Travel Medicine and Infectious Disease 15 (2017) 52–56

Contents lists available at ScienceDirect

Travel Medicine and Infectious Disease

journal homepage: www.elsevierhealth.com/journals/tmid

Original Article

Delayed haemolysis secondary to treatment of severe malaria with intravenous artesunate: Report on the experience of a referral centre for tropical infections in Spain

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ARTICLE INFO

Article history:
Received 19 May 2016
Received in revised form
26 October 2016
Accepted 31 October 2016
Available online 3 November 2016

Keywords:
Malaria
Artesunate
Artemisinins
Haemolysis
Complications

ABSTRACT

Background: Post-artesunate delayed haemolysis is described as hemolytic anemia presenting days after malaria treatment in hyperparasitemic patients. Physiopathological mechanisms and clinical manifestations have not been thoroughly characterised.
Methods: We conducted a retrospective study of hospitalised malaria patients who received artemisinin derivatives from January 1, 2010 to December 31, 2015.
Results: 21 patients were included in the study: 11 travellers, 8 travellers visiting friends and relatives and 2 immigrants. Median age was 35.5 years (IQR: 25.7–44.8) and 11 were men. Eight patients received oral and 13 received intravenous (IV) artemisinin-based drugs. Follow-up after the malaria episode was available for 15 patients (12 with IV treatment). Four patients presented with delayed haemolysis 9–14 days after artesunate treatment; all had been admitted with severe malaria, were treated IV and had hyperparasitaemia (17%–33%). Other than hyperparasitaemia, no other factors were associated with artesunate haemolysis. Patients' outcomes were favourable and the only additional therapeutic measure needed was a blood transfusion.
Conclusions: Delayed haemolysis is a frequent complication in hyperparasitemic malaria treated with IV artesunate. Follow-up is mandatory for at least 2 weeks after treatment initiation. This condition is potentially severe but does not appear to be life threatening.

1. Introduction

According to World Health Organisation (WHO) clinical practice guidelines [1] and Spanish and European guidelines [2,3] among other consensus documents, artesunate is the first-line treatment for severe malaria. A meta-analysis published in 2001 [4] and two randomised controlled trials conducted in Asian adults in 2005 (SEQUAMAT) [5] and in African children in 2010 (AQUAMAT) [6] showed intravenous artemisinin to be superior to quinine in terms of decreased mortality. In addition, intravenous artesunate achieves a much faster reduction in parasitaemia, results in a significantly lower incidence of hypoglycaemia and cardiotoxicity and has the most convenient administration route [7]. This class of drugs drastically reduce parasite biomass by targeting *Plasmodium* spp. immature forms during the erythrocytic phase, inhibiting progression of the disease at an early stage.

The use of intravenous artesunate has been limited due to supply problems and the lack of the good medical practice (GMP) certification, which complicated its use in the USA and Europe. The WHO and the European Medicines Agency (EMEA) approved the use of artesunate made by *Guilin Pharmaceuticals* (Shanghai, PR China) [8,9] even though the GMP certification remains unapproved in Europe and it was not approved in the USA until 2013.

Abbrevations: IV, intravenous; VFR, visiting friends and relatives.

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http://dx.doi.org/10.1016/j.tmaid.2016.10.013
1477-8939/

Malaria parasites invade erythrocytes and consequently destroy them during the schizogony phase. This hemolytic effect may persist even days after the parasite is eliminated [10,11]. Other hemolytic processes such as quinine-associated haemolysis ("blackwater fever") or Glucose-6-phosphate dehydrogenase deficiency-associated haemolysis may occur during the early stages of malaria and may also influence the severity of the disease [12].

A report on intravenous artesunate use for the treatment of severe malaria at seven European centres in 2010 [13], showed the drug could cause delayed extravascular haemolysis between 7 and 21 days after treatment initiation in patients with hyperparasitaemia. While resulting anemia may be relevant and require transfusion, delayed haemolysis appears to have a benign course without an increase in mortality.

The exact process as well as the role played by artesunate in erythrolysis is unknown. Although an immunological mechanism may not be discarded, a plausible hypothesis is that erythrolysis may be caused by a process called *pitting*. Once *Plasmodium* spp. parasites are killed by artesunate they are removed from erythrocytes by the spleen and the 'cleaned' erythrocytes are released back into the circulation. These morphologically altered ('pitted') erythrocytes (or 'once-infected erythrocytes', 'o-iE') remain in circulation but with a considerably decreased life span of 7–21 days. Artemisinin-based drugs are more likely than quinine to result in the creation of significant numbers of o-iE. Patients with higher initial parasitaemia have higher numbers of o-iE after treatment with artemisinin drugs. When these erythrocytes with decreased lifespan are simultaneously cleared *en masse*, a brief haemolytic episode occurs that does not usually recur [14].

The present work evaluates the incidence of delayed haemolysis associated with treatment with artemisinin derivatives in patients with severe malaria at a Spanish reference centre for tropical diseases.

