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 350 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 4ième et 6ième 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 8ième et on été sacrifies a la fin de la 8ième, 12ième 16ième et 20ième semaines respectivement. Les animaux dans le 8ième 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 séminiferroux et volume relatif et absolu de séminiferroux épithelium, stimat et lumen de tubules,
Résultats: Les résultats ont montré qu'il y a une destruction générale des cellules des tubules séminiferroux et l'interstitium testiculaire qui persistait même après la discontuation 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 séminiferroux des rats Sprague-Dawley, bien que le mécanisme du gégâts 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 centuries[1], 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 drugs[2,3]. 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 reactions[4,5].

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 testis. One study[6] reported that QU was effective in inhibiting sperm metabolism and motility while another study[7] showed that QU immobilized 100% of human sperms within 20 seconds.

It has also been reported[8,9] that while QU induced tail angulation in caudal epididymal sperms of the mice in *invitro* studies, the spermatogenic epithelium, interstitial endocrinocytes, sustenocytes and spermatogenesis may be sensitive to the toxic effects of QU in *invivo* studies[10].

To determine the effects of QU on the testis it is nec-

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essary 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 sminiferous 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 200g were procured from the Animal House of the College of Medicine, University of Lagos. They were allowed to acclimatize 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 (10mg/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 infection[11,12]. 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% formol-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 scheme[13].

(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, D_1 and D_2 at right angles where the ratio of $D_1/D2 \geq 0.85$.

(b) Cross-sectional area (Ac) of the seminiferous tubules

Cross-sectional area (Ac) of the seminiferous tubules was determined using the equation $Ac = \pi D^2/4$, 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 (N_A)

The number of profiles of seminiferous tubules per unit area was determined using the unbiased counting frame proposed by Gundersen[14].

(d) Length density (L_V) of seminiferous tubules

The length density of the seminiferous tubules was obtained by applying the equation for a tube model[15]. 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, L_V, relies on the simple fact that test planes 'feel' curve length:

$$L_V = 2 \times N_A$$

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

(e) Volume density (V_V) 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 counted[16]. 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 (N_V) of seminiferous tubules

This is the number of profiles per unit volume and it was determined using the modified Floderus equation: $N_V = N_A/(D + T)$[17] where, N_A 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

	Group 8 rats	Group 4 rats
Initial body weight (g)	190.0 ± 10.0[a]	191.0 ± 9.0
Final body weight (g)	230.5 ± 15.5	219.5 ± 14.5
Weight difference (g)	40.8 ± 5.4	28.9 ± 4.6

Testicular volume

The mean testicular volume of the control group was 1.600 ± 0.150 ml. 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 ± 0.161 g 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 220.52 ± 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 (N_A) and length density (L_V)

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 N_A (165% of control) and L_V (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 93.75 ± 9.85 x 10^{-8} um^{-2} (189% of control) and $187.5 5 \pm 8.92$ x 10^{-8} um^{-2} respectively (Table 2).

Volume density and absolute volume of testicular structures

The mean percentage volume of the seminiferous epithelium was $75.00 \pm 4.51\%$ 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, $72.50 \pm 4.02\%$ 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 lumen in the testis was $12.50 \pm 0.91\%$ 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 lumen of the seminiferous tubules ($14.75 \pm 1.08\%$, 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 $6.25 \pm 0.55\%$ 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 $30.25 \pm 2.79\%$ 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 (N_V)

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

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

G	TV (ml)	D (um)	Ac ($\times 10^3 \mu m^2$)	N_A ($\times 10^{-8} \mu m^{-2}$)	L_V ($\times 10^{-8}$ um^{-2})	N_V ($\times 10^{-10} \mu m^{-3}$)
G1	1.500 ± 0.140[a]	191.58 ± 17.55	28.87 ± 3.37	48.75 ± 4.43	97.55 ± 5.48	24.79 ± 4.65
G2	1.400 ± 0.135[b]	180.50 ± 16.50	25.63 ± 3.18[b]	56.24 ± 8.56[b]	112.52 ± 6.76[b]	30.70 ± 5.89[b]
G3	0.800 ± 0.090[b]	81.67 ± 8.79[b]	5.30 ± 1.08[b]	74.99 ± 7.21[b]	150.03 ± 7.88[b]	86.82 ± 7.09[b]
G4	0.750 ± 0.085[b]	81.33 ± 8.55[b]	5.23 ± 0.85[b]	86.24 ± 8.98[b]	172.52 ± 8.19[b]	103.00 ± 8.87[b]
G5	0.700 ± 0.074[b]	81.29 ± 8.79[b]	5.21 ± 0.090[b]	138.73 ± 11.12[b]	277.49 ± 9.34[b]	162.80 ± 14.34[b]
G6	0.700 ± 0.075[b]	81.67 ± 9.08[b]	5.28 ± 0.80[b]	93.75 ± 9.19[b]	187.54 ± 8.42[b]	111.50 ± 9.84[b]
G7	0.650 ± 0.060[b]	81.65 ± 8.50[b]	5.26 ± 0.91[b]	93.75 ± 9.85[b]	187.55 ± 8.92[b]	111.60 ± 9.76[b]
G8	1.600 ± 0.150[b]	220.52 ± 18.26[b]	38.22 ± 6.31[b]	32.50 ± 2.17[b]	65.04 ± 4.01[b]	14.73 ± 1.50[b]

