Enhanced Antimalarial Activity by a Novel Artemether-Lumefantrine Lipid Emulsion for Parenteral Administration

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Artemisinin and lumefantrine (also known as benflumetol) are difficult to formulate for parenteral administration because of their low aqueous solubility. Cremophor EL as an emulsion excipient has been shown to cause serious side effects. This study reports a method of preparation and the therapeutic efficacies of novel lipid emulsion (LE) delivery systems with artemether, lumefantrine, or artemether in combination with lumefantrine, for parenteral administration. Their physical and chemical stabilities were also evaluated. Furthermore, the in vivo antimalarial activities of the lipid emulsions developed were tested in Plasmodium berghei-infected mice. Artemether, lumefantrine, or artemether in combination with lumefantrine was encapsulated in an oil phase, and the in vivo performance was assessed by comparison with artesunate for injection. It was found that the lumefantrine lipid emulsion (LUM-LE) and artemether-lumefantrine lipid emulsion (ARM-LUM-LE-3) (1:6) began to decrease the parasitemia levels after only 3 days, and the parasitemia inhibition was 90% at doses of 0.32 and 0.27 mg/kg, respectively, with immediate antimalarial effects greater than those of the positive-control group and constant antimalarial effects over 30 days. LUM-LE and ARM-LUM-LE-3 demonstrated the best performance in terms of chemical and physical stabilities and antiplasmodial efficacy, with a mean particle size of 150 nm, and they have many favorable properties for parenteral administration, such as biocompatibility, physical stability, and ease of preparation.

Malaria is one of the most significant causes of morbidity and death worldwide. Every year, nearly one million deaths result from malaria infection, with about 85% occurring among children under 5 years of age. Indeed, one patient dies from malaria every 36 s (1).

Artemisinin and its derivatives are the most important antimalarial drugs; the chemical structures of the four artemisinin compounds and lumefantrine (also known as benflumetol) are presented in Fig. 1. Artemisinin is an effective antimalarial drug isolated from the Chinese herbal medicine Artemisia annua L. It has a special sesquiterpene lactone with a peroxy group. In malaria parasites, artemisinin can be activated by intraparasitic heme iron, which catalyzes cleavage of the endoperoxide (2). Due to its unique structure and antimalarial mechanism, artemisinin has excellent antimalarial activity with low toxicity; therefore, artemisinin-based combination therapy (ACT) is recommended as the first-line strategy to treat malaria (3). After the introduction of ACT interventions, morbidity and mortality rates associated with malaria were decreased in several parts of the world (4). However, antimalarial resistance would probably be increased after long-term application of artemisinin alone. WHO reformed the malaria treatment policy in February 2004, proposing to stop the use of single antimalarial drugs (e.g., artemisinin and quinine) and promoting the use of ACTs. Recently, combination therapies such as artemether in combination with lumefantrine, artesunate in combination with amodiaquine, and artesunate ester in combination with mefloquine were proposed, among which artemether in combination with lumefantrine had the best therapeutic effect. This finding may be because artemether and lumefantrine have complementary advantages. Artemether has an initial burst effect on Plasmodium schizonts and a variety of drug-resistant malaria strains. However, major limitations of artemether have been ascribed to its short duration of action and high recurrence rate. The killing effect of lumefantrine on Plasmodium was less than 95% and the recurrence rate was less than 5%, but the effect was shown slowly. Therefore, the FDA approved the combination oral tablets on 8 April 2009.

The combination oral tablets cannot be used for emergency treatment of malaria in clinical settings due to the oral dosage form. Despite the promising clinical results, lumefantrine suffers from biocompatibility issues, principally poor bioavailability and low solubility in aqueous media. In addition, patients need to eat high-fat foods to realize higher rates of drug absorption and higher blood concentrations to kill malaria when receiving lumefantrine. In order to protect the clinical effects of lumefantrine, the dose and frequency of administration must be increased. Recently, the dose of lumefantrine was increased to 1,840 mg and even to 2,880 mg. In this way, the development of drug resistance would be accelerated, and the economic burden would undoubtedly be increased. In fact, the high cost has become a major barrier for malaria patients (5, 6). In order to overcome the aforementioned drawbacks, it is necessary to develop a new formulation for artemether plus lumefantrine to use for parenteral administration for emergency treatment of malaria in clinical settings.

