article.

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Conflict of interest statement

None declared.

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References

- Kidane G, Morrow RH. Teaching mothers to provide home treatment of malaria in Tigray, Ethiopia: a randomised trial. *Lancet* 2000; **356**: 550-55.
- White NJ. Antimalarial drug resistance and mortality in falciparum malaria. *Trop Med Int Health* 1999; **4**: 469-70.
- White NJ. The pathophysiology of malaria. *Adv Parasitol* 1992; **31**: 83-173.
- White NJ. Clinical pharmacokinetics and pharmacodynamics of artemisinin and derivatives. *Trans R Soc Trop Med Hyg* 1994; **88** (suppl 1): S41-S43.
- Janse CJ, Waters AP, Kos J, Lugi CB. Comparison of *in vivo* and *in vitro* antimalarial activity of artemisinin, dihydroartemisinin and sodium artesunate in the *Plasmodium berghei*-rodent model. *Int J Parasitol* 1994; **24**: 589-94.
- Halpaap B, Ndjave M, Parish M, Benakis A, Kremsner PG. Plasma levels of artesunate and dihydroartemisinin in children with *Plasmodium falciparum* malaria in Gabon after administration of 50mg artesunate suppositories. *Am J Trop Med Hyg* 1998; **58**: 365-68.
- Ribeiro IR, Oliario P. Safety of artemisinin and its derivatives: a systematic review of published and unpublished clinical trials. *Med Trop (Mars)* 1998; **58**: 50-53.
- Looareesuwan S, Wilairatana P, Molunto W, Chalermrut K, Oliario P, Andrial M. A comparative clinical trial of sequential treatments of severe malaria with artesunate suppository followed by mefloquine in Thailand. *Am J Trop Med Hyg* 1997; **57**: 348-53.
- Luxemburger C, Brockman A, Silamut K, et al. Two patients with falciparum malaria and poor *in vivo* responses to artesunate. *Trans R Soc Trop Med Hyg* 1998; **92**: 668-69.
- Gogtay NJ, Kadam VS, Karnad DR, Kanbur A, Kamtekar KD, Kshirsagar NA. Probable resistance to parenteral artemether in *Plasmodium falciparum*: case reports from Mumbai (Bombay), India. *Ann Trop Med Parasitol* 2000; **94**: 519-20.
- Brockman A, Price R, van Vugt M, et al. *Plasmodium falciparum* antimalarial drug susceptibility on the north-western border of Thailand during five years of extensive use of artesunate-mefloquine. *Trans R Soc Trop Med Hyg* 2000; **94**: 537-44.
- Molyneux ME, Taylor TE, Wirima JJ, Harper G. Clinical features and prognostic indicators in paediatric cerebral malaria: a study of 131 comatose Malawian children. *Q J Med* 1989; **71**: 441-59.
- World Health Organization. Severe falciparum malaria. *Trans R Soc Trop Med Hyg* 2000; **94** (suppl 1): S136.
- Day NP, Phu NH, Mai NT, et al. The pathophysiologic and prognostic significance of acidosis in severe adult malaria. *Crit Care Med* 2000; **28**: 1833-40.
- Mansor SM, Taylor TE, McGrath CS, et al. The safety and kinetics of intramuscular quinine in Malawian children with moderately severe falciparum malaria. *Trans R Soc Trop Med Hyg* 1990; **84**: 482-87.
- Plowe CV, Kublin JG, Dzinjalimala FK, et al. Sustained clinical efficacy of sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Malawi after 10 years as first line treatment: five year

- prospective study. *BMJ* 2004; published online Feb, DOI: 10.1136/bmj.37977.653750.EE
- 17 Nosten F, Luxemburger C, ter Kuile FO, et al. Treatment of multidrug-resistant *Plasmodium falciparum* malaria with 3-day artesunate-mefloquine combination. *J Infect Dis* 1994; **170**: 971-77.
 - 18 Luxemburger C, Nosten F, Raimond SD, Chongsuphajaisiddhi T, White NJ. Oral artesunate in the treatment of uncomplicated hyperparasitemic falciparum malaria. *Am J Trop Med Hyg* 1995; **53**: 522-25.
 - 19 Navaratnam V, Mordi MN, Mansor SM. Simultaneous determination of artesunic acid and dihydroartemisinin in blood plasma by high performance liquid chromatography for application in clinical pharmacological studies. *J Chromatography B* 1997; **692**: 157-62.
 - 20 Ranford-Cartwright LC, Taylor J, Umasanthar T, et al. Molecular analysis of recrudescence parasites in a *Plasmodium falciparum* drug efficacy trial in Gabon. *Trans R Soc Trop Med Hyg* 1997; **91**: 719-24.
 - 21 Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown KN. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. *Molec Biochem Parasitol* 1993; **58**: 283-92.
 - 22 Artemether-Quinine Meta-analysis Study Group. A meta-analysis using individual patient data of trials comparing artemether with quinine in the treatment of severe falciparum malaria. *Trans R Soc Trop Med Hyg* 2001; **95**: 637-50.
 - 23 Barradell LB, Fitton A. Artesunate: a review of its pharmacology and therapeutic efficacy in the treatment of malaria. *Drugs* 1995; **50**: 714-41.
 - 24 Gu HM, Warhurst DC, Peters W. Uptake of [³H] dihydroartemisinin by erythrocytes infected with *Plasmodium falciparum* *in vitro*. *Trans R Soc Trop Med Hyg* 1984; **78**: 265-70.
 - 25 Shwe T, Myint P T, Htut Y, Myint W, So, L. The effect of mefloquine-artemether compared with quinine on patients with complicated falciparum malaria. *Trans R Soc Trop Med Hyg* 1988; **82**: 665-66.
 - 26 Looareesuwan S, Kyle DE, Viravan C, et al. Treatment of patients with recrudescence falciparum malaria with a sequential combination of artesunate and mefloquine. *Am J Trop Med Hyg* 1992; **47**: 794-99.
 - 27 Murphy S, English M, Waruiru C, et al. An open randomized trial of artemether versus quinine in the treatment of cerebral malaria in African children. *Trans R Soc Trop Med Hyg* 1996; **90**: 298-301.
 - 28 Taylor T, Wills B, Courval JM, Molyneux, M. Intramuscular artemether vs. intravenous quinine: An open, randomized trial in Malawian children with cerebral malaria. *Trop Med Int Health* 1998; **3**: 3-8.
 - 29 Bunnag D, Viravan C, Looareesuwan S, Karbwang J, Harinasuta T. Double blind randomised clinical trial of two different regimens of oral artesunate in falciparum malaria. *Southeast Asian J Trop Med Public Health* 1991; **22**: 534-38.
 - 30 Ringwald P, Louis FJ, Bickel J, Ringwald C, Daban A, Ravinet L. Efficacy and tolerance in adults of a short (3 days) course of quinine for uncomplicated falciparum malaria. *Ann Soc Belg Med Trop* 1995; **75**: 141-43.
 - 31 Adjuk M, Babiker A, Garner P, et al. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* 2004; **363**: 9-17.
 - 32 Bredekamp BL, Sharp BL, Mthembu SD, Durheim DN, Barnes KI. Failure of sulphadoxine-pyrimethamine in treating *Plasmodium falciparum* malaria in KwaZulu-Natal. *S Afr Med J* 2001; **91**: 970-72.
 - 33 Price R, van Vugt M, Phaipun L, et al. Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *Am J Trop Med Hyg* 1999; **60**: 547-55.
 - 34 Kissinger E, Hien TT, Hung NT, et al. Clinical and neurophysiological study of the effects of multiple doses of artemisinin on brain-stem function in Vietnamese patients. *Am J Trop Med Hyg* 2000; **63**: 48-55.
 - 35 van Vugt M, Angus B, Price R, et al. A case-control auditory evaluation of patients treated with artemisinin derivatives for multidrug-resistant *Plasmodium falciparum* malaria. *Am J Trop Med Hyg* 2000; **62**: 65-69.
 - 36 Johann-Liang R, Albrecht R. Safety evaluations of drugs containing artemisinin derivatives for the treatment of malaria. *Clin Infect Dis* 2003; **36**: 1626-27.
 - 37 Genovese RF, Newman DB, Li Q, Peggins JO, Brewer TG. Dose-dependent brainstem neuropathology following repeated arteether administration in rats. *Brain Res Bull* 1998; **45**: 199-202.
 - 38 Brewer TG, Genovese RF, Newman DB, Li Q. Factors relating to neurotoxicity of artemisinin antimalarial drugs "listening to arteether". *Med Trop (Mars)* 1998; **58**: 22-27.
 - 39 Brewer TG, Peggins JO, Grate SJ, et al. Neurotoxicity in animals due to arteether and artemether. *Trans R Soc Trop Med Hyg* 1994; **88** (suppl 1): S33-36.
 - 40 Brewer TG, Grate SJ, Peggins JO, et al. Fatal neurotoxicity of arteether and artemether. *Am J Trop Med Hyg* 1994; **51**: 251-59.
 - 41 Looareesuwan S, Wilairatana W, Vanijanonta S, Viravan C, Andrial M. Efficacy and tolerability of a sequential, artesunate plus mefloquine, treatment of severe falciparum malaria. *Ann Trop Med Parasitol* 1995; **89**: 469-75.
 - 42 Awad MI, Alkadru AMY, Behrens RH, Baraka OZ, Eltayeb IB. Descriptive study of the safety and efficacy of artesunate suppository in combination with other antimalarials in the treatment of severe malaria in Sudan. *Am J Trop Med Hyg* 2003; **68**: 153-58.
 - 43 Bhatt K, Bhatt S, Omonge E, Otekol L, Andrial M. Efficacy and tolerability of a sequential artesunate treatment in severe malaria. *Jpn J Trop Med Hyg* 1996; **24**: 59-63.
 - 44 Eduardo A, Gomez L. Efficacy of artesunate suppository followed by oral mefloquine in treatment of severe malaria in endemic areas where resistance to chloroquine exists in Ecuador. *Jpn J Trop Med Hyg* 1996; **24**: 17-24.
 - 45 Thwe Y, Than M, Phay S, Zawoo A, YuSoc A. Artesunate - mefloquine tablets (Plasmotrim Rectocaps, Mefloquine Lactab) in the treatment of severe malaria. *Jpn J Trop Med Hyg* 1996; **24**: 25-32.

