Intravenous β-artemether formulation (ARM NLC) as a superior alternative to commercial artemunate formulation

Sushant Patil1, Medha Joshi2, Sulabha Pathak3, Shobhona Sharma3 and Vandana Patravale1*

1Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, N. P. Marg, Matunga, Mumbai 400019, India; 2Chicago College of Pharmacy, Midwestern University, 555, 31st Street, Downers Grove, IL 60515, USA; 3Department of Biological Sciences, Tata Institute of Fundamental Research, Colaba, Mumbai 400005, India

*Corresponding author. Tel: +91-22-33612217; Fax: +91-22-33611020; E-mail: vbp_muict@yahoo.co.in

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Objectives: To compare the in vivo pharmacodynamic efficacy of intravenously administered artemether nanostructured lipid carrier (ARM NLC) with commercial artemunate (C-AST) at different dose levels.

Methods: The study compared the in vivo pharmacodynamic efficacy of ARM NLC with C-AST in a murine model. For this study, the Peters 4 day suppressive test was adopted. Plasmodium berghei was the causative organism for inducing malaria in mice. The efficacies of the formulations were evaluated on the basis of percentage parasitaemia in, and survival of, mice.

Results: In comparison with the C-AST formulation, ARM NLC demonstrated superior activity in terms of reduction in parasitaemia and increased survival.

Conclusions: Although both formulations were found to be effective in reducing parasitaemia in the murine model, ARM NLC was found to be superior. The study clearly demonstrates the effectiveness of this novel alternative to existing artemunate dosage forms.

Keywords: malaria, P. berghei, lipid nanocarriers, antimalarial activity, recrudescence

Introduction

The WHO defines cerebral malaria as unarguable coma in a patient in whom the presence of Plasmodium falciparum asexual parasitaemia has been demonstrated, after other causes of encephalopathy have been excluded. It is the most severe neurological complication, associated with a mortality of 15%–20%.1 Antimalarial drugs are the only interventions that unequivocally reduce mortality in malarial patients. Although, quinine, quinidine and artemisinins are most commonly used, artemisinin derivatives are becoming the drugs of choice for all degrees of severity.2 Artesunate and artemether, in particular, result in more rapid parasite clearance, and are safer and simpler to administer.

Both these drugs are metabolized in vivo to the highly active antimalarial metabolite, dihydroartemisinin. Intravenous (iv) or intramuscular (im) artemunate, a water-soluble artemisinin derivative, and im artemether are increasingly used for the treatment of severe malarial patients. Unlike artemunate, there is no iv preparation of artemether, as artemether is water insoluble and requires to be dissolved in edible oils. Although the oil-based preparation of artemether is administered im, it has very serious drawbacks, such as slow and erratic absorption of the drug, particularly in patients with severe acidosis, and pain at the site of injection resulting in patient non-compliance.3 In terms of in vitro IC90, artemether, artemunate and dihydroartemisinin have relative antimalarial activities of ~0.3, 0.7 and 1.0, respectively.4 Artesunate, a hydrophilic ester derivative of artemisinin, is extremely susceptible to biotransformation (elimination $t_{1/2} = 0.35$ h). In contrast, artemether is a lipophilic methyl ether of dihydroartemisinin having less susceptibility to biotransformation (elimination $t_{1/2} = 0.53$ h). Despite these potentials of artemether, it still remains to be explored in the treatment of cerebral malaria in tropical rural areas.5 Our previous research findings have demonstrated the superior efficacy of artemether nanostructured lipid carrier (ARM NLC) in the treatment of a Plasmodium berghei murine malaria model.6

This formulation was also successful in combating recrudescence, a common drawback with artemether monotherapy. Comparative in vivo efficacy by an intraperitoneal (ip) route in P. berghei-infected Swiss albino mice underlined the superiority of the developed ARM NLC over the marketed formulation.6 In the past few decades, a surge in research activity involving lipidic nanocarriers has elucidated the potential of these formulations in iv delivery of hydrophobic agents.7
aqueous miscibility profile of ARM NLC facilitates easy iv administration. Given the encouraging outcome of the ip lipidic nanoformulation, the present study was undertaken to compare the in vivo pharmacodynamic efficacy of iv-administered ARM NLC with commercial artemesate (C-AST) at different dose levels.

Methods

**Formulation of the artemether-loaded lipid nanoformulation**

Anhydrous ARM NLC preconcentrate was prepared using the microemulsion template technique. The formulation composition and in vitro erythrocyte toxicity studies have been discussed previously.

**Parasite strain**

P. berghei ANKA strain, susceptible to all currently used antimalarials, was used for induction of malaria in the murine antimalarial model.

**Animals**

Inbred, Eperythrozoon coccoides-free male Swiss albino mice (7–8 weeks) weighing 30±5 g were maintained at a temperature of 22±3°C and 65% relative humidity, and were given a mouse diet and water ad libitum. The protocol was approved and the animal studies were carried out in accordance with the ethical standards laid down by the Institutional Animal Ethics Committee of the Tata Institute of Fundamental Research (TIFR) (TIFR/IAEC/2009–2).

**Pharmacodynamic evaluation**

**In vivo antimalarial efficacy testing in P. berghei-infected mice**

Efficacies of both of the formulations were examined from day 1 until the end of the study (day 28). Throughout the study, blood was withdrawn from the tail vein at regular time intervals. Thereafter, blood smears were fixed on a glass slide with methanol, stained with Giemsa’s stain (5%) and parasites were counted. Parasitaemia is reported as the percentage of erythrocytes containing parasites after counting 1000 erythrocytes from each slide. Antimalarial activity was calculated by the following formula by Fidock et al.:  

\[
\text{Activity} = 100 - \left( \frac{\text{mean parasitaemia of treated group}}{\text{mean parasitaemia of control group}} \right) \times 100
\]

The numbers of surviving animals in each group was recorded.

**Comparative pharmacodynamic evaluation of ARM NLC and C-AST formulations at different dose levels**

In this study, a slightly modified Peters 4 day suppressive test was adopted for evaluating the effect of dosage on antimalarial activity of ARM NLC and C-AST formulations. The doses for this study included the mouse equivalent of the human dose for each drug (X), a 2-fold higher dose (2X) and a 4-fold lower dose (1/4X), respectively. Ten groups (n=8) of mice weighing around 30±5 g were employed (Table 1). After 2 h of infection, the mice in each group were treated with a dose of ARM NLC as well as C-AST for four consecutive days as stated in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>control</td>
</tr>
<tr>
<td>II</td>
<td>ARM NLC formulation 0.130 mg (X dose)</td>
</tr>
<tr>
<td>III</td>
<td>ARM NLC formulation 0.260 mg (2X dose)</td>
</tr>
<tr>
<td>IV</td>
<td>ARM NLC formulation 0.033 mg (1/4X dose)</td>
</tr>
<tr>
<td>V</td>
<td>ARM NLC formulation 0.130 mg (single X dose)</td>
</tr>
<tr>
<td>VI</td>
<td>blank NLC, equivalent to 0.260 mg</td>
</tr>
<tr>
<td>VII</td>
<td>C-AST formulation 0.096 mg (X dose)</td>
</tr>
<tr>
<td>VIII</td>
<td>C-AST formulation 0.192 mg (2X dose)</td>
</tr>
<tr>
<td>IX</td>
<td>C-AST formulation 0.024 mg (1/4X dose)</td>
</tr>
<tr>
<td>X</td>
<td>C-AST formulation 0.096 mg (single X dose)</td>
</tr>
</tbody>
</table>

To evaluate single-dose effect of both formulations.

**Statistical analysis**

Analysis of survival of animals and parasitaemia was conducted using Graphpad Instat. Data were compared using an unpaired t-test, differences of P<0.05 being considered significant, and expressed as mean±SD.

