Bull Environ Contam Toxicol (2012) 88:108–111
DOI 10.1007/s00128-011-0460-3

Coartemether Induced Oxidative and Hepatic Damage in *Plasmodium berghei* Strain Anka Infected Mice

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Received: 25 July 2011 / Accepted: 28 October 2011 / Published online: 6 November 2011

Abstract This study investigated the effect of coartemether on antioxidant and hepatotoxic biomarkers in *Plasmodium berghei* infected mice. Erythrocyte, hepatic and renal superoxide dismutase (2.71 ± 0.51; 1.96 ± 0.87; 2.84 ± 0.22 Units/mg protein respectively) and catalase (4.10 ± 0.10; 8.25 ± 1.24; 6.28 ± 0.11 Units/mg protein respectively) activities were significantly ($p < 0.05$) elevated in "parasitized and treated" (PnT) animals. Renal glutathione level (19.02 ± 0.20 µg/mL) was elevated in PnT animals. Glutathione *S*-transferase and malondialdehyde levels in hepatic (8.76 ± 0.49 µmol/min/mg; 527.23 ± 24.56 mmol/dL) and renal (3.35 ± 0.30 µmol/min/mg; 464.42 ± 59.13 mmol/dL) tissues were significantly high ($p < 0.05$) in coartemether-treated animals alone. Plasma aspartate transferase (9.45 ± 3.59 U/L) and alanine transferase (5.78 ± 2.36 U/L) were high in PnT animals. Therefore, data indicates that in the presence of *P. berghei*, coartemether could alter the antioxidant status and induce hepatotoxic damage in mice.

Keywords Antioxidant · Tissues · Mice · *Plasmodium berghei*

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Malaria is a leading cause of mortality and morbidity in developing regions of the world, and remains a major public health problem in endemic regions (Breman et al. 2004). The treatment of malaria has posed great challenge to medicine and development of effective antimalarial agents. This has led World Health Organization to adopt the combination of artemisinin derivative with another effective antimalarial drug that has a complementary mechanism of action and pharmacologic profile to overcome the emergence of drug resistance (Wongsrichanalai et al. 2002). Coartemether (COARTEM®; artemether-lumefantrine; also marketed as Riamet®), is a combination of 20 mg artemether (an artemisinin derivative) and 120 mg lumefantrine (a racemic mixture of a synthetic fluorine derivative formerly known as benflumetol). Coartemether acts as a blood schizonticide and its components have dissimilar modes of action providing synergistic activity against *Plasmodium falciparum* (Cousin et al. 2008). Upon administration of coartemether, artemether is presumed to have a rapid onset of action and rapid elimination, whereas lumefantrine has a slower onset of action and is eliminated more slowly and is expected to provide long-term cure rate after a short treatment course. Lumefantrine is thought to interfere with the polymerization process, while artemisinins generates reactive metabolites-dihydroxyartemisinin (DHA) through its conversion by CYP3A4/5 mostly at the liver which could damage both the host cells as well as the parasites (Anyasor et al. 2009; Mwesigwa et al. 2010). *Plasmodium*, the etiologic agent of malaria, is a unicellular facultative intracellular parasite of the phylum *Apicomplexa*. A hallmark of the malaria parasite is its remarkable capacity to specifically invade and replicate inside the red blood cells (RBC). *Plasmodium* infected erythrocytes are under increased oxidative stress (Becker et al. 2004). This intraerythrocytic proliferation

phase ultimately leads to the disease known as malaria. Due to the high metabolic rates of the rapidly growing and multiplying parasite, large quantities of toxic redox-active by-products are generated. Additional reactive oxygen and nitrogen species are generated by immune effector cells of the host in response to parasite infection and during hemoglobin degradation in food vacuole of the parasite. *Plasmodium* employs multiple biochemical pathways that mediate antioxidant defense and redox-regulation and play a central role in pathogenesis. In most eukaryotic organisms, redox-active enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidases as well as an enzymatic cascade that generates reduced electron donor including glutathione (GSH) and thioredoxin (Trx), sustain the cellular redox homeostasis (Rahlfs and Becker 2006). In this present study, attempt was made to evaluate some of the antioxidant and hepatotoxic biomarkers in *P. berghei* infected mice administered with a fixed dose of coartemether.