Reducing Malaria's Burden

Evidence of Effectiveness
for Decision Makers

Malaria's Challenge: Saving Lives
By Applying Existing Knowledge

Malaria in Children and Pregnant Women

Indoor Residual Spraying and Insecticide-Treated Nets

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The Role of Artemisinin-based Combination Therapy in Malaria Management

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INTRODUCTION

Background

Malaria morbidity and mortality in Africa is rising, and this is principally a result of increasing chloroquine and sulfadoxine-pyrimethamine (SP) resistance and delays in access to effective therapy.^{1,2} Antimalarial resistance results in an increase in treatment failure, prolonged illness, hospitalisation and death as well as increased malaria transmission, particularly of resistant parasites. Drug resistance is generally underestimated and policy change is complex, slow and costly, resulting in significant delays in ensuring an effective malaria treatment policy in many malaria-endemic countries.

In addition to resistance to therapies, the other important reason for the high mortality from malaria is delay in receiving effective medication. Rural health workers and even parents can be taught to identify the symptoms and signs of life-threatening malaria.³ However, many patients with more severe malaria are not able to take medicines orally because they are confused, unconscious or vomiting. Conventional therapy for severe malaria, in addition to the highest level of supportive care available, is injectable (parenteral) quinine. A considerable proportion of people living in malaria-endemic areas do not have ready access to health facilities able to provide parenteral treatment. For patients unable to take oral medication, delay in access to prompt parenteral therapy can be fatal. Progression from being unable to take oral treatment to severe malaria can occur within hours.¹ Once the opportunity for early therapeutic intervention is lost and organ failure has developed, the prognosis worsens considerably and survival depends a great deal on access to a healthcare facility with resources to manage malaria complications.⁵ Such facilities are scarce in malaria-endemic countries.

Rationale for use of artemisinin-based combination therapy

Artemisinin is the active antimalarial component isolated from the medicinal herb *Artemisia annua*, which has been

used for centuries in China as a traditional treatment for fever and malaria. In recent years, artemisinin derivatives have become the focus of worldwide attention in the light of emerging resistance of *Plasmodium falciparum* to first-line drugs; to date resistance to artemisinin has not been demonstrated in malaria parasites. While artemisinin derivatives require at least seven days of treatment when administered alone, when combined with other first-line drugs, artemisinin-based combination therapies (ACTs) can eradicate parasites quickly and protect against the development of resistance to both drugs. Improved cure rates and decreased gametocyte carriage have been confirmed in limited African field trials.^{6,7} Artemisinin derivatives are the only first-line malaria treatments to act on gametocytes (the stage of the malaria parasite's life cycle responsible for ongoing malaria transmission) thereby potentially reducing malaria transmission and particularly the transmission of resistant strains of malaria. The principle of using combination therapy to delay the emergence and spread of resistance is established in the management of tuberculosis and HIV, and is expected to apply equally to malaria.⁸

This chapter will explore the role of artemisinin-based combinations in addressing the increasing morbidity and mortality in malaria-endemic countries, either when administered orally for uncomplicated malaria or as a suppository in more severe malaria.

ROLE OF EVIDENCE

Selection of malaria treatment occurs at two levels, that of policy makers and that of individual health care providers (both formal and informal). Policy makers require evidence to determine when malaria treatment policy should be changed and to select which antimalarial(s) should replace the current treatment. Health care providers are influenced by availability of antimalarial treatments (which in the public sector is often guided by malaria treatment policy and the essential drugs programme) and by perceived effectiveness, safety and affordability.

BOX 1. ARTEMISININ DERIVATIVES CURRENTLY WIDELY AVAILABLE

- ARTEMETHER
- ARTEMISININ
- ARTESUNATE
- CO-ARTEMETHER (artemether plus lumefantrine)
- DIHYDROARTEMISININ

Artemisinin-based Combination Therapy

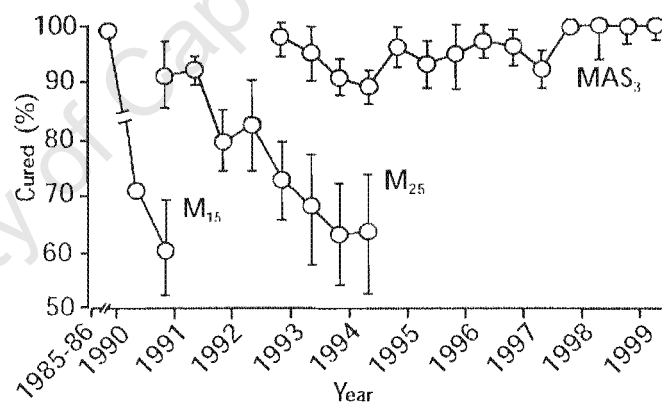
With the possible exception of artemisinin derivatives, resistance of *P. falciparum* to all available alternatives has been frequently documented.⁹ Artemisinin derivatives used alone are associated with high treatment failure rates unless administered for seven days, which is not often achieved as symptoms are generally relieved within two - three days. Thus, artemisinin derivatives should only be used in combination with a second effective antimalarial; poor efficacy of this component significantly compromises the efficacy of the combination. Results presented below focus on currently available combinations that are either packaged together or manufactured as a fixed dose combination. There are a number of artemisinin-based combinations currently under development, with dihydro-artemisinin plus piperazine and artesunate plus chlorproguanil-dapsone most likely to be widely available in the medium term.

