SOME CONSIDERATIONS IN ANTIMALARIAL CHEMOTHERAPY OF SEVERE FALCIPARUM MALARIA

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ABSTRACT

BACKGROUND

Acute severe falciparum malaria is a pernicious and vicious circle of pathophysiologic state and is a medical emergency. The priority of treatment is the parenteral administration of safe and adequate doses of appropriate effective antimalarials given as soon as possible in the setting of the highest possible levels of clinical care. Decreasing mortality is more important than cure of malaria, thus survival of the patients is the key factor. Antimalarial drugs have stage specific actions on plasmodium falciparum. Artemisinin are most rapidly acting of known antimalarials and they have broadest time window of activity. Recent study showed decreased mortality of 34.7% was by intravenous artesunate instead of quinine. Currently, the therapeutic doses of intravenous artemesunate to treat severe malaria are in the range of 2-2.4 mg/kg produces peak drug concentrations (Cmax) with great inter-individual variability with lower Cmax than needed for hyperparasitaemia to efficaciously cover each RBCs as infected cells can concentrate 100-300 fold more dihydroartemisinin (DHA) than the normal RBCs during the first 1-2 hrs. after dosing indicating that intravenous dose should be increased to a higher levels than current loading dose to treat severe malaria with hyperparasitaemia to decrease further morbidity and mortality.

KEYWORDS

Severe Malaria, IV Artesunate, Loading Dose.

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BACKGROUND

Acute severe malaria is a pernicious and vicious circle of pathophysiologic state¹ and is a medical emergency as about 84% of deaths occurs within 24 hrs. despite IV quinine therapy.² Appropriately high initial intravenous dose of an effective antimalarial should be given as soon as possible as survival is key factor than cure of malaria.³ Antimalarial drugs have stage specific actions on plasmodium falciparum.⁴ The decrease in mortality by 34.7% has been the use of intravenous artesunate (IV AS) monotherapy instead of quinine.⁵ Rapid efficacy of IV AS is principally due to its initial high peak drug concentration (Cmax) and its antimalarial potency. The drug exposure time (half-life) and drug exposure level or Area Under the Curve (AUC) tends to be of minor significance.⁶ The current use of initial 120 mg of IV artesunate appears to be insufficient in the treatment of severe malaria with hyperparasitaemia and variance between individual patients in both PK/PD. Successful (100% cure) treatment is found neither in the 3-5 days treatment nor in the higher dose artesunate monotherapy⁷ with recrudescence rates of >52%, 9.8% and 2.5% after 3, 5 and 7 days regimens, respectively, which requires sequential administration of an effective Antimalarial Combination Therapy (ACT) as soon as patients able to take orally.⁸

DISCUSSION

Asexual Erythrocytic Cycle of Plasmodium Falciparum (Figure 1)

Rupture of hepatic meronts of up to 5-100 (usually 8-15) release 2000-40,000 merozoites per hepatic merogony within 24 hrs. duration and infect the RBCs of about 10²-10⁵.⁹,¹⁰ The asexual trophozoites of pf. malaria passes successively through the five stages of development.¹¹

![Figure 1. Erythrocytic Cycle of P. falciparum Malaria](image)

Circulating Ring Stages

After infecting the RBC tiny ring forms (R1) in which the ratio of width of cytoplasm to nucleus is <½ develops within the
first 6 hrs. of the asexual cycle is not visible in light microscopy and not affected by most antimalarial drugs.

The small ring form (R2) in which the ratio of width of the cytoplasm to nucleus is >½ and <1 develops from 6-16 hrs. (10 hrs.) present in the peripheral circulation is visible under light microscopy.

The large ring form (R3) in which the ratio of cytoplasm to nucleus is ≥1 develops from 16-26 hrs. (10 hrs.) present in the peripheral circulation for 2-4 hrs. as sequestration occurs before 24 hrs. of development.

**The Sequestration Stages**
The trophozoite stage- Develop from about 24-26 hrs. and lasted up to 38 hrs. develop into early, mid and late trophozoites stage successively each lasting for 4 hrs. The deadly weapons of pf. malarial knobs begins to appear from 24-26 hrs. of development designed for cytoadherence to vascular endothelium of postcapillary venules and capillary endothelium of vital organs called sequestration and to uninfected RBCs called rosette correlated with severity of complications.

