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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Biochemical Implication of Long Term Administration of Halofantrine Hydrochloride (Halfan) on Estradiol Levels of Female Wistar Rats

C.N.C. Ugochukwu[1], P.E. Ebong[2] and E.U. Eyong[2]
[1]Department of Biotechnology, Federal University of Technology, Owerri, Nigeria
[2]Department of Biochemistry, University of Calabar, Nigeria

Abstract: This study determined the influence of doses of halofantrine hydrochloride, a phenanthrene methanol drug used in the therapeutic treatment of malaria on the estradiol levels of female wistar rats. A suspension of the drug at a dose of 0.5ml/kg and 1ml/kg body weight three times at six hourly intervals were administered orally to different groups of mature female rats for 5 and 10 days duration, control groups received similar treatment doses of normal saline. The animals were sacrificed on the 5th and 10th day after drug administration by cervical dislocation. Whole blood samples were collected for white blood cell count. From the plasma, the hormonal level was determined by radio-immunoassay and the activities of AST, ALT and ALP were also determined. The level of estradiol following 5 and 10 days treatment was higher significantly ($p<0.05$) in both groups compared to the control. The activities of ALT, AST and ALP increased significantly ($p<0.05$). The white blood cell count also increased in a dose dependent manner. These findings suggest that the drug might have some hepatotoxic effects.

Key words: Halofantrine hydrochloride, malaria, estradiol levels, female wistar rats, radio-immunoassay

Introduction

Malaria is the most prevalent of all tropical diseases causing many deaths. About two hundred million people throughout the world are known to suffer from this disease each year. Out of this number, children below the age of five constitute about two million deaths (Halfan Product Data, 1988).

With its attendant high mortality rate, the quest for malarial eradication has received an added attention although there is still no immediate prospect of substantial improvement. In recent years it would appear that the malaria and parasites have made greatest advances than those in malaria chemotherapy (Howells, 1982). The resistance of *P.falciparum* to most of the anti-malarial drugs is spreading. This is due not only to the remarkable adaptability of the parasite, but also to man's own misuse and overuse of drugs for prophylaxis and for inadequate routine treatment of undiagnosed fevers in endemic areas. The spread of drug-resistant *plasmodium falciparum* has encouraged the need to develop new anti-malarial compounds. Chloroquine resistance is now a widespread phenomenon leading to many other drugs used for acute malaria being of limited use.

The 1960s saw the appearance of chloroquine resistance which was suffered by the US troops during the Vietnam War. This stimulated the huge efforts of the Walter Reed Army Institute for Research (WRAIR) Washington DC to develop new compounds for the treatment of malaria. As a result of the research, a compound was tested in humans and found to be effective against strains of P. faciparum which were resistant to chloroquine. Halofantrine hydrochloride was produced in research collaboration with Smith Kline French and WRAIR and it goes with the trade name 'Halfan' (Halfan Product Data, 1988).

Clinical field trials have shown that Halfan has high efficacy and is effective for treatment of falciparum malaria in areas endemic for multi-drug resistance. It cured 88% of the patients who received three doses of 500mg at 6-hourly interval which compared favourably with mefloquine (Ambroise-Thomas *et al*., 1986). The rapid parasite and fever clearance times were also comparable with those seen with mefloquine. Although it has short half life in plasma and risk of developing resistance is reduced. It known to cross the placenta and thus when used in pregnancy and lactation, Halfan has been shown to be embryotoxic but not teratogenic in animal tests (Parkinson *et al*., 1989). Halfan is therefore contraindicated during pregnancy unless benefits are thought to outweigh risk.

It has been shown that the gonads are affected by series of factors like exposure to certain types of drugs and physical agents, irradiation and hypoxia (Heywood and Wardsworth, 1980). Some anti-malarial drugs are much more abused and some have been found to be teratogenic. For example, pyrimethamine has been implicated in several forms of skeletal anomalies when administered to pregnant rats in doses higher than the therapeutic dose (Akpaffiong *et al*., 1986; Akpan *et al*., 1989).

Studies on reproductive and developmental toxicity carried out on male rats using Halfan showed that there was no drug related effects on male fertility or

reproductive performance in low or mid dose groups (Reno, 1982). The epididymus and seminal vesicles were organs of toxicity in the high dose group. Oral administration of excess doses of 80mg/kg Halfan produced scarring and necrosis of the skin (Reno, 1982).

The anti-malarial activity of the phenanthrene methanols was first recognized during the World War 11 (Wiselogle, 1946). The problem of drug resistance experienced in the 1960's and the failure of measures taken to control malaria infection prompted the Walter Reed Army Institute of Research (WRAIR) in Washington DC to discover this drug. Collaboration between WRAIR, Smith Kline and French began in 1983 and World Health Organization (WHO) started conducting clinical trials in Zambia and Columbia in 1987 (WHO, 1988).

It is highly active against multi-drug resistant strains of P. falciparum in vitro and clinical studies have proved its clinical efficacy in humans against such strains. Halfan is used therapeutically in the treatment of malaria and not as a prophylactic agent as this could encourage the development of resistance.

Halfan has the following chemical and structural formulae:

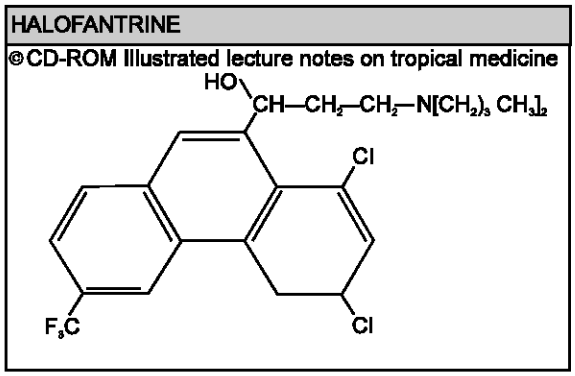

Fig. 1: Chemical structure of halofantrine (Source: CD-ROM Lecture Notes on Tropical Medicine)

3-dibutylamino-1-(1,3-dichloro-6-trifluromethyl-9-phenanthyl)-propan-1-ol hydrochloride. While its molecular formula is $C_{26}H_{30}Cl_2F_3NO.HCl$, with a molecular weight of 536.90.

Halofantrine has a relatively short half-life, much shorter than other anti-malarials, is therefore less likely to develop resistance with this drug (Halfan Product Data, 1988). Most of the drug is eliminated or excreted in the faeces (50%) with very little in the urine (about 1%), suggesting that majority of the drug is not absorbed. Some biliary excretion and enterohepatic recirculation may occur (Halfan Product Data, 1988).

Extensive research has been carried out on this drug, the chemical properties and mode of action can be found in literatures. This study was therefore aimed at studying the effects of halofantrine hydrochloride on the estradiol level, white blood cell counts and the serum enzyme levels of female wistar rats.

Table 1: Effect of 5 and 10 days administration of normal saline and Halfan on estradiol levels female wistar rats

Treatment of group	No. of days	Estradiol level (mg/l)
Group 1 (control)	5	118±4.2
Group 11 (therapeutic dose)	5	78±11.3
Group 111 (toxic dose)	5	177±9.9
Group 1 (control)	10	120±4.9
Group 11 (therapeutic dose)	10	112±5.7
Group 111 (toxic dose)	10	208±2.8

Materials and Methods

Twenty two female albino rats of wistar strain weighing 170-250g obtained from the animal house of the Department of Biochemistry, University of Calabar, Nigeria were used for this research. They were maintained at a temperature range between 25-30°C and were provided with water and feed *ad libitum*.

Halofantrine hydrochloride manufactured by Smith Kline and French Laboratories in suspension form, bought from Rufus Obi Chemist, Aba, Nigeria was administered to the rats on the basis of per kg body weight of the animal, after initial weight. This was done with the aid of an orogastric tube attached to needle and syringe. 5 mL of the suspension contains 100mg halofantrine hydrochloride. The animals were divided into three groups, the first group being the control group, the second and third were the therapeutic dose and the toxic dose groups respectively. These groups were further divided into two sub-groups for the purpose of drug administration for short term (5 days therapeutic dose) and long term (10 days toxic dose).

The control groups were treated with oral administration of physiological (normal) saline (0.9% sodium chloride) solution. The therapeutic dose group was given a dose of 0.05ml/100g (0.5ml/kg body weight) of the drug three times at six hourly intervals giving a maximum dosage of 1.5ml/kg for five days, while the toxic dose group was administered with a dose of 0.1ml/100g (1ml/kg body weight) three times at six hourly intervals giving a maximum dosage of 3ml/kg.

The control group and the treated groups (therapeutic dose and toxic dose) were weighed and sacrificed after five and ten days of drug administration by cervical dislocation. Blood samples were collected both for white blood cell count and enzyme assays, while plasma samples were collected and stored at-20°C for hormonal (estradiol) assay using redio-immunoassay method.

Statistical analysis: One-way analysis of variance was used for data analysis in experimental groups and student't' test was also used to analyze data where

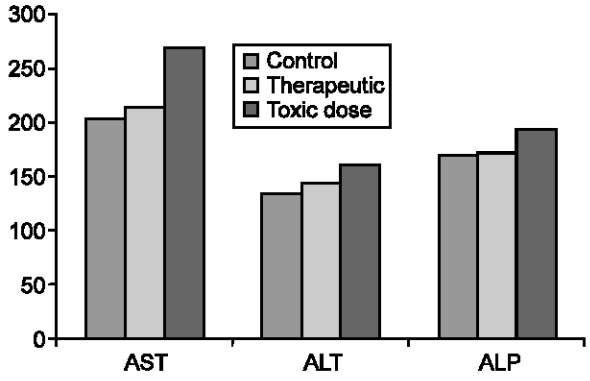

Fig. 2: Effects of Halfan on the levels of AST, ALT and ALP in the serum of female wistar rats treated for 5 and 10 days

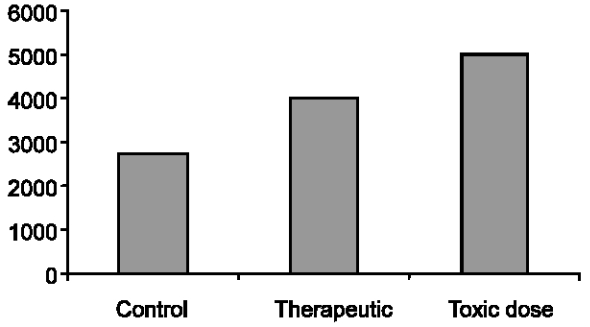

Fig. 3: Effects of Halfan on the white blood cell count of female wistar rat treated for 5 days

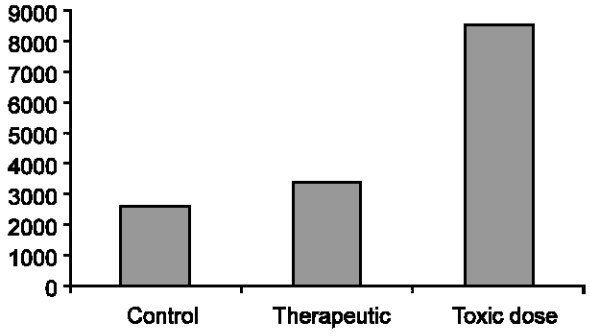

Fig. 4: Effects of Halfan on the white blood cell count of female wistar rat treated for 10 days

probability level of confidence (P) was taken to be significant at $p<0.05$. Experimental data was presented as mean±standard deviation.

