Morphometric and stereological assessment of the effects of long-term administration of quinine on the morphology of rat testis

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Summary
Background and objectives: Quinine (QU) has been used worldwide in the suppression and treatment of malaria for more than 350 years. The aim of this study was to determine the long-term morphological response of the testis to long-term administration of QU using stereological parameters.
Materials and methods: 64 adult male Sprague-Dawley rats weighing 180-200g were used. The animals were randomly divided into 8 groups of 8 rats each. Every experimental animal had intramuscular QU at a dose of 10mg/kg body weight per day (5 times in a week, with the exception of group 1 animals). Group 1 rats had QU for 1 week (7 days consecutively) and were sacrificed on the last day of injection. Groups 2 and 3 rats had QU for 4 and 6 weeks and were sacrificed at the end of the 4th and 6th week respectively. Group 4, 5, 6 and 7 rats had QU for 8 weeks and were sacrificed at the end of week 8, 12, 16 and 20 respectively. Group 8 animals constituted the controls and had equal volume of distilled water intramuscularly for 8 weeks. All sacrifices were by decapitation. The testes were carefully dissected out, their volumes measured, weighed and histological sections prepared. Morphometric assessment was carried out using the diameter, cross-sectional area, number of profiles per unit area, numerical density and volume density of the seminiferous tubules and the relative and absolute volume of the seminiferous epithelium, stroma and lumen of tubules.

Results: The results showed that there was a general destruction of cells of the seminiferous tubules and the testicular interstitium that persisted even after the discontinuation of QU and to the end of our experiment that lasted 20 weeks.

Conclusion: We conclude that QU has deleterious effect on the seminiferous tubules of Sprague-Dawley rats, though the mechanism of damage is unclear.

Key-words: Quinine, Testes, Seminiferous tubules, Seminiferous epithelium

Résumé
Introduction et objectif: Quinine (QU) a été utilisée dans le monde entier dans la suppression et le traitement du paludisme pendant plus de 330 ans. L’objectif de cette étude était de déterminer la réponse morphologique de longue durée de testicule jusqu’à l’administration de la QU à travers l’utilisation du paramètre stéréologique de longue durée.

Matériels et méthodes: 64 rats adultes sexe masculin Sprague-Dawley d’un poids de 180-200g ont été utilisées. Les animaux ont été divisés en 8 groupes de 8 rats chacun au hasard. Chaque animal expérimental avait eu QU intramusculaire avec une dose de 10mg/Kg poids de corps tous les jours (5 fois dans une semaine, à l’exception des animaux dans le premier groupe). Des rats dans le groupe 1 avaient eu QU pendant une semaine (7 jours consécutivement), et ont été sacrifiés pendant le dernier jour à travers l’utilisation d’une injection. Des rats dans les groupes 2 et 3 avaient eu la QU pendant 4 et 6 semaines et ont été sacrifiés à la fin de la 4ème et 6ème semaines respectivement. Des rats dans les groupes 4, 5, 6, et 7 avaient eu la QU pendant 8 semaines et ont été sacrifiés à la fin de la 8ème et on été sacrifiés a la fin de la 8ème, 12ème, 16ème et 20ème semaines respectivement. Les animaux dans le 8ème groupe avaient constitué le groupe de contrôle et avaient un volume égal d’eau distillée intramusculaire pendant 8 semaines. Tous les sacrifices étaient à travers la décapitation. On avait soigneusement disséqué les testicule, on avait mesuré leur volume, pesé et leurs sections histologiques préparés. L’évaluation morphométrique a été effectuée à travers l’utilisation du diamètre, la superficie section transversale, nombre des profils par unité de la surface, densité numérique et volume densité des tubules séminalérux epithélium, stimat et lumen de tubules.

Résultats: Les résultats ont montré qu’il y a une destruction générale des cellules des tubules séminalérux et l’interstitium testiculaire qui persistait même après la discontinuation de QU et jusqu’à la fin de notre expérience d’une durée de 20 semaines.