To the best of our knowledge, a large variety of drug delivery systems have been developed to enhance the bioavailability of poorly water-soluble drugs, including liposomes, micelles, emul-
ions, and polymer microparticles and nanoparticles (7). Lipid emulsions (LEs) with a mean particle size of 200 nm have many favorable properties for parenteral administration, such as biocompatibility, physical stability, and ease of preparation. Deharo et al. (8) found that soybean fat emulsions such as Intralipid and Ivelip, which were found to inhibit the growth of *Plasmodium falciparum* in culture with 50% inhibitory concentration (IC50) values of 8.07 ± 2.13 and 13.32 ± 2.05 mg/ml, respectively, have antimalarial properties. The use of soybean fat emulsions in the treatment of malaria can also have some additional benefits. The administration of lipid emulsions has been shown to cause some reductions in vitamin E levels and to increase linolenic acid levels, effects that may give some additional protection against malaria (9). All of these excellent properties of lipid emulsions make lipid emulsions appropriate carriers for artemether plus lumefantrine.

In this study, artemether lipid emulsion (ARM-LE), lumefantrine lipid emulsion (LUM-LE), and artemether-lumefantrine lipid emulsion (ARM-LUM-LE), containing soybean oil, egg lecithin, α-tocopherol, F68, sodium oleate, glycerin, and water, were prepared. All of the emulsion formulations were characterized in terms of size, polydispersity (Pd), and encapsulation efficacy (EE). In addition, their physical and chemical stabilities were evaluated. The in vivo antimalarial activities of the developed formulations were tested using *Plasmodium berghei*-infected mice, a suitable model for studying malaria because the infection presents structural, physiological, and life cycle analogies with human disease (10).

### MATERIALS AND METHODS

**Chemicals and standards.** The following materials were purchased: artemether and lumefantrine (Xi’an Huisheng Medical Technology Ltd. Co., Shanxi, China), soybean oil, sodium oleate, glycerol, and egg lecithin (98% phosphatidylcholine) (Xi’an Libang Pharmaceutical Ltd. Co., Shanxi, China), poloxamer 188 (Pluronic F68) (Shanghai Qingyuanxing Chemical Technology Ltd. Co., Shanghai, China), and α-tocopherol (Sigma). All chemicals and reagents used were of analytical or chromatographic grade.

**Preparation and characterization of formulations.** The developed formulations were prepared by hot homogenization and ultrasonication processes, and the optimized compositions are shown in Table 1. Briefly, in the case of artemether lipid emulsion (ARM-LE), artemether (0.3%...}

![FIG 1](https://example.com/figure1.png) *Chemical structures of the four artemisinin compounds and lumefantrine.*

| TABLE 1 Compositions of artemether lipid emulsion, lumefantrine lipid emulsion, and artemether-lumefantrine lipid emulsion |
|---|---|---|---|---|---|---|
| Ingredient | Amount (% [wt/vol])<sup>a</sup> | LE | ARM-LE | LUM-LE | ARM-LUM-LE-1 | ARM-LUM-LE-2 | ARM-LUM-LE-3 |
| Artemether | 0 | 0.3 | 0 | 0.1 | 0.3 | 0.1 |
| Lumefantrine | 0 | 0 | 0.6 | 0.3 | 0.1 | 0.6 |
| Soybean oil | 10 | 10 | 10 | 10 | 10 | 10 |
| Egg lecithin | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| α-Tocopherol | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| F68 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 | 2.5 |
| Oleic acid | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Glycerol | 2.25 | 2.25 | 2.25 | 2.25 | 2.25 | 2.25 |

<sup>a</sup> For all emulsions, double-distilled water was used to bring the composition to 100%.
[wt/vol]), soybean oil (10% [wt/vol]), α-tocopherol (0.2% [wt/vol]) as an antioxidant, egg lecithin (1.2% [wt/vol]), F68 (2.5% [wt/vol]), and oleate (0.1% [wt/vol]) as emulsifying agents, glyc erin (2.25% [wt/vol]), and water were used. Preparation of this emulsion was performed in two steps.

In the initial step, egg lecithin was dissolved in dehydrated alcohol and then artemether was added with agitation until it was uniformly dissolved. Soybean oil and α-tocopherol were then added to the solution, and the mixture was heated at 70°C to obtain a clear oil phase. The mixture of glyc erin, F68, oleate, and water was also heated at 70°C, to obtain a clear water phase. The water phase was added slowly to the oil phase, and the mixture was heated for 2 h at 70°C under nitrogen, to obtain a coarse emulsion. The mixture was then cooled at room temperature and adjusted at pH 6 to 9 with NaOH (2 N). In the second step, the coarse emulsion was treated by high-pressure homogenization (NS1001L; Niro Soavi, Italy) at 80°C for eight cycles, to obtain the final emulsion. The temperature was maintained at 40°C for the entire homogenization process. After the treatment, the ARM-LE preparation was sealed in vials and rotated in a 121°C water steam bath for 15 min.

