Evaluation of Selected Biochemical Parameters in Renal and Hepatic Functions Following Oral Administration of Artesunate to Albino Rats

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Abstract: The effect of varying doses of artesunate on function indices of rat kidney and liver was investigated. Twenty white albino rats were randomly divided into 4 groups of 5 rats each; groups 1-3 were orally administered with 2.0, 3.0, and 5.0 mg/kg body weight artesunate respectively, while the fourth group serves as control (distilled water). Result indicated an increased serum creatinine levels in artesunate treated animals compared to untreated control. Urea levels in serum of artesunate treated groups were significantly ($P<0.05$) reduced compared to control in a concentration dependent manner. Sodium, potassium, chloride and bicarbonate levels in artesunate treated groups were significantly elevated ($P<0.05$) compared to control animals in a concentration dependent manner. Furthermore, liver function test indicated no significant difference ($P>0.05$) in the levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in artesunate treated groups compared with untreated control while L-γ glutamyl transaminase (GGT) activity was significantly ($P<0.05$) elevated in all treated groups compared to control. Therefore, result indicated alterations in biochemical parameters investigated with a more pronounced effect on kidney function than liver function tests. This suggests artesunate administration may adversely affect the functional capacities of the kidney and liver.
[Anyasor, God'swill Nduka, Olorunsogo, Olubode Olufunso. Evaluation of Selected Biochemical Parameters in Renal and Hepatic Functions Following Oral Administration of Artesunate to Albino Rats. Researcher. 2011;3(7):30-34]. (ISSN: 1553-9865). http://www.sciencepub.net.

Key words: artesunate, electrolytes, liver, kidney, reactive oxygen species

1.0 Introduction

Artesunate (ART), a dihydroartemisinin -10-α hemisuccinate is a water soluble semi-synthetic derivative of artemisinin with a molecular weight of 384.4 (Abdin et al., 2003; Efferth et al., 2007). Artesunate is the most widely available form of the artemisinin-related compounds; it may be administered parenterally, intravenously, intramuscularly, orally, or rectally. Oral artesunate is used either alone or in combination with other antimalarials (Nosten et al., 1994).

Artesunate and artemisinin derivatives have been reported to be effective against both drug-resistant and cerebral malaria-causing strains of Plasmodium falciparum (Abdin et al., 2003). It was recently demonstrated that ART, apart from its anti-malarial activity, inhibits the growth of leukemic cells and acts in an antiviral manner (Chen et al., 2003; Efferth et al., 2004, 2007).

The mode of action of ART in cancer cells is similar to the cytotoxicity against malaria Plasmodia and it becomes more apparent in the presence of free iron. Iron (II) ion (Fe^{2+}) catalyzes the opening of an endoperoxide bridge in artemisinins, leading to the formation of reactive free radicals, which causes extensive damage to either parasites or cancer cells (Kamchonwongpaisan and Meshnick, 1996; Efferth and Kaina, 2010).

It has been reported that artesunate is toxic to malaria parasites at nanomolar concentration, whereas micro molar concentration is required for toxicity in mammalian cells (Meshnick, 2002). Recently, there have been claims that artesunate have little or no side effect and the most reported defects have been dizziness, nausea, vomiting and anorexia (Price et al., 1996; Ribeiro et al., 1998;Taylor and White, 2004;). Evidences showing the neurotoxicity of artesunate at high doses in laboratory animals (Brewer et al., 1994; Nontprasert et al., 1998, 2000) including the cytotoxicity of artesunate on tumor cell lines have been reported (Efferth et al., 2007). Therefore, this study attempt to investigate the effect of orally administered artesunate on some biochemical parameters of kidney and liver due of their roles in detoxification and eventual elimination of drugs from the host.

2.0 Materials and Methods

2.1 Animals and assay kits

Twenty male white albino rats (Wister Strain) weighing between 200 - 250 g were obtained from the Preclinical Animal House, Physiology

Department, University of Ibadan, Ibadan, Nigeria. Animals were maintained under good laboratory practice. Sodium artesunate powder was obtained from Institute of Medical Research and Training (IMRAT) College of Medicine, University of Ibadan, Ibadan, Nigeria as a gift. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and L-γ glutamyl aminotransferase (GGT) kits were supplied by RANDOX laboratories, Ltd., United Kingdom. All other reagents used were of analytical grade and were prepared using all glass-distilled water.