A considerable clinical evidence base has been established through the largest randomised controlled trials ever conducted on antimalarials, demonstrating that artemisinin-based combinations improve cure rates, decrease gametocyte carriage and are well tolerated with few serious adverse effects (Taylor W, personal communication, 2003). In the first of these multi-centre trials to be published, 941 children who had uncomplicated *P. falciparum* malaria were randomly assigned three days treatment with amodiaquine plus artesunate or amodiaquine plus placebo. Both regimens were well tolerated. The combination of artesunate and amodiaquine significantly improved treatment efficacy in Gabon 85% vs. 71%, ($p=0.02$) and Kenya 68% vs. 41% ($p<0.0001$), although these two were equivalent in S eu egal where the day-28 cure rates for amodiaquine-artesunate versus amodiaquine were 82% vs. 79% ($p=0.5$).¹⁰

The efficacy and safety of artesunate in combination with sulfadoxine-pyrimethamine (SP) has been evaluated in randomised controlled trials involving 2865 patients in sub-Saharan Africa (Taylor W, personal communication, 2003). Results from the first study published from The Gambia,¹¹ where the cure rate with SP monotherapy is 93%, showed that both cure rate and parasite clearance were significantly higher in patients who received 3 days of artesunate plus a single dose of SP compared with those who received SP alone. Gametocyte carriage was 68% following SP treatment in comparison to 21% following combination treatment ($p = 0.001$).

The largest series of therapeutic efficacy studies with artesunate plus mefloquine demonstrate a sustained increased cure rate (almost 100% from 1998 onwards), despite the established resistance pattern seen for high-dose mefloquine alone between 1990-94, prior to deployment of artesunate plus mefloquine (Figure 1).¹²

FIGURE 1. CUMULATIVE CURE RATES (95% CI), ASSESSED AT DAY 28 FOR DIFFERENT REGIMENS FROM PROSPECTIVE STUDIES



M₁₅ = mefloquine (15 mg/kg)
M₂₅ = mefloquine (25mg/kg)
MAS₃ = artesunate (4 mg/kg for 3 days) with mefloquine (25 mg/kg split dose)

Artemether-lumefantrine is the first fixed-dose combination of two antimalarials that was not widely used prior to being marketed. A Cochrane systematic review of randomised and quasi-randomised trials comparing artemether-lumefantrine administered orally with standard treatment regimens (e.g., chloroquine, sulfadoxine-pyrimethamine or mefloquine) found only two trials where participants received the recommended full six doses of artemether-lumefantrine. These showed no difference in cure rates from artesunate plus mefloquine.¹³ Six trials with 1698 participants tested a four-dose regimen. Failure rates for a four-dose regime of artemether-lumefantrine were higher than standard treatment regimens. While artemether-lumefantrine was

better than chloroquine in two studies, the failure rate for chloroquine at the trial sites was over 50%.

Artesunate

Artesunate can be administered as a tablet, an injection, or as a rectal suppository. A comparison of intravenous artesunate and quinine in 113 adults with severe malaria reported mortality of 12% with artesunate and 22% with quinine ($p = 0.22$).¹⁴ In patients with hyperparasitaemia who had no other features of severe malaria but were at an increased risk of developing severe malaria, oral artesunate was found to be superior to intravenous quinine in reducing both clinical symptoms and parasites.¹⁵ Rectally-administered artesunate has been shown to be safe and highly effective in children and adults with uncomplicated or moderately severe falciparum malaria.^{16,17} A number of recent randomised controlled and open label studies of rectal artesunate in Africa and Asia have demonstrated the rapid antimalarial efficacy of a single dose of rectal artesunate (10 mg/kg) in moderately severe falciparum malaria in both children and adults prior to referral for definitive treatment. All patients had evidence of adequate absorption of the drug. Clearance of malaria parasites from the peripheral blood was consistently more rapid with rectal artesunate than with quinine injection. These clinical and parasitological responses suggest that rectal artesunate could provide useful initial management of acute malaria in patients who cannot take medication by mouth and for whom parenteral treatment may not be available. There are also a number of small open label studies, some of which were randomised, demonstrating the clinical and parasitological efficacy of rectal artesunate in adults with severe *P. falciparum* infections¹⁸ where rectal artesunate was administered repeatedly and was combined sequentially with a second oral antimalarial to prevent recrudescence (reappearance) of malaria.

Safety of artemisinin derivatives

Artemisinin and its derivatives have been used extensively, especially in Southeast Asia, for the treatment of *P. falciparum* malaria with very little evidence of toxicity. Most of the concerns regarding the safety of artemisinin derivatives are based on animal studies or on mild to moderate adverse events that have been reported from clinical studies.^{19,20} However, only a few studies have included young children in Africa.²¹ Concerns about potential neurotoxicity stem from animal studies that showed an unusual pattern of damage to the brainstem induced by high dose ($>15\text{mg/kg/day}$ for 14 days) parenteral artemether and arteether.²² Although prolonged coma time was observed in two of three randomised controlled trials comparing artemether with quinine in the treatment of cerebral malaria, a meta-analysis

found no increased risk of prolonged coma in the artemisinin derivative-treated patients.²¹ Autopsy of fatal severe malaria cases exposed to artemisinin derivatives found no evidence of neurotoxicity. There have been sporadic case reports of impaired balance, nystagmus, fine motor coordination, paraesthesia and seizures following treatment of severe falciparum malaria with an artemisinin derivative. However, concomitant administration of mefloquine and malaria itself may have caused these neurological abnormalities.

Clinical studies have reported pruritus and mild skin rash in 1–4% of patients receiving artemisinin-based combination therapy, with a higher incidence observed with the concurrent administration of sulfadoxine-pyrimethamine.^{21,23,26,27} Two cases of severe allergic reactions to artesunate have been reported in Thailand.²⁸

Of 153 children with malaria enrolled in a randomised controlled trial comparing artesunate plus amodiaquine with amodiaquine alone, nine children (6% [95% CI 3–11]) developed a significant decrease in their neutrophil count. Three were on amodiaquine-artesunate and six on amodiaquine alone; all had normal neutrophil counts before treatment.²⁹ A decrease in the number of neutrophil cells in the blood (neutropenia) is associated with increased risk of severe infections.