The Meront or Schizont stage- Appears from 38-48 hrs. in the early Schizont stage (6 hrs.) repeated nuclear divisions take place to form segmentor or Schizont (Meront) and affected by the antimalarial antibiotics. The late mature Schizont stage develop from 44-48 hrs. is not affected by any antimalarials and destine to rupture releasing 6-36 merozoites/RBC to repeat the cycle. Thus, acute severe malaria is a state of vicious and pernicious pathophysiologic state.

**Parasitic Biomass and Broods of Parasitaemia**

**Synchronous Infection**
Natural pf. malaria infections often contain two or more genetically different parasite strains or broods of parasites development relatively synchronous and all the parasites are in the same stage of development in untreated non-immune subjects such that merogony take place within 1-2 hrs. and associated with fever and rigors called the paroxysm.10

**Asynchronous Infections**
Parasites are in different stages of developments and there are more than two broods and one brood may predominate in pf. malaria and another minor brood of parasites cycling 24 hrs. out of phase with the major broods. If two broods of equal size of parasitic biomass oscillating 24 hrs. out of phase and failed to synchronise, daily fever spike occurs. If three broods cycling 24 hrs. out of phase to each other, fever can occur at 24, 36 and 48 hrs. and fever paroxysm overlaps and fever maybe continuous with erratic pattern.

**Significance of Broods of Parasitaemia in p. Falciparum Malaria**
In a synchronous infection, if blood samples taken for microscopy during sequestration phase or in the R1 ring stage parasites cannot be detected despite high parasitaemia. Detection of ring stage parasites during febrile paroxysm is not the cause of that paroxysm and indicate more than two broods of parasites.9

There is loose relationship between parasitaemia and disease severity. A patient with severe or complicated multiorgan failure may have smear negative, because samples may have taken during sequestration phase in a synchronous infection or parasitaemia may be below microscopic detection (<50 parasites/µL) or partially-treated malaria, especially with artemisinins.

In expanding phase of infection, there can be more circulating stage parasites than sequestered parasites14,13 and related to immunity as children of endemic area can tolerate parasitaemia of >50% and an adult up to >10⁵ without symptoms.

**Stage Specificity of Antimalarial Drugs Effect**
(Figure 2)

The most early tiny ring stage (R1) lasting 6 hrs. is relatively resistance to all antimalarial drugs and also the mature Schizont stage lasting 4 hrs. Artesunate having very short half-life (<1 hrs.), if happens to given during these stages will be ineffective.

Artemisinin derivatives have “broadest time window” of activity affecting from small ring (R2) stage to early Schizont stage, i.e. 38/48 hrs. of life cycle.

Quinine, mefloquine, lumefantrine have “narrow time window” of activity affecting from large ring stage (R3) to late trophozoite stage, i.e. 14/48 hrs. of life cycle and kill the parasites in the late trophozoite stage and not prevent cytoadherence and patients may deteriorate for some times after start of these drugs, not suitable for treatment of acute severe malaria.

The antimalarial antibiotics sulfadoxine/pyrimethamine (S/P), tetracycline, clindamycin, azithromycin have “narrowest time window” of activity act only in the early Schizont stage (6/48 hrs.) are not suitable for severe malaria.
The timing of starting an antimalarial drug is important, if quinine-like drugs start at the stage of R1 even with high-density parasitaemia, cytoadherence can be prevented, but started at R3 stage cannot prevent cytoadherence and it’s pathological consequences indicating constant exposure time required from R1 stage to decrease knobs formation.\textsuperscript{15,13} Whereas starting of artesunate prevent cytoadherence even with high density of R1 to R3 as it acts from R1 to early Schizont stages and prevent cytoadherence within 2 hrs. of exposure. Thus, artesunate is the drug of choice in acute severe malaria.

**Efficacy of Different Antimalarial Drugs**
The Parasite Reduction Ratio (PRR) is the fractional reduction in parasitaemia per asexual life cycle by an antimalarial drug expressed as killing power or rate.\textsuperscript{12} It is increased by background immunity and decreased with parasite resistance. There is considerable differences in PRR between antimalarial agents reflecting the differences in intrinsic activity (Emax) between the drugs.

