

## Assessment of side-effects of administration of artemether in humans

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### Abstract

In animal in-vivo study, we have reported some significant changes such as reduced sperm counts, degeneration of seminiferous epithelium and interstitial leydig cells in the testicle. Therefore human study was designed to assess the effects of artemether on some biochemical parameters in individuals treated with artemether. Moderate and transient side effects were observed after administration. This implies that administration of artemether is still tolerated and the moderate side effects are not comparable with the debilitating effects of malaria infection it is meant to cure. Artemisinin is still a tolerable antimalaria drug with no major side effects on human.

**Keywords:** Artemether, Antimalaria, Biochemical parameters, Side-effects, Humans.

### Introduction

Artemisinin is the most rapid-acting class of antimalaria drugs for both uncomplicated and severe malaria. They act against the asexual stage gametocytes and they block sexual stage. Artemisinin is a natural antimalaria drug derived from the plant *Artemisia annua*. It is used in traditional Chinese drug for the management of fever resulting from malaria. To improve its bioavailability, its derivatives such as artemether, artesunate etc have been developed. Artemether, (methyl ether of dihydroartemisinin) is derived from artemisinin (Chinese plant *Quinghaosu*), and extract of the herb *artemisia annua* L.

Artemether is characterized by its novel structure and unique antimalaria action, which are totally different from the conventional antimalaria drugs. Artemether is very effective against multidrug resistant malaria. An animal pharmacodynamics showed that artemether is a strong schizonticide. Parasitemia clearance occurs rapidly with stable efficacy after administration. It is also effective against chloroquine resistant *Plasmodium falciparum* malaria. The mode of action is that endoperoxide bridge in artemether introduced by free iron decomposed by hemoglobin, produces unstable free radicals and/or other electrophilic intermediates, which form covalent compound with the protein of plasmodia hence make the plasmodia killed (Meshnick et al, 1989).

Furthermore, artemether, has been shown to induce transient and moderate

elevations in liver transaminases, bradycardia. Also, dihydroartemisinin had been demonstrated to have caused morphological changes in the testes and suppressed spermatogenesis (Nwanjo, et al., 2007). In animal in-vivo study, we have reported some significant changes such as reduced sperm counts; degeneration of seminiferous epithelium and interstitial leydig cells in the testicle. Therefore human study was designed to assess the effects of artemether on some biochemical parameters in individuals treated with artemether.

### Materials and Methods

#### *Study population*

Healthy adult males between age 20 and 40 years and who satisfied the enrolment criteria were recruited into the study. None of the subjects had any history of testicular or erectile dysfunction e.g. aspermia, hyperspermia or suffering from liver or any other diseases that may affect the parameters assessed.

#### *Study performed*

After enrolment, each adult male was placed on artemether therapy for 5 days. Each adult was bled 3 times on days 0, 7 and 14. Blood was collected at day zero (baseline), on the 7th and 14th (after treatment) days. Each patient served as his own control. Serum concentrations of testosterone, total cholesterol, alanine aminotransferase, gamma glutamyl transferase, albumin, and total proteins were determined.

#### *Inclusion criteria*

- 1) Healthy adult male aged 20 years to 40 years
- 2) A febrile temperature less than 37.5°C.
- 3) Willingness to provide written or witnessed verbal informed consent by study volunteers (being adults).

#### *Exclusion criteria*

- 1) History of hypersensitivity to any of the artemisinins.
- 2) Clinical features of severe malaria (WHO, 2003).
- 3) Treatment within the preceding seven days with any anti-malaria drug other than chloroquine.
- 4) Concomitant infections, malnutrition or any other chronic diseases e.g. sickle cell disease or infections that may mask evaluation of response to treatment.
- 5) Perceived inability to comply with study protocol.

#### *Withdrawal criteria*

Serious adverse event.  
Withdrawal of consent.  
Violation of study protocol.

#### *Drug dosing*

Oral artemether tablet (Artem<sup>TM</sup>) in a supervised regimen for 5 days was given as follows: 200mg once on day 1, followed by 100mg once daily for subsequent 4 days.

#### *Follow up*

At each visit, the following parameters were assessed: axillary temperature using a digital thermometer, Pulse rate (c/m).

#### *Determination of alanine transaminase*

Alanine transaminase was determined by spectrophotometric method. This method couples the production of pyruvate in the reaction catalysed by alanine transaminase to a second reaction whereby pyruvate is reduced by NADH in a reaction catalyzed by lactate dehydrogenase. The disappearance of NADH because of its oxidation to NAD is then monitored at 340nm.

#### *Estimation of alkaline phosphatase*

Alkaline phosphatase activity was determined using an optimized standard method. Into 1cm light path cuvette 1.0ml of reagent was pipetted. The initial reading was taken, 0.02ml of serum was then added into the cuvette and timer was started simultaneously. Readings were taken

against air at 1, 2 and 3 minutes at Hg 405nm x 2760.

#### *Estimation of total proteins*

Protein concentration was determined by the Biuret method. Cupric ions, in an alkaline medium interact with protein peptide bonds resulting in the formation of coloured complex. (Weichselbaum, 1946).

#### *Estimation of testosterone*

Serum testosterone level was determined by ELISA method which is a quantitative determination of testosterone in human serum or plasma.

#### *Statistical analysis*

Analysis of variance (ANOVA) was used for general comparison and student's t-test was used for comparison between various groups. Level of significance was taken at  $p < 0.05$ .

#### **Results**

Table 1 showed the physical parameters of the study volunteers during the study. All the male volunteers are between 20 – 40 years of age. They have a febrile temperature. There was no difference in the physical parameters through the study period. Table 2 shows analysis of variance of selected serum biochemical parameters. Table 3 shows pair wise comparisons of selected biochemical parameters in volunteers before and after administration of artemether. Total protein and albumin were reduced in volunteers on day 7 when compared with the corresponding values at baseline, however, the differences did not reach level of statistical significance ( $p > 0.05$ ). The serum concentrations of gamma glutamyl transferase on day 7 was significantly higher ( $p < 0.05$ ) when compared with the corresponding value at baseline. The serum concentrations of alanine transaminase, total cholesterol, and testosterone were higher at day 7 when compared with the corresponding values at baseline, however, none of these differences reached level of statistical significance ( $p > 0.05$ ).

At day 14, the serum concentrations of total protein and albumin were higher when compared with values at day 7, however, the difference did not reach level of statistical significance ( $p > 0.05$ ). The serum concentrations of alanine transaminase, gamma glutamyl transferase, total cholesterol and testosterone were reduced when compared with the corresponding values at day 7, however, none of

the differences reached level of statistical significance ( $p>0.05$ ). At day 14, the changes

observed in the serum concentrations of biochemical parameters become normalised.

**Table 1:** Demography of enrolled study volunteers.

Sex	Male
Mean age (yrs)	25.2±8.2
Range (yrs)	20-40
Mean weight (kg)	70.4±15
Mean temperature (°C)	36.1±0.5

**Table 2:** Analysis of variance of selected biochemical parameters in malaria patients before and after treatment with artemether.

PARAMETERS	Day 0	Day 7	Day 14	F-value	P-value	Remarks
TP(g/dl)	77.15±14	64.13±17.02	74.45±14.95	7.88	0.00	S
ALB(g/dl)	29.35±4.35	28.73±4.93	31.45±5.27	3.45	0.04	S
ALT(IU/l)	19.60±7.37	25.10±17.58	24.08±12.25	1.76	0.18	NS
GGT(IU/l)	<sup>a</sup> 23.24±7.99	<sup>b</sup> 36.16±19.06	26.86±10.04	10.10	0.00	S
T.C.(mg/dl)	158.80±45.02	217.5±28.91	188.7±29.29	1.197	0.31	NS
Testo.(nmol/l)	22.53±7.52	28.16±12.16	25.96±9.92	2.95	0.06	NS

b compared with a is significant. TP = total proteins, ALB= albumin, ALT= alanine transaminase  
GGT= gamma glutamyl transferase, TC = total cholesterol, Testo. = testosterone.

**Table 3:** Pairwise comparisons of serum concentrations of parameters between groups before and after drug administration.

	TP(g/dl)	ALB. (g/dl)	ALT(iu/l)	GGT(iu/l)	T.Chol. (mg/dl)	Testo. (nmol/L)
Baseline vs Day 7	NS	NS	NS	S	NS	NS
Baseline vs day 14	NS	NS	NS	NS	NS	NS
Day 7 vs Day 14	NS	NS	NS	NS	NS	NS

**Discussion**

In human study, the increased serum concentrations of alanine transaminase (ALT) & gamma glutamyl transferase (GGT) (i.e. liver enzymes) observed on the 7th day may be suggestive of the side effects of artemether on the liver. However, the reduction in the serum concentrations of the two enzymes by the 14th

day when compared with the 7th day may be suggestive of reversible side effects of the drug on the liver. The effects on the liver are evident by the increased serum concentrations of alanine transaminase, alkaline phosphatase and gamma glutamyl transferase. These enzymes are retained within the cells of origin by the plasma membrane surrounding the cells. Direct

attack on the cell membranes by organic chemicals such as drugs is an obvious cause of their release and this is of particular importance to the liver. Slight or moderate elevations of these enzymes activities may be observed after administration of certain drugs. Jaspers et. al., 1996 reported that antimalaria mefloquine induces liver enzymes i.e. transaminases in Dutch marines who took mefloquine during 3 months in Cambodia, and who were not drinking alcohol at the time.

Reactive derivations oxidants are generated during the process of drug biotransformation. The reactive species generated can bind and/or react with cellular components in the liver, and cause liver injury which may result in impairment of liver functions. Reaction of reactive species with cellular antioxidants causes antioxidant depletion that may result in oxidative stress (Jeong, 1999, Timbrel et al; 1980). Recent studies have indicated the existence of a strong correlation between hepatic injury and oxidant stress in experimental animals treated with anti-tuberculosis drugs (Tasduq et al, 2005, Attri et al; 2001). Biochemical tests related to the hepatocellular integrity can be checked to follow hepatocellular integrity and liver injury. In this study, administration of artemether caused an elevation in the activities of ALP, ALT and GGT. Increased activities of these enzymes showed that the integrity of hepatocytes was abnormal, resulting in the release of intracellular enzymes into the systemic circulation. Decrease in albumin and total protein levels showed that administration of artemether has caused impairment of liver function e.g. its capacity to synthesize albumin. Treatment of malaria with artemether has been reported to cause transient elevations in the serum concentrations of alanine transaminase & aspartate transaminase in malaria patients. If any, the changes in ALT & GGT appear to be transient in this study. In volunteers given artemether, on day 7, total cholesterol tended to show an increase and by the 14th day, (when short-term treatment had stopped), the tendency for increase still persisted. It is also very striking that the tendency for increase in total cholesterol was accompanied by a tendency for increase in testosterone concentrations at the 7th and 14th days when compared with the baseline values.

The implication of the high testosterone concentrations (if this persist for a longer period) in respect to suppression of spermatogenesis needs special mention, especially in the light of

previous studies indicating that high circulating testosterone concentrations may in fact inhibit the synthesis of follicle stimulating hormone and luteinizing hormone (hormones required for regulation of testosterone) from the pituitary gland via a feedback mechanism. Therefore, prolonged or long-term use of this drug may require follow-up. The transient decrease in the serum concentrations of total proteins and albumin in the malaria patients treated with artemether was an indication that the drug may have effect on the liver. Proteins are synthesized by the liver; however, the synthetic capability may be affected by any chemical that may have either moderate or extensive hepatotoxic effects. Most drugs bind to albumin & globulins; however, it is the free fraction of the drugs that elicits the desirable biological effects observed during drug treatment. However, any condition that may reduce the circulating concentrations of proteins, particularly albumin will alter binding capability of the drug thereby leading to a high concentration of the free fraction which may be higher than required by the body thereby making the drug to exert undesirable effects. The altered circulating concentration of albumin in the human study may be due to side effects of artemether on the liver cells.

Finally, these results suggest that artemether could be used as an antimalaria drug, since the observed reversible side effects are not comparable with the disease it is intended to cure, however, prolonged or long-term use should be with adequate follow-up particularly in developing countries where self-medication is especially common and sometimes at dosages higher than therapeutic dose. Repeated doses without appropriate recommendation should be discouraged. However, these findings deserve more research in view of the fact that the study was performed in a small population. The monitoring needed for adverse reactions to drugs used depends on which drugs are being used and how they are used. It may also vary with nutritional status and between populations exposed to different infections. The main reason for monitoring adverse reactions is to ensure the safety of individuals taking treatments and identify those who need to stop treatments that are damaging their health and change to other treatments, if available. The drug safety regulatory bodies in many countries operate reporting schemes for doctors and pharmacists to report adverse events which they suspect may be linked to treatments. This is known as

'Pharmacovigilance' and is important for identifying what may be rare but serious side effects of drugs that are not picked up in clinical trials. When drugs are taken in combination, deciding what contribution each of those drugs has to effects that are seen as well as trying to decide what effects are due to illness, or are completely unrelated either to the drugs or to the illness, is a challenge. To monitor drug reactions, it is essential that baseline assessments and measurements are made before the drug is first taken. Good medical record keeping is fundamental for effective monitoring. Some people may be more vulnerable to particular side effects from drugs than others. Some of these factors can be identified through a careful clinical history. Being overweight, being malnourished or drinking alcohol can predispose some people to particular drug side effects.

Many drugs have been shown, apart from curing the particular diseases they were meant for, to have toxic effects on certain body functions. Some are known to affect the synthesis, metabolism and storage of certain biochemical parameters. There are reported cases of interference of some drugs in the metabolic processes required for the normal functioning of the body. Some drugs are equally known to affect the ultrastructure of organs such as liver, kidney etc. In view of this, effects of drugs on normal body metabolic processes and ultrastructure of some organs should be studied as these will greatly help in monitoring and improving the management strategies of different diseases.

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