In order to obtain lumefantrine lipid emulsion (LUM-LE), lumefan- trine (0.6% [wt/vol]) was dissolved in soybean oil, egg lecithin, α-tocopherol, F68, oleate, glyc erin, and water, and then the emulsion was prepared as described above. In the case of artemether-lumefantrine lipid emulsion (ARM-LUM-LE), artemether (0.1% or 0.3% [wt/vol]) and lumefantrine (0.1%, 0.3%, or 0.6% [wt/vol]) were weighed together with soybean oil, egg lecithin, α-tocopherol, F68, oleate, glyc erin, and water, and then the emulsion was prepared as described above.

The particle size, polydispersity, and ζ-potential of the developed emulsions were measured with a Zetasizer Nano ZS system (Malvern Ltd., England). The emulsion samples were diluted 1:1,000 with purified water before measurements. The pH values of the developed emulsions were measured at room temperature (25 ± 2°C) using a pH meter (Professional Meter PP-50; Sartorius Ltd., Germany) fitted with a microelectrode.

The encapsulation efficiency (EE) of the developed emulsions was determined by measuring the concentration of artemether or lumefantrine in the dispersion phase. The emulsions were subjected to ultracentrifugation (Optima L-100XP; Beckman Coulter Ltd.) at 50,000 rpm for 1.5 h at 4°C, before measurements. The aqueous phase was collected, and the concentration of artemether or lumefantrine in the aqueous phase was estimated using high-performance liquid chromatography (HPLC). The EE was calculated according to the following equation: EE (%) = ([Ctotal - Cwater] / Ctotal) × 100.

The artemether-loaded concentration was assayed by HPLC analysis performed using a Waters 2695 quaternary gradient pump, a Waters 2996 UV detector, and a Waters 717 autosampler (Waters Ltd.). The column employed was an Agilent HC-C18 column (250 mm by 4.6 mm; particle size, 5 μm), the mobile phase was acetonitrile/double-distilled water (60:40), the flow rate was set at 1.0 mL/min, the column temperature was 25°C, the wavelength of the UV detector was set at 216 nm, and the injection volume was 20 μL. The lumefantrine-loaded concentration was assayed by HPLC analysis. The column employed was an Agilent HC-C18 column (250 mm by 4.6 mm; particle size, 5 μm), the mobile phase was methanol/double-distilled water/glacial acetic acid/diethylamine (94.5:4:10:0.5), the flow rate was set at 0.8 mL/min, the column temperature was 25°C, the wavelength of the UV detector was set at 334.5 nm, and the injection volume was 20 μL. The emulsions were stored at 4°C, and physicochemical characterization was carried out according to the methods described above. All of the measurements were performed in triplicate.

Stability investigation. The artemether-loaded, lumefantrine-loaded, and artemether-lumefantrine-loaded lipid emulsions were stored at 4 ± 2°C. After 3 months, the samples were removed and brought to room temperature. Then, their physical and chemical stabilities were evaluated by monitoring of physical appearance, particle size distribution, polydispersity, ζ-potential, pH, and remaining drug. The study was performed in triplicate.

 Animals. Kunming mice (18 ± 2 g; one-half male and one-half female) were obtained from the Laboratory Animal Science Department of Beijing University of Technology. The animals were kept in a facility with a 12-h light/dark cycle, a temperature of 22 ± 1°C, and 60% relative humidity, were fed a standard mouse diet, and were provided with clean drinking water ad libitum throughout the experiments. Animal experiments were carried out according to the European Communities Council Directive of 24 November 1986 (86/609/EEC) for experimental animal care. All efforts were made to minimize the numbers of animals used and their suffering.

