Body and Testicular Weight Changes in Adult Wistar Rats Following Oral Administration of Artesunate

A.M. Izunya, A.O. Nwaopara, A. Aigbiremolen and G.A. Oaikhena

INTRODUCTION

Malaria is a leading cause of mortality and morbidity in developing areas of the world, and remains a major public health problem in endemic regions (Breman et al., 2004). Resistance to available drugs is increasing, creating a need for new drugs that are well tolerated and simple to use. In the face of this ominous situation, artesinin and its derivatives (artesunate, arteether, artemether, and dihydroartemisinin) have given renewed hope for combating resistant malaria (Hein, 1994; Harinasuta and Karbwang, 1994). These drugs have gained considerable prominence in the chemotherapy of both uncomplicated and severe falciparum malaria by demonstrating high activity against multidrug-resistant falciparum strains with low toxicity profiles (Chanthaph et al., 2005).

Artesunate is one of the numerous drugs for malaria intervention in Nigeria. It is a semi-synthetic derivative of artemisinin, the active compound of the Chinese herb Artemisia annua which consists of the sodium succinyl salt of dihydroartemisinin (Ittarat et al., 1999). Artesunate and its active metabolite dihydroartemisinin are potent blood schizonticides, highly effective against multi-drug resistant strains of Plasmodium falciparum hence its increasingly wide usage for the treatment and management of malaria (Van Agtmael et al., 1999). It is used in combination therapy and is effective in cases of uncomplicated P. falciparum.

Serious concern has been raised about uncontrolled use of artemisinin derivatives because at the moment they are the last resort in the combat against multi-drug resistant P. falciparum malaria (White, 1994). The use of these drugs should be controlled and restricted to proven multi-drug resistance on severe malaria in order to preserve their efficacy (Mulenga, 1998) 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 (Nwano and Oze, 2007). 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 (Udonna, 2000).

Several studies have shown that high doses of artesunate can produce neurotoxicity such as selective...
damage to brainstem centres, gait disturbances in mice and rats (Nonprasert et al., 1998, 2002; Genovese et al., 2000) and loss of spinal cord and pain response mechanisms in animals (Genovese et al., 1995; Dayan, 1998). Others showed some varying degree of cell clustering, cellular hypertrophy, and intercellular vacuolations in the stroma of the superior colliculus of artesunate treated animals (Eweka and Adjene, 2008a). Another showed some degenerative and necrotic changes, cellular hypertrophy, and increase intercellular vacuolations in the stroma of the stomach of rats (Eweka and Adjene, 2008b). More importantly, despite several studies on the effects of artemisinin-based antimalarial on male reproductive functions, there appears to be little or no information in the literature on the impact of an ACT drug, artesunate, on body and testicular weights. This study was therefore undertaken to examine the effects of artesunate on the body and testicular weights of adult wistar rats.

MATERIALS AND METHODS

Location and duration of study: This study was conducted at the histology laboratory of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The preliminary studies, animal acclimatization, drug procurement, actual animal experiment and evaluation of results, lasted for a period of one month (January, 2010). However, the actual administration of the drug to the test animals lasted for one week (15, January to 21, January 2010).

Animals: Fifteen adult wistar rats weighing between 100-150 g were used for this experiment. They were obtained and maintained in the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State. They were divided into three groups A, B, and C of five rats each. Groups A and B were the treatment groups, while Group C served as the control. They were kept in each group per cage and fed with grower’s mash produced by Bendel Feeds and Flour Mills Limited, Ewu, Nigeria. Water was given ad libitum. They were allowed to acclimatise for one week before commencement of the study. Ethical approval was sought and received from the Department of Anatomy, College of Medicine, Ambrose Alli University, Ekpoma, Edo State on the need to observe completely the rules guiding the employment of rats for scientific studies.

Drug administration: The artesunate tablets used for this experiment, were manufactured by Mekophar Chemical Pharmaceutical Join-Stock Company, Ho Chi Minh City, Vietnam and purchased from Irrua Specialist Hospital, Irrua, Edo State. The drug solution was made with distilled water (1mg.ml⁻¹) and administered to the animals by orogastric tube for a period of seven days. The dosage of artesunate was as per WHO recommendation of 4 mg.kg⁻¹ body weight daily for 3 days followed by 2 mg.kg⁻¹ body weight daily for the remaining 4 days. All the animals were weighed before the experiment. The drugs were administered to the groups as follows:

Group A: Four mg.kg⁻¹ body weight of artesunate daily for 3 days followed by 2mg.kg⁻¹ body weight daily for the remaining 4 days.

Group B: Eight mg.kg⁻¹ body weight of artesunate daily for 3 days followed by 4 mg.kg⁻¹ body weight daily for the remaining 4 days.

Group C (Control): Distilled water.

The graded daily doses gave us the opportunity of studying the effect of the normal and higher doses of the drug.

Body and testicular weights determination: The animals were weighed and autopsied by cervical dislocation 24 h after the last dose on the 8th day of the respective treatment. The testes were dissected out, freed from adherent tissues, and weighed up to the nearest 0.001g on a mettler analytical balance (PE 1600, Mettler Instrument AG; Switzerland).

Statistical analysis: The data for body & testicular weights were expressed as the mean ± SD. The treated groups were compared to control using the Student’s t test. Differences with values of p<0.05 were considered statistically significant (Mahajan, 1997).