2.2 Animal grouping and drug administration

The animals which had been acclimatized for 14 days and allowed access to water and rat chow ad libitum were randomly distributed into four groups of five rats each. Group 1: rats serve as control group and received 1 ml sterile distilled water. Group 2 – 3 rats were administered orally with artesunate doses of 2.0, 3.0, and 5.0 mg/kg body weight respectively for 7 consecutive days in accordance to the artesunate regime for severe malaria therapy (Utzinger et al., 2005).

2.3 Plasma preparation

Experimental rats were subjected to cervical dislocation and blood samples were drawn using 5 ml hypodermal syringe by cardiac puncture into clean, dry centrifuge tube, allowed to stand for 30 min to clot and further centrifuged at 3000 rpm for 10 min (Ogbu and Okechukwu, 2001), serum was separated from clot with Pasteur pipette into sterile sample tube, kept frozen and used for renal and liver function tests within 12 h of collection.

2.4 Determination of biochemical parameters

Serum urea concentration was determined by the methods of diacetyl monoxime method of Natelson (1957). The serum samples were heated with diacetyl monoxime, a colored compound formed was measured colorimetrically at 500 nm. Serum creatinine was estimated by using the method of Broad and Sirota (1948) modified. Serum sodium and potassium ions concentrations were evaluated with the aid of flame photometer as described by Bassir (1971). Serum bicarbonate concentration was determined using titrimetric method while serum chloride level was determined using mercuric nitrate method (Schales and Schales, 1941)

2.5 Statistical analysis

This was done with the aid of SPSS for windows; SPSS Inc., Chicago, Standard version 14.0 to determine difference between mean using One Way Analysis of Variance (ANOVA). Data were reported as mean ± standard deviation.

3.0 Results

Tables 1 and 2 depict the effect produced on some selected functional indices of rat kidney and liver respectively following repeated administration of sodium artesunate. Administration of artesunate resulted in a significant elevation of creatinine in all artesunate treated animals compared to untreated control animals ($P<0.05$). Urea levels in plasma of artesunate treated groups were found to be significantly ($P<0.05$) reduced compared to untreated control group in a concentration dependent manner. Sodium, potassium, chloride and bicarbonate levels in artesunate treated groups were found to be significantly elevated ($P<0.05$) compared to untreated control group in a concentration dependent manner (Table 1).

The liver function test indicated significant reduction ($P<0.05$) in the activities of ALT, AST and ALP in artesunate treated animals compared to untreated control animals while GGT activity was significantly ($P<0.05$) high in all treated groups compared with untreated control group (Table 2).

Table 1. Effect of various doses of artesunate on kidney function

Groups	Creatinine (mg/dl)	Urea (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Control	0.20±0.20	42.75±0.10	124.50± 1.90	3.9±0.10	102±0.80	22±1.40
2.0 mg/kg/day	0.21 ± 0.00	42.0 ± 0.00	132.0 ±5.60	6.0 ±2.60	102.0 ±5.20	22.8 ±0.50
3.0 mg/kg/day	0.35 ±0.06	40.25±0.96	125.75±9.50	7.88±0.44	103.5±5.45	20.25±0.50
5.0 mg/kg/day	0.30±0.00	36.5±1.91	142.25±12.18	7.55±1.57	114.25±0.95	21.25±0.96

Table 2. Effect of various doses of artesunate on liver function

GROUPS	ALT (IU/L)	AST (IU/L)	ALP(IU/L)	GGT(IU/L)
Control (normal saline)	0.79±0.11	1.04±0.01	3.45±0.53	0.01±0.03
2.0 mg/kg/day	0.70±0.05	0.90±0.22	2.055±.95	0.02±0.01
3.0 mg/kg/day	0.66±0.50	0.91±0.05	1.38±14.75	0.03±0.00
5.0 mg/kg/day	0.64±0.45	0.84±0.86	0.12±4.55	0.05±0.00

4.0 Discussion

Artesunate, an antimalarial drug was introduced by world health organization (WHO) to combat the problem of multidrug-resistant P. falciparum malaria (Efferth, 2001; Philip, 2008; Efferth and Kaina, 2010).

In this study, effects of orally administered artesunate on indices of kidney and liver function tests were investigated. Kidney function tests are required either to demonstrate the presence or absence of active lesion in kidney, or to assess the normal functioning capacity of nephron (Yakubu et al., 2006). Inorganic electrolytes occur in large quantities in cellular fluids and can dissociate readily into their constituent ions or radicals in the extracellular and intracellular compartments (Zilva, 1991). The increased serum Na^+ concentration in artesunate treated animals is an indication of alteration important biochemical parameters, such as an increase production of aldosterone and other mineral corticoids which will in turn increase the tubular reabsorption of Na^+ or decrease production of either antidiuretic hormone or decreased tubular sensitivity to the hormone (Tietz et al., 1994). Hypernatraemia could in a way serve as an indicator of liver disease (Ziva, 1991).

