New Antimalarial Drug Development: Pre-clinical Status of $\alpha$ and $\beta$ Artelinate as Fast Acting Blood Schizontocides

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The development of new fast-acting water soluble artemisinin derivatives which can be administered intravenously to severe complicated and comatose cerebral malaria cases, continues to receive high priority in global tropical diseases programme. The present communication reports the fast-acting antimalarial potential of both $\alpha$ and $\beta$-artelinate that are stable in bicarbonate solution and can be given intravenously to quickly achieve high blood levels. Both these derivatives have been evaluated against highly virulent Plasmodium knowlesi infection in Rhesus monkey (Macaca mulatta) which is considered as one of the experimental models for cerebral malaria. Using the rigorous criteria for total parasite clearance upto 60 days of follow up, it has been established that both $\alpha$- and $\beta$-sodium artelinate administered intravenously at 20 mg/kg/day x 3 days are 100% curative against P. knowlesi (W1) infection in 4/4 monkeys treated with $\beta$-artelinate and 2/2 monkeys treated with artelinate whereas at the lower dose i.e. 15 mg/kg x 3 days, both $\alpha$ and $\beta$-artelinate showed 50% cure rate (1/2 monkeys cured). Further tests at lower doses i.e. 5 and 10 mg/kg doses of $\beta$-artelinate showed that these doses were not curative. The curative efficacy of both $\alpha$ and $\beta$ epimers of sodium artelinate against mefloquine resistant P. knowlesi (W1) infection strongly support further drug development with these potential candidate drugs for clinical use against MDR/mefloquine resistant P. falciparum infections. Although both $\alpha$ and $\beta$ anomers are equally effective against P. knowlesi, $\alpha$-artelinate has a higher safety margin on the basis of its higher LD$_{50}$ (>1g/kg) dose in mice, in comparison to $\beta$-artelinate whose LD$_{50}$ dose is 670 mg/kg. Besides fast blood schizontocidal activity, artelinate also exhibits strong gametocytocidal action and could be used to stop malaria transmission.

Key Words: Malaria, $\alpha$ and $\beta$-Artelinic acid, Artelinate, Artelinic acid-L-lysine salt (AL-LY), Blood schizontocide, Plasmodium knowlesi, Macaca mulatta, Toxicity, Pharmacokinetics, Mefloquine resistance.

Introduction

According to WHO, Plasmodium falciparum malaria is becoming increasingly resistant to chloroquine, sulfadoxine/pyrimethamine, quinine and mefloquine (Eyles et al. 1963, WHO (1984); Nosten et al. (1991); Hurwitz et al. (1981); Timmermanns et al. (1982); Looareesuwan et al. (1992); Boudreau et al. (1982). Besides, most seriously affected areas include Thai-Myanmar Burmese border, where resistance to nearly all available drugs (chloroquine, sulfadoxine-pyrimethamine, mefloquine, quinine) has already got established (WHO, 1999). Mefloquine is currently recommended by WHO (2004) as one of the ATC combination drug which is reported to produce neuropsychiatric side-effects in adults who develop tonic clonic fits. Besides psychosis, delusions and hallucinations anxiety sleep disturbances were also reported after mefloquine treatment (Panisiko and Keystone, 1990; Jha et al. 2006).

Artelinic acid $\beta$ was synthesized by Lin, Klayman and Mihous (1988-U.S Patent 4,791,135) and its first water soluble formulation in sodium bicarbonate 5% solution was tested for antimalarial activity in mice and the results based on this study were published by Lin et al. (1987) who showed that it was a promising water soluble and stable antimalarial derivative of artesinin, which showed curative blood schizontocidal activity at 40 mg/kg x 3 days against Plasmodium berghei in Swiss mice when the compound dissolved in 5% sodium bicarbonate was administered subcutaneously in mice and the compound was safe upto 640 mg/kg base. In comparison to artelinic acid, the artesunic acid which is also water soluble showed weak antimalarial activity; its curative dose being 640 mg/kg x 3 days. In vitro studies have confirmed the antimalarial action of artelinic acid against drug resistant P. falciparum isolates (Lin et al. 1987; Basco and Le Bras (1993) and van Vianen et al. (1990).

Transdermal absorption of artelinic acid and sodium $\beta$-artelinate from transdermal patch has also been reported to cure P. berghei infection at 480 mg/kg total
dose administered in 3 days treatment (Lin et al. 1994).