Parasite and inoculation. The Plasmodium berghei strain used was originally from the Faculty of Life of Beijing University of Technology (Beijing, China) and was maintained by serial passage of blood from mouse to mouse. The strain was used for evaluation of antimalarial activity because it provides a good model to estimate survival times and antimalarial efficacy in reducing parasitemia. It is sensitive to all currently used antimalarial drugs. Samples of infected blood were collected in heparinized tubes through the ocular venous sinus and were diluted with isotonic saline solution. Each animal was inoculated by intraperitoneal injection of 1 × 107 infected erythrocytes. Parasitemia was evaluated using peripheral blood samples taken from the tail vein. The samples were stained with a 10% solution of Giemsa stain in phosphate buffer (pH 7.2) and were examined with a light microscope under ×100 magnification with oil immersion. Levels of parasitemia for each subject were determined by counting the number of red blood cells with parasites among 1,000 red blood cells. Infected mice with parasitemia levels of 25 to 35% were allocated to several groups of 10 mice each.

Drug treatment protocol and antiplasmodial activity. Plasmodium berghei-infected mice were randomly divided into 36 groups of 10 mice each and were treated through the 4-day suppressive test. The 4-day suppressive test was performed with P. berghei-infected mice according to the procedure detailed by Deharo et al. (8). The different treatments were administered starting 24 h after infection. Mice in one negative-control group were treated with 0.2 mL of saline solution. Mice in another negative-control group were given 0.2 mL of LE once daily for 3 days. Positive-control mice were treated with injections of artesunate at 0.8, 0.4, 0.28, 0.196, 0.137, or 0.096 mg/kg of body weight once daily, through the tail vein, for 3 days. Mice were treated with ARM-LE, LUM-LE, ARM-LUM-LE-1, ARM-LUM-LE-2, or ARM-LUM-LE-3 administered once daily, through the tail vein, for 3 days, at the same dosages as the positive-control group. Parasitemia was measured by microscopic quantitation from Giemsa-stained thin blood smears made from tail vein blood samples and observed with an immersion optical microscope.

The average level of parasitemia of each group of mice was used to calculate the percent reduction of parasitemia using the following formula: \( \text{Percent Reduction} = \left( \frac{A - (B/A)}{A} \right) \times 100 \), where A is the mean level of parasitemia in the negative-control group (untreated) and B is the level of parasitemia in each treated group (11). Treatment was considered effective if the level of parasitemia was reduced by 30% or more (12).

If treatment was effective, then the mice were carefully monitored with thin blood smears to determine the levels of parasitemia at days 7, 9, 14, and 30. Parasitemia was measured by microscopic quantitation from Giemsa-stained thin blood smears made from tail vein blood samples and observed with an immersion optical microscope. Throughout the test, the general condition of the animals, in terms of behavior and clinical signs, was also evaluated; the survival of recovered mice was observed until day 30.

Statistical analysis. All values in the present study are reported as means ± standard deviations from at least three independent experiments. The significance of treatment effects was evaluated using SPSS 11.5, with significance set at \( P < 0.05 \). All treated groups were compared with the negative-control group. One-way analysis of variance (ANOVA) was used to test statistical differences in single-group analyses, followed by Tukey’s test for multiple comparisons. Two-way ANOVA was used for grouped analyses of statistical differences, followed by post hoc tests.
TABLE 2 Size, polydispersity, \( \zeta \)-potential, and encapsulation efficiency of LE, ARM-LE, LUM-LE, ARM-LUM-LE-1, ARM-LUM-LE-2, and ARM-LUM-LE-3

<table>
<thead>
<tr>
<th>Emulsion</th>
<th>Size (nm)</th>
<th>Pd</th>
<th>( \zeta )-potential (mV)</th>
<th>Artemether</th>
<th>Lumefantrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>LE</td>
<td>154.20 ± 1.12</td>
<td>0.11 ± 0.01</td>
<td>−32.80 ± 2.11</td>
<td>82.35 ± 3.22</td>
<td>85.14 ± 1.23</td>
</tr>
<tr>
<td>ARM-LE</td>
<td>128.20 ± 2.51</td>
<td>0.12 ± 0.01</td>
<td>−27.60 ± 1.12</td>
<td>82.12 ± 2.43</td>
<td>84.56 ± 1.66</td>
</tr>
<tr>
<td>LUM-LE</td>
<td>130.60 ± 2.32</td>
<td>0.14 ± 0.01</td>
<td>−23.80 ± 3.24</td>
<td>81.89 ± 1.50</td>
<td>83.21 ± 2.71</td>
</tr>
<tr>
<td>ARM-LUM-LE-1</td>
<td>126.60 ± 3.54</td>
<td>0.16 ± 0.01</td>
<td>−25.30 ± 2.17</td>
<td>82.12 ± 3.43</td>
<td>84.56 ± 1.66</td>
</tr>
<tr>
<td>ARM-LUM-LE-2</td>
<td>125.10 ± 1.46</td>
<td>0.15 ± 0.01</td>
<td>−24.20 ± 1.13</td>
<td>82.63 ± 2.71</td>
<td>84.44 ± 2.03</td>
</tr>
<tr>
<td>ARM-LUM-LE-3</td>
<td>133.00 ± 2.11</td>
<td>0.07 ± 0.01</td>
<td>−27.60 ± 1.42</td>
<td>82.12 ± 2.43</td>
<td>84.56 ± 1.66</td>
</tr>
</tbody>
</table>