Infants born to 287 women inadvertently exposed during pregnancy to a mass administration of a single dose of artesunate plus SP, had higher birth weights than those not exposed, and there were no differences in the proportion of congenital abnormalities, stillbirths, abortions or neonatal deaths between exposed and unexposed women.³⁰ In a prospective open-label study of pregnant women treated with artemisinin derivatives (mostly artesunate) for 539 multidrug-resistant falciparum malaria episodes, there was no evidence of adverse effects. Birth outcomes did not differ significantly from community rates for abortion, stillbirth, or congenital abnormality.³¹ Although these studies suggest that artemisinins may have a relatively good safety profile during pregnancy, only 80 of the women studied were exposed to the drug during the first trimester.

Implementation Issues

The gaps between scientific knowledge and health policy as well as between theoretical health policy and practice continue to widen.³² Although local efficacy data is recognised as a necessary pre-requisite for an appropriate and evidence-based malaria policy, efficacy data alone are not sufficient to ensure effective policy-making or implementation. Even with adequate data on the status of antimalarial drug resistance, enormous challenges face those attempting to use research results to determine policy and/or influence practice.³³

Effectiveness vs. efficacy

Effectiveness of treatment may be lower than the efficacy reported in clinical trials as a result of poor adherence to therapy, sub-therapeutic dosing (usually done to limit cost to the patient), poor quality medication or greater disease severity than the group studied. For example, artemether-lumefantrine needs to be administered with a food or drink containing fat to ensure adequate absorption, a particular challenge for patients suffering from nausea or who cannot afford or access such foods. Studies are usually conducted in well-equipped facilities that also provide further supportive care, which is generally scarce in malaria endemic countries.

Diagnostic challenges

Malaria diagnosis is usually based on clinical symptoms, often resulting in misdiagnosis in patients presenting with a disease caused by another pathogen that will not respond to malaria treatment. In areas with higher intensity malaria transmission, even the introduction of definitive diagnosis would not fully address this problem, as many malaria infections are asymptomatic. Thus the presence of parasites does not necessarily imply that malaria is the cause of the presenting disease.

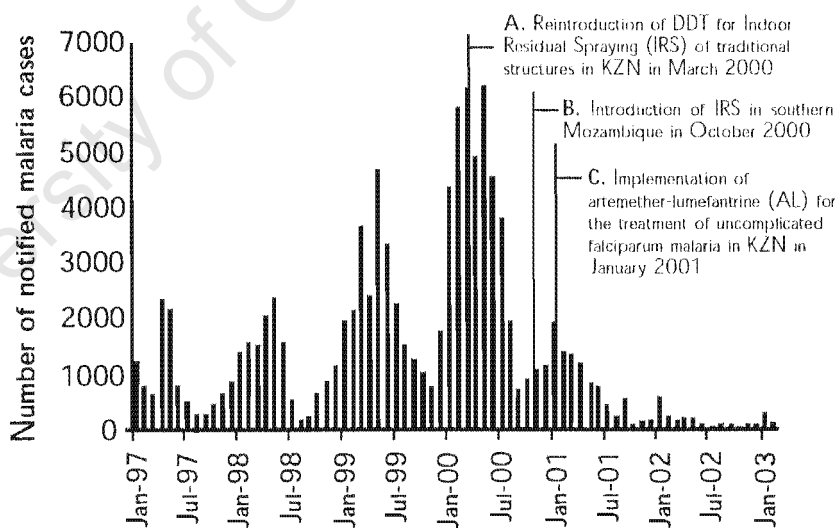
Adverse events

Rare serious adverse effects of treatment are unlikely to be detected in randomised trials involving hundreds of patients. However, a systematic review of over 10,000 patients reported no instances of severe adverse events or toxicity.³¹ It is important to note that those at highest risk, including pregnant women, infants and the immunocompromised (as a result of HIV-AIDS or malnutrition) may be systematically excluded from therapeutic efficacy studies. This is particularly important for potentially life-threatening adverse effects that are not clinically apparent, such as neutropenia or hepatotoxicity. Both require routine laboratory monitoring for early detection, which is not achievable in most health care facilities situated in the tropics.

Curtailing resistance and transmission

As clinical trials may overestimate the value of ACT in the real world, research is needed to determine the net benefit of wide-scale deployment of these drugs. A prospective study of malaria incidence and treatment was conducted on the northwest border of Thailand to assess the incidence of *P. falciparum* malaria and the therapeutic responses to mefloquine treatment over 13 years. During this time, mefloquine resistance was decreased following the widespread deployment of artesunate plus mefloquine, and there has been a sustained decline of at least six-fold in the incidence of *P. falciparum* malaria in the study area. Laboratory studies confirmed that the susceptibility of falciparum malaria parasites to mefloquine improved significantly following the addition of artesunate to mefloquine ($p=0.003$).³⁰ Although such a study would take years to replicate, the significantly decreased malaria case load observed in Vietnam and KwaZulu-Natal, South Africa following the large-scale implementation of ACTs is encouraging (Figure 2).

FIGURE 2. NUMBER OF NOTIFIED MALARIA CASES IN KWAZULU-NATAL, SOUTH AFRICA BY MONTH IN RELATION TO TIMING OF SIGNIFICANT MALARIA CONTROL INTERVENTIONS



Source: KwaZulu-Natal Department of Health, South Africa

Treatment-seeking behaviour

The beneficial effects on antimalarial resistance and transmission depend on ensuring that the majority of falciparum infections are treated with artemisinin-based combinations and that the use of either component alone is curtailed. This is influenced by the treatment-seeking behaviour of patients and their adherence to completing malaria treatment, as well as by the intensity of malaria transmission. In areas of high intensity malaria transmission, individuals carrying malaria parasites are frequently asymptomatic and are thus less likely to seek treatment, potentially resulting in lower ACT coverage. It is encouraging to note that despite having to carry the cost themselves, the majority (88.5%) of symptomatic patients in a Myanmar study preferred artesunate plus mefloquine over the drugs they had used before; perception of higher drug efficacy was the reason given for the preference by most.³⁶

Costs

Despite growing international evidence that ACT is one of the few effective measures available to "Roll Back Malaria", the costs of ACTs are an important obstacle to widespread implementation. This reflects the poor documentation of the high societal and economic costs associated with antimalarial resistance, and the perceptions of healthcare providers and governments that ACT would not be affordable or sustainable in their resource-constrained environments. However, decreasing malaria transmission would result in a decrease in malaria cases and hospital admissions. Delaying the development of resistance should prolong the effective life of these antimalarials, resulting in fewer treatment failures and therefore fewer deaths and potentially lower future costs. Economic evaluations are underway to measure the cost and cost-effectiveness of implementing an ACT malaria treatment policy. Biological and economic modelling are also being used to explore how to optimise the deployment of antimalarials as well as to assess their effectiveness and impact on direct and indirect costs of malaria.^{37,38}

Rectal artesunate

Rectal administration of artesunate as initial treatment prior to referral makes it possible to provide a rapidly acting and effective drug to patients with acute malaria who are unable to take oral medication and have no access to healthcare facilities where parenteral antimalarial therapy is available. Suppositories can be safely administered in minimally-equipped health care facilities and even at home. Provided that early administration of rectal artesunate does not deter patients from reaching a health care facility able to provide further malaria treatment and appropriate supportive care, this intervention has the potential to reduce malaria-related morbidity and mortality in rural areas in

the tropics. Among the artemisinin derivatives, artesunate was selected on the basis of its rapid and broad spectrum of antimalarial activity, reliable absorption and reassuring safety and efficacy profiles in comparison to other available treatments.^{39,40} A thermostable suppository formulation of artesunate was chosen because unrefrigerated storage and ease of administration are essential where facilities are rudimentary.