The second Artelinic acid β formulation designated as artilinic acid-L-lysine salt (AL/LY) for intravenous use, was cited in U.S. Patent Application no. 20030171424 filed by Lin, Van-Hamont and Milhous (2003).

Li et al. (2003) reported the development of a new artelinate formulation, namely artilinate -L-lysine salt (WR255663) and compared its antimalarial efficacy with that of artemesunate using rodent malaria P. berghei-infected Sprague-Dawley rats. Both the compounds (at 191.2 mmoles per kg. dose) were not curative in rat model. The investigators clearly point out that their goal was only to achieve parasite clearance and not complete cure. The short course AL-L-Lysine and artemesunate injection must be followed by full dose of mefloquine as curative drug.

In all of the earlier studies on artelinate, the rodent malaria model was used to establish the antimalarial profile of β sodium artelinate, while α sodium artelinate had not been examined for its antimalarial potential. Using P. knowlesi – rhesus monkey infection, a rigorous animal model for selection of candidate antimalarials for drug development, the authors had very briefly published the first report on the antimalarial potential/curative dose data of both α and β artelinate in abstracts published by Vishwakarma et al. (1992) and Tripathi et al. 1992 a) respectively. Both the compounds were freshly dissolved in sterile 5% sodium bicarbonate solution for intravenous injection. Authors had also stressed the need to under take detailed preclinical evaluation of both these water soluble artelinate compounds (α and β anomers) which could be administered by intravenous route and developed as potential antimalarial (blood schizontocides) for emergency treatment of cerebral malaria. The synthesis of β artelinate was published earlier by Lin et al. (1987) and that of α-artelinate was reported by authors (Vishwakarma et al. 1989 ; 1992). Subsequently, the authors had also reported an additional activity of sodium β-artelinate namely its gametocytocidal activity against Plasmodium cynomolgi B. – rhesus monkey model indicating its potential to completely block the transmission of malaria infection to vector (Anopheles stephensi) by both intravenous and oral route of drug administration, though the drug had no sporontocidal effect in vector (Tripathi et al. 1996).

The present communication deals with the preclinical status of artilinic acid (sodium β-artelinate) and also reports the fast acting blood schizontocidal efficacy of both α and β sodium artelinate against a highly virulent mefloquine resistant P. knowlesi (W1) infection in rhesus monkey. It is hoped that the preclinical antimalarial efficacy data of α and β-artelinate against P. knowlesi which is an accepted cerebral malaria model (Migasena and Areekul, 1987) would stimulate further drug development efforts with both α and β-artelinate for their possible use in cerebral malaria cases.

Materials and Methods

Plasmodium knowlesi (W1 strain) – a non-gametocyte producing strain received in 1980 from U.K. through the courtesy of late Professor P.C.C. Garnham, was used in this study. This P. knowlesi strain is resistant to mefloquine and produces a virulent fatal infection in rhesus monkey (Macaca mulatta) following intravenous inoculation of 1 x 10^4 - 1x10^6 parasitized RBC. Rhesus monkeys used in the study were maintained at the Primate malaria facility of the Central Drug Research Institute, Lucknow under standard conditions, and fed on pellet diet, seasonal fruits and vegetable diet, and water ad libitum, under 12 hour photoperiodicity. The animals were quarantined for 45 days, chest x-rayed and tuberculin tested. Blood smears of healthy animals are examined weekly for 4 weeks to ensure that they are free of any haemoprotozoan infections.

The mortality rate of naive monkeys infected with P. knowlesi is 100%. In the present study each monkey was inoculated with 1x10^6 infected RBCs and the parasitaemia was recorded daily by examining Giemsa-stained thick and thin blood smears. α and β Sodium artelinate were administered to the monkeys when they became patent. These derivatives dissolved in sterile 5% sodium bicarbonate were evaluated for their blood schizontocidal activity by intravenous route. Freshly prepared solution of α and β sodium artelinate was given intravenously on three consecutive days. Blood smears from each monkey were obtained for at least 60 days after the end of the treatment and the recrudescence, if any, was recorded. Monkeys that remained negative on blood smear examination for 60 days were considered as cured. A total of ten infected monkeys were treated with β artelinate and four monkeys were treated with α artelinate.

Both α and β artelinate synthesized at CIMAP Lucknow (Vishwakarma et al. 1989) were freshly dissolved in sterile 5% sodium bicarbonate solution and injected intravenously in the leg vein of rhesus monkeys (using 5ml drug volume). Besides β artelinate from WRAIR was also used for comparison and dissolved in 5% sodium bicarbonate solution for intravenous injection in infected monkeys.