Keys
G = Groups of rats (G1 to G8)
TV = Testicular volume
D = Diameter of seminiferous tubule
Ac = Cross -sectional area of seminiferous tubule
N_A = Number of profiles of seminiferous tubules
per unit area
Lv = Length density
Nv = Numerial density
a = Mean ± standard deviation
b = p < 0.05
AV = Absolute volume

Table 3 Effects of quinine on the seminiferous epithelium, lumen and interstitial stroma of Sprague-Dawley rats

| G	Seminiferous epithelium		Stroma		Lumen	
	Vv (%)	AV (ml)	Vv (%)	AV (ml)	Vv (%)	AV (ml)
G1	73.75 ± 4.18^a	1.106 ± 0.191	11.50 ± 0.85	0.173 ± 0.029	14.75 ± 1.08	0.221 ± 0.047
G2	72.50 ± 4.02	1.015 ± 0.165	16.50 ± 1.17	0.231 ± 0.042	11.00 ± 0.81	0.154 ± 0.029
G3	50.00 ± 3.53^b	0.400 ± 0.099^b	43.75 ± 3.01^b	0.350 ± 0.094^b	6.25 ± 0.53^b	0.050 ± 0.009^b
G4	50.00 ± 3.61^b	0.375 ± 0.078^b	43.75 ± 3.21^b	0.328 ± 0.091^b	6.25 ± 0.55^b	0.047 ± 0.008^b
G5	41.25 ± 3.02^b	0.289 ± 0.065^b	37.50 ± 2.97^b	0.262 ± 0.077^b	21.25 ± 2.02^b	0.149 ± 0.027^b
G6	30.25 ± 2.76^b	0.212 ± 0.053^b	39.50 ± 3.01^b	0.276 ± 0.092^b	30.25 ± 2.79^b	0.212 ± 0.053
G7	30.50 ± 2.90^b	0.198 ± 0.099^b	39.00 ± 3.01^b	0.254 ± 0.020^b	30.50 ± 2.81^b	0.198 ± 0.097
G8	75.00 ± 4.51^b	1.200 ± 0.201^b	12.50 ± 0.92^b	0.200 ± 0.031^b	12.50 ± 0.91^b	0.200 ± 0.031^b

Keys
Vv = Volume density
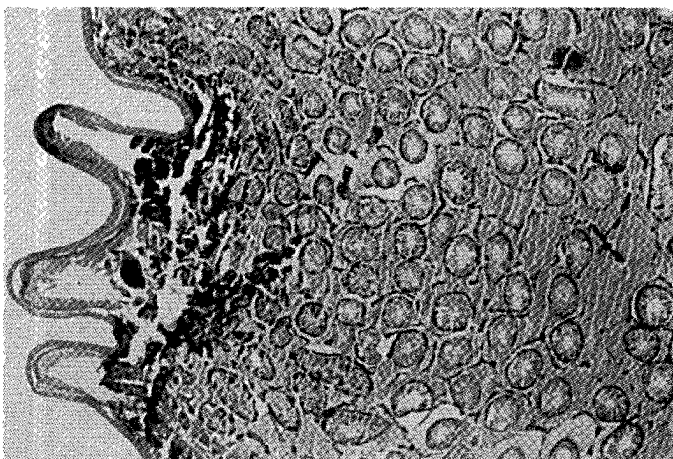*AV = Absolute volume*

a = Mean ± standard deviation
b = p < 0.05
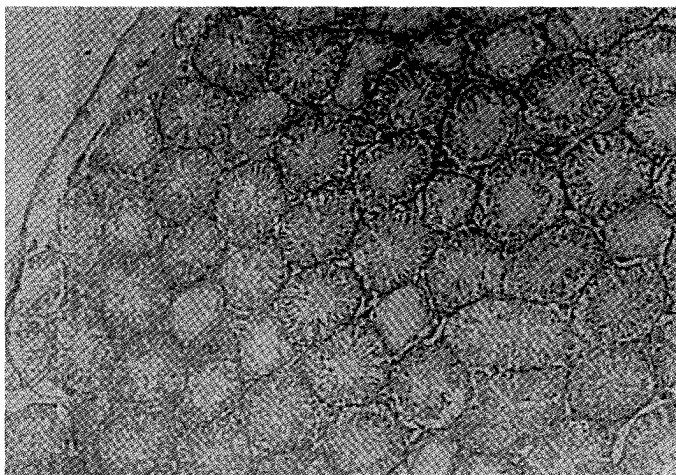
Fig. 1 *Cross section of the seminiferous tubules of normal Sprague-Dawley rats (Group 8) stains: haematoxylin & eosin. Magn. x 40*

Fig. 3 *Cross section of the seminiferous tubules of Sprague-Dawley rats treated with QU for 8 weeks and sacrificed at the end of the 8th week (Group 4). Stains: haematoxylin and eosin. Mag. x 40*

Fig. 2 *Cross-section of the seminiferous tubules of Sprague-Dawley rats treated with QU for 6 weeks and sacrificed at the end of 6th week (Group 3): Stains: haematoxylin & eosin. Mag. x 40*

Fig. 4 *Cross section of the seminiferous tubules of Sprague-Dawley rats treated with QU for 8 weeks and sacrificed at the end of the 12th week (Group 5). Stains: haematoxylin and eosin. Mag. x 40*

Interstitium

There was a significant (P < 0.05) increase (64 % of control) in the absolute volume of the connective tissue stroma from 0.200 ± 0.031 to 0.328 ± 0.091 ml during the period of QU administration. By the 12th week post QU-administration the mean absolute volume of connective tissue stroma had declined to 0.254 ± 0.020 ml (Table 3).

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
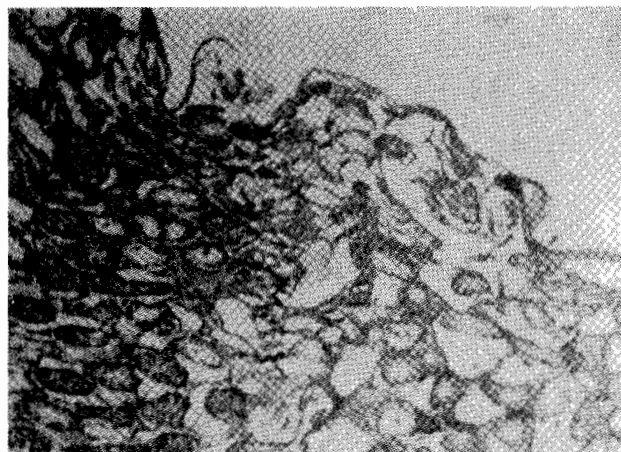
Fig. 5 *Cross section of the seminiferous tubules of Sprague-Dawley rats treated with QU for 8 weeks and sacrificed at the end of 20th week (Group 7). Stains: haematoxylin and eosin. Mag. x 40*

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)[18] 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[19] 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[20] suggests that QU compromises the process of spermatogenesis.

It has been reported that QU is a wide spectrum ion blocker[21]. It may be postulated that QU disrupts the ion channels leading to a disturbance in ionic equilibrium across membranes and ultimately to a disruption in cellular morphology. As shown in the current study, experimental disruption of tubular morphology is also accompanied by a general destruction of testicular interstitium 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?[22]. 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/or 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[23]. 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[24]. 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[25]. 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[5, 26, 27] 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

1. Robert A, Benoit-Vical F, Dechy-Cabaret O and Meunier B. From classical antimalarial Drugs to new compounds based on the mechanism of action of artemisinin. *Pure Appl. Chm.,* 2001; 73: 1173-1188.

2. Wilairatana P, Krudsood S, Treeprasertsuk S, Chalermrut K and Looareesuwan S. The future outlook of antimalarial drugs and recent work on the treatment of malaria, *Arch Med Res* 2002; 33: 416-221.

3. Lopes D, Rungsihirunrat K, Nogueira F, Seugorn A, Gil J, Do Rosario VE and Cravo P. Molecular characterization of drug-resistant Plasmodium falciparum from Thailand. *Malar J.* 2002; 1: 12.

4. Goldsmith RS. Antiprotozoa drugs. In: Bertram K (editor). Basic and clinical Pharmacology.7th edition. Connecticut: Ap-

pleton and Lange. 1998: 838-61.

5. Rang HP, Dale MM and Ritter JM. Pharmacology 4th edition, London: Churchill Livingstone, 1999; 725-738.

6. Trifunac NP and Berstein GS. Inhibition of the metabolism and motility of human spermatozoa by various alkaloids. *Contraception* 1982; 25: 69-87.

7. Garg S, Doncel G, Chabra S, Upadhyay SN and Talwar GP. Synergistic spermicidal activity of neem seed extract, reetha saponins and quinine hydrochloride. *Contraception* 1994; 50: 185-190.

8. Yeung CH, Sonnenberg-Riethmacher E, Cooper TG. Infertile spermatozoa of c-ros tyrosine kinase receptor knockout mice show flagellar angulation and maturational defects in cell volume regulatory mechanisms. *Biol Reprod 1999;* 61: 1062-1069.

9. Petrunkina AM, Harrison RA, Hebel M, Weitze KF and Topfer-Petersen E. Role of quinine-sensitive ion channels in volume regulation in boar and bull spermatozoa, *Reproduction.* 2001; 122: 327-336.

10. Borovskaya TG, Gol'dberg ED, Abramova EV, Fomina TI and Tkachenko. Effect of quinine on the morphology of mouse testes. *Bull Exp Biol Med* 2000; 130:994-996.

11. Woodruff AW and Wright SG. A synopsis of infectious and tropical diseases. 3rd edition. Bristol: IOP Publishing Limited. 1987: 217-32.

12. Lesi A and Meremikwu M. High first dose quinine regimen for treating severe malaria. *Cochrane Database Syst Rev.* 2002; 3: CD003341.

13. Gundersen HJG and Jensen EB. The efficiency of systematic sampling in stereology and its Prediction. *J Microsc* 1987; 229-226.

14. Gundersen HJG. Notes on the edge of the numerical density of arbitrary profiles: the edge effect. *J. Microsc.* 1977; 111: 219-23.

15. Nyengaard JR. Stereologic methods and their application in kidney research. J. Am Soc Nephrol 1999; 10:1100-1123.

16. Weibel ER and Gomez DM. A principle for counting tissue structures on random   sections. *J Appl Physiol* 1962; 17: 343-348.

17. Gilliland KO, Freel CD, Lane CW, Fowler WC, Costello MJ. Multilamellar bodies as potential scattering particles in human age-related nuclear cataracts *Molecular Vision* 2001; 7:120-130.

18. Okanlawon AO and Ashiru OA. Sterological estimation of seminiferous tubular dysfunction in chloroquine treated rats. *Afr J Med Med Sci.* 1998; 27: 101-106.

19. Fisher D. New light shed on fluid formation in seminiferous tubules of the rat. *J. Physio* 2002; 542.2:445-452.

20. Jegou B. The Sertoli cell. *Baillier's Clinical Endocrinology and Metabolism.* 1992; 6:273-311.

21. Yeung CH and Cooper TG. Effects of the ion-channel blocker quinine on human sperm volume, kinematics and mucus Penetration, and the involvement of potassium channels. *Mol Hum Reprod* 2001; 819-828.

22. Caroppo E, Niederberger C, Iacovazzi PA, Correale M, Palagiano A and D'Amato G. Human chorionic gonadotropin free beta-subunit in the human seminal plasma: a new marker for spermatogenesis? Eur *J Obstet Gynecol Reprod Biol.* 2003: 106: 165-169.

23. Harpe RM, KLerr JB, McKinnell C and Millar M. Temporal relation between androgen-dependent changes in the volume of seminiferous tubule fluid, lumen size and seminiferous tubule protein secretion in rats. *J. Reprod. Fertil.* 1994; 101: 193-198.

24. Okanlawon AO and Ashiru OA. Morphological changes in rat seminiferous tubules produced by chloroquine. *Niger Postgrad Med J* 1996; 3: 105-9.

25. Okanlawon A, Kusemiju O and Dym M. Direct toxic effects of clinical doses of chloroquine on transferrin secretion in immature rat Sertoli cells *in-vitro. Nig Postgrad Med J.* 2001; 8: 165-169.

26. Agrawal R, Tripathi R, Tekwani BL, Jain SK, Dutta GP and Shukla OP. Haem polymerase as a novel target of antimalarial action of cyproheptadine *Biochemical Pharmacology,* 2002; 9: 1399-1406.

27. Leed A, DuBay K, Ursos LM, Sears D, De Dios AC and Roepe PD. Solution structures of antimalarial drug-heme complexes. *Biochemistry* 2002; 41:10245-10255.