*Data are shown as means ± standard deviations (n = 3).*

### RESULTS

**Characterization of lipid emulsions.** The dispersions of all lipid emulsions were analyzed in terms of size, polydispersity (Pd), and \( \zeta \)-potential, as summarized in Table 2. The mean diameters of all lipid emulsions were <200 nm, suitable for parenteral administration. Pd is a dimensionless measure of the broadness of the size distribution calculated from distribution analysis, and Pd was calculated for each peak as peak width/mean diameter. Pd values range from 0 to 1, with smaller values indicating more narrow distributions. The mean Pd values for all lipid emulsions were <0.2, and the results show that the lipid emulsions had good distribution.

The \( \zeta \)-potential is an important parameter for the determination of emulsion stability. It reflects the size of the electrostatic repulsion between particles, thus affecting the aggregation of the particles and the stability of the dispersion system. The values of the \( \zeta \)-potential generally range from −20 to −45 mV. In our study, the \( \zeta \)-potential values for all prepared formulations were around −25 mV, which indicates that the emulsions were stable (Table 2). LE had a \( \zeta \)-potential value of around −30 mV, which illustrates that LE had greater electrostatic stabilization of dispersion.

The artemether and lumefantrine loading efficiencies were >80% for all prepared formulations, as evaluated by HPLC analysis (Table 2). The results indicated that artemether and lumefantrine had good solubility in soybean oil, and they were suitable for preparation as lipid emulsions.

**Stability studies.** The stability of artemether-loaded, lumefantrine-loaded, and artemether- plus lumefantrine-loaded lipid emulsions was studied over 1 month. Physical stability was checked by monitoring the size and polydispersity over time by dynamic light-scattering analysis. In particular, the vesicle size variations were up to 3.18%, 12.39%, 13.39%, 10.11%, 9.03%, and 5.83% of the original values for LE, ARM-LE, LUM-LE, ARM-LUM-LE-1, ARM-LUM-LE-2, and ARM-LUM-LE-3, respectively (Fig. 2). Furthermore, the polydispersity (Pd) of all liposomal formulations was stable over time, as reported in Fig. 3. Indeed, the Pd variations were up to 11.66%, 13.33%, 11.11%, 16.67%, 11.36%, and 4.76% of the original values for LE, ARM-LE, LUM-LE, ARM-LUM-LE-1, ARM-LUM-LE-2, and ARM-LUM-LE-3, respectively. According to these results, no vesicle size alterations occurred during the test period.

Chemical stability was checked by quantification of the drug contents after the disruption of purified vesicles. As described in Materials and Methods, the contents of artemether and lumefantrine in the lipid emulsions were quantified by HPLC analyses. In the case of the combined lipid emulsions, we found that artemether maintained a residual percentage of 89.67% after 90 days in ARM-LE, while 91.33% of the artemether content in ARM-LUM-LE-1 and ARM-LUM-LE-2, respectively (Fig. 4). After 90 days, artemether maintained residual percentages of 87.33% and 87.00% in ARM-LUM-LE-1 and ARM-LUM-LE-2, respectively (Fig. 4). After 90 days, lumefantrine maintained residual percentages of 86.67% and 85.33% in LUM-LE and ARM-LUM-LE-3, respectively (Fig. 5); after 60 days, lumefantrine maintained residual percentages of 86.33% and 82.33% in ARM-LUM-LE-1 and ARM-LUM-LE-2, respectively (Fig. 5).