Results

In the present investigation sodium salts of both α and β artelinate have been evaluated for their blood schizontocidal activity against mefloquine resistant strain of Plasmodium knowlesi (W1) infection in rhesus
monkey in order to establish their curative doses by intravenous route of drug administration. Rigorous criteria were used to establish the curative dose by checking the blood smears stained in Giemsa stain upto 60 days post-treatment and the dose showing no recrudescence upto 60 days was considered as curative dose. Both α and β-arthelinate were dissolved daily in sterile 5% sodium bicarbonate solution for intravenous administration. β-arthelinate doses ranging from 5.0, 10.0, 15.0 and 20.0 mg/kg. and that of α arthelinate 15.0 and 20.0 mg/kg x 3 iv injections on three consecutive days, were administered to monkey after potency was established and the results of parasitaemia clearance/cure or recrudescence are summarized in Tables 1 and 2. Treatment with 5.0 and 10.0 mg/kg x 3 doses of β-arthelinate (iv) failed to control the P. knowlesi infection and the monkeys died following recrudescence of parasitaemia. The dose of 15.0 mg/kg x 3 doses cured one of the two monkeys (50% cure) with α as well as β arthelinate. At highest dose i.e. 20 mg/kg x 3 doses (iv), both α and β arthelinate were curative in 2/2 monkeys each. Additional 2 infected monkeys treated with β-arthelinate were also cured. No recrudescence was recorded in cured monkeys which were followed up for 60 days. At 20 mg/kg x 3 dose level, α-arthelinate cured 2/2 monkeys (100% cure rate) while β-arthelinate cured 4/4 monkeys (100% cure rate). 

LD₅₀ dose of α-arthelinate determined in mice was >1.0 g/kg, while the LD₅₀ dose of β-arthelinate was 670 mg/kg (ip). The study shows the α-arthelinate has higher safety margin compared to β-arthelinate, although both the anomers are equally effective blood schizontocides. No vascular necrosis at the site of intravenous injection was observed in monkeys treated with α- and β-arthelinate formulations used in the study.

Discussion

Earlier studies in which rodent malaria screening model was used, led to the identification of artemisinin derivative namely artelinic acid (and its water soluble sodium salt β-arthelinate) as a potential antimalarial compound for drug development (Lin et al. 1987). Even after 20 years of its discovery, the compound sodium β-arthelinate has not cleared animal pharmacology/regulatory toxicology and teratogenicity or clinical safety and tolerance evaluation in human subjects primarily because of the lack of preclinical antimalarial data to provide curative dose/safety profile of sodium β-arthelinate in simian malaria models which forms the basis of selection of new compounds for drug development. (Dutta and Tripathi 1996, 2003). Since the publication of Lin et al. (1987) and Brossi et al. (1988) major thrust in drug development efforts was focussed towards development of oil soluble injectible β-arteether which was registered in October 2000 (TDR News No. 63) and is in clinical use. Synthesis of another oil soluble injectible compound - β-arteether had been reported by Lin et al. (1987) and Brossi et al (1988), and it was approved by WHO (1996) for clinical use. Synthesis of third oil soluble injectible compound α and β-arteether

Table 1: Blood schizontocidal activity of sodium b-arthelinate intravenous formulation against P. knowlesi in rhesus monkeys

<table>
<thead>
<tr>
<th>S.No. of monkey</th>
<th>Drug dose (mg/kg) x 3 days</th>
<th>% Parasitaemia</th>
<th>Recrudescence (R)</th>
<th>Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>After last dose</td>
<td>after last dose</td>
</tr>
<tr>
<td>1.</td>
<td>5.0</td>
<td>2.5</td>
<td>Nil</td>
<td>Day 5</td>
</tr>
<tr>
<td>2.</td>
<td>5.0</td>
<td>5.2</td>
<td>Nil</td>
<td>Day 4</td>
</tr>
<tr>
<td>3.</td>
<td>10.0</td>
<td>2.7</td>
<td>Nil</td>
<td>Day 9</td>
</tr>
<tr>
<td>4.</td>
<td>10.0</td>
<td>5.5</td>
<td>Nil</td>
<td>Day 5</td>
</tr>
<tr>
<td>5.</td>
<td>15.0</td>
<td>1.3</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>6.</td>
<td>15.0</td>
<td>5.7</td>
<td>Nil</td>
<td>Day 9</td>
</tr>
<tr>
<td>7.</td>
<td>20.0</td>
<td>0.1</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>8.</td>
<td>20.0</td>
<td>0.2</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>9.</td>
<td>20.0</td>
<td>0.02</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>10.</td>
<td>20.0</td>
<td>0.06</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>11.</td>
<td>Control</td>
<td>4.2</td>
<td>75.0 on day 8</td>
<td>Died on day 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0%)</td>
</tr>
</tbody>
</table>

Table 2: Blood schizontocidal activity of sodium a-arthelinate intravenous formulation against P. knowlesi in rhesus monkeys

<table>
<thead>
<tr>
<th>S.No. of monkey</th>
<th>Drug dose (mg/kg) x 3 days</th>
<th>% Parasitaemia</th>
<th>Recrudescence (R)</th>
<th>Cure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>After last dose</td>
<td>after last dose</td>
</tr>
<tr>
<td>1.</td>
<td>15.0</td>
<td>0.05</td>
<td>Nil</td>
<td>Day 8</td>
</tr>
<tr>
<td>2.</td>
<td>15.0</td>
<td>0.06</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>3.</td>
<td>20.0</td>
<td>0.5</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>4.</td>
<td>20.0</td>
<td>0.1</td>
<td>Nil</td>
<td>No</td>
</tr>
<tr>
<td>5.</td>
<td>Control</td>
<td>0.03</td>
<td>55.0 on day 5</td>
<td>Died on day 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0%)</td>
</tr>
</tbody>
</table>
(30:70) was reported from India in 1989 (Vishwakarma et al. 1989) and this drug was successfully marketed in India in 1997 and is currently in clinical use in India and African countries (Dutta and Tripathi 2003). Another water soluble fast acting artemisinin derivative namely artesunic acid/sodium artesunate for intravenous injection developed by Lin et al. (1987, 1988) and Brossi et al. (1988) is currently a life saving drug for cerebral malaria if used in combination with full dose of mefloquine.

Comparative studies on two water soluble artemisinin derivatives namely artelinic acid and artesunic acid by Lin et al. (1987, 1988) had clearly established that artelinic acid (iv) in bicarbonate solution gave 16-fold superior blood schizontocidal activity against P. berghei in mouse model as compared to artemunic acid. Stability tests in 5% NaHCO3/ID2O further confirmed the superiority of artelinic acid because of its long term stability in solution as compared to artesunic acid (half life about 45 days for artelinic acid vs <1 day for artesunate). Grace et al. (1999) had reported the efficacy of artelinic acid was partly due to its metabolism to antimalarial metabolite dihydroartemisinin by cytochrome P 450 3A4/5 in the liver. In another comparative study on antimalarial efficacy of β-artelinate and artesunate, Li et al (2003) used P. berghei - Sprague-Dawley rat model for antimalarial screening and reported that though β-artelinate was superior to artesunate on the basis of parasite clearance, however, after both the treatments, recrudescence was 100% in rats indicating failure of both treatment doses used in rat model. These investigators, however, argued that it would be necessary to combine intravenous artelinate/artesunate 3 dose iv therapy, with a follow-up of oral mefloquine as additional antimalarial therapy to achieve curative action. Li et al (2003) also reported vascular necrosis at the site of injection in rats as side-effects of iv β-artelinate-L-lysine formulation. In U.S. Patent Lin et al (2003) reported the development of new improved intravenous formulation of β-artelinic acid - L-lysine salt for intravenous treatment of severe and complicated malaria. According to Lin et al. (2003), the dose of 2-4 mg/kg/day x 3 times/day of above formulation proposed for several days by iv administration in severe malaria patients might not be curative; this formulation was recommended for iv administration together with quinine as an additional second antimalarial to produce complete cure. The new formulation has half life of 1.5-3 h.

In another study in monkeys (Lin et al. 2003) also pointed out that unexpectedly the AL-L-lysine formulation had encountered numerous toxicity problems. In this primate study, ten spleenectomized rhesus monkeys infected with mild P. coatneyi malaria treated with 11.8mg/kg first iv dose followed by 5.9mg/kg for 2 more days, resulted in 3 severe adverse events causing death of 2 monkeys from shock–like syndrome. The authors recommended coadministration of quinine with AL-L-lysine intravenous formulation for treating severe complicated malaria. The overall mortality with AL-L-lysine treatment in P. coatneyi infected monkeys was 56% regardless of the doses of drug administered ranging from 1.5 - 47.2mg/kg. The pathology observed in treated monkeys included renal failure, hepatic necrosis and hemolysis.

The new formulation artelinic acid-L-lysine salt, does not appear to be safe, in view of adverse preclinical toxicities reported by subsequent investigators (Xie et al. 2003; Li et al. 2003; Li et al. 2005; Xie et al. 2005; Li et al. 2007)

Xie et al. (2003) and Li et al. (2005) also reported that following second AL-L-lysine dosing the urinary output in rat was reduced by 73% and after 3rd dose urinary out put was 60% below normal. Histopathology showed renal lesions in rats treated with 40.6 mg/kg dose for 3 days. Signs of nephrotoxicity, involving 5-20% of renal tubules were observed in the male rats while females showed more than 40% renal tubular damage with multicellular necrosis and degeneration. Li et al. (2005) (coated the unpublished work of Q. Li et al.) which showed that the rats given 40.6 mg/ kg of artelinic acid L-lysine salt given for 3 days by intravenous route, produced acute renal failure with haemoglobinuria, decreased urinary excretion and renal damage.

Li et al. (2005) conducted the toxicokinetics of artelinate L-lysine salt and also referred to earlier toxicity profile of AL-L-lysine conducted by Xie et al. (2003). These investigators had reported that AL-L-lysine salt caused a mild to moderate renal failure and urinary excretion inhibition at 40.6 mg/kg dose in malaria infected rats (Xie et al. 2003) which could alter the pharmacokinetics, efficacy and toxicity of AL-L-lysine formulation.

Risk assessment and therapeutic indices of new L-lysine formulations of both artesunate (AS) and artelinate (AL) in P.bergheri ANKA infected rat, have been compared (Xie et al., 2005). Using L-lysine salts of AL and AS, the therapeutic index at SD50 dose was found to be 9.3 with AL and 32.6 with AS formulation. All the animals had parasite recrudescence even at MTD dose of 240 mg/kg. On the basis of therapeutic indices, L-lysine salt of artelinate was reported to be more toxic than AS (Xie et al., 2005). Although both the salts showed transient parasite clearance, nevertheless, above authors reported 100% recrudescence with 3 doses of AL/AS L-lysine salt formulations. Further, irreversible vascular irritation, reversible nephrotoxicity and acute renal failure at 40.36 mg/kg x 3 doses of AL L-lysine salt...
formulations have been reported. They also reported 3 times higher toxicity with AL lysine complex than artesunate lysine formulation (Li et al., 2007).

Pharmacokinetic evaluation of water soluble formulation of artelanic acid given by intravenous, intramuscular, oral and rectal administration in rabbits at 20 mg/kg dose was carried out by Titulaer et al. (1993) and after intravenous route of drug administration the artelanic acid blood plasma level was reported to be highest (Co=76 ±15 mg/l) as compared to the other routes of drug administration, and the drug was rapidly eliminated with half life 15±3 min. Li et al (1998b) had carried out extensive study on toxicology, pharmacology and pharmacokinetics of β-artelanic acid in animal models. The LD₅₀ dose of artelanic acid (IM) was reported to be 535.2 mg/kg in rats. Toxicity evaluation of single IM dose of 100-200 mg/kg artelanic acid was found to be safe in rats, however, higher doses of 400 mg/kg produced 20% mortality, 600 mg/kg produced 60% mortality and 800 mg/kg dose produced 100% mortality of rats. Pharmacokinetic study in dog established that following 10 mg/kg dose of artelanic acid, the C max was highest (17763 ± 4152 ng/mL) by iv route, with t½ β-elimination of 2.62 ± 0.58 h. Bioavailability (%) was also highest (100%) by iv route in comparison to IM (90.2 ð 10.3%) and intragastric (79.7 ± 13.5%) routes. Li et al. (1998) also reported that artelanic acid achieved markedly higher concentrations within human red blood cells (cell-plasma ratio = 0.44) than β- arteether and artemether (0.23 and 0.28 cell: plasma ratio), suggesting that artelanic acid could be a more potent fast acting blood schizontocide in humans. Lower toxicity of artelanic acid could be due to very low (4.3%) conversion of total artelanic acid to its antimalarial metabolite namely dihydroartemisinin (DHA) which has much higher toxicity. In comparison to artelanic acid with only 4.3% conversion to DHA, other derivatives show very high rate of conversion to DHA, e.g. artesunate shows (upto 73%) DHA metabolite, arteether (upto 16%) and artemether (upto 12%) DHA conversion in rat, which may account for lower toxicity of artelanic acid. DHA had been reported to be most toxic causing anorectic toxicity in rat than its analogues (Brewer et al. 1993). On the other hand artelanic acid was much safer as it produced minimum anorexia at 25 mg/kg/day administered for 7 days which could be due to very low conversion rate to DHA.

Genevese et al. (2000) further investigated the neural toxicity of sodium β-artelinate in rats and concluded that 36 mg/kg x 7 doses of the drug was safe when compared to β-artether (25 mg/kg x 7 doses) as it did not produce any brain stem damage.

The present study has established the curative blood schizontocidal activity of both α and β-artelinate against mefloquine resistant P. knowlesi in rhesus monkey model. Both the drugs are fast acting and equally curative at 20 mg/kg x 3 doses when the drugs are administered intravenously for 3 days and no recrudescence was observed upto 60 days of observation. Curative efficacy of both α and β- sodium artelinate against a highly virulent simian malaria model justifies its potential use in cerebral malaria cases. LD₅₀ data in mice show higher safety of artelinate. Pharmacokinetic data generated by Li et al. (1998b) presented above, justified its intravenous route of administration, as the drug provides very high blood levels compared to other routes of administration. The drug also is free of neurotoxicity. Earlier studies had also suggested absence of neurotoxicity with β-artelanic acid (Wesche et al. 1994; Li et al. 1998b).

Wesche et al. (1994) used in vitro models for toxicity studies of artemisinin derivatives using rat derived neuroblastoma NG 108-15 and mouse- derived neuroblastoma Neuro-2a cultures. IC₅₀ (mM) assays against NG 108-15 neuronal cell cultures showed that artemisinin, α-arteether and artelanic acid were least toxic with high IC₅₀ values = 100, 96.78 and 68.73 mM respectively while dihydroartemisinin (DHA) and artesunic acid were most toxic as shown by very low IC₅₀ = 0.47 and 0.46 mM respectively. Assay with Neuro-2a cell cultures also showed that artemisinin, α-arteether and artelanic acid with high IC₅₀ =100, 77.02 and 66.12 mM respectively, were least toxic, while DHA and artesunic acid with very low IC₅₀ =0.84 and 0.54 mM respectively, were most neurotoxic to cell lines. Wesche et al. (1994) finally concluded that artesunate and its metabolite DHA exhibited most potent neurotoxic potential in comparison to artelanic acid. Li et al. (1998b) had explained that it was very rapid conversion/metabolism of artesunate to DHA (half life 2-4 min) which led to accumulation of DHA in the plasma and consequently could be responsible for neurotoxic potential of artesunate. Wesche et al. (1994) had proposed that if high level of DHA crossed the blood brain barrier to reach the cerebrospinal fluid, it could cause occult brain stem neurotoxicity reported in literature.

In the present study intravenous administration of artelanic acid (both α and β) into the leg vein of the rhesus monkeys did not show any necrosis at the site of injection.

The present investigation justified further pre-clinical development of α and β-sodium artelinate individually or as (50 : 50) α and β sodium artelinate combination for treatment of MDR/complicated P. falciparum comatose case.
Another major advantage in developing sodium artelinate (α- and β- or α-/β-epimers) as drug for clinical use will be its strong gametocytocidal potential and its ability to sterilize gametocytes and stop malaria transmission. Tripathi et al (1996) have reported earlier that even a single dose of β- artelinate (15-25 mg/kg IV or 10-30 mg/kg oral) produces gametocytocidal effect against human transmissible Plasmodium cynomolgi B infection in rhesus monkey. Gametocytocidal activity of artemisinin (Dutta et al. 1989) and α/β arteether (Tripathi et al. 1990, 1992) against simian malaria and against P. falciparum has also been confirmed in clinical trials (Dutta et al. 2001). Sodium artelinate, thus will have dual application in malaria control strategy as a fast acting blood schizontocide and as a transmission blocking safe drug which could be a candidate to replace primaquine. Price et al. (1996) have also proposed that artemisinin derivatives offer a promising alternative for the treatment and control of malaria transmission. Combination of artemisinin derivatives which are effective gametocytocides e.g. sodium β-artelinate, with standard antimalarials (blood schizontocides/sporontocides) has also been proposed to stop malaria transmission (Hogh et al. 1998).

It is hoped that the above update and pharmacokinetic data (Li et al. 1998 b) on sodium artelinate (epimers) would help to promote further development of this drug.

This paper is dedicated to the memory of Late Dr. DL Klayman of Walter Reed Army Institute of Research, Washington, DC, who supplied the compound β artelinc acid.

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