Histopathological And Biochemical Effects Of Chloroquine Phosphate On The Testes Of Male Albino Wistar Rats

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Citation

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Abstract

Histolpathological and biochemical effects of chloroquine phosphate were investigated. Rats weighing 180-200g selected from the animal stock of Anatomy department, University of Calabar, Nigeria were randomly divided into three groups (1, 2 and 3). Groups 2 and 3 received 2mg/kg and 4mg/kg body weights of chloroquine respectively for 4 weeks. Group 1 animals served as control and were given normal saline in place of the drug. All the animals had free access to rat chow and tap water during the period of the experiment. Seminiferous tubules of the treated groups were shrunken compared to control. There was degeneration of the interstitial tissues and associated loss of interstitial cells of Leydig. Poorly developed spermatozoa were lying freely in the lumen of the seminiferous tubules accompanied with cell debris. We observed decrease in the diameter of the seminiferous tubules and development of necrospermia. Sertoli and Leydig cells were found to have regressed in the test groups. Biochemical observations revealed an increase in the tissue activity of alkaline phosphatase and serum glucose concentration. Treatment of experimental animals with 2mg and 4mg/kg body weight significantly reduced the level of DNA and RNA when compared to those of controls. These findings seem to unfold the toxicological implication of chronic application of chloroquine phosphate treatment on male reproductive organ.

INTRODUCTION

Chloroquine is used for the treatment of amoebasis, discoid lupus, erythematosis and rheumatoid arthritis (1) and for the treatment of all species of malaria as well as sensitive Plasmodium falciparum. Other less common uses of chloroquine are amoebic liver abscess, polymorphous light eruption, solar urticaria and chronic cutaneous vasculitis (2). Literature abounds on the adverse effects of chloroquine on tissues (3-5). Adverse reactions include, mental disturbances, bleaching of hair and gastrointestinal symptoms (6). Chloroquine has been misused over the years in Nigeria because of the ease of acquisition and its availability, such that any likely symptoms suggestive of malaria or headache are concluded to be malaria, and in most cases, self diagnosis is usually followed by self medication. Chloroquine has been implicated as an anti-fertility agent (7). Preliminary investigation has shown disruption of the process of spermatogenesis following toxic administration of chloroquine (8). A reduction in Leydig cell population and concomitant decline in plasma testosterone level has also been reported (9).

In the treatment of malaria, chloroquine is given for a short period of time, but in malaria endemic region of tropical Africa, treatment is often repeated in a period as short as 2 weeks due to repeated attack of the sickness (10). Chloroquine therapy may last up to 12 weeks in the treatment of discoid lupus, extra intestinal ameobiasis and rheumatoid arthritis (1). We therefore have considered the toxicity of chloroquine therapy on both histopathological and biochemical evaluation of the testis of male albino Wistar rats.

MATERIALS AND METHODS

Twenty-four (24) mature male albino Wistar rats weighing 180-200g, obtained from the animal house of the Human Anatomy department, Faculty of Basic Medical Sciences, University of Calabar were kept in well ventilated experiment section of the animal house. Animals were randomly distributed into 3 experimental groups of 8 rats each and were acclimatized for 3 days before the commencement of treatment. Group 1 animals served as control, while groups 2 and 3 received daily oral dose of 2 and 4mg/kg body weight of chloroquine phosphate respectively for 4 weeks. Equal volume of normal saline, which served as placebo was given to animals in the control group. Twenty-four hours after the last administration, the animals were anesthetized under chloroform vapour. Blood

samples for sera preparation were collected by cardiac puncture into sterile plan tubes. Sera were separated from the clot by centrifugation at 3000xg for 5 minutes using a bench-top centrifuge (MSE minor, England). Glucose concentrations and ALP activity in the sera were determined immediately thereafter.

The animals were dissected; testes were removed and fixed in 10% buffered formalin ready for histological studies. Trimmed down tissue sample measuring 3mm x 3mm were processed into slides with Haematoxylin and Eosin. Photomicrographs and their interpretations were carried out in the Histology Laboratory of the Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar. Analysis of serum glucose was by standard method (11). Serum alkaline phosphatase activity was determined by the method of REC (12). RNA and DNA levels were determined by the method of Fleck (13) and Williams (14) as modified by Akintowa et al (15). Absorbance measurements were done using UV- spectrophotometer. Statistical analysis of data employed the standard analysis of variance (ANOVA). Paired comparison was done using the student's t-test and values of P< 0.05 were considered as significant.

RESULTS

Plate 1 shows the results of the histological sections of the seminiferous tubules of the control animals. Germinal epithelial lining of the tubules consists of spermatogenic and Sertoli cells. Numerous spermatozoa were seen lying freely in the lumen of the coiled tubules. Prominent and closely packed polyhedral shaped Leydig cells were also present. (Plate1).