**In Vivo Pharmacodynamic PRR of Different Antimalarial Drugs**
Artemisinins have higher PRR of all antimalarials available with killing power of $10^3$-$10^5$ (10$^3$) cycle or 99.99% per asexual life cycle and artesunate reduce parasitic biomass of roughly 10,000 fold need to be given for at least three parasite life cycle (>6 days) to remove all parasites from the body of 10$^{12}$ in non-immune patients. For quinine-like drugs with killing power of 99.9% or 10$^2$-$10^3$/cycle reduce parasitic biomass by 100-1000 fold requires maintenance of paraciticidal therapeutic drug concentration for >4 life cycle (>8 days) and for mefloquine with 97% killing rate requires >9 days. If the killing power falls below 85%, then parasitaemia will continue to increase despite continuation of therapy in therapeutic concentrations.\textsuperscript{12} After 3 days of artesunate therapy, maximum of parasite remains is about 0.000001% of initial parasitaemia. Recrudescence is invariably proportional to duration of therapy. Therefore, ACT with one slow and long-acting effective component must be given sequentially after few days of initial monotherapy to eradicate all the parasites from the body and to prevent development of resistance.

**Percentage Inhibition of Cytoadherence and Rosetting Properties by Different Antimalarial Drugs at Different Time after Drug Exposure**
Artesunate inhibit cytoadherence and rosetting by 80%, >80%, >90% and >100% at 2, 4, 8 and 24 hours, respectively after starting therapy. Artemether/Areteether inhibit by >40%, 10%, 70% and 100% at 2, 4, 8 and 24 hours, respectively, whereas quinine requires at least >4 hrs. to reduce rosetting by 50%, but does not prevent cytoadherence significantly, i.e. 10- <20%, 0%, <10% and <40% at 2, 4, 8 and 24 hours, respectively.\textsuperscript{14}

**Changes in Parasitaemia Immediately Following Initiation of Antimalarial Chemotherapy**
None of the antimalarial drugs act instantaneously even if given IV, the pattern of parasitaemia in the first few hours following the start of treatment will be same as that which would have occur without treatment.\textsuperscript{12} When the majority of sequestrated parasites are at the mature Meront stage, the parasitaemia may rise alarmingly on the hours following start of treatment as the mature meronts and the liberated merozoites are not affected by any antimalarials and invade the new RBCs and developed into tiny ring form misled as parasite resistance. Artesunate having short half-life of <1 hrs. if happens to given during these stages are ineffective. Conversely, a rapid decline in parasitaemia immediately following initiation of therapy as a result of sequestration, misled that the drug is being rapidly effective and can occur with use of quinine-like drugs when the majority of parasites are in large ring stages (R3) and trophozoite stages not prevented from cytoadherence and patients may actually deteriorate before parasites are killed in late trophozoite stage.\textsuperscript{12}

**Artemisinins**
There are five artemisinin derivatives; all are highly effective against blood stage of pf. malaria. They are Artesunate (AS), Dihydroartemisinin (DHA), Arteether (AE), Artemether (AM) and Artelinc acid (AL).

Stage specific sensitivity and growth inhibition of artemisinin derivatives with different drug-resistant isolates and clones of Plasmodium falciparum in vitro.

AS and DHA are most effective artemisinin in a study of W2 and D6 clones of different drug-resistant pf. malaria in vitro and in vivo efficacy among the five analogues compared to the relative antimalarial potency of artemisinin (QHS) as potency 1. DHA had potency of 2.59-4.96, AS 3.29-4.14, AM 1.84-2.04 and AE 2.24-3.11. Since, AS is prodrug of DHA in vitro activity among five artemisinin is drug of choice in treatment of severe malaria.\textsuperscript{16} AS have gametocidal effect against immature gametocytes and prevent disease transmission.\textsuperscript{17}

**PK/PD Evaluation of Artemisinins**
Rapid efficacy of artemisinins have principally due to their peak drug concentration ($C_{\text{max}}$), dependent killing and relative antimalarial potency. Drug exposure levels (AUC) and drug exposure time (half-life) tend to be minor significance and AS is superior to all other artemisinins. Quinine-like drugs have above MIC and AUC dependent killing effect requires maintenance of constant therapeutic drug concentrations throughout the treatment period.\textsuperscript{15}