Many patients with potentially life-threatening malaria have more severe disease than those enrolled in clinical studies, with deeper levels of coma, severe acidosis, severe anaemia and impairment of pulmonary and renal function. Although minimally trained personnel can administer artesunate suppositories safely, staff (and parents) should be informed of the need to observe for the expulsion of suppositories. The time to clear a fever has sometimes been reported to be shorter with artemisinin derivatives than quinine. Although rapid fever clearance is an important clinical benefit, it does not necessarily reflect cure and may create a false sense of security; additional definitive curative treatment remains essential even if symptoms are relieved. Health care providers need to ensure that patients or caregivers understand the need for further curative treatment at a referral centre.

Scaling up ACT

The scaling up of artemisinin-based combination therapy as first-line treatment policy has followed the improved cure rates, decreased malaria transmission and delayed resistance documented on the western border of Thailand.⁴¹ There is now growing international consensus that wide-scale systematic implementation of ACT is one of few effective measures that will enable malaria-endemic countries to achieve the ambitious goals set in Abuja to Roll Back Malaria, particularly the goal of cutting malaria morbidity and mortality in half by 2010.⁴²

A statement from the World Health Organisation (WHO) released on 20 February 2002, recommended that:

"Governments...rapidly adopt more effective treatments. The aim is to provide effective treatment against malaria and to slow the spread of drug resistance... In particular, WHO recommends the use of artemisinin-based combination therapy."⁴³

The widespread use of ACTs was initially adopted in Southeast Asia, in Thailand and Vietnam and more recently in Cambodia, Bhutan and Myanmar. ACTs are increasingly being recommended elsewhere as first-line treatment for uncomplicated malaria in South America (western Peru, Surinam, Bolivia, Guyana and French Guyana) and in Africa (South Africa, Zambia, Gabon, Burundi and Zanzibar). Funding by the Global Fund to Fight AIDS, Tuberculosis and Malaria has largely facilitated these policy changes.

Policy Implementation

The successful translation of policy into practice is challenging, and improvements to the public health care infrastructure required for facilitating this are beyond the scope of this chapter. However, the most important aspects include: the proactive guarantee of supplies through the early involvement of pharmaceutical services, intensive training in small groups, on-site structured monitoring visits, and the physical removal from each facility of the anti-malarial drug being withdrawn and its replacement with the new treatment. The implementation of changes in malaria treatment policy has been compromised in countries without the staff and resources to do this on the scale needed to insure a complete switch in drugs (Williams HA, unpublished data 2002). Thus, implementation plans should be drafted well in advance of the date of implementation, so that adequate funds are identified to assist with additional demands on already limited health care resources.

CHALLENGES/NEXT STEPS

There has been a welcome increase recently in funding for both the wide-scale public sector implementation of effective case management and for the comprehensive evaluations of such interventions within the normal context of use. Consensus is growing regarding the important role of artemisinins in malaria case management, particularly for the use of ACT as first-line treatment of uncomplicated malaria and for the rectal administration of artesunate to patients who are unable to tolerate oral treatment and do not have access to injectable treatment. There are, however, a number of questions that need to be resolved to optimise the public health benefits of these strategies.

Despite the evidence available to date, concern has been expressed that the purported benefits of ACT have not yet been proven in Africa.^{11,15} This has motivated large-scale comprehensive evaluations of the staged introduction of ACT that are currently underway in the public sector in Southern and East Africa.¹⁶ These studies aim to establish whether ACT is cost effective (or even cost-saving) as well as whether ACT improves treatment cure rates, reduces malaria transmission, decreases morbidity and mortality and delays the emergence of resistance to affordable first-line anti-malarial therapy within the normal context of use in Africa. These studies also hope to establish how treatment-seeking behaviour and patient adherence influence the public health impact of ACTs.

Tools for monitoring drug safety and adherence with treatment need strengthening, particularly in resource-poor

settings. Although more safety data is available regarding artemisinin derivatives than other antimalarials, concerns regarding safety in high-risk groups, specifically pregnant women and infants, require further monitoring. As these special risk groups carry the highest burden of malaria, addressing this need is urgent. The efficacy and safety of ACT for routine intermittent treatment through antenatal and vaccination programmes are currently being studied.

Rectal artesunate has been shown to clear parasites more rapidly than parenteral quinine, although this does not necessarily lead to a clinical advantage or a decrease in mortality. Large randomised placebo-controlled studies are underway in Africa and Asia to establish whether the administration of rectal artesunate to patients unable to tolerate oral treatment in basic health care facilities where parenteral treatment is not available, decreases malaria-related hospital admissions and deaths. These studies are being conducted without other interventions, so they will reflect benefits within the normal context of use where health care providers face numerous challenges. These include the use of presumptive or clinical rather than definitive diagnosis of malaria, the high prevalence of HIV/AIDS and malnutrition, dual infections at presentation (e.g., meningitis), and the widespread use of concomitant medication (traditional and Western medicines). Concern that the rectal administration of artesunate may delay seeking definitive treatment and supportive care if needed, or that rectal artesunate may be less reliably absorbed in patients with more severe malaria, should also be answered through these studies.

Changing national malaria treatment policy could be the single intervention that is most likely to enhance access to ACT, whether administered orally for uncomplicated malaria or rectally for more severe malaria. However, what is not known is how much evidence is required to change existing national treatment policy. In countries that have different transmission profiles, evidence from one or two local studies may not be sufficient to convince policy makers of the need for a national level change of policy. The guidelines most widely used are those of the WHO, which recommends implementing a malaria treatment policy change when first-line treatment fails in 25% of patients; this recommendation is considered to contribute to the prolonged use of ineffective malaria treatment, particularly since the recommended 14-day follow-up underestimates resistance.¹⁷ Up-to-date international guidelines on what constitutes adequate sentinel surveillance for determining malaria treatment efficacy and the levels of resistance at which the change process should be initiated, would facilitate rational antimalarial drug policy making. Global agencies tasked with malaria control should take a lead in preparing such guidelines. Once a minimum standard of surveillance is agreed upon, adequate resources will need to be earmarked to support this pivotal activity.

The most frequently cited obstacle to enhancing access to ACT has been its substantially higher cost per treatment course when compared to chloroquine or SP. Economic and societal costs of ineffective treatment policies need to be defined so that these can be compared with the costs of an ACT policy change. However, it is likely that the cost of such a policy change will not be affordable to many malaria endemic countries, and international funding to support this change is needed. It is of concern that the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) is currently spending more in the procurement of chloroquine than of ACTs in Africa, despite established widespread chloroquine resistance and an associated increase in mortality.¹⁶ This funding should rather be targeted to ensure that communities suffering from malaria are

provided with effective malaria treatment. This will require revision of the procedure for technical review of funding applications so that the mechanisms currently in place within the GFATM to ensure rational funding of second-line tuberculosis treatment can also be applied to malaria treatment.

Through dedicated partnership between malaria endemic countries and international research and funding organisations, the ambitious goals set in Abuja to roll back malaria are achievable. The widespread use of ACT is one of the few tools currently available that can reduce morbidity and mortality from malaria and make malaria a preventable and treatable condition.

