Hepatotoxic and hemolytic effects of acute exposure of rats to artesunate overdose

Omotuyi, I. O. 1, Nwangwu S. C. 2, Okugbo, O. T. 1, Okoye, O. T. 2, Ojieh, G. C. 3 and Wogu, D. M. 1

1Department of Basic and Applied Science, Benson Idahosa University, Benin Nigeria.
2Department of Biochemistry, Igbinedion University, Okada Nigeria
3Department of Medical Biochemistry, Ambrose Alli University, Ekpoma, Nigeria

Accepted 23 April, 2008

Hepatotoxic and hemolytic effects of artesunate overdose were examined in rats. Forty (40) rats were grouped randomly into four designated as A, B, C, and D, and were given oral administration of artesunate as follows: 0 mg/kg (control), 1 mg/kg (Under-dose), 2 mg/kg (Normal dose) and 4 mg/kg (Overdose) respectively. The administration was continued for 5 days. Hepatotoxicity was monitored in the rats as a function of changes in serum levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total serum albumin and malondiadehyde (MDA) level. The hemolytic effect of this drug was monitored by changes in the packed cell volume (PCV), total bilirubin, conjugated bilirubin and malondiadehyde (MDA) levels of erythrocyte. For group D (overdose group) subjects when compared with the control (group A), there was significant (p<0.05) decrease in serum albumin and hematocrit, but significant increase in serum levels of total bilirubin, and conjugated bilirubin. An increased hepatocyte and erythrocyte malondiadehyde level was also observed in group D. The result also shows increased activities for the serum enzymes in all the groups when compared with control group but significant increase was recorded for groups C and D. There is a clear indication that hepatotoxicity and hemotoxicity are associated with artesunate administration at both required and overdose conditions however these effects are magnified in overdose conditions.

Key words: Artesunate, hepatotoxicity, hemotoxicity, serum enzymes, bilirubin, malondialdehyde.

INTRODUCTION

Malaria has indeed become a pandemic disease in most part of the world, and its pandemic nature has been fortified with the advent of mutant strains of malaria parasite which defy chemotherapy, spurring researchers into seeking new chemotherapy or making modification to the pre-existing ones. One of the most recent phytochemical agents isolated and biologically characterized to exhibit anti-malaria potency is artemisinin; this compound was isolated from Artemisia annua. Recently, there have been other synthetic derivatives of artemisinin which includes: artemether, arteether (artemotil), artesunate and arteminol (α-dihydroartemisinin, DHA). Artemisinin and its derivatives are known to exhibit potency against the asexual, and erythrocytic forms of Plasmodium falciparum and Plasmodium vivax Pukrittayakamee et al., 2004). The generally recommended effective doses in humans with uncomplicated falciparum-caused malaria range between 10 mg/kg/day for artemisinin and 2 - 5 mg/kg/day for other artemisinin derivatives. The underlying mechanism for the parasiticidal activity of these agents is a free radical reaction (Olliaro et al., 2001). This reaction is initiated with the reaction of artemisinin with iron present in the hem prosthetic group of hemoglobin, leading to the generation of activated oxygen species, such as oxygen radicals and superoxide radicals. These radicals then propagate free radical reaction capable of inducing oxidative damage in the parasite (Meshnick et al., 1996; Olliaro et al., 2001). The mechanism of action of these drugs and their ability to accumulate in the red blood cell being its primary site of action, had raised questions regarding the safety of artemisinin and its derivatives, especially artesunate which is fast gaining wide-spread acceptance in most malaria endemic regions of the world. This research evaluated the toxicological influence of different artesunate dosage administration on
on the biochemical integrity of the liver and the red blood
cells (erythrocyte is the primary site of action, the site of
metabolism is the liver). The parameters used in the
evaluation of hepatotoxicity include serum levels of
aspartate transaminase (AST), alanine transaminase
(ALT), alkaline phosphatase (ALP), total serum albumin
and malondiadehyde (MDA) level. The parameters
estimated to determine the hematotoxicity are the packed
cell volume (PCV), total bilirubin, conjugated bilirubin and
MDA levels of erythrocyte.

MATERIALS AND METHODS

Chemicals and assay kits

Artesunate® (Tianji Pharma and Chem- China). Copper sulphate
pentahydrate, di-sodium potassium phosphate anhydrous and
sodium dihydrogen phosphate (anhydrous) were products of British
Drug House (Poole, England). Malondialdehyde (MDA), Trichlo-
roacetic acid (TCA), thiobarbituric acid (TBA), and ferrous sulfate
hexahydrate, were products of Sigma Chemicals Co.(St Louis, Mo,
USA). ALT, AST and ALP assay kits (Randox), Quantichrom® BCG
Albumin assay kit, Follin-ciocalteau, sodium-potassium tartrate
were products of Patel industries (Mumbai). All others chemicals
used were of analytical grade.