**Drug Exposure Level (AUC) and Time Effect on Efficacy of Artemisinins**
DHA is highly effective against almost all stages of parasites achieving 100% growth inhibition within 2-4 hrs. of exposure.\textsuperscript{18,19} In ivin vitro dose range of artemisinin of $10^7$-$10^9$M (30-3000 ng/mL) is extremely effective against rings to early Schizonts and 100% inhibition of parasites growth
within 4-6 hrs. At drug concentration of \(10^6\text{-}10^9\text{M} (280-2800\text{ ng/mL})\) requires 3 hrs. of exposure time to show parasitocidal effect and at \(10^7\text{M} (28\text{ ng/mL})\) requires 24 hrs. of exposure time for cidal effect.\(^{19}\) At \(10^8\text{-}10^9\text{M} (3-30\text{ ng/mL})\) requires 24-96 hrs. of constant exposure to kill all parasites (Ic99). At \(10^6\text{M} (2.8\text{ ng/mL})\) had no appreciable effect on parasites. If this concentration increased to 3 times (8.4 ng/mL) with drug exposure time extending to 72-96 hrs. parasites level decreased to 0.5\%, the long exposure time to lower concentrations could be as effective as high concentrations.\(^{19}\) Thus, low dose will increase the treatment period and higher dose will shorten the treatment period. Therefore, low-dose artemisinin regimens cannot be selected for treatment of severe malaria and longer exposure time is the principal factor leading to fatal neurotoxicity with AE and AM.\(^{20}\) Thus, artemisinin plasma concentrations should be much more in the range of \(10^6\text{-}10^9\text{M} (300-3000\text{ ng/mL})\) or even higher for the first exposure to cover all RBCs.\(^{19}\)

**PK/PD PARAMETERS OF ARTESUNATE, AE AND AM**

**Intravenous (IV) AS PK/PD-**

The current 120 mg or 2.4 mg/kg initial dose of IV AS produce a \(C_{\text{max}}\) of 11343 ng/mL with t\(\frac{1}{2}\) of 0.05 hrs. and \(C_{\text{max}}\) of DHA of 2646 ng/mL with t\(\frac{1}{2}\) of 0.67 hrs. (total AS+DHA=13987 ng/mL) in trials. Intravenous AS is very fast and efficient on killing parasites at a mean time of 3.1 hrs. for clearance of half of parasites (Pc 50) is the lowest Pc 50. The time lag phase in parasitaemia curve was 1.9 hrs. is the lowest lag time. The Emax of parasitaemia % estimated to be 0.00111% in the first day. With oral AS the Emax parasitaemia was 0.00115% and the time lag phase in parasitaemia was 2.8 hrs. is still shorter than other derivatives. The Emax is the principal PD parameter to determine antimalarial maximal effect, which is very steep concentration effect relationship.\(^{21}\)

**After 2.4 mg/kg Dose of Intramuscular (IM) AS-**

The \(C_{\text{max}}\) of IM AS was 532-3211 ng/mL (median 2193 ng/mL) with t\(\frac{1}{2}\) of 30 min. and rapidly hydrolysed to DHA \(C_{\text{max}}\) of 488-2011 ng/mL with t\(\frac{1}{2}\) of 52 min. The \(C_{\text{max}}\) of AS was 198 ng/mL and DHA 1052 ng/mL with 100 mg oral AS with t\(\frac{1}{2}\) of 0.75 hrs. versus 3.2 mg/kg of IM. AM produce \(C_{\text{max}}\) of 171 ng/mL with t\(\frac{1}{2}\) of 10 hrs. and little conversion to DHA.\(^{22,23}\) Thus, above finding indicate that IM AS is superior to IM AM and \(C_{\text{max}}\) of 2193 ng/mL with IM AS is 13 times higher than IM AM contributing to greater efficacy and reducing mortality.\(^{23}\)