RESULTS

General findings: No gross differences were observed between the two groups of animals on day 8 at the completion of experimental procedure. There were no expected deaths recorded.

Effect of artesunate on body weight: The data obtained from the mean body weights of the control and artesunate-treated rats are given in Table 1. A comparison of the mean body weight of the rats before and after treatment showed that both the artesunate-treated and control rats manifested the same increase in body weight. The percentage increase in mean body weight of rats was 25%.

Effect of artesunate on testicular weight: The data obtained from the mean testicular weights of the control and artesunate- treated rats are given in Table 1. Using t-test analysis technique there was no significant difference in the mean testicular weights of rats in groups
Table 1: Effect of artesunate on the body and testicular weights of rats following oral administration of normal and double normal doses

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Testicular weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>A</td>
<td>100.00</td>
<td>125.00</td>
</tr>
<tr>
<td>B</td>
<td>100.00</td>
<td>125.00</td>
</tr>
<tr>
<td>C</td>
<td>100.00</td>
<td>125.00</td>
</tr>
</tbody>
</table>

Values are expressed as means: SD; *: Significantly different statistically from the control at p<0.05, T-test.

C and A, but there was a significance increase in mean testicular weight of rats in groups C and B.

DISCUSSION

Our investigation on gross/morphological changes indicated that there were no difference between the control and experimental rats. The observed normal body weight gain in the control and experimental groups, implied that oral administration of normal and double normal doses of artesunate had no negative effect on somatic growth. This finding agrees with the work of Nwanjo and Oze (2007) in which artesunate administration caused no significant increase in body weight of guinea pigs.

Although there was no difference in mean testicular weight between the control and normal dose artesunate treated rats, a significant increase in the mean testicular weight of the double normal dose treated rats was however observed. In fact, it has been reported that an increase or decrease in relative or absolute weight of an organ after administering a chemical or drug is an indication of the toxic effect of that chemical (Simons et al., 1995; Maina et al., 2008). Also, the weight of male reproductive organs usually provides a useful reproductive risk assessment in experimental studies (Raji et al., 2005; Simons et al., 1995) and testicular size is the best primary assessment for spermatogenesis, since the tubules and germinal elements account for approximately 98% of the testicular mass (Sherines and Howards, 1978). Thus, the observed no-testicular-weight change in the testis of both the control and the normal dose treated rats suggests that the administration of artesunate at normal dose had no toxic effect on this organ. But the observed increase in testicular weight in the double normal dose treated rats indicate that the drug may have toxic effect on the testis at that dose.

Interestingly, there are studies that have shown that the administration of a herbal tea mixture containing Gynostemma pentaphyllum, Radix polygoni multiflori, Semen cassiae, Green tea and Folium nelumbinus caused testicular oedema which has been linked to factors such as testosterone (Maddocks and Sharpe, 1989), Human Chorionic Gonadotrophin (hCG) or endogenous LH (Bergh et al., 1990), direct effect of GnRH agonists, vascular abnormality (Bergh et al., 1988), inflammation, salt retention and lymphatic obstruction (Robbins and Cotran, 2004). It has also been shown that testicular fluids reside in two compartments, the seminiferous tubules and the interstitium (Setchell et al., 1994). Seminiferous tubule fluid is produced by the Sertoli cells, and its volume is regulated by reabsorption in the efferent ductules (Turner et al., 1984). Interstitial fluid originates primarily from the testicular vasculature, and its volume is influenced by changes in blood flow, vascular permeability, physiological osmotic pressure differences, and lymphatic drainage (Bergh et al., 1988). Testicular interstitial fluid production has been shown to increase after radiation (Laporte et al., 1985) and increase in volume after the administration of drugs such as busulfan (Udagawa et al., 2001), procarbazine (Delic et al., 1986) and dibromochloropropene (DBCP) (Kluwe, et al., 1983). Similarly it has been observed that blockage of the efferent ducts by cells sloughed from the germinal epithelium or the efferent ducts themselves can lead to an increase in testis weight due to fluid accumulation (Hess et al., 1991; Nakai et al., 1993), an effect that could offset the effect of depletion of the germinal epithelium on testis weight.

Generally, artesunate exerts its anti-malarial activity by generation of reactive, alkylating, oxygen free radicals from its endoperoxide bond (Maggs et al., 1988) leading to lipid peroxidation (Robert et al., 2001). The accumulation of lipid peroxides is toxic to the membrane structure, leading to a change in permeability and to disintegration of cellular organelles (Muler and Ohnesorge, 1982). Based on these reports therefore, it is possible that the artesunate used in this study may have acted in a similar manner to induce the testicular weight gain observed in the double dose artesunate treated group.

Moreover, the damage to the testes can be detected as a weight change only at doses higher than those required to produce significant effects in other measures of gonadal status (Berndtson, 1977; Foote et al., 1986; Ku et al., 1993). The effects seen in this study might have been due to the ability of the artesunate to induce such effects in the testes of the double dose treated rats. It is uncertain however if these changes are reversible.

CONCLUSION

Our study suggests that artesunate at normal dose has no effect on testicular weight but at double normal dose, it causes a significant increase in testicular weight. It is however uncertain, if these changes are reversible. Thus, there is a need to determine if these observations in wistar rats may be applicable to humans and in this regard, one can say that overdosage in humans may likely produce a reproductive toxicological risk. We therefore recommend...
that further studies be carried out in humans to corroborate these findings and that self-medication involving artesunate should be discouraged.

REFERENCES