Conclusion: Nous concluons disant que QU a un effet nuisible sur les tubules séminalérux des rats Sprague-Dawley, bien que le mécanisme du dégâts soit incertain.

Introduction
Quinine (QU), a quinoline-methanol derived from the bark of the Cinchona tree has been used in the suppression and treatment of malaria for more than three centuries1. Although once superseded by other antimalarials, QU, has again become an important anti-malarial as a result of the widespread development of resistance to chloroquine and other drugs2-3. A number of adverse effects have been reported during QU administration. These include nausea, blurring of vision, hypotension, cardiac arrhythmias, severe central nervous system disturbances, hypoglycaemia, blood dyscrasias, and hypersensitivity reactions4-5.

However, there is a dearth of publication on the effects of QU on the male reproductive system and the few available ones lack detailed morphological analysis on the testes. One study6 reported that QU was effective in inhibiting sperm metabolism and motility while another study7 showed that QU immobilized 100% of human sperms within 20 seconds.

It has also been reported8-9 that while QU induced tail angulation in caudal epididymal sperms of the mice in invitro studies, the spermatogenic epithelium, interstitial endocrinocytes, sustencocytes and spermatogenesis may be sensitive to the toxic effects of QU in invitro studies10.

To determine the effects of QU on the testis it is necc-
ecessary to probe the organ with geometric entities: points, lines, volumes and planes. Meaningful results have been obtained by the use of these probes on isotropic uniform random (vertical) sections. The results obtained show that QU causes a continuous decrease in the mean testicular volume and volume density, diameter and cross-sectional area of the seminiferous tubules during the entire experimental period (20 weeks) in all groups which is not reversed after drug cessation. In contrast the concomitant increase in the number of profiles per unit area and numerical density is reversed after the period of administration. The data suggest that QU is deleterious to seminiferous tubular morphology.

Materials and Methods
Sixty-four adult (6-8 weeks old) male Sprague-Dawley rats weighing between 180 and 200 g were procured from the Animal House of the College of Medicine, University of Lagos. They were allowed to acclimate for two weeks in the Rat Control Room in the Department of Anatomy with an ambient temperature maintained between 26 and 28°C. They were allowed unrestricted access to water and food. The animals were maintained under standard photoperiodic regime of 12 hours of light alternating with 12 hours of darkness. The animals were randomly divided into 8 groups (Groups 1-8) of 8 rats each. Each experimental animal (Groups 1-7) had intramuscular QU (100 mg/kg body weight per day, 5 times a week), with the exception of group 1 animals which had it for only 7 consecutive days). We have chosen this dosage since this is the maintenance dose of QU in human malarial infection11. Group 1 rats had QU for 1 week and were sacrificed at the end of the week. Group 2 rats had QU for 4 weeks and were sacrificed at the end of the 4th week. Group 3 rats had QU for 6 weeks and were sacrificed at the end of the 6th week. Groups 4, 5, 6 and 7 rats had QU for 8 weeks and were sacrificed at the end of weeks 8, 12, 16 and 20 respectively. Group 8 animals served as control and had an equal volume of distilled water (DW) intramuscularly for 1, 4, 6 and 8 weeks and were sacrificed at the end of weeks 1, 4, 6 and 8 respectively. The testes were carefully dissected out, trimmed of all fat, their weight determined and volume measured by water displacement method. Each testis was fixed in 10% formal saline and histological slides prepared. However, prior to embedding, it was ensured that the sections were orientated such that the long axis of the testis was in the horizontal plane. Sections of 5 microns perpendicular to the horizontal were cut. These were designated as 'vertical sections.'

Morphometric and stereological analysis
For each testis, five vertical sections from the polar and the equatorial regions were sampled and the following morphometric parameters (diameter, cross-sectional area, volume density and absolute volume of the seminiferous epithelium, testicular interstitium and seminiferous tubular lumen, number of profiles of seminiferous tubules per unit area of testis, length and numerical density of the seminiferous tubules) determined using a systematic random scheme13.

(a) Diameter of seminiferous tubules
The mean tubule diameter (D) was derived in 25 round transverse sections of seminiferous tubules per animal by taking the average of two diameters, D1 and D2, at right angles where the ratio of D1 / D2 ≥ 0.85.

(b) Cross-sectional area (Ac) of the seminiferous tubules
Cross-sectional area (Ac) of the seminiferous tubules was determined using the equation Ac = πD2/4, where π is equivalent to 3.142 and D the mean diameter of the seminiferous tubules.

(c) Number of profiles of seminiferous tubules in a unit area (Nn)
The number of profiles of seminiferous tubules per unit area was determined using the unbiased counting frame proposed by Gundersen14.

(d) Length density (Ln) of seminiferous tubules
The length density of the seminiferous tubules was obtained by applying the equation for a tube model15. Briefly the length of a structure is represented as profiles of the structure and is sampled uniformly by isotropic area probes. The formula below for estimating length density, Ln, relies on the simple fact that test plates ‘feel’ curve length:

\[ L_n = 2 \times N_n \]

where Nn is the number of profiles per two-dimensional test frame area.

(e) Volume density (Vn) of testicular components
Volume density was determined using the principle of point counting. Briefly the ratio of the number of the points hitting each testicular component was obtained dividing the sum of the points falling on each component by the total number of points counted16. This ratio was multiplied by 100 to give the percentage of volume density.

(f) Absolute volume of each testicular component
The absolute volume of each testicular component was obtained by multiplying its relative volume by the testicular volume obtained by the water displacement method.

(g) Numerical density (Nn) of seminiferous tubules
This is the number of profiles per unit volume and it was determined using the modified Flerodius equation: Nn = Ns / (D + T)17 where, Ns is the number of profiles per unit area, D is the mean diameter of the seminiferous tubule and T the average thickness of the section.

Statistical analysis
Results were expressed as mean ± standard deviation. The means of all the parameters were subjected to statistical analysis using the analysis of variance (ANOVA) and the Scheffe's post-hoc test.

Results
Microscopic examination of the testis showed a general destruction of the interstitium and the seminiferous epithelium of the testes of rats treated with QU (Figures 1-5). This destruction is also apparent 8 weeks after post-injection of QU despite the similarity in the body weight of the experimental and control rats. There were no significant differences in the body weight of the experimental and control animals both at the beginning and end of the experiment (Table 1).
Table 1: The effects of QU on body weight of Sprague-Dawley rats at the end of 8 weeks

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial body weight (g)</th>
<th>Final body weight (g)</th>
<th>Weight difference (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 rats</td>
<td>190.0 ± 10.0a</td>
<td>230.5 ± 15.5</td>
<td>40.8 ± 5.4</td>
</tr>
<tr>
<td>4 rats</td>
<td>191.0 ± 9.0</td>
<td>219.5 ± 14.5</td>
<td>28.9 ± 4.6</td>
</tr>
</tbody>
</table>

**Testicular volume**

The mean testicular volume of the control group was 1.600 ± 0.150ml. The testicular volume of rats injected for 8 weeks showed a steady and significant decline beginning from the first week of administration. This decline in testicular volume continued for the next 12 weeks (40.63% of control) after cessation of QU (Table 2).

**Testicular weight**

The mean testicular weight of the control group (G 8) was 1.611 ± 0.161g while those of the experimental groups (G 1-7) were 1.522 ± 0.154, 1.413 ± 0.138, 0.812 ± 0.087, 0.752 ± 0.082, 0.712 ± 0.074, 0.704 ± 0.069 and 0.651 ± 0.061 g respectively.

**Tubular diameter and cross-sectional area of the tubules**

The diameter and cross-sectional area of the tubules in the control rats were 220.5 ± 18.26 and 38.22 ± 6.31 respectively. There was a gradual decline in tubular diameter (18% of control) and cross sectional area of the tubules (23% of control) over 4 weeks during the administration of the drug which was followed by a sharp decline in tubular diameter (63% of control) and cross sectional area (86% of control) during the next 2 weeks of drug administration (Table 2). During the post-drug administration phase both parameters remain relatively constant.

**Number of profiles of tubules per unit area (Np) and length density (Lp)**

The mean number of tubular profiles per unit area and tubular length density in the control group were 32.50 ± 2.17 x 10^8 um^-2 and 65.04 ± 4.01 x 10^8 um^-2 respectively. QU significantly increased Np (165% of control) and Lp (165% of control) during the period of administration. Both parameters continued to increase (327% of control) 4 weeks post-administration. This was followed by a decline to 95.75 ± 9.55 x 10^8 um^-2 (189% of control) and 187.5 ± 8.92 x 10^4 um^-2 respectively (Table 2).

**Volume density and absolute volume of testicular structures**

The mean percentage volume of the seminiferous epithelium was 75.00 ± 4.51% in the control animals with no significant differences between control and rats treated with QU for 1 and 4 weeks at each time point (73.75 ± 4.18, 72.50 ± 4.02% respectively). The mean seminiferous epithelial volume per testis was 1.200 ± 0.201 ml in the control which steadily and significantly declined to 0.375 ± 0.078 ml by the 8th week of QU administration. The decline continued for another 12 weeks to 0.198 ± 0.099 ml (16.5% of control) after cessation of QU administration (Table 3).

In the control the percentage volume of the tubular lumina in the testis was 12.50 ± 0.91% and the absolute luminal volume per testis was 0.200 ± 0.031 ml (Table 3). There was an initial increase in the mean volume density and absolute volume of the lumina of the seminiferous tubules (14.75 ± 1.08%, 0.221 ± 0.047 ml respectively) at the end of the first week of QU treatment which was followed by a steady and significant (p < 0.05) decline to 6.25 ± 0.55% and 0.047 ± 0.008 ml respectively at the end of the 8th week. However the mean volume density and absolute luminal volume recovered and increased to 30.25 ± 2.79% and 0.212 ± 0.053 ml respectively over 8 weeks post QU-administration period (Table 3).

**Number of tubular profiles per unit volume of testis (Nv)**

The Nv of the tubules increased significantly (P < 0.05) from 14.73 ± 1.50 to 103.00 ± 8.87 x 10^-6 um^-3 (Table 2) during the 8-week of drug administration. This rise continued to 162.80 ± 14.34 x 10^-6 um^-3 in the initial 4-week post administration but declined the next 8 weeks to 111.60 ± 9.76 x 10^-6 um^-3.

Table 2: The effects of quinine on the seminiferous tubules of Sprague-Dawley rats