**Auto-Induction of Artemisinin Drug Metabolism**

The plasma drug concentrations of daily AS doses were one-third less on day 3 than day 1 suggesting auto-induction of hepatic drug metabolism enzymes. Thus, it is important to avoid low dose and to give high initial dose for few days to kill maximum parasites and to avoid auto-induction of metabolism and less killing of parasites due to low drug levels.\(^{24,25}\)

**Injectable as for Severe and Complicated Malaria**

The IV AS can provide sufficiently high peak concentrations and provide fastest efficacy in killing parasites and injectable AS is superior to other artemisinins in PK/PD in various regimens. The current IV AS dose of 120 mg (2.4 mg/kg) results in the \(C_{\text{max}}\) ranging from 605-18,909 ng/mL in clinical trials. These dose regimens are still not enough to cure severe and complicated malaria in 100%.\(^{11,17}\) It is the antimalarial activity with high killing capability in the first few hours following the first administration of a parenteral drug that is critical in severe malaria in whom mortality can reach between 15% and 20% despite appropriate antimalarial and supportive care.

The total drug concentrations of AS+DHA were calculated as 0.51 \(\times\) \(10^6\) molecules/RBC in the 100 mg oral AS subjects (\(C_{\text{max}}\) of 1250 ng/mL) and 4.67 \(\times\) \(10^6\) molecules/RBC in IV AS subjects (\(C_{\text{max}}\) of 13,989 ng/mL). In a culture incubation study, the 99% inhibitory concentration (Ic99) of AS was 0.31-0.49 \(\times\) \(10^6\) molecules/RBC and 0.39-0.73 \(\times\) \(10^6\) molecules/RBC for DHA. In other words, the minimum concentration of AS or DHA to kill 99% of culture parasites was in the range of 0.31-0.73 \(\times\) \(10^6\) molecules/RBC. The 0.51 \(\times\) \(10^6\) molecules/RBC of AS+DHA in patients with 100 mg oral AS reached the just minimum concentration needed to exterminate 99% parasites with 0.8-1% parasitaemia, whereas after 120 mg IV AS has 4.67 \(\times\) \(10^6\) molecules/RBC was about 10-fold higher than the Ic99 level (0.31-0.73 \(\times\) \(10^6\) molecules/RBC).\(^3\)

The \(C_{\text{max}}\) 1052 ng/mL of oral AS is just equal to the minimal level for killing 99% parasites is not enough to efficaciously cover all RBCs (infected and uninfected) and with low curative rate of 81.3% following 5 days regimen.\(^{26}\) Even the 120 mg IV AS produce 10-fold higher \(C_{\text{max}}\) than oral AS is still a big clinical failure with 7.5% recrudescence.\(^{27}\) In order to have efficacious blood concentrations, there are three factors that should be considered.

**Factor 1- Effect of High Parasitaemia on Drug Concentration**

Uptake of DHA in infected erythrocytes has been 100-300 times more than uninfected erythrocytes in vitro in some reports\(^{28}\) most probably due to DHA binding proteins are present on the cytoplasmic face of parasitised RBCs and may contain clefts/pores and alterations of RBCs membrane phospholipids between inner and outer sheath favouring entry of DHA.\(^{29,30}\) Thus, levels of parasitaemia affect the drug concentrations and at 1% parasitaemia drug concentration of 100 mg oral AS is just minimum concentration to inhibit 99% parasites with efficacious equilibrium of drug between infected and uninfected RBCs.\(^{31}\) At >1-2% parasitaemia, uptake of DHA will increase in infected RBCs and free plasma concentrations will decrease and the 1250 ng/mL \(C_{\text{max}}\) of oral AS is not sufficient to distribute to all RBCs.

In an in vitro study of AS+DHA response curve consistently affected by parasite burden. There was 6-fold increase of AS+DHA Ic99 from 1-5% parasitaemia. The minimum concentration for hyperparasitaemia could be at
least of 7500 ng/mL to exterminate 5% parasitaemia and 30,000 ng/mL for 20% parasitaemia.³

**Factor 2- Effect of the Protein and RBC Binding on Drug Concentration**

The RBCs and plasma binding ratio of five artemisinins are 0.23, 0.28, 0.44, 0.52 and 0.72 for AE, AM, AL, DHA and AS, respectively indicating higher binding of AS to RBCs followed by DHA indicating advantage of AS and DHA readiness to entering the human RBCs due to higher free drug in the plasma. The plasma binding affinity are higher for AE and AM and there is less free drug available to enter the infected RBCs, so less effective in severe malaria.³²

