LABORATORY REPORT

Influence of Chronic Administration of Chloroquine on Leydig Cell Integrity and Testosterone Profile of Albino Wistar Rats

PE Ebong,[1] EU Eyong,[1] MU Eteng[1] and CN Ukwe[1]

ABSTRACT

The effect of chronic administration of chloroquine on leydig cells and plasma testosterone level was examined. Twenty-five albino Wistar rats were divided into five groups — A, B, C, D, and E. Group A animals received a normal dose of 0.57mg per kg body weight of chloroquine for 3 days. Groups B, C and D received chronic doses of 0.57mg per kg body weight of chloroquine for 4, 5 and 6 days respectively. Group E animals, which served as control, were administered normal saline. Histological examination of the processed sections of groups B, C and D indicated numerical reduction of the leydig cells when compared with the control group. Group A appeared normal. The basement membrane of the seminiferous epithelium in groups B, C and D were disrupted, leading to the detachment of many spermatocytes. Groups B, C and D recorded reduced level of plasma testosterone when compared with the control group. However, the concentration of plasma testosterone in group A (2.15 ± 1.63μg/ml) and control (2.40 ± 1.48μg/ml) were similar. Chronic administration of chloroquine reduced the number of leydig cells with concomitant reduction of testosterone production. It also disrupted seminiferous epithelium, leading to the detachment of spermatocytes. (*Afr J Reprod Health* 1999; 3(2):97-101)

RÉSUMÉ

L'influence de l'administration chronique de la chloroquime sur les cellules interstitielles du testicule et sur le profil testostérone des rats Wistar albinos. L'effet de l'administration chronique de la chloroquine sur les cellules interstitielles du testicule et sur le testostérone plasmique a été étudié. Vingt-cinq rats Wistar albinos ont été divisés en cinq groupes – A, B, C, D et E. Les animaux du groupe A ont reçu une dose normale de 0,57 mg par kg du poids corporel de la chloroquine pendant trois jours. Les groupes B, C et D ont reçu des doses chroniques de 0,57mg par kg du poids de la chloroquine pendant 4, 5 et 6 jours respecttivement. Les animaux du groupe E qui servaient des cas témoins ont reçu du salin normal. Une étude histologique des sections traitées des groupes B,C, et D a révélé une réduction numérique des cellules interstitielles, comparée au groupe témoin. Le groupe A semblait être normal. La membrane basale de l'épithélium seminifère dans les groupes B,C, et D a été rompue aboutissiant au décollement de nombreux spermatocytes. Les groupes B,C et D ont enregistré un nieveau réduit de testosterones en comparaison avec le groupe témoin. Pourtant, la concentration de testostérone plasmique dans le groupe (2,15 ± 163 μg/ml) et dans groupe témoin (2,40 ± 1,48μg/ml) étaient similaires. L'administration chronique de la chloroquine a diminué le nombre des cellules interstitielles avec une réduction concomitante de la production de la testostérone. Elle a également rompu l'épithéluim seminifère, ce qui a abouti au détachement des spermatocytes. (*Rev Afr Santé Reprod* 1999:3(2):97-101)

KEY WORDS: Chloroquine, leydig cells, testosterone, seminiferous epithelium

[1]*Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar*

Correspondence: *P E Ebong, Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar*

Introduction

Malaria is widespread in tropical and subtropical countries. It is caused by infection with parasites of the genus *Plasmodium*, transmitted through the bite of an infested female anopheline mosquito. It is characterised clinically by recurrent paroxysms of chills, fever and sweating, and it affects hundreds of millions of people. It is estimated that over 2 billion people (over 40% of the world's population) living in more than 100 countries are exposed to the risk of malaria, and that 270 million of these are infected with malaria parasites. About 110 million clinical cases occur annually with about 1 million deaths yearly.[1]

Chloroquine is a synthetic derivative of 4-aminoquinoline. It is presently one of the drugs of choice for the control and cure of malaria[2] and it is highly effective against the erythrocytic parasite. Although it does not eliminate the exoerythrocytic forms of *P. vivax*, it effectively terminates the clinical attack by this parasite.[3] Apart from its anti-malarial activity, chloroquine proves useful in the treatment of gastrointestinal amoebiasis, fluke infections, giardiasis, systemic lupus erythematosis, discoid lupus erythematosis and rheumatoid arthritis.[4]

Literature abounds on the adverse effects of chloroquine on tissues.[3,5-10] Adverse reactions to chloroquine include rashes, itching and other allergic reactions, mental disturbances, bleaching of hair and gastrointestinal symptoms.[11]

Preliminary investigation by Ihejirika[12] had shown a reduction in the number of leydig cells following chronic administration of chloroquine. Okanlawon *et al*[13] have also reported the disruption of the process of spermatogenesis following chronic toxic administration of chloroquine. The present study was designed to correlate leydig cell reduction with levels of plasma testosterone following chronic administration of chloroquine to rats.

Materials and Methods

Drug

Chloroquine phosphate (May and Baker, Lagos, Nigeria) was purchased from Kamel Pharmacy, Calabar, Nigeria.

Animal

Twenty-five male Wistar rats weighing 180–240g were obtained from the animal house of the Biochemistry Department, University of Calabar. The animals were divided into five groups (A, B, C, D and E) of five animals each and acclimatised. They were housed in well-ventilated cages and allowed normal daylight cycles. The temperature of the animal house was 26±2°C. They were fed normal rat pellets (Pfizer Feeds (Nig) Ltd., Lagos, Nigeria) and water *ad libitum*.

Drug Administration

The drug was administered intraperitoneally to groups A, B, C and D. Group E, which served as control, was administered normal saline intraperitoneally. Groups A, B, C and D were daily administered 0.57mg/kg body weight of chloroquine in 0.1ml normal saline for 3, 4, 5 and 6 days respectively. Group E received 0.1ml of normal saline for 6 days. At the end of the experimental period, the rats were anaesthesised in a chloroform chamber, dissected and blood samples obtained through cardiac puncture into clean labelled heparinised sample bottles. The blood samples were immediately centrifuged, the plasma obtained into clean sample bottles and stored in a freezer (–20°C) for subsequent hormonal assay.

Histological Studies

The testes of the rats in each group were removed, washed with physiological saline and immediately fixed in 10% buffered formalin solution, processed, embedded in paraffin wax and cut into ribbon sections of 5µm thickness. The sections were stained with hematoxylin and eosin and mounted, using DPX, onto a light microscope slide for histological examination with a light microscope.

Hormonal Assay

Plasma levels of testosterone were assayed in duplicates by radioimmunoassay at Raykon Laboratories, Lagos, Nigeria. The sensitivity of the assay was reported as 0.85ng/ml.

Statistical Analysis

Pairwise comparison of plasma testosterone level was done using the students' *t*-test and the analysis of variance (ANOVA). Values of $p<0.05$ were regarded as significant.

Results

Histological Observations

The ultra-structural integrity of sections of the testes of animals in all groups was normal with irregularly closely packed polyhedral shaped leydig cells, which appeared elongated when viewed individually. Numerically, the leydig cells appeared to be fewer in the testes of groups B, C and D, which were treated with 0.57mg/kg body weight of chloroquine for 4, 5 and 6 days respectively. Group A animals, which received the dose for a shorter duration, showed no observable numerical reduction as compared with the control group E animals. Marked disruption of the inter-tubular stroma together with the seminiferous epithelium of the testes in groups B, C and D, as compared with the control and group A animals, was also observed (Figures 1, 2, 3 and 4).

