

## Impaired Clinical Response in a Patient with Uncomplicated *Falciparum* Malaria Who Received Poor-Quality and Underdosed Intramuscular Artemether

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**Abstract.** We describe an adult with uncomplicated *Plasmodium falciparum* malaria who did not improve clinically despite 5 days of intramuscular artemether therapy. He was prescribed a lower dose (kg body weight) than that recommended, and a vial from the packet contained only 74% of the artemether dose as stated by the manufacturer. The combination of underdosing, poor-quality drug, and the intrinsic low bioavailability of artemether may have contributed to his poor clinical response. Analysis of the packaging and chemical “fingerprinting” of the artemether suggested that the drug was genuine but was either substandard or had deteriorated after manufacture.

### INTRODUCTION

Intramuscular artemether has been widely used for the treatment of severe *Plasmodium falciparum* malaria, and clinical trial evidence suggests that it is has similar efficacy to parenteral quinine<sup>1–3</sup> but is probably inferior to parenteral artesunate.<sup>4–6</sup> Intramuscular artemether is also used inappropriately to treat uncomplicated malaria in the absence of vomiting.

Malaria remains a clinical problem in the Lao PDR (Laos),<sup>7</sup> and we report a patient with uncomplicated falciparum malaria who, despite 5 days of intramuscular artemether therapy, remained unwell and febrile. Although the most commonly counterfeited antimalarial in SE Asia is oral artesunate,<sup>8–12</sup> counterfeit intramuscular artemether and substandard intramuscular artemether have been described from Burma (Myanmar) and Kenya, respectively.<sup>13,14</sup> As there is no evidence for resistance to the artemisinin derivatives in Laos,<sup>15</sup> the artemether was suspected to be of poor quality. Therefore, we performed chemical analysis of a vial of intramuscular artemether from the same packet as the vials taken by the patient and inspected the accompanying packaging material.

### CASE REPORT

A 30-year-old Chinese male itinerant trader presented in May 2006 to a military hospital in Savannakhet, southern Laos, with 2 days of fever, chills, headache, and a dry cough. The patient had spent the previous 2 months trading in Savannakhet Province. The medical history was otherwise unremarkable. He had slide-positive falciparum malaria and was treated with intravenous infusions and intramuscular artemether 80 mg for 3 days, which he had brought from China as standby therapy, but did not improve. He returned to Vientiane where he was admitted to a private Chinese clinic,

where he continued another 2 days of intramuscular artemether from the same personal supply he had brought from China. The fever persisted, mild jaundice developed, and he was therefore transferred to Mahosot Hospital, Vientiane. On admission he was febrile (39.5°C) and conscious but had nausea, dry cough, moderate dehydration, chest pain, and abdominal tenderness. He was malaria smear-negative but was Paracheck-positive (Orchid Industries, Goa, India) for *Plasmodium falciparum* malaria HRP-2. His serum creatinine and glucose were normal with ALT 301 U/L, AST 230 U/L, alkaline phosphatase 470 U/L, total bilirubin 14 µmol/L, and direct bilirubin 6.4 µmol/L.

He had received a total calculated dose of 400 mg of intramuscular artemether, representing—with a body weight of 62 kg—6.5 mg artemether/kg over 5 days. The instructions accompanying the artemether advise 160 mg followed by 80 mg once a day from Day 2 to 5, but he had not received the loading dose. His fever and symptoms had not cleared after 120 hours of therapy. The patient was treated with oral quinine sulfate 10 mg/kg/day every 8 hours and doxycycline 100 mg every 12 hours for 7 days. His fever cleared 35 hours after starting this therapy, and he was discharged well.

Concerned that the patient remained unwell despite 5 days of artemether therapy, we analyzed for artemether content a vial of the artemether (study code 06/09, 80 mg/vial, Artemether Injection, Ratification No. GYZZ H10900011, Kunming Pharmaceutical Corp., People’s Republic of China) purchased by the patient at an unknown location in China. We also purchased an additional 5 boxes of the same product in Vientiane (study code 07/11) for comparative analysis of vial contents as well as visual examination of the packaging material.

### ANALYSIS OF ARTEMETHER

**Methods.** Artemether injectables were prepared for high-performance liquid chromatographic (HPLC) analysis by diluting the sample 1/160 with 2-propanol. After they had been thoroughly mixed and filtered, 5 µL of the preparations was injected into an Agilent 1100 Series HPLC system (Agilent, Palo Alto, CA) with diode array detection. Chromatographic separation was achieved using a 150 mm × 4.6 mm C18 col-

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umn and a mobile phase consisting of 70% acetonitrile and 30% 0.05 M perchlorate buffer (pH 2.5). Flow rate was 1 mL/min, and the column temperature was maintained at 30°C. Detection wavelength was set to 220 nm, and peak purity was assessed by comparing the UV spectra of the component peaks with a reference standard.