REFERENCES

- 1 Trape JE, Pison G, Preziosi MP, Enel C, Desgrées du Lou A, Delaunay V, Samb B, Lagarde E, Moïez JE, Simoneton E. Impact of chloroquine resistance on malaria mortality. *Comptes rendus de l'Académie des Sciences. Serie III, Sciences de la vie.* 1998; 321: 689-697.
- 2 Snow W, Trape J, Marsh K. The past, present and future of childhood malaria mortality in Africa. *Trends in Parasitology.* 2001; 17: 593-597.
- 3 Kidanè G, Morrow RH. Teaching mothers to provide home treatment of malaria in Tigray, Ethiopia: a randomised trial. *Lancet.* 2000; 356: 550-55.
- 4 White NJ. Antimalarial drug resistance and mortality in falciparum malaria. *Trop Med Int Health.* 1999; 4: 469-470.
- 5 White NJ. The pathophysiology of malaria. *Adv Parasitol.* 1992; 31: 83-173.
- 6 von Seidlein L, Milligan P, Pinder M, et al. Efficacy of artesunate plus pyrimethamine - sulfadoxine for uncomplicated malaria in Gambian children: a double blind, randomized, controlled trial. *Lancet.* 2000; 355: 352-357.
- 7 Adjuik M, P. Agnancy, A Babiker, et al. Amodiaquine and artesunate versus amodiaquine for uncomplicated falciparum malaria in African children: a randomized multi-centre trial. *Lancet.* 2002; 359 (9315): 1365-72.
- 8 White NJ. Preventing antimalarial drug resistance through combinations. *Drug Resistance Updates.* 1998; 1:3-9.
- 9 Op cit 4.
- 10 Op cit 7.
- 11 Op cit 6.
- 12 Nosten F, van Vugt M, Price R, Luxemburger C, Thwai KI, Brockman A, McGready R, ter Kuile F, Looareesuwan S, White NJ. Effects of artesunate-mefloquine combination on incidence of Plasmodium falciparum malaria and mefloquine resistance in Western Thailand: a prospective study. *Lancet.* 2000; 356: 297-302.
- 13 Omari AAA, Preston C, Garner P. Artemether-lumefantrine for treating uncomplicated falciparum malaria (Cochrane Review). In: *The Cochrane Library* (2003), Issue 4. Chichester, UK: John Wiley & Sons, Ltd.
- 14 Newton PN, Angus BJ, Chierakut W, Dondorp A, Ruangveerayuth R, Silawat K, Iserapong P, Suputtamongkol Y, Looareesuwan S, White NJ. Randomised comparison of artesunate and quinine in the treatment of severe malaria. *Clin Infect Dis.* 2003; 37(1): 7-16.
- 15 Luxemburger C, Thwai KI, Raimond SD, Chongsuphajaisiddhi T, White NJ. Oral artesunate in the treatment of uncomplicated hyperparasitaemic falciparum malaria. *Am J Trop Med Hyg.* 1995; 53: 522-525.
- 16 Karunajeewa HA, Kemiki A, Alpers MP, Lorry K, Barry KT, Ilett KF, Davis TM. Safety and therapeutic efficacy of artesunate suppositories for treatment of malaria in children in Papua New Guinea. *Pediatr Infect Dis J.* 2003; 22(3): 251-6.
- 17 Sabchareon A, Attanath P, Chanthavanich P, Phamuksook P, Prarinyanopharb V, Poonpanich Y, Mookmanee D, Teja-Isavadharm P, Heppner DG, Brewer TG, Chongsuphajaisiddhi T. Comparative clinical trial of artesunate suppositories and oral artesunate in combination with mefloquine in the treatment of children with acute falciparum malaria. *Am J Trop Med Hyg.* 1998; 58: 11-16.
- 18 Awad MI, Alkadru AMY, Behrens RH, Baraka OZ, Fitayeh IB. Descriptive study of the efficacy and safety of artesunate suppository in combination with other antimalarials in the treatment of severe malaria in Sudan. *Am J Trop Med Hyg.* 2003; 68(2): 153-8.
- 19 Ribeiro IR, Olliaro P. Safety of artemisinin and its derivatives: A systematic review of published and unpublished clinical trials. *Med Trop (Mars)* 1998; 58, 50 - 53.
- 20 Price R, van Vugt M, Phaipun L, et al. Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *Am J Trop Med Hyg.* 1999; 60, 547-555.
- 21 Newton P, Proux S, Green M. Fake artesunate in southeast Asia. *Lancet.* 2001; 357 (9272): 1948-50.
- 22 Brewer T.G., Grate S.J., Peggins J.O., Weina P.J., Petras J.M., Levine B.S., Heiffer M.H., Schuster B.G. Fatal neurotoxicity of arteether and artemether. *Am J Trop Med Hyg.* 1994; 51: 251-259.
- 23 McIntosh HM, Olliaro P. Artemisinin derivatives for treating severe malaria. In: *The Cochrane Library* (2003), Issue 3: 1-25; Oxford: Update Software.
- 24 Balshi R, Hermeling-Fritz I, Gathmann I, Alteri E. An integrated assessment of the clinical safety of artemether-lumefantrine: a new oral fixed-dose combination antimalarial drug. *Trans R Soc Trop Med Hyg.* 2000; 94(4): 419-424.
- 25 Price R, van Vugt M, Phaipun L, Luxemburger C, Simpson J, McGready R, ter Kuile F, Kham A, Chongsuphajaisiddhi T, White NJ, Nosten F. Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *Am J Trop Med Hyg.* 1999; 60, 547-555.
- 26 Tjitra E, Suprianto S, Currie BJ, Tjitra E, Suprianto S, Currie BJ, Morris PS, Saunders JR, Anstey NM. Therapy of uncomplicated falciparum malaria: a randomised trial comparing artesunate plus sulfadoxine-pyrimethamine versus sulfadoxine-pyrimethamine alone in Irian Jaya, Indonesia. *Am J Trop Med Hyg.* 2001; 65(4): 309-317.

²⁷ van Vugt M, Looareesuwan S, Wilairatana P, McGready R, Villegas L, Gathmann I, Mull R, Brockman A, White NJ, Nosten F. Artemether lumefantrine for the treatment of multidrug resistant falciparum malaria. *Trans R Soc Trop Med Hyg* 2000; 94: 545-548.

²⁸ Leonardi E, Gilvary C, White NJ, Nosten F. Severe allergic reactions to oral artesunate: a report of two cases. *Trans R Soc Trop Med Hyg*. 2001; 95(2): 182-183.

²⁹ Op cit. 7.

³⁰ Deen JL, von Seidlein L, Pinder M, Welraven GE, Greenwood BM. The safety of the combination of artesunate and pyrimethamine-sulfadoxine given during pregnancy. *Trans R Soc Trop Med Hyg*. 2001; 95(4): 424-8.

³¹ McGready R, Cho T, Keo NK, Titwai KL, Villegas L, Looareesuwan S, White NJ, Nosten F. Artemisinin antimalarials during pregnancy: a prospective treatment study of 539 episodes of multidrug resistant *Plasmodium falciparum*. *Clin Infect Dis*. 2001; 33(12): 2009-2016.

³² Kitua AY. Antimalarial drug policy: making systemic change. *Lancet*. 2000; 354, 32.

³³ Bloland PB. A contrarian view of malaria therapy policy in Africa. *American Journal of Tropical Medicine and Hygiene* 2003; 68, 125-126.

³⁴ Op cit. 19.

³⁵ Op cit. 12.

³⁶ Op cit. 33.

³⁷ Hastings IM. Modelling parasite drug resistance: lessons for management and control strategies. *Trop Med Int Health* 2001; 6(11): 883-90.

