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Hypothesized cause of delayed hemolysis associated with intravenous artesunate

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ABSTRACT

In recent publications, investigators described cases in which there was a delayed hemolysis following intravenous (IV) artesunate treatment. The delayed hemolysis event occurred at the nadir of blood hemoglobin concentration, i.e., at the time when blood hemoglobin concentration was switching from a progressive decline to a progressive increase. It is hypothesized that this nadir indicates the time when red cell production is resuming after having been arrested, the delayed hemolysis event is due to lysis of the first (aberrant) reticulocytes released once production is resumed and, therefore, that the hemolysis signals the resumption of red cell production. Since this delayed hemolysis has not been associated with a significant decrease in blood hemoglobin, the hemolytic event is not of particular concern even if it could be attributed to artesunate. More important than this hemolysis event was the preceding progressive anemia that lasted for up to 19 days. Both a decrease in reticulocyte production and a shortened life span of previously infected red cells likely contributed to the anemia. The question that remains to be answered is whether the progressive anemia that lasted 2–3 weeks in these patients was attributable solely to their severe malaria or was possibly enhanced and prolonged by the high plasma concentrations of artesunate associated with IV administration. Controlled clinical studies addressing this question may be needed.

Introduction

In recent publications [23], and [17], investigators described cases in which there was a delayed hemolysis following intravenous (IV) artesunate treatment (described in detail in Section 'Finding'). A mechanism to explain this phenomenon and its medical significance are suggested here. Background information is presented first.

Effects of artemisinins on erythroid cells in laboratory animals

In pregnant rats and monkeys, artesunate causes reticulocytopenia and embryotoxicity at doses (6–17 mg/kg) that caused no other detectable toxicity [4,5]. It was suggested that these findings (reticulocytopenia and embryotoxicity) have the same cause – the killing of circulating erythroid cells active in hemoglobin synthesis and therefore containing mitochondria with high concentrations of ferrous iron. Furthermore, it was suggested that ferrous iron reduces artemisinins to free radicals that bind within mitochondria and, if the dose of artesunate is high enough, leads to cell death. In the case of embryos, nearly all of the circulating red cells can be killed in which case the embryo dies. In adults, the effects are minor and transient even if the majority of reticulocytes, likely those containing mitochondria, are killed.

Anemia has been observed in animals at higher doses. Mild anemia was observed in monkeys treated orally for 14 days with 40 mg/kg artesunate [6]. There was mild anemia in rats after 2 weeks of oral treatment at 100 mg/kg/day and after 4 weeks of treatment at 50 and 100 mg/kg/day [11,12]. The mild anemia was accompanied by increased reticulocyte counts. Synthesizing information from numerous studies, the pattern in rats seems to be that continuous daily oral treatment at high doses results in decreases in reticulocyte count followed by a mild anemia together with increased reticulocyte count indicating that artesunate is eliminating some red cells but that the impact is diminished by increased production of red cells (i.e., regenerative anemia). There were no microscopic findings in the bone marrow in the 2- and 4-week rat toxicity studies even though the radiolabeled derivatives of artesunate concentrate in the bone marrow and high concentrations remain 24 h after dosing [7].

In rat whole embryo culture, dihydroartemisinin (DHA) caused three effects on embryonic erythroblasts – arrest of cells in the later stages of the cell cycle, unequal nuclear division and apoptosis [16]. DHA also caused apoptosis in the leukemia cell line HL-60 [14] and the human lung adenocarcinoma cell line A549 [22].

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Effects of artemisinins on erythroid cells in humans

Artesunate-related decreases in reticulocyte count have been reported in humans with effects being more pronounced in healthy volunteers than in malaria patients [5]. Some of the most severe decreases in reticulocyte count were observed following a single IV dose of 4 mg/kg artesunate in healthy volunteers [21]. In this study in which subjects received a single dose of 4 mg/kg artesunate i.v. on Day 1, there was a 69% decrease in absolute reticulocyte count compared to baseline at the nadir on Day 4 ($N = 6$) and a 60% decrease compared to control ($N = 2$). On Day 4, five of the six subjects had absolute reticulocyte counts less than 25×10^9/L. Based on the rate that cells proceed through erythropoiesis, Weina et al. hypothesized that:

> This decrease in reticulocytes is most likely related to a temporary arrest of the basophilic erythroblast stage of the developing red blood cells which results in a brief "hole" in the release of reticulocytes from the bone marrow followed by a robust rebound of the "backed up" developing red cells.

