Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60083-5

Effect of artemether on hematological parameters of healthy and uninfected adult Wistar rats

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ARTICLE INFO

Article history:
Received 10 October 2011
Received in revised form 25 November 2011
Accepted 18 December 2011
Available online 28 June 2012

Keywords:
Artemether
Anemia
Antimalaria
Hematology
Hematological profile
Malaria
Anemic tendency

ABSTRACT

Objective: To evaluate the effect of short term artemether administration on some blood parameters in adult male Wistar rats. **Methods:** Sixty five albino rats with body weight of 190-220 g were used for the four-phased study. The animals were randomly divided into five groups. The first-four groups of 15 rats were further divided into 3 subgroups of 5 rats. The drug was administered orally at sub-optimal, therapeutic, and high doses of 25, 50 and 75 mg/kg bw, respectively to the rats for 1 day, 2 days and 3 days. Blood samples were collected by cardio-puncture from the rats for hematology at the end of each phase. The last group served as control, and they were given water *ad libitum*. **Results:** Artemether caused significant reduction ($P<0.05$) of the hematological profile of the animals in a dose dependent manner. Discontinuation of the drug use however showed gradual recovery of the depressed indices of the blood parameters. **Conclusions:** The results suggest that artemether can induce reversible changes in hematological profiles of rats by extension man. This can probably aggravate anemia when artemether is administered to malaria patients. Hence, the study supports the use of the drug with caution especially in patients prone to anemic tendencies.

1. Introduction

Hematological disorders are ailments of major public health concern globally especially in developing countries including Nigeria, where diagnostic facilities are not readily available and accessible. Anemia has been identified as the commonest of them all. It also forms a common presenting feature of malaria and tends to worsen it[1-4].

Malaria is the world most important protozoan disease of which at least 300 million people are infected worldwide yearly, causing 1.0 to 1.5 million deaths per year. It is a major cause of morbidity and mortality in West Africa. Ninety percent of malaria related deaths occur in Sub-Saharan Africa[5,6]. The disease is endemic in sub-Saharan Africa and children below the age of five suffer the greatest burden of the disease[6,7]. Despite efforts to control the disease, malaria is among the top three deadly communicable diseases and the most deadly tropical disease. The disease also poses economic burden on developing countries, cutting across all socioeconomic groups but with greater effect on the poor[8].

Anti-malaria drugs are drugs that are used for prophylactic and therapeutic treatment of malaria. The recent adoption of artemisinin combined therapy as treatment of choice has proven to be a potent resort for uncomplicated malaria[6].

This therapy usually contains artemisinin or any of its derivatives, one of which is artemether. Artemether is a lipid soluble methylether of dihydro artemisinin with rapid schizonticidal activity against *Plamodium falciparium* parasites in blood and parasitemia clearance rate of 30-84 h[5].

Toxicity studies in dogs and rats revealed dose-dependent and potentially fatal neurotoxic effects after intramuscular injection of artemether at higher and multiple doses. These changes affect areas associated with vestibular, motor and auditory functions. Other toxic effects include embryotoxicity, gonadotoxicity, genotoxicity, immunotoxicity, cardiotoxicity, nephrotoxicity, and allergic reactions[9-11].

Therefore, this study was aimed to determine the potentially toxic effect of artemether on some blood parameters using albino rats.

2. Materials and methods

2.1. Animals

Sixty five albino rats of the Wistar strain (190-220 g)

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obtained from the Animal House of Department of Physiology, University of Ibadan, Nigeria were used for the study. All the animals were males to disallow hormonal influence or any other gender related physiological factors that can obliterate the pharmacological action of the drug. The animals were fed with standard rat cubes (Ladokun Feeds Nig. Ltd.) and water *ad libitum*. Five animals were housed per cage in the Animal House of Department of Physiology, Olabisi Onabanjo University, Ikenne at room temperature under photo-period controlled environment (12 h light: 12 h darkness cycles), where they were acclimatized for a period of seven days.

2.2. Experimental procedure

The study was a four-phased study involving 65 albino rats randomly divided into five groups. The first-four groups contained fifteen rats in each group. The fifth group contained 5 rats and served as control. Artemether (Rhone-Poulene Rorer International, France) was obtained from Olabisi Onabanjo University Health Center, Ago-Iwoye, Nigeria. The drug was administered orally at sub-optimal, therapeutic, and high doses of 25, 50 and 75 mg/kg bw, respectively to the rats for 1 day, 2 days and 3 days. Approval for the use of the rats was obtained from the departmental committee on the ethical use of animals.

Rats in group 1 (n=15) were divided into three subgroups of five rats each and treated with 25, 50 and 75 mg/kg bw of artemether, respectively for 1 day.

Rats in group 2 (n=15) were divided into three subgroups of five rats each and treated with 25, 50 and 75 mg/kg bw of artemether, respectively for 2 days.

Rats in group 3 (n=15) were divided into three subgroups of five rats each and treated with 25, 50 and 75 mg/kg bw of artemether, respectively for 3 days.

Rats in group 4 (n=15) were divided into three subgroups of five rats each and treated with 25, 50 and 75 mg/kg bw of artemether, respectively for 3 days and were allowed to recover from the drug effects for another 3 days.

Rats in group 5 (n=5) were given sterile water throughout the study, and served as the control in all phases.

2.3. Analytical procedure

The rats were weighed prior to treatment and at the end of each phase differential weight gains (if any) relative to basal values were obtained. The animals were anesthetized with 25% urethane administered at a dosage of 0.6 mL/100 g bw. Blood sample was collected *via* cardio-puncture for analyses. Hematology was done according to standard methods[12].

2.4. Statistical analysis

All calculations were done using the SPSS-V11 statistical software package for analysis of the data. The data were presented as means ± standard deviation (SD), and statistical analyses were carried out using the Student's t-test and ANOVA. Differences were considered to be of statistical significance at an error probability of less than 0.05 (P<0.05).

3. Results

There was no significant change in body weights of artemether treated rats when compared with the controls (data not shown). This trend was also observed in the recovery group.

There was significant reduction in all the hematological parameters measured after the administration of artemether at the various dosages (Table 1-4). Drug withdrawal resulted in gradual restoration of the hematological values.

Table 1
Blood parameters of experimental animals in 1 day treatment (mean±SD) (n=5).

Groups	Dosages (mg/kg bw)	PCV (%)	Hb (× 10 g/L)	WBC (10^9/L)	RBC (10^{12}/L)
Control	Sterile water	43.07±0.35	14.00±0.20	6.45±0.18	7.02±0.20
Sub-optimal	25	40.67±0.50*	13.03±0.25*	4.90±0.13*	6.54±0.22*
Therapeutic	50	39.33±0.39*	12.60±0.19*	4.50±0.15*	6.40±0.17*
High	75	38.66±0.44*	12.10±0.12*	3.20±0.12*	6.01±0.19*

PCV: Pack cell volume; Hb: Hemoglobin concentration; WBC: White blood cell count; RBC: Red blood cell count; *: P<0.05 as compared with the control.

Table 2
Blood parameters of experimental animals in 2 days treatment (mean±SD) (n=5).

Groups	Dosages (mg/kg bw)	PCV (%)	Hb (× 10 g/L)	WBC (10^9/L)	RBC (10^{12}/L)
Control	Sterile water	43.07±0.35	14.00±0.20	6.45±0.18	7.02±0.20
Sub-optimal	25	39.66±0.25*	11.60±0.45*	4.70±0.14*	6.40±0.14*
Therapeutic	50	36.33±0.22*	10.70±0.26*	3.50±0.12*	6.10±0.20*
High	75	33.66±0.48*	9.00±0.20*	2.80±0.19*	5.50±0.15*

PCV: Pack cell volume; Hb: Hemoglobin concentration; WBC: White blood cell count; RBC: Red blood cell count; *: P<0.05 as compared with the control.

Table 3
Blood parameters of experimental animals in 3 days treatment (mean±SD) (n=5).

Groups	Dosages (mg/kg bw)	PCV (%)	Hb (× 10 g/L)	WBC (10^9/L)	RBC (10^{12}/L)
Control	Sterile water	43.07±0.35	14.00±0.20	6.45±0.18	7.02±0.20
Sub-optimal	25	35.66±0.25*	11.00±0.45*	4.15±0.10*	6.30±0.12*
Therapeutic	50	33.33±0.22*	10.40±0.26*	3.17±0.12*	5.98±0.15*
High	75	30.00±0.24*	9.00±0.20*	2.50±0.19*	4.90±0.10*

PCV: Pack cell volume; Hb: Hemoglobin concentration; WBC: White blood cell count; RBC: Red blood cell count; *: P<0.05 as compared with the control.

Table 4
Blood parameters of experimental animals in 3 days treatment and 3 days recovery (mean±SD) (n=5).

Groups	Dosages (mg/kg bw)	PCV (%)	Hb (× 10 g/L)	WBC (10^9/L)	RBC (10^{12}/L)
Control	Sterile water	43.07±0.35	14.00±0.20	6.45±0.18	7.02±0.20
Sub-optimal	25	37.66±0.40*	11.50±0.10*	6.00±0.14*	6.37±0.12*
Therapeutic	50	35.66±0.25*	11.30±0.46*	4.93±0.19*	6.19±0.15*
High	75	31.33±0.20*	9.50±0.20*	4.12±0.10*	4.95±0.14*

PCV: Pack cell volume; Hb: Hemoglobin concentration; WBC: White blood cell count; RBC: Red blood cell count; *: P<0.05 as compared with the control.

4. Discussion

Our study made an attempt to investigate the effect of short term administration of different oral doses of artemether on red blood cell, white blood cell (WBC) and hemoglobin concentration of albino rats. We demonstrated that administration of artemether significantly (P<0.05) reduced these measured parameters. The results further show that this observed reduction is dose dependent. Similar results were documented by previous workers[13-15] on pathological effects on body organs caused by oral administration of artemether and some other antimalaria agents. Some studies however discovered a rise in WBC count[10,16]. This disparity may be attributed to dosage and route of administration of the drug. Higher than human therapeutic doses are needed to attain equivalent dose in animal models because they metabolize these drugs faster[17].

We have also demonstrated that the anemic and leucopenic effect of artemether is reversible as noticed in the recovery group. This concurs with previous work that depicts the effect of artemether wanes with time[11] upon recovery. The reversal is observed to be time and dose dependent and this may not be unconnected with clearance of the drug (by the liver's cytochrome p450 system) and subsequent excretion. Artemether is thought to be less toxic compared to other artemisinin derivatives[16,18].

The characteristic peroxide lactone structure in the artemisinins is indispensable for their anti-malarial activity. Splitting of this endoperoxide bridge by heme iron species results in the release of reactive oxygen species (ROS) that eventually cause parasite's death[18,19]. Although, this process takes place within plasmodium-infected erythrocytes, artemisinins distributed in other parts of the body could also be oxidized to generate ROS that will induce oxidative stress and cause toxicity as observed in this study.

In addition to attacking the causative organism of malaria, *Plasmodium falciparum*, the use of artemether may potentiate hematological abnormalities such as anemia. Caution should therefore be observed in its use in people with anemic tendencies.

Conflict of interest statement

We declare that we have no conflict of interest.

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