<table>
<thead>
<tr>
<th>G</th>
<th>TV (ml)</th>
<th>D (um)</th>
<th>Ac (x10^6 µm²)</th>
<th>Np (x10^9 µm²)</th>
<th>Ly (x10^6 um²)</th>
<th>Nv (x10^-6 um³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1</td>
<td>1.500 ± 0.140a</td>
<td>191.58 ± 17.55</td>
<td>28.87 ± 3.37</td>
<td>48.75 ± 4.43</td>
<td>97.55 ± 5.48</td>
<td>24.79 ± 4.65</td>
</tr>
<tr>
<td>G 2</td>
<td>1.400 ± 0.135b</td>
<td>180.50 ± 16.50</td>
<td>25.63 ± 3.18b</td>
<td>56.24 ± 8.56b</td>
<td>112.52 ± 6.76b</td>
<td>30.70 ± 5.89b</td>
</tr>
<tr>
<td>G 3</td>
<td>0.800 ± 0.090b</td>
<td>81.67 ± 8.79b</td>
<td>5.50 ± 1.08b</td>
<td>74.99 ± 7.21b</td>
<td>150.03 ± 7.88b</td>
<td>86.82 ± 7.09b</td>
</tr>
<tr>
<td>G 4</td>
<td>0.750 ± 0.085b</td>
<td>81.33 ± 8.55b</td>
<td>5.23 ± 0.85b</td>
<td>86.24 ± 8.98b</td>
<td>172.52 ± 8.19b</td>
<td>103.00 ± 8.87b</td>
</tr>
<tr>
<td>G 5</td>
<td>0.700 ± 0.087b</td>
<td>81.29 ± 8.79b</td>
<td>5.21 ± 0.90b</td>
<td>138.73 ± 11.12b</td>
<td>277.49 ± 9.34b</td>
<td>362.80 ± 14.34b</td>
</tr>
<tr>
<td>G 6</td>
<td>0.700 ± 0.075b</td>
<td>81.67 ± 9.08b</td>
<td>5.28 ± 0.80b</td>
<td>93.75 ± 9.19b</td>
<td>187.54 ± 8.42b</td>
<td>111.50 ± 9.84b</td>
</tr>
<tr>
<td>G 7</td>
<td>0.650 ± 0.060b</td>
<td>81.65 ± 8.50b</td>
<td>5.26 ± 0.91b</td>
<td>93.75 ± 9.85b</td>
<td>187.55 ± 8.92b</td>
<td>111.60 ± 9.76b</td>
</tr>
<tr>
<td>G 8</td>
<td>1.600 ± 0.150b</td>
<td>220.52 ± 18.26b</td>
<td>38.22 ± 6.31b</td>
<td>32.50 ± 2.17b</td>
<td>65.04 ± 4.01b</td>
<td>14.73 ± 1.50b</td>
</tr>
</tbody>
</table>

**Keys**

G = Groups of rats (G1 to G8)
TV = Testicular volume
D = Diameter of seminiferous tubule
Ac = Cross-sectional area of seminiferous tubule
Np = Number of profiles of seminiferous tubules
Ly = Length density
Nv = Numerical density
Np = Absolute volume
### Table 3: Effects of quinine on the seminiferous epithelium, lumen and interstitial stroma of Sprague-Dawley rats

<table>
<thead>
<tr>
<th>G</th>
<th>Seminiferous epithelium</th>
<th>Stroma</th>
<th>Lumen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vv (%)</td>
<td>AV (ml)</td>
<td>Vv (%)</td>
</tr>
<tr>
<td>Gl</td>
<td>73.75 ± 4.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.106 ± 0.191</td>
<td>11.50 ± 0.85</td>
</tr>
<tr>
<td>G2</td>
<td>72.50 ± 4.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.015 ± 0.165</td>
<td>16.50 ± 1.17</td>
</tr>
<tr>
<td>G3</td>
<td>50.00 ± 3.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.400 ± 0.099&lt;sup&gt;p&lt;/sup&gt;</td>
<td>43.75 ± 3.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G4</td>
<td>50.00 ± 3.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.375 ± 0.078&lt;sup&gt;p&lt;/sup&gt;</td>
<td>43.75 ± 3.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G5</td>
<td>41.25 ± 3.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.289 ± 0.065&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.50 ± 2.97&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G6</td>
<td>30.25 ± 2.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.212 ± 0.053&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.50 ± 3.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G7</td>
<td>30.50 ± 2.90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.198 ± 0.099&lt;sup&gt;b&lt;/sup&gt;</td>
<td>39.00 ± 3.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G8</td>
<td>75.00 ± 4.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.200 ± 0.201&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.50 ± 0.97&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Keys**

- *Vv* = Volume density
- *AV* = Absolute volume

*<sup>a</sup>* = Mean ± standard deviation
*<sup>b</sup>* = p < 0.05

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**Discussion**

We have used simple but precise stereological parameters to determine the effect of QU on the 3-dimensional properties of the testis. Stereology offered a set of practical tools which can derive 3-dimensional geometry and topological parameters from measurements made on 2-dimensional sectional images. The present study
demonstrated a continuous decrease in mean testicular volume, diameter and cross-sectional area of seminiferous tubules and volume of the seminiferous epithelium in rats administered QU for 8 weeks. While the mean testicular volume and volume of seminiferous epithelium continued to decrease after cessation of treatment for another 12 weeks, the diameter and cross-sectional area of the tubules remained relatively constant. In contrast, QU administration produced a concomitant increase in the mean number of profiles per unit area, length and numerical density of seminiferous tubules during the 8 weeks of treatment. Although all these latter parameters continued to increase during the next 4 weeks post-administration this was followed by a decline that did not approach normal values during the next 4 weeks (Table 2).

The continuous decrease in mean testicular volume, the relative and absolute volume of the seminiferous epithelium in rats administered QU for 8 weeks suggest that the normal morphology of the germinal epithelium (responsible for the unique composition of the luminal fluid) is compromised. This is in concert with the significant reduction (p < 0.05) in the tubular lumen induced by QU which also suggests a loss of fluid secretion into the lumen by the Sertoli cells as these cells continuously secrete luminal fluid into the seminiferous tubules. The fact that this unique composition of the luminal fluid is an essential prerequisite for the process of spermatogenesis suggests that QU compromises the process of spermatogenesis.

It has been reported that QU is a wide spectrum ion blocker. It may be postulated that QU disrupts the ion channels leading to a disturbance in ionic equilibrium across membranes and ultimately to a disturbance in cellular morphology. As shown in the current study, experimental disruption of tubular morphology is also accompanied by a general destruction of testicular interstitial and spermatogenesis. Could QU be acting via a reduction in the synthesis of testosterone from the Leydig cell which is responsible for the integrity of the germinal epithelium? Although the relative and absolute volume of the testicular interstitium significantly increased from the 6th week of QU treatment, the interstitium was almost entirely replaced by connective tissue stroma. The disruption of the synthesis and secretion of testosterone by the Leydig cells in the testicular interstitium could also have led to disturbances in the regulation of fluid dynamics of the testis. Presently we do not know whether QU affects testicular and/or plasma testosterone level since radioimmunoassay studies were not carried out.

The disruption of spermatogenesis may also arise from a disturbance in the functions of Sertoli cells with or without a disturbance or disruption in the physiology and/or morphology of the blood-testis barrier. Also alterations in the function of Sertoli cells, either directly or resulting from changes in paracrine signals from the seminiferous tubules could contribute toward the expression of toxicity. Since the germ cells develop in the microenvironment provided by the Sertoli cells, it is possible that the full control of germ cell differentiation probably mediated by the Sertoli cell is disrupted by QU as chloroquine has been demonstrated to significantly decrease the secretion of transferrin by rat Sertoli cells. It is also probable that the mechanism of QU-induced testicular damage is via inhibition of protein synthesis as QU has been demonstrated to form hydrogen bonded complex with double-stranded DNA thereby inhibiting strand separation, transcription, and protein synthesis both in Plasmodium falciparum and lymphocytes. Taken together, morphometric analysis shows that QU induces a progressive destruction of the seminiferous tubules and partial recovery occurred 12 weeks after cessation of QU treatment.

Although none of our propositions on the pathophysiology could be fully substantiated now, they are the subjects of on-going research projects in our department.

Conclusion

The present study has provided quantitative evidence that therapeutic doses of QU are capable of inducing cell degeneration in both the seminiferous epithelium and testicular interstitium in Sprague-Dawley rat. This cytotoxic effect may not be completely reversible (at least not within 12 weeks of the discontinuation of QU). However, further work is needed to elucidate the mechanism of damage and to explore the prospect of QU as a male contraceptive.

References