Microscopic examination of the seminiferous tubules of both experimental groups showed loose arrangement. The seminiferous tubules of group 2 animal contained cell debris (Plate2). In the group 3 animals, the tubules appeared as isolates throughout the testis. Spermatogenic and Sertoli cells were observed to have been degenerated. Total loss of the interstitial tissues and cells of Leydig was also observed. Poorly developed spermatozoa were seen in the lumen of the seminiferous tubules of the treated animals. These effects were pronounced throughout the testis (at the peripheral and central regions) (Plate 3).

The result of the biochemical investigation is reported in table 1. the results did not show any significant (P< 0.05) increase in the serum glucose concentration and alkaline phosphatase activity. The values for DNA and RNA were significantly (P<0.05) decreased by the administration of chloroquine phosphate, with the decrease being dose dependent. Statistical analysis of results was done using student's t-test.

Figure 2

Parameter	Group A	Group B	Group C	Treatment effects
ALP(mol/l)	103.30± 0.73	106.30±1.09	108.50±2.18	NS
DNA(mg/ml)	307.70±7.43	241.70±4.45	296.40±6.50	*
RNA(mg/ml)	130.02±4.24	108.14±2.07	86.13±5.37	*
PG (mmol/l)	5.50±0.74	6.15±0.55	6.98±0.89	NS

NS- Not significant
*- significant (P<0.05)

ALP-Alkaline phosphatase
DNA -Deoxyribonucleic acid
RNA -Ribonucleic acid
PG -Plasma glucose

{image:2}

Plate 1: Micrograph of control rats showing well defined spermatogenic cells,

Spermatogonia, Sertoli cells and Leydig cells (H & E X 400).

Plate 2: Micrograph of 2mg/kg chloroquine treated rats showing shrunken

Seminiferous tubules, reduced spermatocytes and spermatids (H &EX400).

Plate 3: Micrograph of 4mg/kg chloroquine treated rats showing degenerated

Sertoli and Leydig cells and interstitial tissues (H&EX400).

DISCUSSIONS

The toxic effects of chloroquine on the testis have been documented (7, 9, 16, 17). In the present study, long term treatment with chloroquine for weeks revealed derangement of the seminiferous tubules in the treatment groups. Regression of Sertoli cells may inhibit spermatogenesis since these cells are responsible for the production of androgens binding protein and fluids into the lumen of seminiferous tubules essential for normal spermatogenesis (18). Impairment of Sertoli cells may have caused the inhibition of spermatogenesis and is line with the findings of Ebong et al (9). Total loss of Leydig cells would probably lead to a deficiency in testosterone which might ultimately lead to an arrest in the function of male accessory organs

since it is known to stimulate the growth and activity of accessory sex organs and secondary sexual characteristics (19). The loose arrangement observed in the experimental groups may be due to reduction in the diameter of the tubules. Lumen of tubules that contained cell debris may also be caused by broken sperm tails, degeneration of spermatocytes and spermatids. Loss of cells of Leydig would inhibit the production of testosterone and its function, since testosterone has been shown to enhance and maintain the motility and fertility power of sperms (20). Adegoke et al (21) had reported a decline in sperm motility of rats treated with chloroquine. Our findings throw more light on this, since the lack of production of testosterone needed for the enhancement and maintenance of motility of sperm has reduced. Following degeneration of Leydig cells, damage to the seminiferous tubules occurred at both regions (peripheral and central). During chloroquine therapy, the observed uniform response to the drug may be due to its ability to penetrate the blood-testis barrier. Our result is of special interest because it contradicts earlier report which stated that damage to the seminiferous tubules is at the peripheral region of the testis than the central region due to the tightness of the blood-testis barrier (22).

The serum activities of ALP were elevated in both experimental groups in this study. This elevation in ALP activities may be due to damage caused to spermatocytes and sperms after chronic chloroquine therapy. This is in accordance with earlier findings which showed an elevation in ALP activity during endocrine manipulation (23). Elevated activity of ALP may be as a result of cell injury and disturbances of membrane integrity. ALP has been reported to be involved in the transport of metabolites across cell membranes, protein synthesis and secretory activities (24). Prolonged administration of chloroquine led to gradual increase in ALP, which shows that chloroquine may have a consequential effect on membranous components of cells, and may gradually lead to molecular cell death and necrosis.

DNA is synthesized in the nucleus of cells and its physiological function is to determine the growth characteristics of cells and also when or whether these cells divide to form new cells (25). The significant decrease of DNA in experimental animals correlated well with the regressive changes observed in the spermatogenic cells after treatment with chloroquine. The integrity of nuclear DNA is one of the most extensively used biomarkers for cell death (26). Chloroquine has been reported to interfere with cell proliferation through inhibition of DNA replication (27, 28). This interference might be the cause of degenerating nuclei observed in treated animals and might affect the normal functions of the testis involved in spermatogenesis and production of testosterone.

This study indicates the prolonged administration of chloroquine to male Wistar rats induced adverse effects in their testes and its cumulative effect on ALP and DNA which led to inhibition of spermatogenesis. We therefore call for caution of males/ females in the use of chloroquine in their reproductive age.

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