**Factor 3- Effects of High PK/PD Variability on Drug Concentration**

PK Variability- Following 120 mg of IV AS, high Cmax occurs with first exposure time and the Cmax variability ranges from 735-1890 ng/mL (AS+DHA). This variability is 25-fold from different trials and with oral AS it varies from 453-1718 ng/mL is 4-fold variable in different trials. Thus, there is large inter-individual variability and such low drug concentrations presenting in some patients may explain treatment failure.³³

PD Variability- Stages and synchronicity of the infecting parasites population and the multiplication rate of mature Schizonts determine the initial parasitaemia profiles after drug administration. Some patients have very different parasitaemia profiles, i.e. asynchronous infections have considerable inter-individual variance in parasite clearance profiles. Patients with severe complicated malaria have many critical and variable conditions and comorbidities, i.e. cerebral malaria, acute kidney injury, hepatopathy and multiorgan failure, etc. with variable severity scores that may determine the drug’s PK/PD characteristics and prognosis.

**High-Loading Dose IV as should be used in Treatment of Severe Malaria**

To treat severe malaria, the monotherapy of IV AS is needed in the first couple of days in order to rapidly rescue lives and possibly reduce mortality. The current dose of 120 mg of IV AS produce variable Cmax from 735-1890 ng/mL with greater inter-individual variability is still a lower Cmax. There is also no uniform Good Manufacturing Practice (GMP) of artemunate.³ Patients with hyperparasitaemia requires higher Cmax of about 7,500-30,000 ng/mL or 4-8 mg/kg body weight to efficaciously cover all RBCs within first exposure time of 1-2 hrs. Lower dose IV AS requires longer exposure time with low peak levels not able to cover all RBCs giving rise to some untreated infected RBCs, which maybe the reason for treatment failure, recrudescence and future development of resistance. The 4-8 mg/kg loading dose is safe as the Maximum Tolerated Dose (MTD) of AS is 240 mg/kg in rats used for 3 days without neurotoxicity. In a current phase I study with IV AS of 4-8 mg/kg loading dose was extremely well tolerated in humane volunteers and malaria patients and for the consideration of WHO experts and investigators in the further development and treatment.³ To prevent resistance and recrudescence, sequential ACT should be used as soon as patient able to take orally to maintain therapeutic drug levels for ≥7 days. Following initial IV AS loading dose (0 hr.), the second IV AS loading dose should be given at 12 hrs. as it may happen that the first dose has been given at the stage of tiny ring or mature Schizont stages of a synchronous infection.

**CONCLUSION**

The antimalarial drug actions have stage specific effects. The tiny ring and mature Schizonts of falciparum malaria is relatively resistance to all antimalarial drugs. Intravenous AS with its short half-life of <1 hr. if happens to given at this stages may not be effective. Artemisinin derivatives have broadest time window of activity (38 hrs.) and artesunate prevent cytoadherence and rosetting within 1-2 hrs. of exposure is its biggest advantage in severe malaria to rescue life, whereas quinine-like drugs have narrow time window of activity (14 hrs.) and requires constant exposure of therapeutic drug concentrations and kills the parasites at late trophozoite stage and does not prevent cytoadherence for about 24 hrs. Artesunate is superior to all regards in PK/PD profiles with killing power of 99.99% (10⁸/cycle). Artesunate-reduced parasitaemia by 10,000 fold and quinine 100-1000 fold per asexual life cycle. Antimalarial drugs even with 99.99% killing power needed to be given for at least >3 life cycle (7 days) to remove all parasites from the body of 10,¹² while quinine requires 4 life cycle (>8 days) and mefloquine requires >4 life cycles (>9 days). Short course artesunate monotherapy has high recrudescence rate requires sequential ACT for cure of malaria. The current dose regimen of IV AS appears to be insufficient in treatment of severe malaria and larger loading dose of 4-8 mg/kg maybe used for few days to ensure that maximum effect is obtained in all patients with severe and complicated malaria to rescue life and decrease further morbidity and mortality.

**REFERENCES**