Samples for mass-spectrometric analysis were diluted in methanol as 1% v/v by vortexing for 20 minutes and then serially diluted to a final concentration of 0.01% in 50:50 MeOH/H<sub>2</sub>O, 0.1% CH<sub>3</sub>COOH. Analysis was done using electrospray ionization mass spectrometry in positive ion mode at an electrospray voltage of 4 kV and a solution flow rate of 1 μL/min. Samples were analyzed with and without addition of 100 μM dodecylamine (DDA) to the spray solution. In the presence of DDA, it has been shown that artemisinin derivatives produce stable complexes, with more sensitive ionization.<sup>16</sup> Experiments were performed on an LCQ Deca XP+ ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA) autotuned for optimum detection of the precursor ion of interest. Data were collected via Xcalibur software (version 2.0, Thermo Finnigan) and averaged for 1 minute. The ion-transfer capillary was set at 300°C. The instrument was set to collect spectra in automatic gain mode for an ion-trap injection time of 200 ms at 2 microscans/spectrum.

**Results.** Examination of the vial and packaging of the suspect sample (batch number 20051134-01, manufacture date November 2005, expiry date October 2009) did not reveal any overt differences from the genuine samples bought in Vientiane. Kunming Pharmaceutical Corporation kindly examined photographs of the vial and packaging and did not find any features suggesting that the medicine was counterfeit. None of the features of fake injectable artemether described from

Burma<sup>13</sup> (differences in packet color, absence of manufacturing and expiry dates and Myanmar Registration Number on the packet, and presence of a watery, rather than an oily, fluid in the ampoules) was evident. The batch numbers of the samples bought in Vientiane were 20041139 (manufactured November 2004, expiry date October 2008), 20060628 (manufactured June 2006, expiry date May 2010), and 20060739 (manufactured July 2006, expiry date June 2010).

HPLC analysis of the suspect sample (06/09) demonstrated that it contained only 59 mg artemether (74% of that stated on the vial) while the other 10 samples from 5 packets contained a median (range) of 84.5 (83–91) mg of artemether, or 105.6% (103.8–113.8%) of the stated 80-mg artemether content. The electrospray mass spectra (Figure 1, a and b) demonstrated that both samples were identical in terms of their qualitative chemical composition, i.e., their “fingerprints,” but differed in active ingredient content. Artemether was the only component identified in the samples, as shown by the peaks at *m/z* 321.0 and 619.1 corresponding to [artemether + Na]<sup>+</sup> and [2 artemether + Na]<sup>+</sup>, respectively (Figure 1a). In these experiments, the peaks at *m/z* 221.0, 239.1, and 267.1 correspond to various artemether fragments. The presence of artemether was verified by performing electrospray experiments in the presence of DDA (Figure 1b), where a peak with *m/z* 484.0 was observed, corresponding to the [artemether + DDA + H]<sup>+</sup> non-covalent complex ion. The ion at *m/z* 186.2 corresponds to the free protonated amine ion [DDA + H]<sup>+</sup>. An ion at *m/z* 652.3 was also observed in the spectra of both samples but could not be identified.

The relative amounts of artemether in samples 06/09 and 07/11 were determined from the intensity of the ion at *m/z* 321 ([artemether + Na]<sup>+</sup>) in the electrospray mass spectrum (Figure 1a), showing that sample 06/09 contained only 72% of