Potassium ion plays an important role during transmission of nerve impulses along the nerve cells to receptor cells. Sodium pump maintains the intracellular K^+ concentration of 5 mM (Horton et al., 1993). The hyperkalaemia observed in artesunate treated animals suggests a possible adverse effect on the pump that maintains the homeostasis of Na^+ in extracellular concentration. Hyperkalaemia is a more dangerous condition because of its effect on the heart, but rarely occurs unless renal function is depressed completely.

The presence of reduced serum urea in ART treated animals occurred in a dose dependent manner. Urea is the major nitrogen containing metabolic product of protein degradation. The significant reduction in plasma urea concentration as the dose increases may be attributed to liver damage (Philips, 1994) or impairment of urea cycle leading to reduced production of the metabolic product. This reduction in plasma urea could also indicated reduction in serum aminotransferases (ALT and AST) in artesunate treated animals. Biosynthesis of urea involves transamination of amino acids, oxidative deamination of glutamate and reactions of the urea cycle stages, it seems logical that artesunate may be affecting the transamination stage of the urea cycle. This is an abnormality in the physiological excretion of urea caused by a non renal factor (Segasothy et al., 1994). Previous works reported that artesunate undergoes bioactivation in the liver to artenimol, the active antimalarial agent (Cumming et al., 1996), this results in generation of reactive oxygen species or free radicals (Li et al., 2005).These radicals could damage enzymes and tissues such as the kidney and liver (Nontprasert et al., 2000; Anyasor et al., 2009). Consequently, since urea synthesis convert toxic ammonia to non toxic urea, defect in urea synthesis as observed in this study may result in ammonia intoxication.

The reduction in creatinine, another product of protein metabolism in the treated animals may indicate compromised renal function. Artesunate might have interfered with the metabolism of creatinine leading to the observed reduction, an indication of partial loss in functional tubular excretion (Musa et al., 2003).

High serum chloride ion and bicarbonate ion levels indicated artesunate might have caused an imbalance in the concentration of these ions in treated animals. Chloride (Cl-) ion is the most abundant anion in the extracellular fluid. Chloride ion concentration in the plasma generally follows those of Na+. However, fluctuations in serum or plasma Cl- and bicarbonate (HCO3-) may have little consequence but are often signs of an underlying disturbance in cellular fluid and acid–base homeostasis (Philips, 1994).

In this study, the hepatotoxic effect of artesunate was subsequently investigated based on the examination of the inducibility of enzymes ALT, AST, ALP and GGT. The activities of ALT, AST and ALP in all treated animals were slightly reduced (non significant, $P>0.05$) compared to control while the activity of GGT was significantly elevated compared

to those of untreated control animals. Similar result has been reported by China Cooperative Research Group (2003). Although, the activities of these serum liver function enzymes may appear not affected, there could be some degree of damage to liver tissue as indicated by elevated GGT activity in treated animals. Artesunate have been previously reported to damage mitochondria membrane of tumor cells and normal liver cells through reactive oxygen species (ROS) mediated apoptosis (Efferth et al., 2007, Anyasor et al., 2009; Efferth and Kaina, 2010) and could as well induce cellular, lipid and protein damage (Cheng et al., 2003; Ravindra et al., 2004). Reduced serum ALP could also serve as an index for severe ascorbate deficiency (Philips, 1994). Ascorbate deficiency may also exercebate artenimol free radical attack resulting in tissue damage (Parthikrit et al., 2008). Furthermore, increase in GGT activity might further suggest other hepatic damage, particularly an obstruction of bile ducts; however, it could indicate an induction of enzyme synthesis in response to artesunate treatment (Philip, 1994; EMEA, 2008).

Results of the present investigation have shown that artesunate is capable of producing alteration in some biochemical parameters. The alteration seem to be more pronounced with the kidney function parameters probably because the oral artesunate usually, undergoes a biotransformation in the liver cells to a free radical artenimol that might subsequently unleash damage to cellular macromolecules such as enzymes, membranes, and genetic materials. These results suggest that the use of antimalarial such as artesunate should be monitored especially in patient with history of renal and hepatic dysfunctions.

Acknowledgement: With sincere gratitude, we thank Babcock University Administration for the financial support. We also thank the Head of Department Chemical and Environmental Sciences, School of Science and Technology, Babcock University Prof. Edward B. Esan for providing material and technical support. Our appreciation goes to Anyasor C.O. for the manuscript typesetting and editing. The authors declare no competing interest

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Submission date: 13/6/2011