The basophilic erythroblasts are the next cell in the erythroid line after the proerythroblasts which represent the first stage that starts acquiring and storing large amounts of iron and synthesizing hemoglobin.

Malaria-induced anemia

Malaria causes anemia during the blood stages of infection as a result of both decreased erythropoiesis in the bone marrow and increased removal of erythrocytes ([10]). The inhibition of erythropoiesis results not from deficient erythropoietin production but rather from mediators of inflammation including IL6 which induces hepcidin expression leading to decreased iron available for erythropoiesis.

As reported by [17], hemolytic anemia is a characteristic finding in acute malaria:

> The aetiology of malarial anaemia is multifactorial and includes destruction of infected and uninfected erythrocytes as well as impaired erythropoiesis. Both, parasite toxicity as well as host immune mechanisms are causally involved [13]. Impaired erythropoiesis is reflected by a low reticulocyte production index (RPI) ... Usually, however, RPI rises concurrently with parasite clearance [20,15].

There is active removal of parasites from infected cells (possibly in the spleen) without destruction of the red cells [1]. Artesunate (and not quinine) apparently stimulates this process since there were more parasite-free, malaria antigen-positive cells following artesunate treatment [3].

New reports of hemolytic anemia

Findings

In two articles, there were 9 case reports (6 from [23], and 3 from [17] in which investigators reported delayed hemolytic anemia associated with treatment with intravenous artemisinin. An additional case report reports delayed hemolytic anemia induced by oral artemether–lumefantrine [8]. In each of the cases, the attribution of the anemia to hemolysis was based on the observation of elevated plasma LDH. In 3 patients (patients 2 and 3 in [17] and the 1 patient from [8], low haptoglobin was also found.

Table 1 summarizes the relevant data from the three patients in [17], and the two most severely affected patients from [23]. In these 5 patients, there was a progressive decline in blood hemoglobin starting the day after the first dose of artesunate and extending to 15–19 days after the first dose. This period of declining hemoglobin was then followed by a period of increasing hemoglobin. Transient spikes of increased serum LDH and a rebound in the reticulocyte production index occurred at about the same time as the shift from decreasing to increasing hemoglobin. An idealized version of these findings is shown in Fig. 1.

The life span of a normal red cell in circulation is 120 days so each day that the release of reticulocytes into circulation is stopped results in a decrease in red cell count of approximately 0.8%. As stated above, both infected and noninfected erythrocytes can be killed as the result of malarial infection. An estimate of the loss of erythrocytes due to the shutdown of production and the loss of infected cells (based on percent parasitemia) for 5 patients is shown in the far right column in Table 1. This estimate was sometimes less than the actual decrease in hemoglobin suggesting that there was also loss of uninfected cells.

Interestingly, there were no drops in hemoglobin that coincided with the LDH spikes. LDH is not a specific marker for red cell hemolysis but can also indicate liver damage or myocardial infarction. Low haptoglobin levels were observed in 3 patients but low haptoglobin can also indicate liver damage as haptoglobin is produced in the liver. Nevertheless, it is considered that hemolysis is the most likely cause of the elevated LDH and low haptoglobin.

Hypothesis

In the reported cases of delayed hemolytic anemia, the peak of the hemolytic event as measured by a transient increase in LDH was preceded for about 2 weeks by declining blood hemoglobin concentrations and a low reticulocyte production index and was followed by sustained increases in reticulocyte production index and blood hemoglobin. These findings can be reconciled by the following hypothesis:

1. The nadir in blood hemoglobin concentration indicates the time when red cell production is resuming after having been arrested;
2. The delayed hemolysis event is due to lysis of the first (aberrant) reticulocytes released once red cell production is resumed; and,
3. Therefore, the hemolysis signals the resumption of red cell production.

This would explain why there is no impact on blood hemoglobin levels as it is only new red cells that are dying. There is precedent for thinking that artesunate would cause aberrant red cells from the observation that DHA caused unequal nuclear division in embryonic erythroblasts [16].

Critical question

If this hypothesis is correct, then the occurrence of hemolysis two weeks after the initiation of treatment with IV artesunate is not clinically significant. The episode of hemolysis does not indicate a marked worsening of the anemia but rather signals that the production of reticulocytes is resuming. Malaria itself causes anemia. The critical question is "during recovery from malaria, does IV artesunate cause a more prolonged and severe anemia than other curative drugs?"

Possible rationale for iv artesunate contributing to anemia

As described above, the derivatives of artesunate concentrate in the bone marrow and cause decreases in reticulocyte count. It is not unreasonable to consider that IV artesunate could contribute to anemia based on the following factors: