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

PE Ebong, EU Eyong, MU Etieng and CN Ukwe

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 0.57 mg per kg body weight of chloroquine for 3 days. Groups B, C and D received chronic doses of 0.57 mg 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 ± 0.63 μg/ml) and control (2.40 ± 1.48 μg/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

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

1Department 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-aminquinoline. It is presently one of the drugs of choice for the control and cure of malaria² 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 erythematosus, discoid lupus erythematosus and rheumatoid arthritis.⁴

Literature abounds on the adverse effects of chloroquine on tissues.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 Ihejirika¹² 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 Bio-

chemistry 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 anaesthetised 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 0.57 mg/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](image1.png) Photomicrograph of testes showing reduction of Leydig cells in Group B treated rats. H and E × 400

![Figure 2](image2.png) Photomicrograph of testes showing reduction in Group C treated rats. H and E × 400

![Figure 3](image3.png) Photomicrograph of testes showing reduction of Leydig cells in Group D treated rats. H and E × 400

![Figure 4](image4.png) Photomicrograph of testes showing reduction of Leydig cells in Group E (control) rats. H and E × 400

(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.

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

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

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. Okanalwo et al.\(^3\) 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 spermatogenesis \textit{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. Okanalwon et al.\(^3\) 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 Okanalwon et al.\(^3\) 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 precedes 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


17. Ashiru OA, Okanlawon AO and Norouha CC. Application of the point-sample intercepts to the seminiferous tubules: evidence of decreased tubular size following chronic chloroquine administration. J Scanning Mi-
crosc (Scanning) 1991; Vol. 13 (Suppl1), in press.