Results and Discussion

Hormonal profile: From the table above, it can be seen that the group treated for 5 days with therapeutic dose of 0.5ml/kg body weight of halfan (78±11.3) showed no significant difference from the control group (118±4.2), while there was a significant increase ($p<0.05$) in estradiol levels in the group treated with the toxic dose of 1ml/kg body weight of halfan (177±9.9).

Then following a ten-day treatment of the animals with 0.5ml/kg body weight of halofantrine hydrochloride (halfan), differences in estradiol levels were observed. The control group had a level of 120.5±4.9mg/l, which was higher compared to the therapeutic dose group (112±5.7mg/l). The toxic dose of 1ml/kg body weight of halfan increased significantly to 208±7.8mg/l.

Halofantrine hydrochloride caused significant increases in the levels of serum enzymes studied (Aspartate Transferase, Alanine Transfarase and Alkaline Phosphatase) using the control, therapeutic and toxic group doses of 0.5ml/kg body weight. Aspartate Transferase (AST) increases was very significant compared to Alanine Transferase (ALT) and Alkaline Phosphatase (ALP) at the toxic dose point (Fig. 2).

There were significant increases in the white blood cell count of the rats after 5 and 10 days of drug administration (Fig. 3 and 4). This shows that Halfan causes an increase in the white blood cell count of female wistar rats.

A number of factors such as genetic, environmental and psychological factors could affect the ovary like any other organ in the body. Emotion and stress have been found to affect both menstrual as well as ovarian cycles as this may delay or prevent menstruation (Singh and Paduanadhan, 1978). Drugs constitute a substantial environmental factor, which could affect ovarian hormones. Hormones play very important roles in the body of animals, which includes the maintenance of hormonal body homeostasis as well as in the reproductive life of animals. Female sex hormones have particular roles in the development of follicles and ovulation. Estradiol is needed for the maintenance of the normal menstrual cycle. A study carried out by Adjene and Agoreyo (2003) shows that halofantrine hydrochloride caused reduction in follicle size and cytoplasmic vacuolation in the ovary of the rats. These findings are in agreement with the report of Donham *et al.* (1990) on the effect of monosodium glutamate on the ovary, in which the ovaries were characterized by abundant interstitium, arrested follicular development, fibrotic ovaries and permanent sterility. Studies also carried out with some laboratory animals revealed the extent to which various substances such as hormonal defect affect the reproductive system.

The results showed that following 5 and 10 days treatment with 0.5ml/kg and 1ml/kg body weight of halfan, there was significant increase ($p<0.05$) in estradiol levels in both groups. The results therefore appear to suggest that halfan may be inducing the steroidgenic enzymes thereby causing high levels of estradiol. Espay (1980) also found that pre-ovulatory gonadotropic surge may induce mammalian ovulation of initiating.

Inflammatory processes in the wall of mature ovarian follicle. This would influence both endocrine balance and reproductive activities, thereby affecting both anatomical and physiological functions of the ovary (Adjene and Agoreyo, 2003). Evidence from female users of this drug has shown that the drug administered at a normal human therapeutic dose induces menstrual flow, which could probably be attributed to increase in estradiol levels. However, more work that is detailed needs to be done to confirm this proposition. Conversely, reproductive and developmental toxicity studies carried out on male rats using Halfan showed no drug related effects on male fertility or reproductive performance in low or mid dose groups (Reno, 1982), while oral administration of excess doses of 80mg/kg Halfan produced scarring and necrosis of the skin (Reno, 1982).

It was discovered from this study that halofantrine hydrochloride caused a significant increase in the white blood cell counts of the treated wistar rats. This could signify toxicity of the drug on the bone marrow of the rats, the increase in the white blood cell counts indicates a pathological condition. The drug was also discovered to cause increases in the serum enzyme levels in the experimental rats, suggesting a possible hepatotoxicity of this drug. However, other parameters of liver function were not assayed in this study, but this preliminary study appears to indicate that overdose of halofantrine hydrochloride may be hepatotoxic.

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