**Results**

**Comparative pharmacodynamic evaluation of ARM NLC and C-AST formulations at different dose levels**

The control group showed the highest parasitaemia (Figure 1a), followed by blank NLC and C-AST single dose. On the seventh day, the C-AST single dose had reached a similar parasitaemia load as that of the control group on the fifth day. On the seventh day, the ARM NLC single dose and the ARM NLC 1/4X dose showed 1.2- and 1.3-fold lower parasitaemia loads (P<0.05) than the C-AST single dose and the C-AST 1/4X dose, respectively. However, the single dose of ARM NLC demonstrated antimalarial activity up to 9 days with 44.6% parasitaemia. Among the two formulations, the ARM NLC X dose exhibited a 2.5-fold lower parasitaemia (P<0.05) than the C-AST X dose, and the ARM NLC 2X dose exhibited no parasitaemia, whereas the C-AST 2X dose showed 6.9% parasitaemia.

In terms of activity, at single dose there was a reduction in activity for ARM NLC (below 56%) after 4 days, whereas for C-AST it was observed after 3 days (Figure 1b). The repeated administration of ARM NLC at X and 2X dose showed activity >60% and 100% on the 7th day while for C-AST at X and 2X dose, it was found to be 44% and 60%, respectively. Although, after 4 days at 1/4X dose, both C-AST and ARM NLC showed decreased activity, it should be noted that ARM NLC showed 1.6-fold greater activity than C-AST.

The survival plot (Figure 2) shows that survival of the various groups was in the order ARM NLC 2X>ARM NLC X>C-AST 2X>ARM NLC 1/4X>C-AST X>C-AST 1/4X>blank NLC>ARM NLC single dose>C-AST single dose>control.

**Discussion**

A lipidic nanoformulation of artemether was developed to cater for the need for a rapidly acting parenteral antimalarial
formulation. Though artemether has a longer half-life (0.53 h) than artesunate ($t_{1/2}$ of 0.35 h upon iv administration), the drug has not been explored as an alternative for cerebral malaria, mainly due to its lipid solubility, necessitating im administration of the drug, which results in patient non-compliance. As is evident from the literature, lipidic nanoformulations have shown good potential for delivering hydrophobic drugs with improved therapeutic efficacy. This was the rationale of our investigation. We formulated ARM NLC, which retained nanosize (63 ± 28 nm) upon dilution with isotonic saline solution. The particulate nature of ARM NLC provided sustained release of artemether for a prolonged period. These observations suggest a high bioavailability of artemether for a prolonged period, though this remains to be confirmed by pharmacokinetic studies. This hypothesis is well supported by 100% activity shown by the ARM NLC 2X dose for 10 days despite the cessation of treatment after the first 4 days. Further, it is well documented that particles <100 nm can evade the mononuclear phagocyte system, resulting in prolonged circulation of the particles. This attribute could also result in significant reduction of parasitaemia and possibly avoidance of recrudescence.

As artesunate is the most widely used artemisinin derivative for cerebral malaria, C-AST was used for establishing the iv acceptability of the developed ARM NLC formulation. As clearly shown in Figure 1(b), ARM NLC showed significantly better activity than C-AST. Although the 1/4X dose of ARM NLC showed similar activity to the X dose of C-AST, it still showed parasitaemia in later stages of the study. Predictably, at the X dose, ARM NLC showed better activity than C-AST. The study demonstrates the superiority of ARM NLC over C-AST. The activity of ARM NLC (X dose) was >60% at the end of 7 days, whereas that of C-AST (X dose) had reduced to <50% on day 7 (shown in Figure 1b). While the activity of ARM NLC (2X dose) was 100% at the end of 7 days, the activity of C-AST (2X dose) dropped to 60% on day 7. Further, the ARM NLC 2X dose showed 62% survival for 28 days, while none of the commercial formulations showed survival beyond 20 days. Although ARM NLC showed parasitaemia, it showed a better survival rate than C-AST at the 2X dose level.
This study clearly demonstrates that the developed ARM NLC can be considered as a superior alternative to the existing therapeutic interventions in cerebral malaria. Additionally, this study presents a huge opportunity for developing combinational therapeutic dosage regimens with artemether as a partner molecule.

Conclusions

ARM NLC significantly reduced parasitaemia in a murine model and was found to be superior to C-AST. The study clearly demonstrates the efficacy of an iv alternative to conventional artesunate delivery.

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Transparency declarations

None to declare.

References