Materials and Methods

Twenty male Swiss albino mice weighing between 19 and 24 g were obtained from Nigerian Institute of Medical Research (NIMR) Yaba, Lagos. The animals in cages were acclimatized for 2 weeks in accordance to good laboratory animal care practice at departmental animal house. Tap water and commercial pelleted feed were provided under standard conditions of temperature 28 ± 2°C and 12-h dark/light cycle. *P. berghei* (Anka strain) obtained from NIMR was maintained by blood passaging in mice and a dose of 10^5 parasitized RBC was inoculated intraperitoneally (IP) following the method of Makinde et al. (1993). The parasite density was assessed using giemsa-stained blood smear preparation from infected animals 6–10 days after inoculation. Infected RBC were counted 5 times using the haemocytometer. Oral coartemether tablets (Norvatis, Pharma AG, Switzerland) were dissolved in sunflower oil as vehicle and administered orally to the test groups using an oral dosing needle for 3 days in accordance to WHO recommendation for treatment of uncomplicated malaria. Animals were assigned randomly into four groups of five rats each. Group I: (control) normal rats were given oral sunflower oil (1 cm^3), group II rats were pre-inoculated IP with *P. berghei* parasite, group III normal rats were administered with oral coartemether (28 mg/kg body weight) while group IV rats were pre-inoculated with *P. berghei* parasite and given oral dose coartemether (28 mg/kg body weight). The animals were euthanized 24 h after fasting overnight by cervical dislocation at the end of treatment period. Blood samples were collected with 5 mL hypodermal syringes from animals through cardiac puncture into EDTA bottles. Whole blood was centrifuged immediately at 3,000*g* for 10 min to obtain plasma for liver function assays. The blood cells were washed three times in cold saline and RBCs' were then suspended in equal volume of 0.9% saline and used for the estimation of antioxidant activity. Liver and kidney organs excised from rats were blotted of blood stain, encapsulated and perfuse with 11.5% potassium chloride, the organs were homogenized in a phosphate buffer (pH 7.0) and the homogenate were centrifuged at 10,000*g* for 20 min to obtain post-mitochondrial fraction used for the antioxidant assay. Protein content was determined by biuret method using bovine serum albumin (BSA) as standard (Gornall et al. 1949). Erythrocyte and tissue SOD activity were determined by the method of Mistra and Fridovich (1972). Erythrocyte and tissue catalase activity were determined according to the method of Sinha (1972). Glutathione *S*-transferase (GST) activity was estimated by the method of Habig et al. (1974). Reduced GSH was measured by the method of Sedlak and Lindsay (1968) and Jollow et al. (1974). Lipid peroxidation was estimated using the method of Stocks and Dormandy (1971). Plasma assays for liver alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined spectrophotometrically with RANDOX (USA) diagnostic kits with 2,4-dinitrophenylhydrazine as substrate. Results were analysed with the aid of SPSS for windows, SPSS Inc., Chicago, Standard version 17.0 to determine difference between mean using Analysis of Variance (ANOVA). Data were reported as mean ± standard deviation.

Results and Discussion

Figures 1 and 2 showed that the SOD and CAT activities were significantly high ($p < 0.05$) in the parasitized and treated (PnT) animals compared to other treatment groups. This is in consonance with other reports that *Plasmodium* parasites and bioactivated DHA increased free radical and reactive oxygen species (ROS) generations in the body. Consequently, this would trigger the elevation of endogenous antioxidant defense mechanism including SOD and CAT to scavenge ROS (Pham-Huy et al. 2008). Catalase is an antioxidant enzyme that converts the H_2O_2 generated from SOD activity to water and elemental oxygen thereby mopping up the peroxide (Iyawe and Onigbinde 2009). Hepatic GSH levels in parasitized and PnT animals were significantly reduced ($p < 0.05$) while it was elevated in coartemether treated group compared to other control (Table 1). Kidney GSH level was significantly elevated in PnT group compared to other treatment groups. Reduced GSH, a non-enzymatic antioxidant serves as sulfhydryl buffer which protects the –SH groups of protein from the

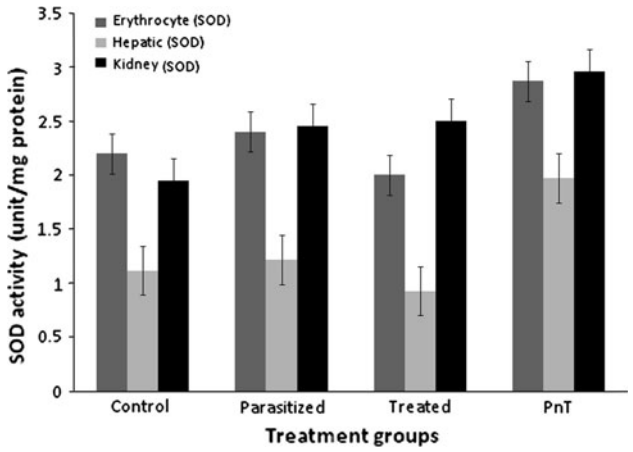

Fig. 1 Erythrocyte, hepatic and kidney superoxide dismutase (SOD) activity in parasitized, treated, "parasitized and treated" (PnT) and control mice