**Drug treatment protocol and antiplasmodial activity.** The in vivo antimalarial efficacy of the developed lipid emulsions was determined by the classic 4-day suppressive test, commonly known as the Peters test (13, 14). The antiplasmodial activities of

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**FIG 2** Physical stability of the developed lipid emulsions (LE, ARM-LE, LUM-LE, ARM-LUM-LE-1, ARM-LUM-LE-2, and ARM-LUM-LE-3) in terms of mean diameter, as determined by dynamic light-scattering analysis. The lipid emulsions were stored at 4 ± 2°C for 3 months. Data are shown as means ± standard deviations (n = 3). LE, lipid emulsion; ARM-LE, artemether lipid emulsion; LUM-LE, lumefantrine lipid emulsion; ARM-LUM-LE, artemether-lumefantrine lipid emulsion.
all of the developed lipid emulsions were evaluated in *P. berghei*-infected mice and compared with the performance of artesunate. Mice were treated with 1 x 10^7 infected red blood cells injected intraperitoneally on day zero. Then, mice were treated with LE, ARM-LE, LUM-LE, ARM-LUM-LE-1, ARM-LUM-LE-2, ARM-LUM-LE-3, or artesunate once daily from day 1 to day 3. Blood smears were obtained on day 4. Figure 6 shows the progression of parasitemia for all groups investigated. An untreated group was evaluated in parallel.

As expected, the negative-control group treated with saline solution registered increasing levels of parasitemia in the blood. The negative-control group treated with LE exhibited similar levels of parasitemia and was omitted in Fig. 6. In contrast, the positive-control group maintained the level of parasitemia under 35% with the dosage of 4 mg/kg/day for 3 days. LUM-LE, ARM-LUM-LE-1, and ARM-LUM-LE-3 at the dosage of 4 mg/kg/day for 3 days began to decrease parasitemia levels to under 25%. In the case of ARM-LE and ARM-LUM-LE-2, the parasitemia levels were maintained at 60% or more after 3 days at the same dosage (Fig. 6). Furthermore, the treatment of mice with LUM-LE and ARM-LUM-LE-3 had immediate antimalarial effects, and the parasitemia levels were controlled at 5% or less with the dosage of 8 mg/kg/day for 3 days. In contrast, the treatment of mice in the positive-control group yielded a parasitemia level of 25%, higher than the levels in the LUM-LE and ARM-LUM-LE-3 groups (Fig. 6). In the case of ARM-LE and ARM-LUM-LE-2, the parasitemia levels were maintained at 45% or more, higher than the levels in the positive-control group, although the animals survived. In addition, 50% suppressive dose (SD_{50}) and 90% suppressive dose (SD_{90}) values were calculated by simplified probit analysis. The SD_{50} and SD_{90} values for all lipid emulsions are given in Table 3. The SD_{50} values for LUM-LE and ARM-LUM-LE-3 were approximately one-half of the dosage for the positive-control group. Furthermore, the SD_{90} values for LUM-LE and ARM-LUM-LE-3 were approximately one-fourth of the value for the positive-control group. The results showed that the antimalarial actions of LUM-LE and ARM-LUM-LE-3 were greater than the results for the positive-control group.

The animals were also evaluated in terms of behavior and clinical signs throughout the test period, and the survival of the recovered mice was observed until day 30. All of the animals in the negative-control group died by 12 days postinoculation, showing parasitemia levels of more than 40% of the initial value. All of the liposomal treatments extended the period of survival of the mice until 30 days postinoculation.

Finally, Fig. 7 presents a statistical comparison of LUM-LE-
treated, ARM-LUM-LE-1-treated, and ARM-LUM-LE-3-treated groups and the positive-control group over 15 days. No remarkable differences between the positive-control group and the ARM-LUM-LE-1-treated group were observed until day 10. However, LUM-LE and ARM-LUM-LE-3 seemed to give the most pronounced and statistically significant therapeutic effects in this murine model of malaria at day 7 (P < 0.05), and the parasitemia levels were controlled at 2% or less until day 15. The parasitemia levels were controlled at 10% and 8% in the positive-control group and the ARM-LUM-LE-1-treated group, respectively.

**DISCUSSION**

Malaria continues to impose significant global health and socioeconomic burdens in regions in which it is endemic. While substantial investments in the delivery of frontline artemisinin-based combination therapies and the use of insecticide-impregnated bed nets have yielded decreases in the mortality rates attributed to malaria in recent years, data from 2009 show that this disease still has significant effects in terms of both morbidity (~255 million cases) and death (~781,000 deaths) each year (15).

