Acute Hepatotocixity Following Administration Of Artesunate In Guinea Pigs

H Nwanjo, G Oze

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Abstract

Artesunate is an antimalarial drug commonly employed in chloroquine resistant cases of Plasmodium falciparum infection. The hepatotoxic potentials of artesunate was studied in guinea – pigs; 0, 2.0, 4.0, 8.0 and 16.0mg/kg were given orally to 4 groups (n = 5) for 7 days. The mean values in body weight of the guinea pigs before and after the drug administration and the serum aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin (TB) and conjugated bilirubin (CB) levels were determined. There was no significant difference (P>0.05) in weight loss or gain of the animals in all dose level at the end of the experiment. The results also showed that there were significant increases of AST only at highest dose (16mg/kg): 21.71 ± 6.9 control(c), 30.81 ± 5.56 text(t) IU/L (P<0.05); ALT: 14.81 ± 3.41 (c), 21.85 ± 1.20(t) IU/L (P<0.05); ALP: 50.101 ± 13.03(c), 85.60 ± 38.20(t) (P<0.05) and CB: 0.30 ± 0.03(c), 0.50 ± 0.0.16(t) mg/dl (P<0.05); but no significant increase of TB: 0.61 ± 0.16(c), 0.71 ± 0.28(t) mg/dl (P>0.05). The outcome suggests compromise of liver function and possible damage to the hepatocytes at the highest dose level tested.

INTRODUCTION

Artesunate is a semi-synthetic drug used to treat malaria, especially chloroqine resistant malaria in Nigeria. Malaria is and continues to be a major health problem in some parts of the world especially in the tropics ([1]). It is a particular blight on progress in tropical or sub-tropical regions. It affects mainly areas where the average temperature is 16 $^{\circ}$ C or higher ([2]). In sub-Sahara Africa, over a million persons die annually from the disease ([1]). This infection is caused by various single celled protozoans in the genus plasmodium and is transmitted by the female anopheles mosquitoes. The plasmodium species that infect humans are Plasmodium falciparum, Plasmodium malaria, Plasmodium vivax and Plasmodium ovale. Characteristically the infection causes recurring attacks of severe chills, high fever and sweating.

Delay in treating this infection may result in rapid deterioration in the patients' conditions, together with the development of a number of life threatening complications. Consequently, chemotherapy is initiated using antimalarial drugs. Drug resistance by the causative strains of plasmodium has made the treatment of malaria expensive because money is wasted in treating cases of recrudescence and relapse. Chloroquine and quinine have been generally used as antimalarials ([3]). They are active against erythrocytic forms of the parasites, sporozoites and the exo-erythrocytic forms. However, the chloroquine resistant strains of the malaria parasites are much more prevalent mainly in the tropics. WHO reported that chloroquine resistance is now virtually global and recommended the treatment of patients with severe malaria using quinine or where appropriate an artemisinin derivative ([4]).

Artemisinins are derived from leaves of a plant called sweet wormwood or sweet Annie (Artemisia annua) by Chinese scientists. In china, where they were discovered, “qinqhao” extracts were reported to have antipyretic properties more than 1500 years ago. In 1967 an outstanding coordinated programme was started by the Chinese government to discover antimalarial principles in various medicinal herbs including qinghao. In 1971, a highly active chemical from qinghao known as qinghaosu was obtained and is now called artemisinin ([5]). Since this initial discovery, an array of semi-synthetic oil and water soluble derivatives of artemisinin have been developed with variety of formulations entering clinical studies.

Artemisinin derivates are semi-synthetic compounds derived from a biologically active chemical known as Quighaosu or Artemisinin. These compounds have impressive parasiticidal properties in vivo and in vitro ([6]). They rapidly arrest parasite metabolism and kill parasites more quickly than

other antimalarial drugs ([7]). Artemisinin, which is the parent compound, is the antimalarial principle of these compounds and is derived from the leaves of a plant called sweet wormwood (Artemisia annua). Artemisinin was isolated by the Chinese scientists from Artemisia annua leaves ([8]). This antimalaria principle is highly crystalline and does not dissolve in polar or non-polar solvents, hence it is modified chemically to yield these derivatives: artesunate, artemether, arteether, artelinic acid and dihydroartemisinin. Artesunate is a water-soluble artemisinin derivative. It is the most widely used member of the artemisinin derivative ([9]). Artesunate is effective against Plasmodium falciparum resistant to other operationally used antimalarial drugs ([10]).

Serious concern has been raised about uncontrolled use of these drugs because at the moment they are the last resort in the combat against multi-drug resistant P. falciparum malaria ([7]). The use of these drugs should be controlled and restricted to proven multi-drug resistance on severe malaria in order to preserve their efficacy ([11]) and avoid emergence of resistant strains. In malaria endemic areas such as Nigeria, self medication is quite common and purchase of antimalarials in the open market is rampant. The possibility of administering overdose and misappropriation in the usage of antimalarials are very common. Drugs though useful in the treatment of disease conditions could also produce untoward effects in the individual. The untoward or toxic effect may be harmful to the patient ([12]). Studies on brain stem showed preclinical evidence of brain stem toxicity in animals ([13]). Fetotoxicity studies based on animals are going on. However, not much investigations or information have been documented on the adverse effect of artesunate on the liver since it is the organ of metabolism of drugs and other substances. Therefore, the present study was aimed at investigating the possible toxic potentials of the drug on the liver using the hepatospecific enzymes marker.