2. Methods

Artesunate is used as the standard first-line therapy for severe malaria since 2010 at the Ramon y Cajal Hospital, a referral centre for tropical infectious diseases. A retrospective observational study of patients with a final diagnosis of malaria, who were hospitalised and received oral and intravenous artemisinins between January 1, 2010 and December 31, 2015 was performed. Cases were identified from patients registered in the Tropical Medicine Unit database, which has been approved by the hospital's Ethics committee. Inclusion in the database is offered to all patients and only those signing informed consent are included.

A malaria episode was considered when a patient presented with fever or other suggestive clinical symptoms along with a confirmatory microbiological test. Both a Giemsa stained peripheral blood smear and thick blood smear are performed for all patients with suspected malaria for the diagnosis and quantification of the parasitaemia. A rapid antigen detection test and a PCR for molecular detection of the parasite are also performed in most cases.

Criteria used to classify severity of malaria were those proposed by the WHO [1]. WHO recommendations were also followed for dosing artesunate: an initial dose of 2.4 mg/kg of IV artesunate, and the same dose repeated at 12 h and then daily with a maximum total dose of 12 mg/kg (3 mg/kg instead of 2.4 mg/kg were prescribed for children under 20 kg). Once the patient could tolerate oral medication, a full course of an oral antimalarial was administrated. All patients who initiated treatment with intravenous artesunate received at least three doses, according to WHO recommendations. Patients only treated with oral artemisinin derivatives received artemether + lumefantrine or dihydroartemisinin + piperaquine adjusted for weight and age.

Post-artesunate hemolytic anemia was defined as altered laboratory parameters suggestive of extravascular haemolysis (such as decreased hypoglycaemia haemoglobin, increased LDH or bilirubin) along with 0% parasitaemia (on thick and thin peripheral smears) between 7 and 21 days after treatment initiation.

For the descriptive analysis, categorical variables were expressed as absolute and relative frequencies whilst quantitative variables were expressed as medians and interquartile ranges (IQR: P_{25}–P_{75}).

3. Results

A total of 21 patients hospitalised with malaria during the study period received artemisinin drugs: 13 intravenously and 8 orally. There were 31 additional patients who were hospitalised and treated with other antimalarial drugs (mainly atovaquone-proguanil and chloroquine) and were not included in this report. Among these 21 patients, 8 were born or were resident in Spain and visited relatives in malaria-endemic regions (VFR, visiting friends and relatives), 3 were short-stay travellers, 8 were long-stay travellers (missionaries or expatriates), and 2 were recently arrived immigrants (one of them a young girl from Equatorial Guinea who came to spend her summer holidays in Spain). The median length of stay in malaria-endemic regions among VFR and short-stay travellers was 35 days (IQR: 30–90 days). No patients took correct antimalarial chemoprophylaxis: only four had initiated prophylaxis but this was abandoned a few days after departure. The median age of patients was 35.5 years (IQR: 25.7–44.8) and 11 out of 21 patients were male. Nine of the patients experienced the first symptoms before arriving to Spain. For another 9, symptoms started after arrival in Spain, and for the remaining 3, on the day of arrival. The median time between first symptoms and diagnosis was 5 days (IQR: 2–9). *Plasmodium falciparum* was diagnosed in 17 patients, *P. vivax* and *P. ovale* were detected in another two patients respectively, and there was a mixed infection due to *P. falciparum* and *P. malariae* in two patients. All cases were confirmed by PCR.

Fourteen of the patients had at least one criterion of severe malaria. All patients regarded as having severe malaria on arrival to the emergency department were prescribed intravenous artesunate except for one, infected by *P. vivax*, who received treatment with oral artemisinin-based drugs.

Patients treated only with oral artemisinin drugs were administered artemether + lumefantrine for three days. Four of the patients with severe malaria had received other antimalarial drugs (quinine, mefloquine or atovaquone and proguanil) prior to artesunate, although no more than one dose.

The median parasitaemia was 3% (IQR: 0.1%–17%), and 12 of the treated patients had hyperparasitaemia ($\geq$2%). The median time until parasite clearance after the initiation of artesunate treatment was 2 days (IQR: 2–3 days). The median haemoglobin concentration at the time of malaria diagnosis was 11.9 mg/dl (IQR: 10.1–14.3).

Among the 21 patients included in the study, five of the 8 patients who received oral artemisinin drugs did not have any follow-up visit or analysis after finishing antimalarial treatment (all with low parasitaemia). These patients were either foreign-born not living in Spain or patients in transit. Another patient was not followed-up either because he returned to his home country (Canada) after being treated with intravenous artesunate. The remaining 15 patients are described in Table 1. Three of the cases only had up to one week of follow-up after hospital discharge: cases number 5, 9 and 14.

Four patients presented with haemolysis associated to treatment with IV artesunate (Table 2). One patient was a 61-year-old missionary woman residing in Cameroon who presented with