In recent years, many compounds, including natural products derived from plants and marine organisms, have been developed to inhibit the growth of parasites (16, 17). Dependensin and its analogs were subjected to antimalarial growth inhibition assays against *Plasmodium falciparum* and were found to have IC50 values ranging between 1.9 and 3.9 μM (18). Sesamatin and artemetin, isolated from the aerial parts of the Cape Verdean shrub *Artemisia gorgonorum* Webb (Asteraceae), were carefully investigated and were the most active compounds (IC50 values of 3.37 and 3.50 μg/mL, respectively), without noticeable toxicity on normal mammalian cells (19). In addition, 1,2,3-triazole-tethered β-lactam and 7-chloroquinoline bifunctional hybrids were synthesized and showed reasonable antimalarial activities, with IC50 values ranging from 1.1 to 5.9 μM (20). A series of 5-phenyliminobenzo [a]phenoxazine derivatives were synthesized, and the N,N-dimethyl-5-[[(4-methoxyphenyl)limino]-5H-benzo[a]phenoxazin-9-amine showed an IC50 of 0.040 μM, with a selective index of 1,425 against *Plasmodium falciparum* K1 (21). 6-[2-(3,4-Dimethoxy- phenyl)ethenyl]-4-methoxy-2H-pyran-2-one methysticum, isolated from *Alpinia speciosa* and *Polygala sabulosa*, exhibited antimalarial activity (IC50 of <10 μM) (22). The antiplasmodial activity of VA1 (peptide sequence, Ac-K-W-ΔF-W-ΔF-V-K-ΔF-A-K-NH2), alone and in combination with chloroquine, was determined; the IC50 values of VA1 and of VA1 in combination with 40 nM chloroquine were 10 μM and 2.5 μM, respectively (23).

Some new compounds have been synthesized to inhibit the growth of parasites *in vitro*; in addition, the *in vivo* effects of some new compounds have been investigated. Indole alkaloids isolated from various *Strychnos* species of plants have been demonstrated to show *in vitro* antiplasmodial activity (0.96 μM); *in vivo* inhibition of parasitemia at the dosage of 15 mg/kg was 87% at most (24, 25). A series of novel ketoneamine chalcone-chloroquine-based hybrids were synthesized, and compounds 25 and 27 each showed 99.9% *in vivo* suppression of parasitemia at a dosage of 100 mg/kg/day by the oral route (26). A series of novel 4-anilinoquinoline Mannich base molecules were synthesized, and the *in vivo* inhibition of *Plasmodium yoelii* N67 in Swiss mice by compound BM-1 [4-(7-chloroquinolin-4-ylamino)-2-morpholin-4-ylmethylphenol] at a dosage of 500 mg/kg by the oral route was 100% (27). A series of compounds containing bivalent imidazolium rings and one triazolium analog were synthesized; parasitemia was reduced by ~50% in mice receiving the lower dose of compound 39 of 3.75 mg/kg and by ~75% in mice receiving the higher dose of 15 mg/kg (28). Ethanol and aqueous leaf extracts of *Aloe* sp. caused 73.94% and 58.10% parasitemia suppression, respectively, at a dose of 650 mg/kg. An ethanol extract of *Azadirachta indica* leaves induced

**TABLE 3** SD50 and SD90 against *P. berghei* for positive control, ARM-LE, LUM-LE, ARM-LUM-LE-1, ARM-LUM-LE-2, and ARM-LUM-LE-3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg/day)</th>
<th>SD50</th>
<th>SD90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>0.2230</td>
<td>1.2762</td>
<td></td>
</tr>
<tr>
<td>ARM-LE</td>
<td>0.6088</td>
<td>4.8467</td>
<td></td>
</tr>
<tr>
<td>LUM-LE</td>
<td>0.1190</td>
<td>0.3236</td>
<td></td>
</tr>
<tr>
<td>ARM-LUM-LE-1</td>
<td>0.2008</td>
<td>0.7303</td>
<td></td>
</tr>
<tr>
<td>ARM-LUM-LE-2</td>
<td>0.4853</td>
<td>3.7761</td>
<td></td>
</tr>
<tr>
<td>ARM-LUM-LE-3</td>
<td>0.1021</td>
<td>0.2716</td>
<td></td>
</tr>
</tbody>
</table>

*Data are shown as means ± standard deviations, considering the average parasitemia of each group of mice.*

**FIG 6** Parasitemia progression for groups of mice infected with *P. berghei*. Positive-control mice were treated with artesunate at a dosage of 0.8, 0.4, 0.28, 0.196, 0.137, or 0.096 mg/kg once daily, through the tail vein, for 3 days. Mice were treated with ARM-LE, LUM-LE, ARM-LUM-LE-1, ARM-LUM-LE-2, or ARM-LUM-LE-3, at the same dosages as for the positive-control group, once daily, through the tail vein, for 3 days. Data are shown as means ± standard deviations. ARM-LE, artemether lipid emulsion; LUM-LE, lumefantrine lipid emulsion; ARM-LUM-LE, artemether-lumefantrine lipid emulsion.