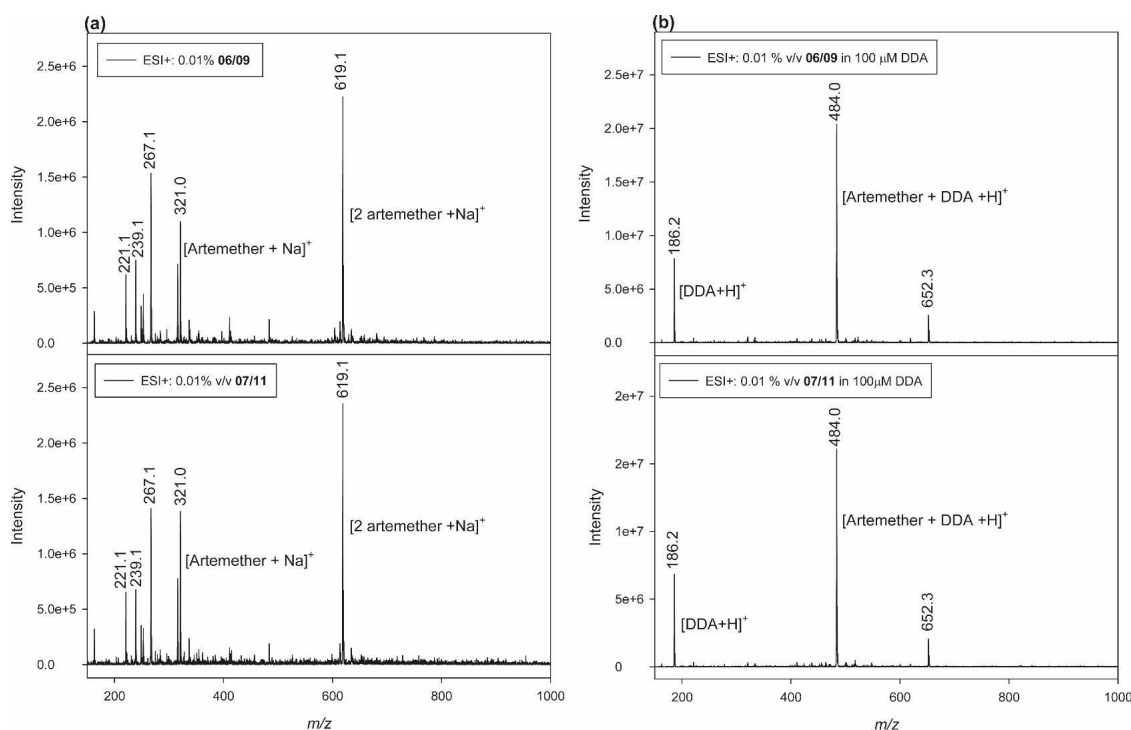


FIGURE 1. Positive ion mode electrospray mass spectrum of samples 06/09 and 07/11, (a) without dodecylamine and (b) with addition of 100 μM dodecylamine.

artemether compared with the amount in sample 07/11. This confirms the HPLC findings by an independent method.

## DISCUSSION

The artemether taken by our patient was of poor quality, containing only 74% of the stated amount of artemether. The analysis of packaging and mass-spectrometric "fingerprints" suggests that the drug was a genuine product. The artemether may have been substandard, but it could also have been issued as a good-quality genuine product that deteriorated during storage and transport from China. We have been unable to find published information on the long-term stability of intramuscular artemether or arteether in tropical climates, making it difficult to reach a conclusion as to the reason for the vial having reduced amounts of active ingredient. Important limitations of the report are that we do not know the storage conditions of the artemether after it left the factory and that only one vial of artemether from the suspected poor-quality sample was available for testing, and therefore standard pharmacopoeial procedures could not be exactly followed.

A sample of probably substandard arteether from Kenya contained 77% of the active ingredient.<sup>14</sup> Assuming that all the vials in the packet taken by our patient contained approximately 74% artemether, the patient would have received only  $\approx 296$  mg artemether or 4.8 mg/kg over 5 days. The conventional dose of intramuscular artemether is 3.4 mg/kg stat followed by 1.6 mg/kg/day.<sup>17</sup> Although the instructions that came with the artemether advised a 5-day course, monotherapy is not recommended, but when it is necessary, it should be continued for 7 days.<sup>17</sup> Therefore, in addition to the artemether being of poor quality, the patient was underdosed, receiving a prescribed 1.3 mg/kg/day without a loading dose and a probable actual dose of  $\approx 1.0$  mg/kg/day, and was treated with monotherapy of inadequate duration. The common error of package inserts advising 5 days, rather than the international guidelines of 7 days, of monotherapy has recently been highlighted.<sup>18,19</sup>

As intramuscular artemether is not usually used for therapy of uncomplicated malaria, there are few estimates of fever clearance in this situation. The median (range) fever clearance time among Vietnamese adults with severe malaria treated with intramuscular artemether was 127 (0–756) hours,<sup>1</sup> but our patient had no evidence of severe disease. In one small study of patients with uncomplicated malaria ( $N = 22$ ), the median (range) fever clearance time was 52 (8–136) hours.<sup>20</sup> Our patient remained febrile at 120 hours, and, although recognizing that this is within the upper range of 136 hours (see above), he continued to exhibit persistent clinical symptoms after 120 hours of intramuscular artemether. Although no malaria parasites were detected on the blood film, the poor clinical response suggests that he was failing treatment.

In comparison to oral artemether and the water-soluble intramuscular/intravenous artesunate, intramuscular artemether has the important disadvantages of greatly reduced  $C_{\max}$  (maximum serum antimalarial activity) and prolonged  $t_{\max}$  (time to  $C_{\max}$ ), in both uncomplicated and severe malaria.<sup>5,6</sup> It is likely that intramuscular/intravenous artesunate is superior to intramuscular artemether in the treatment of

severe malaria because of the much more rapid absorption of artesunate, but results of clinical trials are not yet available. The use of underdosed, poor-quality, artemether in our patient and the poor inherent intramuscular absorption of this drug probably resulted in suboptimal blood concentrations of artemether and its main metabolite, dihydroartemisinin, and hence poor clinical response.<sup>5</sup>

The patient would probably have recovered rapidly if oral co-artemether (artemether–lumefantrine) had been given, as specified in the Lao national treatment guidelines (2005).<sup>21</sup> This case emphasizes the importance of appropriate therapy and of checking the quality of a medicine taken by a patient if expected improvement does not occur. More information is needed on the stability of artemisinin derivatives and other anti-infectives in tropical climates, and these data should be used to advise on the appropriate storage conditions of standby treatment carried by patients. There have been false reports of antimalarial drug resistance, in both Africa and Asia, which further investigation showed to be due to poor drug quality.<sup>22–24</sup> The frequency of poor-quality artemether used by patients is unknown, as few have looked to check the quality of the supply in the field.<sup>8</sup> The use of artemisinin derivative combinations with subtherapeutic drug content, whether fake, substandard, or deteriorated, and drug prescriptions for inadequate doses or duration raises concern that these factors may engender the spread of resistance to this vital class of antimalarials.

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