³⁸ Goodman CA, Coleman PG, Mills AJ. Changing the first line drug for malaria treatment- cost-effectiveness analysis with highly uncertain inter-temporal trade offs. *Health Econ*. 2001; 10(8): 731-49.

³⁹ White NJ. Clinical Pharmacokinetics and pharmacodynamics of artemisinin and derivatives. *Trans. R. Soc. Trop. Med. Hyg.* 1994; 88 (Suppl 1): S41 - S43.

⁴⁰ Op cit. 19.

⁴¹ Op cit. 12.

⁴² Yamay G. African heads of state promise action against malaria. *BMI*. 2000; 320: 1228.

⁴³ Ibid.

⁴⁴ Op cit. 33.

⁴⁵ Dorsey G, Njama D, Kanya MR, Cattamanchi A, Kyabayinze D, Staedke SG, Gasasira A, Rosenthal PJ. Sulfadoxine/pyrimethamine alone or with amodiaquine or artesunate for treatment of uncomplicated malaria: a longitudinal randomised trial. *Lancet*. 2002; 360, 2031-2038.

⁴⁶ Kachur SP, Abdulla S, Barnes KI, Mshinda H, Durrheim DN, Kitua A & Bloland P. Complex, and large, trials of pragmatic malaria interventions. *Tropical Medicine and International Health*. 2001; 6, 324-325.

⁴⁷ White NJ. The assessment of antimalarial drug efficacy. *Trends Parasitol*. 2002; 18(10), 458-64.

⁴⁸ Op cit. 1.

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POTENTIAL CONFLICT OF INTEREST

None.

| | |
|----------|--|
| Code | |
| Initials | |

Subject Information and Consent Form: Artesunate study

You have malaria, which is a serious disease. We are conducting a study on a new drug for treating malaria which is called artesunate, and we would like to include you in this study. You will be treated for this disease whether or not you choose to participate in this study. You are free to ask any questions about the study at any time.

This new medicine, artesunate, has been found to be effective and safe in treating malaria in other countries, but has not yet been used in South Africa. This study may help artesunate to become more available in South Africa and other countries in Africa. We plan to include 100 patients with malaria in this study. Patients entered into the study will be closely monitored. Regular blood tests will be taken to see what is happening to these medicines in their body, and how quickly the malaria is going away.

Some patients will be given the usual malaria treatment, so that we can compare the results of the old and new treatments. The sickest patients will be given quinine (the usual treatment) in a drip at once, with or without the new medicine, artesunate suppository. These patients will need to stay in hospital for a week.

The less sick patients, will be given either artesunate suppositories OR quinine injections on the first day followed by sulphadoxine / pyrimethamine tablets (the usual treatment for patients who are well enough to take tablets) on the second day. Anyone who does not get better will be given a quinine drip. These less sick patients only need to stay in hospital until they are well enough to go home.

Artesunate has been found to be safe in the patients who have been treated with this drug in other countries. But, since this is a new medicine, we will examine you very carefully for any side effects. These may include changes in your nervous system, which have been seen in animals (given much higher doses than we will be using), but not in humans. The new treatment may also cause nausea and vomiting.

We would like you to come back for a check up after one, two and six weeks, to make sure that you are well and that the malaria has not come back. We will pay you R50 at each visit to cover your expenses.

Please note:

- Women who may be pregnant should not enter the trial.
- Those who have taken other malaria medicines in the last 24 hours should not enter the trial.
- Only patients between 16 and 65 should enter the trial.
- Those allergic to quinine or artemisinin derivatives (e.g. artemether) should not enter the trial.
- Those patients who have had any rectal surgery or disease of the rectum should not enter the trial.

By signing this form, I agree to participate in this study; or the next of kin / legal guardian agrees that the patient named participates in the study; or the witness agrees that the patient has given verbal consent to participate in this study.

Patient surname

Patient first name

Next of kin / legal guardian / witness surname

Next of kin / legal guardian / witness first name

Patient / next of kin / legal guardian / witness signature. ***Please specify which***

Date

Normal Ranges for Laboratory Values

| | Lower limit | Upper Limit | Units |
|---------------------------------------|-------------|-------------|-------------------|
| Haematology (Mosvold hospital) | | | |
| Haemoglobin | 11.6 | 17.3 | g/dl |
| Haematocrit | 0.32 | 0.53 | L/L |
| MCV | 80 | 95 | fl |
| White cell count | 3 | 11 | $\times 10^9 / L$ |
| Platelets | 155 | 415 | $\times 10^9 / L$ |

| Chemistry (Study lab) | | | |
|------------------------------|-----|-----|------------|
| Sodium | 136 | 144 | mmol/L |
| Potassium | 3.5 | 5.5 | mmol/L |
| Ionised Calcium | 1.1 | 1.2 | mmol/L |
| Glucose | 3.9 | 5.6 | mmol/L |
| Lactate | 0.7 | 1.8 | mmol/L |
| Urea | 1.7 | 6.7 | mmol/L |
| Creatinine | 75 | 115 | micromol/L |

| Blood Gas Analysis (Study Lab) | | | |
|---------------------------------------|------|------|--------|
| pH | 7.36 | 7.44 | |
| Standard bicarb | 22 | 27 | mmol/L |
| Base deficit | -3 | 0 | |

| Liver Function Tests (Groote Schuur hospital) | | | |
|--|----|----|-----------|
| Total protein | 60 | 80 | G/L |
| Albumin | 35 | 50 | G/L |
| Total bilirubin | 1 | 17 | U mol / L |
| Conj. bilirubin | 0 | 4 | U mol / L |
| Alk. Phos. | 30 | 70 | Units / L |
| AST | 7 | 25 | Units / L |
| ALT | 1 | 25 | Units / L |
| Gamma GT | 0 | 40 | Units / L |
| CRP | 0 | 5 | Mg/L |

1. ANALYTICAL METHODOLOGY

Artesunate (AS) and dihydroartemisinin (DHA) were determined in plasma by a specific and sensitive high-performance liquid chromatographic method with electrochemical detector operating in the reductive mode (HPLC-ECD) method [1]. However the published method has been modified and is briefly summarised below; a detailed description of the revised methodology is given in the Standard Operating Procedures of the Centre of Drug Research, Universiti Sains Malaysia (USM) (CDR-SOP.ALU2). The lower limit of detection for both AS and DHA was 4.0ng/0.5ml. The assays were conducted according to Good Laboratory Practice (GLP) procedures and requirements at the Centre for Drug Research, USM, Penang, Malaysia.

After extraction from plasma, AS and DHA were analyzed using Hypersil Butyl C₄ column and a mobile phase of acetonitrile– 0.05 M acetic acid (40:60 v/v) adjusted to pH 5.0 with electrochemical detection in the reductive mode. The mean recovery of AS and DHA over a concentration range of 50-200 ng/ml was 75.5% and 93.5%, respectively. The within-day coefficients of variation were 4.2-7.4 % for AS and 2.6-4.9% for DHA. The day-to-day coefficients of variation were 1.6-9.6% and 0.5-8.3%, respectively. The minimum detectable concentration for both AS and DHA in plasma was 4.0 ng/0.5ml. The limit of quantification for both AS and DHA in plasma was 10ng/0.5ml.

1.1 COMPOUNDS

Artesunate

Decahydro-3, 6, 9-trimethyl-3, 12-epoxy - 12H - pyranol [4,3-*j*]-1, 2 - benzodioxepin-10-ol, hydrogen succinate.