**FIG 7** Statistical comparison of positive-control, LUM-LE-treated, ARM-LUM-LE-1-treated, and ARM-LUM-LE-3-treated groups (treatment dose, 0.8 mg/kg/day) with the negative-control group over 15 days. Data are shown as means ± standard deviations, * significant at P < 0.05. LUM-LE, lumefantrine lipid emulsion; ARM-LUM-LE, artemether-lumefantrine lipid emulsion.
54.79% parasitemia suppression at a dose of 650 mg/kg, and a water extract induced 21.47% parasitemia suppression at a similar dose. A water extract of the fruits of *Tamarindus indica* showed the highest level of parasitemia suppression (81.09%) at a dose of 650 mg/kg. For the *in vivo* tests, all of the plant extracts were given to mice orally (29).

The use of lipid nanocarriers such as liposomes and nanocapsules to improve the delivery of antimalarial compounds has also been established. Among these investigations, Isacchi et al. studied mice treated with liposomal artemisinin at a dosage of 50 mg/kg/day, alone or with curcumin as a partner drug administered at a dosage of 100 mg/kg/day. The percent reduction of parasitemia with liposomal artemisinin was 96.01% and that with liposomal artemisinin plus curcumin was 98.84% on day 5 (30). Yamégó et al. reported that artemisinin-containing colloidal suspensions (nanosphere or nanoreservoir-type systems to associate artemisinin) inhibited the growth of cultured *Plasmodium falciparum*, both multiresistant K1 and susceptible 3D7 strains, with IC50 values (2.8 and 7.0 ng/ml, respectively) close to those of the reference artemisinin solution (31).

Among these reported compounds, the most promising compounds were indole alkaloids, with *in vitro* antimalarial activity (0.96 µM) and *in vivo* parasitemia inhibition at the dosage of 15 mg/kg of 87% at most. In the present study, the parasitemia inhibition by LUM-LE and ARM-LUM-LE-3 was 90% at dosages of 0.32 and 0.27 mg/kg, respectively (Table 3), which was the most remarkable observation. This is because the antimalarial effect of artemether or lumefantrine was enhanced by the lipid emulsions, and the passive targeting produced by the lipid formulations was more effective for the combination of artemether and lumefantrine. The bioavailability of artemether or lumefantrine was increased to 100% by intravenous administration, with an immediate antimalarial effect. Furthermore, in the case of ARM-LUM-LE-3, a synergistic or additive effect of artemether and lumefantrine was found.

In conclusion, the work presented in this paper represents a successful attempt to formulate artemether or lumefantrine alone or artemether combined with lumefantrine in lipid emulsions. The lipid emulsions have proper physical characteristics, in terms of particle size, polydispersity, encapsulation efficacy, and ζ-potential, to function as drug carriers for parenteral administration. In addition, they simultaneously provide an appropriate solvent for the drugs, a stabilizing system for maintenance and storage for a substantial time after production, and drug targeting to *P. berghei*-infected organs.

The antimalarial activities of artemether or lumefantrine alone or artemether combined with lumefantrine in lipid emulsions were evaluated in *P. berghei*-infected mice, a suitable *in vivo* model. The pharmacological results indicated that LUM-LE and ARM-LUM-LE-3 began to decrease the parasitemia levels only 3 days after the start of treatment and had immediate antimalarial effects greater than those of the positive-control group; in contrast, ARM-LUM-LE-1 had an immediate antimalarial effect similar to that of the positive-control group. The treatment of mice with both conventional ARM-LE and ARM-LUM-LE-2 appeared to have lower antimalarial effectiveness.

Furthermore, the LUM-LE and ARM-LUM-LE-3 formulations showed less variability in plasma concentrations and, as a consequence, constant antimalarial effects over time. The best performance, in terms of chemical and physical stability and antiplasmodial efficacy, was obtained with LUM-LE and ARM-LUM-LE-3.

These results demonstrated the optimization of the efficiency of existing drugs through innovative formulation strategies. Further research on drug delivery systems might offer novel therapeutic approaches for malaria chemotherapy.

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**REFERENCES**