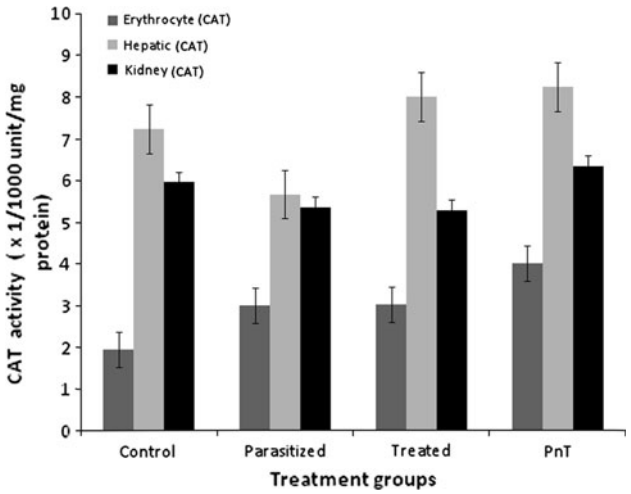

Fig. 2 Erythrocyte, hepatic and kidney catalase (CAT) activity in parasitized, treated, "parasitized and treated" (PnT) and control mice

Table 1 Liver and kidney GSH, GST, MDA and plasma AST and ALT levels in parasitized, treated, "parasitized and treated" (PnT) and control mice

	Control	*P. berghei*	Treated (25 mg/kg coartemether)	Parasitized and treated (25 mg/kg coartemether)
Liver				
Glutathione level (μg/mL)	18.75 ± 0.50	17.50 ± 0.50*	19.02 ± 0.03*	18.50 ± 0.61*
Glutathione *S*-transferase activity (μmol/min/mg)	5.79 ± 0.91	4.07 ± 0.70*	8.76 ± 0.49*	5.47 ± 0.18$^{\#}$
Malondialdehyde (nmol/dL)	438.66 ± 27.83	211.22 ± 10.46*	527.23 ± 24.56*	301.92 ± 52.10*
Kidney				
Glutathione level (μg/mL)	18.01 ± 0.10	18.05 ± 0.02	18.00 ± 0.50	19.02 ± 0.20*
Glutathione *S*-transferase activity (μmol/min/mg)	0.64 ± 0.14	2.17 ± 0.55*	3.35 ± 0.30*	1.60 ± 0.41*
Malondialdehyde (nmol/dL)	392.70 ± 72.22	205.77 ± 56.11*	464.42 ± 59.13*	259.61 ± 76.46*
Plasma				
AST (U/L)	4.25 ± 1.55	11.80 ± 4.80*	4.65 ± 0.58*	9.45 ± 3.59*
ALT (U/L)	2.38 ± 0.76	8.52 ± 1.41*	2.39 ± 1.01*	5.78 ± 2.36*

* Indicate significantly different from control at $p < 0.05$, $^{\#}$ indicate significantly different from *P. berghei* and treated at $p < 0.05$

damaging effects of ROS and depletion of hepatic GSH has been associated to predispose liver cells and other tissues to chemical or drug toxicity (Kidd 1997). The increased renal GSH may be related to the de novo GSH synthesis or GSH regeneration (Hassan et al. 2010). GST catalyzes the initial reaction involving the conjugation of xenobiotics having electrophilic constituent with GSH thereby removing reactive electrophiles and hence protecting vital nucleophilic groups in macromolecules such as proteins and nucleic acids. GST activity in hepatic and renal tissues was elevated in the coartemether treated group compared to other treatment groups (Table 1). The increase activity of GST in non-parasitized coartemether-treated animals may have resulted from the response of GST to detoxify the coartemether drug. However, there have also been claims that *Plasmodium* parasites withdraw the host antioxidants to protect itself from the deleterious effect of hemozoin and deplete the host antioxidants (Hassan et al. 2010). This may have accounted for the observed response of GST in both the parasitized and PnT animals. Malondialdehyde levels were high in the liver and kidney of the non-parasitized coartemether-treated group (Table 1). Increased MDA indicates that coartemether increased production of ROS in these tissues. Malondialdehyde which is a by-product of lipid peroxidation is one of the major outcomes of free radical-mediated injury to tissue. Peroxidation of fatty acyl groups occurs mostly in membrane lipid bilayers, resulting in severe cellular dysfunction (Andy et al. 2004).

In this study, the hepatotoxic effect of coartemether and *P. berghei* was examined based on inducibility of hepatic function biomarkers. AST and ALT are two of the numerous enzymes that catalyses the initial step in amino acid degradation called transamination reaction. These hepatic enzymes are originally present in higher concentrations in the cytoplasm. The presence of hepatopathy causes these enzymes leak into the blood stream in conformity with the extent of hepatic damage. Table 1 showed that AST and ALT levels were high in the parasitized and PnT animals suggesting a possible damage to the liver (Iyawe and Onigbinde 2009). In conclusion, this study implicates the dual action of malaria parasite and coartemether in altering antioxidant status in vivo and possibly inducing hepatic damage.

Acknowledgments We express gratitude to Babcock University Administration for their support. We are grateful to the Head of Department of Chemical and Environmental Sciences, Babcock University Prof. E. B. Esan for constant encouragement, support and critique of the manuscript. Our appreciation goes to Anyasor Chiamaka O. for typesetting and editing of the manuscript. We declare no conflict of interest and are solely responsible for the writing and content of this work.

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