MATERIALS AND METHODS

ANIMALS

The experimental animals used were guinea pigs (Cavia perellus). Thirty, guinea pigs obtained from Michael Okpara College of Agriculture Umuagwo, Nigeria and moved to the Animal House of the College of Medicine and Health Sciences, Imo State University, Owerri, Nigeria. They were housed in ambient temperature under 12 hours light-dark cycle. They were fed with vital growers feed produced by Grand Cereals and Oil Mills Ltd {GCOML} Bukuru, Jos, Nigeria. Water was given ad libitum. They were allowed to acclimatize for one week.

DRUGS

The artesunate tablets (Adamsnate) were manufactured by Adams Pharmaceutical Group Co {Anhui} Ltd, China. The drug solution was made with distilled water (2mg/ml) and administered to the animals by oral compulsion for a period of seven days.

EXPERIMENTAL DESIGNS

The experimental animals were divided into five groups of six guinea pigs each. Groups I to IV were the treatment Groups while Group O was the control group. The drugs were administered to the groups as follows: Group O: Distilled water, Group I: 2mg/kg, Group II: 4mg/kg, Group III: 8mg/kg, Group IV: 16mg/kg. The animals were sacrificed 24 hours after the last dose on the 7 th day. Five milliliters of blood sample was collected from the common carotid artery. The sample was allowed to clot and centrifuged at 1000 rpm for 5mins using Uniscope centrifuge and serum separated for analysis.

ANALYTICAL METHODS

Estimation of aspartate aminotransdferase {AST} activities and alanine aminotransferase {ALT} activities were done using Reitman-Frankel method ([14]). Estimation of alkaline phosphatase activities using King and King method ([15]). Bilirubin (Total and conjugated} were determined using Malloy and Evelyn method ([16]).

RESULTS

Orally administered artesunate at the different doses did not cause mortality in the animals. Table 1 shows the mean values in body weight of the guinea pigs before and after the drug administration. It also showed the mean differences in weight at the different doses at the end of the experiment. There was no significant difference (P>0.05) in weight loss or gain of the animals in all dose level at the end of the experiment. The mean differences of the treated groups when compared with the control were also not statistically significant (P>0.05).

Figure 1

Table 1: The Mean values of the Body Weight of Guinea Pigs before and after 7 days of Artisunate Administration.

	Doses (mg/kg) (n =6)				
Weight (g)	0.0	2.0	4.0	8.0	16.0
Initial	520.00±108.44	371.67±93.68	423.33±94.80	416.67±94.59	463.30±78.08
Final	525.00±101.73	374.33±87.04	428.33±92.88	421.00±102.56	468.33±80.42
Diff ± S.D.	5.00 ±29.50	3.33±19.66	5.00 ±14.14	4.33 ±17.51	5.03 ±10.49

There were transient weight changes.

Table 2 shows the mean serum concentration of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphates (ALP), total bilirubin (TB) and conjugated bilirubin (CB) of the control and treated groups. The AST activities increased from 21.71± 5.18 (IU/L) in the control group to 23.14 ± 11.90 (IU/L) in group I; 22.30 ± 6.90 (IU/L) in group II; 23.56 ± 9.90 (IU/L) in group III and 30.81 ± 5.56 (IU/L) in group IV. Statistically, there was only significant increase (P<0.05) of AST in group IV over the control.

The ALT activities increased from 14.81± 3.41 (IU/L) in the control group to 16.80 ± 3.73 (IU/L) in group I; 16.26 ± 6.05 (IU/L) in group II; 15.55 ± 4.88 (IU/L) in group III and 21.85 ± 1.20 (IU/L) in group IV. The ALT increase was statistically significant (P<0.05) of AST in group IV over the control.

The ALP activities increased from 50.01 ± 13.03 (IU/L) in the control group to 64.71 ± 60.00 (IU/L) in group I; 67.41 ± 25.32 (IU/L) in group II; 66.37 ± 44.65 (IU/L) in group III and 85.60 ± 38.10 (IU/L) in group IV. It was observed that ALP showed statistically significant increase (p<0.05) over the control in group IV (16mg/kg) while in other groups (I, II and III) the increases were merely numerical with no statistical difference (P>0.05).

The total bilirubin (TB) level also increased from 0.61 ± 0.16 (mg/dl) in the control group to 0.67 ± 0.26 (mg/dl) in group I; 0.62 ± 0.20 (mg/dl) in group II; 0.69 ± 0.43 (mg/dl) in group III and 0.71 ± 0.22 (mg/dl) in group IV. TB showed no statistically significant increase (P>0.05) over the control in group I (2mg/kg); group II (4mg/kg); group III (8mg/kg) and group IV (16mg/kg).

The conjugated bilirubin (CB) level also increased from 0.30 ± 0.03 (mg/dl) in control group to 0.29 ± 0.10 (mg/dl) in group I; 0.31 ± 0.13 (mg/dl) in group II; 0.33 ± 0.20 (mg/dl) in group III and 0.50 ± 0.16 (mg/dl) in group IV. The increases were significant in group IV (16mg/kg) (P<0.05) when compared with the control, while group I, II and III (2, 4 and 8 mg/kg respectively) were not (P>0.05).

Figure 2

Table 2: The Mean values of the Serum Hepato-specific Marker in both Control and Experimental Animals

Group	Dose(mg/kg)	AST	ALT	ALP	TB (mg/dl)	CB (mg/dl)
control	0	21.71±6.90	14.81±3.41	50.01±13.03	0.61±0.16	0.30± 0.03
I	2.0	23.14±11.70	16.08±3.73	64.71±60.00	0.67± 0.26	0.29±0.10
II	4.0	22.30± 6.90	16.26±6.05	67.41±25.32	0.62± 0.20	0.31± 0.13
III	8.0	23.56±9.90	15.55±4.88	66.37±44.65	0.69± 0.43	0.33± 0.20
IV	16.0	30.81±5.56*	21.85±1.20*	85.60±38.20*	0.71±0.22	0.50±0.16*

* Indicates significant (P<0.05) difference when compared with control.

DISCUSSION

Artesunate comes in 50mg tablets. The commonly used effective adult dose in Nigeria is 6-8 mg/kg in three divided daily doses. This informed the use of graded daily doses of 2-16 mg/kg body weight in the guinea pigs (Table 2). This dose range gave us the opportunity of studing the effect of the submaximal and higher doses of the drug.

This study showed that there was no significant difference (P>0.05) in weight loss or gain of the animals in all dose level at the end of the experiment. The administration of 16.0 mg/kg artesunate caused increases in the activities of aspartate aminotransferase, (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and concentration of conjugated bilirubin (P<0.05) when compared with control. The significant increases in AST, ALT and ALP are indicative of damage to hepatocytes ([17,18]). There was no significant increase in the mean values of the serum hepato-specific markers at the other dose levels (p>0.05) when compared with control.

The current investigations suggest toxicity of the liver cells of the guinea pigs upon artesunate administration. The findings in this study agree with the work of Ngokere et al ([8]), in which artesunate administration caused significant increase in the liver marker enzymes in rabbit. They also agree with Woodrow et al, ([9]) in which artesunate caused a transient rise in the liver transaminases. The results are also in agreement with other results ([19,20,21]). The liver cell damage may have been caused by free radicals generated by artesunate, which are also responsible for their antimalarial actions. The deleterious effects were considered to be caused by free radicals produced during peroxide formation. Precisely, the level of hydroxyl and peroxide radicals induced by artesunate treatment may be responsible for the hepatotoxicity to the guinea pigs.

Artesunate metabolism involves hepatic cytochrome P_{450} and other enzymes. Moreover, artesunate increases the O-

xanthine oxidase ($_{22}$). This enzyme is found in the liver and other parts of the body. Both cytochrome P_{450} reductase and xanthine oxidase cause the induction of superoxide dismutase to form hydrogen peroxide and oxygen ($_{23}$). However, the rate of this reaction is dependent on the action of superoxide dismutase. The O-xanthine oxidase can mobilize iron from ferritin by a mechanism largely dependent on the generated superoxide ions. The hydroxyl species are the most biologically toxic of most free radicals. The molecules are very reactive, and act in-situ to the point of origin. They can react with proteins, nucleic acids, lipids, cell membranes and other cell structures to produce tissue damage. The free radical species may be responsible for the various toxic effects on the liver as observed in this study. Elevation of the serum enzymes indigenous to the liver is an indication of hepatotoxicity. Transaminases are important useful markers for hepatocellular damage ($_{17}$). However, ALT is specific for hepatocellular damage because they are predominantly produced within the liver cells

It is then concluded that the raised liver enzyme levels at highest dose (16mg/kg) of artesunate administration suggests hepatocellular damage consequent upon artesunate administration on the guinea pigs. Moreover, the significant serum concentration of both the liver transaminases and conjugated bilirubin suggest that there may be hepatic dysfunction as a result of overdose of the drug.

These observations in guinea pigs may be applicable to humans. It is therefore suggested that the drug be prescribed with caution in patients with hepatic dysfunction, especially as the drug is fast replacing chloroquine in the Nigerian market in the treatment of chloroquine-resistant malaria infection. Self-medication involving artesunate should be discouraged as this may possibly lead to overdose and liver damage.

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Author Information

H. U. Nwanjo
Department of Medical Laboratory Sciences, Imo State University

G. Oze
Department of Medical Biochemistry, Imo State University