Experimental animal

Experimental animals (Rattus norvegicus) weighing 80 - 100g used
for this research were obtained from the animal house of the
college of Health Sciences, Igbinedion University, Okada. They
were housed in wooden cages [Protected with wire mesh]. They
were fed and given water ad libitum on normal rat cube, the room
temperature was maintained at 25 ± 2°C throughout the
experiment.

Experimental design

The animals were assigned randomly to four groups, each with ten
(10) rats. Group A: The control, the animals were placed on
placebo (normal saline, 0.85% sodium chloride solution). Group B:
These animals were administrered with oral doses of 1 mg/kg/day of
artesunate in normal saline (Under dose). Group C: These animals
were administrered with oral doses of 2 mg/kg/day of artesunate in
normal saline (Normal dose). Group D: These animals were
administrered with oral doses of 5 mg/kg/day of artesunate in normal
saline (Over dose). The administration was continued for 5 days
(normal duration of treatment in humans). After the experiment, the
animals were sacrificed after cervical dislocation method; the
thoracic region was opened to expose the heart. Blood samples
were drawn from the heart via cardiac puncture. The liver was
decapsulated and perfused in physiological saline, the organ was
homogenized in 0.1 M phosphate buffer (pH 7.2)

Hematological assay

The hematocrit (packed cell volume)

Non-heparinized capillary tubes (75mm long with a bore of 1.155 ±
0.085 mm) sealed at one end was filled up to 2/3 length with whole
blood, was spun at 12,000 x g for five minutes in a micro-hematocrit
centrifuge. The packed cell volume was determined using the
gradation in the standard hematocrit chart and recorded as % total
blood

Biochemical parameters

Alkaline phosphatase activity (ALP)

This enzyme acts on esters of phosphoric acid and catalyse hydro-
lysis and phosphate group release. For enzymatic assay, P-nitro-
phenol phosphate was used as the substrate, a colorless solution
which turns yellow upon hydrolysis. The yellow coloured complex
was measured spectrophotometrically at 405 nm.

Activity [U/L] = 3300 x change in absorbance at 405nm/change in
time [min].

Alanine transaminase (ALT)

ALT catalyses the transamination of alanine to alpha-keto glutarate
to form glutamate and pyruvic acid, which then reacts with 2,4-
dinitro-phenylhydrazine to form the hydrazone derivative of pyru-
vate, a coloured complex which can be measured at 546 nm. The
activity (U/L) was determined from the standard calibration graph.

Aspartate transaminase (AST)

ALT catalyses the transamination of aspartate to alpha-keto gluta-
rate to form glutamate and oxaloacetate, which then reacts with 2,4-
dinitro-phenylhydrazine to form the hydrazone derivative of oxalo-
acetate, a coloured complex which can be measured at 546
nm. The activity (U/L) was determined from the standard calibration graph.

Determination of total serum albumin (BCG)

Bromcresol green forms a colored complex specifically with albu-
min. The intensity of the color, measured at 620nm, is directly
proportional to the albumin concentration in the sample. The
concentration of total serum protein (g/dl) is determined from the
calibration graph.

Malondialdehyde assay

Principle of assay

Lipid peroxidation generates peroxide intermediates which upon
cleavage release malondialdehyde, a product which reacts with
thiobarbituric acid. The product of the reaction is a coloured
complex which absorbs light at 532 nm. The MDA levels of samples
were determined from the calibration graph.

Procedure of assay

1 ml aliquot of each homogenate supernatant was incubated for 2 h
at 37°C with 3 ml of ferrous sulphate/ phosphate buffer solution. 1
ml of each mixture was added to a tube containing 2ml trichlo-
roacetic acid (TCA). After centrifugation at 700 x g for 10 min, 2 ml
of each supernatant was added to 1 ml of 2-thiobarbituric acid (TBA)
solution. The tubes were boiled for 10 min and allowed to cool, and
the absorbance at 532 nm was measured

Calculation

Thiobarbituric Acid Reactive Substance of the samples were calcu-
lated as malondialdehyde (MDA) equivalents, using serial dilutions of Malondialdehyde standard (10 nmol/ml).

**Assay for total bilirubin**

**Principle**

Conjugated bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

**Calculation**

\[
\text{Total bilirubin [mg/dl]} = 10.8 \times A_{578 \text{ nm}}
\]

\[
\text{Conjugated bilirubin [mg/dl]} = 14.4 \times A_{546 \text{ nm}}
\]

**Statistical analysis**

The results are expressed as mean ± standard deviation. The difference between mean values was assessed for significance by student T-test (SPC-XL, 2000) and considered significant at 0.05.

**RESULTS AND DISCUSSION**

Table 1 shows the effect of artesunate administration on the total serum albumin (g/dL), serum enzyme activity (U/L) and Malondialdehyde level of the liver (nmol/L).

<table>
<thead>
<tr>
<th>Plasma enzymes</th>
<th>Alkaline phosphatase (U/L)</th>
<th>Aspartate transaminase (U/L)</th>
<th>Alanine transaminase (U/L)</th>
<th>Total serum albumin (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>4.83 ± 1.09</td>
<td>1.30 ± 0.02</td>
<td>1.40 ± 0.98</td>
<td>1.72 ± 1.90</td>
</tr>
<tr>
<td>Group B</td>
<td>6.28 ± 1.76</td>
<td>2.68 ± 0.94</td>
<td>1.73 ± 0.43</td>
<td>1.89 ± 0.78</td>
</tr>
<tr>
<td>Group C</td>
<td>15.87 ± 3.54</td>
<td>3.20 ± 1.02</td>
<td>2.83 ± 0.02</td>
<td>0.88 ± 0.54</td>
</tr>
<tr>
<td>Group D</td>
<td>26.22 ± 3.59</td>
<td>4.80 ± 1.47</td>
<td>4.63 ± 1.13</td>
<td>0.29 ± 0.21</td>
</tr>
</tbody>
</table>

Values are reported as mean +/- standard deviation from 10 rats. *Values differ significantly when compared with the control (p< 0.05).

The observed dose-dependent increase in the activities of alkaline phosphatase, aspartate transaminase, and alanine transaminase, can be traced to possible necrosis of the where these enzymes are native, since they are not classified among the secretory enzymes. Isoenzyme analysis of these enzymes would have given a clearer detail of their tissue origin. Since the assay was not conducted, the reported tissue distribution of the drugs would be correlated with the likely tissue origin of these enzymes. The drug was reported to accumulate significantly in three tissues; liver, erythrocyte and to a lesser extent the kidney (Li et al., 2006). And the erythrocyte is known to have a low activity of ALT and AST, the kidney would rather play an excretory role than metabolic leaving the liver as the potential site of substantial of artesunate and its metabolites which also represent the site of detoxification for most xenobiotic chemicals (Amin and Geoffrey, 2007). These enzymes can therefore be assumed to have originated from the liver, although with little contribution from extrahepatic tissues.

Meanwhile the proposed necrotic effect of artesunate on the liver was demonstrated by the observed dose-dependent increase in malondialdehyde level of the liver. The increased MDA level of the liver is possibly due to lipid peroxidation. The source of biological oxidant which could induce this peroxidation of lipid is central to the mechanism of action of the drug; it is known to generates reactive oxygen species (Olliaro et al., 2001). The normal response of hepatocyte to lipid peroxidation would have been the induction of antioxidant enzymes, artesunate is however known to inhibit protein synthesis in mammalian cells (Xu et al., 2007) or an additive influence of both factors.

The increased MDA level of the liver is possibly due to lipid peroxidation. The source of biological oxidant which could induce this peroxidation of lipid is central to the mechanism of action of the drug; it is known to generates reactive oxygen species (Olliaro et al., 2001). The normal response of hepatocyte to lipid peroxidation would have been the induction of antioxidant enzymes, artesunate is however known to inhibit protein synthesis in mammalian cells (Xu et al., 2007) or an additive influence of both factors.
of the red blood cells. Total bilirubin increased significantly in groups B and D, while conjugated bilirubin levels increased significantly. The PCV levels of overdose decreased significantly, while MDA levels increased significantly in normal and overdose groups. The decreased PCV observed for overdose group agrees with the published work of (Xie et al., 2005). The increased level of bilirubin also confirms the increased level of red blood cell destruction associated with artesunate administration, while the increase in the total bilirubin can be accounted for by the heam degradation that follows red blood cell destruction (Krücken et al., 2005).

### Conclusion

From the data available from this research, artesunate is not significantly toxic at the recommended dosage but an overdose may be potentially toxic to the liver and the Red blood cell.

### REFERENCES