Molecular Formula: C₁₉ H₂₈ O₈

Molecular Weight: 384.4

Control number : 2019261

Supplier: National Institute of Drug Quality Control, Ministry of Health, Vietnam.

Dihydroartemisinin

Decahydro-3, 6, 9-trimethyl-3, 12-epoxy - 12H - pyranol [4,3-*j*]-1, 2 - benzodioxepin-10-ol

Molecular Formula: C₁₅ H₂₄ O₅

Molecular Weight: 284.4

Lot Number: DHA0010

Supplier: National Centre for Genetic Engineering and Biotechnology, Bangkok, Thailand.

Artemisinin

(used as an internal standard)

Octahydro-3, 6, 9-trimethyl-3, 12-epoxy - 12H - pyranol [4,3-*j*]-1, 2 - benzodioxepin-10(3H) - one.

Molecular Formula: C₁₅ H₂₂ O₅

Molecular Weight: 282.3

Lot Number: 09029JF

Supplier: Aldrich

1.2 QUALITY CONTROL SAMPLES

For within-study assay validation, quality control (QC) samples were prepared one day prior to study sample analyses, by spiking freshly thawed drug free plasma (0.5ml) with known amounts of both AS and DHA. A minimum of 6 QC samples was prepared. The resulting concentrations were in the range of 80ng to 600 ng/0.5ml. The QC samples were stored below -70°C in silanized tubes pending analyses. For each batch of study samples one set of QC samples was analyzed.

1.3 CALIBRATION SAMPLES

Calibration samples (C) were prepared one day prior to sample analyses by spiking freshly thawed drug free plasma (0.5ml) with known amounts of both AS and DHA. The resulting individual values of concentrations of artesunate and dihydroartemisinin were always in the range of 10ng to 800ng/0.5ml, above the lower limit of quantification. The calibration samples were prepared in a similar manner for each batch of study samples. The calibration samples were stored below -70°C in silanized tubes pending analyses. Prior to sample analysis, three calibration curves with three sets of QC samples were analysed for a pre-study validation exercise. The results of these experiments are documented on site in CDR File (Pre-study Validation: Artesunate). On the day of batch sample analyses, QC samples, calibration samples and study samples (clinical samples) were spiked with known amount of internal standard (artemisinin) before undergoing sample processing.

Different stock solutions of AS and DHA were used for the preparation of QC and calibration samples. A qualified person not involved in the study did the preparation of the stock solutions for QC.

1.4 SAMPLE PROCESSING

Tubes containing 0.5ml of QC samples and calibration samples, which were stored below -70° C together with study samples (clinical samples), were thawed at room temperature. Aliquots of 0.5ml study samples were pipetted into silanized tubes. 5 µl of the working solution of the internal standard (40ng/µl) was spiked into all the tubes containing QC, calibration and study samples. The mixture was vortex-mixed for 30 seconds. Appropriate amounts of ultra pure water were pipetted into each of the QC, calibration and study samples in order to obtain a final volume of 1.0ml. The mixture was vortex-mixed for 30 seconds. The samples were then loaded onto C₁₈ SPE cartridges, which had been conditioned with acetonitrile and water (each 1ml x 2). Each cartridge was washed with 1-ml water. Elution was carried out with 3 sequential applications of 0.5ml acetonitrile. The eluents were collected into a test tube and dried in a gentle stream of nitrogen at room temperature. The residue was reconstituted in 100µl of ethanol-water (50:50 v/v) and left for 18hr at 4°C in order to allow stabilization of the ratio of the α and β isomers of DHA. Twenty microlitres was injected into the HPLC for quantification.

The chromatographic conditions were as follows:

Stationary Phase: Hypersil butyl (C₄) column, 250mm x 4.6mm i.d. x 5µm
Flow rate: 1.5 ml/min
Mobile Phase: Acetonitrile: 0.05M acetic acid (40:60, v/v) adjusted to pH 5.0 with 1.0M sodium hydroxide
Detection: Electrochemical detector operating in the reductive mode. The detector was set at 50nA.

1.5 DATA PROCESSING

The concentrations of unknown and QC samples are determined by linear regression analysis (function $y = a + b \cdot x$; weighting factor $1/y^2$). The peak height ratios of the drug (AS and DHA) to the internal standard (y) were plotted against the concentrations (x) of the drug (AS and DHA). Calculation of results was carried out by use of a specific worksheet programmed in SPSS® software (SPSS for Windows®, version 6.0, SPSS Inc., USA).

The laboratory personnel reviewed all analytical data for completeness and accuracy. The Principal Investigator for this Bioanalytical part of this study, Dr. Surash Ramanathan, finally checked the raw data (chromatograms). CDR Quality Assurance Group crosschecked data entered manually into a database against the source data. Concentrations below the limit of quantification are designated as '<LOQ'. The abbreviations 'SS' and 'PS' were used to designate concentrations that could not be generated. 'SS'-sample spoilt referred to samples which, following extraction, were not suitable for injection into HPLC i.e. the sample was not clean and if injected into the HPLC, would damage the analytical column, as well as, generate a chromatogram that would be of difficult integration. 'PS'-peak spoilt referred to samples that were injected into the HPLC, but the peaks generated following a HPLC run were not well resolved to baseline, not symmetrical or properly integrated by the integrator in order to be accepted as valid drug peak. Samples designated as 'PS' or 'SS' were required to undergo repeat analysis if there was a sufficient amount of plasma volume for extraction.

All concentrations related to AS and DHA. The final concentrations were rounded to one decimal place.

1.6 CRITERIA FOR ASSAY PERFORMANCE AND SAMPLE RE-ANALYSIS

An individual sample was subjected to repeat analysis if the sample was dirty and not suitable for injection, sample concentrations fell out of calibration range, there was peak spoilage owing to interference from endogenous peak or/and peak of interest does not resolve to baseline. As for batch sample analysis, it was to occur if less than five calibration points used to construct the calibration curve and/or less than 4QC samples passed.

2. DRUG ASSAY AND VALIDATION: RESULTS

2.1 Assay Performance

Calibration

The calibration curves for artesunate (AS) and dihydroartemisinin (DHA) were linear with a coefficient of correlation ranging from 0.980057 to 0.999997 and 0.979582 to 0.999998 respectively.

Within-study assay validation

Within-study assay validation was performed by analyses of the QC samples together with the study samples.

The method validation data are summarized in the tables below:

Table 1 a: Summary of within-study assay validation for artesunate

| Nominal conc. In QC sample [ng/0.5ml] | Number of determinations | Mean recovery [% of nominal conc.] | Precision* [%] |
|---------------------------------------|--------------------------|------------------------------------|----------------|
| 80 | 106 | 104.3 | 8.4 |
| 300 | 136 | 99.8 | 9.7 |
| 600 | 128 | 97.6 | 10.2 |

* coefficient of variation (100 * standard deviation/mean)

Table 1 b: Summary of within-study assay validation for dihydroartemisinin

| Nominal conc. in QC sample [ng/0.5ml] | Number of determinations | Mean recovery [% of nominal conc.] | Precision* [%] |
|---------------------------------------|--------------------------|------------------------------------|----------------|
| 80 | 121 | 107.4 | 7.9 |
| 300 | 146 | 100.9 | 8.2 |
| 600 | 142 | 99.1 | 9.0 |

* coefficient of variation (100 * standard deviation/mean)