Figure 1 **Photomicrograph of testes showing reduction of leydig cells in Group B treated rats. H and E × 400**

Figure 2 **Photomicrograph of testes showing reduction in Group C treated rats. H and E × 400**

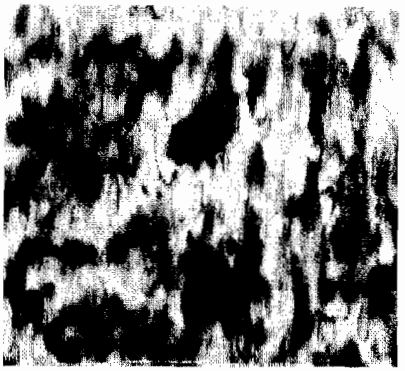

Figure 3 **Photomicrograph of testes showing reduction of leydig cells in Group D treated rats. H and E × 400**

Figure 4 **Photomicrograph of testes showing reduction of leydig cells in Group E (control) rats. H and E × 400**

Testosterone Level

Radioimmunoassay analysis of the plasma testosterone level showed a reduction in testosterone level in groups B, C and D when compared with the control and group A animals. This reduction in testosterone level was however not significant ($p>0.05$). The reduction in testosterone level correlated with the numerical decline in leydig cell population observed on histological examination. Table 1 shows the effect of chloroquine on plasma testosterone level of all experimental animals.

Table 1 **Effect of Chloroquine (0.57mg/kg body weight) on Plasma Testosterone Level of Wistar Rats**

Group	Duration of administration (days)	Testosterone level (μg/ml)
A	3	2.15 ± 1.63
B	4	1.21 ± 0.99
C	5	1.16 ± 0.60
D	6	1.12 ± 1.42
E (Control)	7	2.40 ± 1.48

Mean ± SD (n=5)

Discussion

Subsequent to the administration of 0.57mg/kg body weight of chloroquine phosphate to adult male wistar rats, numerical decline in leydig cell population and corresponding decreases in the plasma level of testosterone has been observed to correlate with the duration of drug administration. This numerical decline in leydig cell population may be attributed to the prolonged administration of the drug, which also resulted in the disruption of intertubular stroma culminating in decreases in the level of plasma testosterone that is principally produced by the leydig cells.[14,15] Although the decrease in the level of plasma testosterone was not significant, it may hinder the development and maintenance of male secondary sexual characteristics, leading to reduced sexual capabilities. The observed decline in leydig cell population is consistent with the previous observation of Ihejirika.[12] It has not been conclusively established whether the reduced plasma testosterone level may result from a reduced testosterone production due to the decline in leydig cell population or a direct inhibition of testosterone synthesis. Further work is in progress to elucidate this aspect.

Dixon[16] had previously reported that chloroquine reduces male reproductive function. However, these reports lack quantitative information on reproductive toxicities. Investigations by Ashiru *et al*[17] showed the erosion of the leydig cells of rats and a loss of 64% of the mean tubular volume of seminiferous tubules following the administration of a chronic toxic dose of chloroquine for 16 weeks. The result indicates that chloroquine causes a reduction in tubular diameter and probably tubular length. Okanlawo *et al*[13] have also shown that chronic toxic administration of chloroquine for 16 weeks disrupts the process of spermatogenesis and results in an increased number of spermatocytes in a dose-related manner. Similarly, Thomas[18] had earlier reported that chloroquine inhibits spermatozoa *in vitro*, thus having an inhibitory effect on sperm motility. From the present study, the observed decline in leydig cell population and reduced level of plasma testosterone may ultimately lead to the inhibition of spermatogenesis. However, the presence of spermatogonia in all seminiferous tubules points to the fact that there may not be a total suppression of spermatogenesis, probably due to the duration of drug administration. Okanlawon *et al*[13] had earlier suggested that the suppression is most likely reversible although the mechanism of action is not fully understood. The presence of spermatocyte may also result from the disruption of the basement membrane of the seminiferous epithelium, resulting in the detachment of many spermatocytes from the epithelial line, culminating in an increase in the number of spermatocytes. This is consistent with the results obtained by Ashiru *et al*,[17] Ihejirika[12] and Okanlawon *et al*[13] on chronic toxic administration of chloroquine.

From the foregoing, it is evident that chronic chloroquine administration results in an alteration of testicular morphology and a concomitant decline in plasma testosterone levels. It has not been

ascertained which preceeds the other, but this effect may result in the inhibition of the development and maintenance of the secondary sexual characteristics, leading to reduced virility and consequent infertility. The results of this study suggest caution in the use of chloroquine especially in rural areas where it is sold across the counter.

REFERENCES

1. World Health Organistion. Practical chemotherapy of malaria. *Techn. Rep. Ser.* 1990; No. 805, Geneva.
2. Bisseru, D. Chloroquine resistance in Africa. *Postgraduate Doc* (Africa) 1985; 7:58–64.
3. Goth A. *Medical Pharmacology.* 11th Edition. Toronto: The C. V. Masby Company St. Louis, 1984, 678–695.
4. Huskinsson EE. Penicillamine and drugs with specific action on rheumatoid arthritis. *Handb Exp Pharmacol* 1979; 50.399–414.
5. Elueze EI, Edafiogho IO and Osisanya JOS. Chloroquine pharmacology. *Pharmacy World J* 1982; 6:4.
6. Bruce-Chawatt LJ. Lessons learned from applied field research activities in Africa during the malaria eradiction era: applied field research in malaria in Africa, WHO, Geneva, 1984, 19–29.
7. Laurence DR and Benneth PN *Clinical Pharmacology.* 5th Edition. Edinburgh, London and New York, Churchill Livingstone, 1986, 286–291.
8. Ratliff NB, Esther MC, Myes JL *et al.* Diagnosis of chloroquine cardiomyopathy by endomyocardial biopsy. *N Engl J Med* 1987; 316: 191–193.
9. Okpako DT and Aziba PJ. A dual effect of chloroquine on muscle contraction evoked by different agents. *Eur J Pharmacol* 1989; 183 (186): 2429.
10. Obiaoime AW and Brambaifa N. Chloroquine ainoay smooth muscles and sparmogen-induced contraction of the cat trachea. *Proceedings West Afr Soc Pharmac* 1991; 20:43.
11. Rook A, Wilkinson DS and Ebling FJG. *Textbook of Dermatology.* Oxford, 2nd Edition, 1972, 1034.
12. Ihejirika CB. Effect of chloroquine on the leydig cells in the rat testis. B.Sc Thesis, Department of Anatomy, University of Calabar, Calabar, Nigeria, 1992.
13. Okanlawon AO, Noronha CC and Ashiru OA. Increase in germ cell population and seminiferous tubular volume following chloroquine administration — sereological study using the disector method. *Nig J of Physoil Sci* 1992; 8(1–2): 102–106.
14. Dufau ML and Catt KJ. Gonadotrophic stimulation of intestinal cell functions of the rat testes in vitro. *Methods in Enzymology* 1975; 39:252–271.
15. Ebong PE and Peddie MJ. Comparison of the biological activity of gonadotrophin preparations using the steroids secreted by leydig of granulosa cells. *Nig J Physiological Sciences* 1990; 6(1): 57–61.
16. Dixon RC. Toxic responses of the reproductive system. In: Casarett and Doulls toxicology — the basic science of poisons, 3rd Edition (Eds. CD Klassen, MO Amdur and J Doull) New York: Macmillan Pub. Company, 432–477.
17. Ashiru OA, Okanlawon AO and Noronha CC. Application of the point-sample intercepts to the seminiferous tubules: evidence of decreased tubular size following chronic chloroquine administration. *J Scanning Microsc (Scanning)* 1991; Vol. 13 (Suppl.1), in press.
18. Thomas NO. Chloroquine infertility. *Pharm. Wld. J.* 1989; 6(7): 5.