

Short Communication

Prevention of red cell lysis in artesunate-treated rats: A role for glucose-6-phosphate dehydrogenase

Toyin M. Salman

Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.
E-mail: drsalman111@yahoo.com. Tel: +2348032337422.

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The effects of artesunate were investigated on red blood cell count and glucose-6-phosphate dehydrogenase activity in male rats. Twelve (12) male rats were divided into two groups of six (6) rats each. Group 1 rats were control rats which received normal saline while group 2 rats were treated with artesunate orally for five (5) days. 2.90 mg/kg of artesunate was given on the first day of treatment and 1.45 mg/kg was given on the subsequent four days. The results showed that artesunate induced a 25-fold increase ($p < 0.001$) in glucose-6-phosphate dehydrogenase activity while there was no significant reduction in the red blood cell count. The results suggest a role for glucose-6-phosphate dehydrogenase in the prevention of lysis of the red blood cells that may result from treatment with artesunate.

Key words: Artesunate, red blood cell, glucose-6-phosphate dehydrogenase, rat.

INTRODUCTION

Malaria is a devastating disease worldwide and despite the extensive programme for the drug control and eradication instituted by the World Health Organization, malaria remains one of the greatest causes of illness and death in the world and particularly in Africa (WHO, 1985). Although, several drugs are available for the treatment of malaria, their efficacy has been limited by rapid development of resistant strains of malaria parasites, especially *Plasmodium falciparum* during the last 30 years (White, 1999). Chloroquine is becoming ineffective and resistance to other agents such as mefloquine and antifolates has also emerged, especially in *P. falciparum* (Meshnick et al., 1996). With the wide spread establishment of chloroquine resistance, there are only two classes of compounds that are useful to manage severe malaria, the cinchona alkaloids (quinine and quinidine) and artemisinin. However, in some parts of the world, there is evidence of increasing quinine resistance (Purkrittayakamee et al., 1994) such that artemisinins are now used as first line of treatment for severe malaria.

Artemisinin is insoluble and can only be used orally. Analogs are however been synthesized to increase solubility and improve its antimalarial efficacy. The most important of these analogs is artesunate. Artesunate is a water soluble hemisuccinate derivative of hydroartemisinin and it is a potent blood schizonticide active

against the ring stage of the malaria parasite (Bertram, 2004).

Generally, anti-malarials are known to release reactive oxygen species (ROS). These reactive oxygen species then accumulate in cells causing oxidative stress and reducing the cell population (Eitan et al., 2005). For instance, administration of chloroquine for four days in mice caused a decrease in the nucleated cell counts in the peripheral blood, the spleen and liver and, to a certain extent, the bone marrow and the peritoneum. A follow-up however revealed that the total number of nucleated cells returned to normal within ten days, except in cases where chloroquine administration continued for more than four days. Similarly, antimalarial medicinal plants such as *Enantia chlorantha*, *Ocimum gratissimum*, *Azadirachta indica* and *Morinda lucida* have been reported to cause slight increases in the number of nucleated cell counts in the liver and peripheral blood. However, prolonged administration of the medicinal plants for more than four days brought about a decrease in cell counts and subsequent anaemia (Agomo et al., 1992).

The red blood cells are very susceptible to oxidative stress and they become hemolyzed when the oxidant stress becomes too high. However, glucose-6-phosphate dehydrogenase (G-6-PD), a rate-limiting enzyme of the pentose phosphate pathway has been shown to prevent

the red blood cell from oxidative stress and its deficiency results in lysis of the red blood cells (Wiese et al., 1995). Recent report had shown that chloroquine-induced deficiency of glucose-6-phosphate dehydrogenase was responsible for lysis of red blood cells due to the release and accumulation of excess reactive oxygen species in the cells which in turn lead to oxidative stress (Eitan et al., 2005). With the wide spread establishment of resistance to chloroquine, quinine, mefloquine and antifolates (Meishnick et al., 1996; Purkrittayakamee et al., 1994), artesunate is now one of the commonest drugs used as first line of treatment for severe malaria. The evaluation of artesunate for possible hemolytic effects therefore becomes worthwhile.

The present study was therefore designed to investigate the effects of artesunate on red blood cell count and glucose-6-phosphate dehydrogenase activity in male rats.

MATERIALS AND METHODS

Animal model

Wister strain albino rats (160-200 g) obtained from the Central Animal House, College of Medicine, University of Ilorin, were used for the study. The rats were housed in wire mesh cages under standard conditions (Temperature, 25-29°C, 12 h light and 12 h darkness cycles) and fed with standard rat pelleted diet and water. The study was generally conducted in accordance with recommendations from the declaration of Helsinki on guiding principles in the care and use of animals.

Drugs and reagents

Tablets of artesunate (Tuyil Pharmaceutical Industry, Nigeria) and G-6-PD kit (Randox Laboratory Ltd, Canada) were used for the study. The artesunate was dissolved in normal saline and administered orally.

Experimental design

Twelve (12) male rats were divided into two (2) groups of six (6) animals per group. Group 1 consists of rats which received normal saline and served as the control. Group 2 rats were treated with artesunate for five (5) days. The artesunate was administered orally at 2.90 mg/kg on the first day and 1.45 mg/kg on the subsequent four days. The dosage and duration of treatment were chosen to mimic those in humans.

Red blood cell count and G-6-PD estimation

The rats were anaesthetized using ether 24 h after the last administration of the treatment and blood samples were collected into sample bottles for red blood cell count and serum G-6-PD activity. RBC count was done using the improved Neubauer haemocytometer while the G-6-PD activity was estimated using the G-6-PD kit. The procedures for the assay as contained in the manufacturer's manual were strictly followed. Absorbance was read at 340 nm using a spectrophotometer. A standard curve was also plotted from which the activities of the test samples were obtained.

Table 1. RBC counts and G-6-PD activity in control and artesunate-treated rats.

Groups	RBC count (X10 ⁶ /mm ³)	G-6-PD (mU/ml)
Control	5.13 ± 0.32	0.52 ± 0.39
Artesunate	5.08 ± 0.13	13.28 ± 4.10

Statistical analysis

Data were expressed as mean ± SEM and analyzed using the student's t-test. P<0.05 was considered significant.

RESULTS AND DISCUSSION

The results are presented in Table 1 below. The results showed that artesunate had no significant change on the red blood cell count as the value of 5.23 ± 0.32 (x10⁶ mm³) observed in the control rats was similar to 5.08 ± 0.13 (x10⁶ mm³) recorded for the treated rats. However, there was a highly significant (p<0.001) increase in G-6-PD activity in the treated rats when compared with the control. The G-6-PD activity was 0.52 ± 0.39 mU/ml in the control rats while 13.28 ± 4.10 mu/ml was recorded in the treated rats. This represents about 2454% increase in the activity of G-6-PD.

The present study observed a very significant increase in the activity of G-6-PD without a significant reduction in the red blood cell count of the artesunate-treated rats. These findings are not consistent with observations made with chloroquine which had been reported to cause deficiency of G-6-PD and subsequent hemolysis due to the release and accumulation of excess reactive oxygen species (ROS) in cells which in turn lead to oxidative stress (Eitan et al., 2005).

It is noteworthy that the reactive oxygen present in the artemisinin molecule due to its internal peroxide group (Ames et al., 1985) established its linkage with oxidative stress. Thus, the highly significant increase in G-6-PD activity could be a response to oxidative stress resulting from the reactive oxygen species released from artesunate. Glucose-6-phosphate dehydrogenase has been suggested to be an antioxidant enzyme because it provides NADPH to maintain glutathione reductase. Increased G-6-PD activity may restore cellular glutathione reductase levels after its depletion by oxidative stress. Glucose-6-phosphate dehydrogenase also regulates the production of nitric oxide in endothelium and other types of cells (Leopold et al., 2003).

The increase in G-6-PD activity could therefore be construed as a feedback response to a depleted glutathione reductase level during rigorous antioxidant activity. In addition, artesunate might have altered the levels of NAD⁺ and NADPH because both are involved in the regulation of the activity of glucose-6-phosphate dehydrogenase (Luzzatto, 1967). Both NADH and gluta-

thione reductase are necessary for the reduction of hydrogenperoxide, therefore preventing red blood cell lysis. The overexpression of G-6-PD and subsequent protection of the red cells observed in this study is consistent with earlier report that G-6-PD overexpression decreases endothelial cell oxidant stress (Leopold et al., 2003). These findings have shown that the red blood cells are not susceptible to lysis when artesunate is given at a normal dose and duration. This implies that artesunate is better tolerated and safer than chloroquine.

The results suggest a critical role for glucose-6-phosphate dehydrogenase in the prevention of oxidative stress and lysis of red blood cells in artesunate-treated rats. Further studies on the activities of G-6-PD and glutathione reductase in artesunate-treated rats will be required.

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