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Full Length Research Paper

Effects of anti-malarial alkaloids on the sperm properties and blood levels of reproductive hormones of adult men

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The effects of treatment with the anti-malarial alkaloids quinine and chloroquine on sperm properties and blood levels of selected reproductive hormones (testosterone, follicle stimulating hormone and luteinizing hormones) of adult men were determined. Informed consents were obtained from twenty healthy adult volunteers who were subsequently allotted to groups A and B with 5 subjects each .While group C had 10 subjects. Group A received 600 mg of quinine 8 hourly for 5 days; group B subjects had 4 tablets of chloroquine (250 mg each) daily for 2 days then 2 tablets for one day. Group C subjects had neither of these drugs in the study period of 65 days. Venous blood and masturbation specimens of semen were obtained from the subjects before treatment, immediately post-treatment and by the 65th day from commencement of treatment. Blood levels of follicle stimulating hormones, leutinizing hormone and testosterone were determined by Enzyme Linked Imuno Assay. Seminal Fluid Analysis was carried out on the semen specimens to determine sperm count, percentage forward motility and percentage abnormal sperm morphology. The means of all the variables assessed were within the limits of normal for their respective method of analysis. No statistical significant effect of these drugs on sperm count, percentage sperm forward motility and blood levels of testosterone were observed when pre-treatment results were compared with post-treatment and 65th day results as well as when results of quinine and chloroquine treated groups were compared with those of control group. The suggestion by disparate *in vivo* animal and *in vitro* studies that the short term use of these drugs to treat malaria may be associated with fertility changes as a result of their inherent anti-spermatogenic effects have not been collaborated by this study in adult men.

Key words: Anti-malarial alkaloids, quinine, chloroquine, sperm properties, reproductive hormones, adult men.

INTRODUCTION

Malaria is caused by any of the four protozoan species of the genus plasmodium. *Plasmodium falciparum* is the deadliest of all the species and is the most common cause of malaria in sub-Saharan Africa (Ajayi et al., 2005; Kumar and Clark, 2002). The alkaloidal agents quinine and chloroquine are widely used in the treatment of malaria (Olatunde, 1980).

The emergence and spread of multi-drug resistance in the parasite have not negated the popularity of the use of these two drugs in the treatment of malaria fever in endemic areas. Chloroquine, for instance, has been reported to still be a preferred first-line anti-malarial drug in Nigerian practice (Solako et al., 1990; Isah et al., 1997; Ebong and Adiele, 2001). Recently, World Health Organization has been in the vanguard of promotion of the use

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of Artesunate based combination drugs as first line intervention for malaria therapy in patients living in areas of high level of resistance. Increasing acceptance and use of the Quinghahosu anti-malarial drug has been observed in this part of the world (Akoria and Isah, 2004). Quinine and chloroquine, however, continue to be widely used. Perhaps their continued popularity may not be unrelated to their ready availability and relative affordability (Bloland et al., 2000).

Both drugs have been associated with several different adverse effects but their patient compliance or acceptability in clinical use is especially determined by the experience of two different types of side effects: The generalized body itch or puritus that affect certain persons following the ingestion of chloroquine (Ekpechi and Okoro, 1964; Osifor, 1980, 1984) and the ringing sensation in the ears or tinnitus in patients following ingestion of quinine.

Of recent, the toxic effects of these anti-malarial alkaloids on human and animal spermatocytes have been investigated by biomedical researchers using both *in vivo* animal and *in vitro* techniques. The inhibitory effect of both quinine and chloroquine on sperm metabolism (measured by production of lactic acid and CO_2) and motility have been reported (Trifunac and Berstein, 1982). In another study assessing the spermicidal activity of quinine, 100% of human spermatozoa were immobilized within 20 s of incubation with the alkaloid (Garg et al., 1994). Washed human spermatozoa have also been observed to undergo changes in size and shape in response to incubation with quinine that was accompanied by reduced straight line velocity and linearity of the swim path or percentage forward motility (Yeung et al., 1999). Similar observations were made when spermatozoa in semen and artificial mucus were incubated with quinine and marked reduction in mucus penetration and migration was also reported. Apart from the above *in vitro* studies several *in vivo* studies in mice and rats also seem to suggest that these anti-malarial alkaloids may have anti-spermatogenic capacities. In the study in mice, the gonocytes in all the layers of the spermatogenic epithelium, Interstitial cells of Leydig and Sertoli cells were reportedly sensitive to the toxic effects of quinine (Borovskaya et al., 2000). Another *in vivo* study in rats determined the effects of short term administration of quinine on the seminiferous tubules of the Sprague Dawley rats. It concluded that quinine had deleterious effects on the seminiferous tubules of Sprague Dawley rats and may possibly disrupt spermatogenesis and suggested the need for the exploration of the place of quinine as a male contraceptive agent in humans (Osinubi et al., 2004).

A third *in vivo* study in Sprague Dawley rats also reported degenerative histological changes in the germinal epithelium as well as statistically significant decrease in sperm count, sperm activity and percentage normal morphology (Nwangwa et al., 2007). Ashiru et al. (1991) also reported that the injection of rats with chloroquine for 16 weeks eliminated all Leydig cells. The elimination of Leydig cells by chloroquine may eliminate testosterone and other Leydig factors that may be required for spermatogenesis (Abayomi et al., 1992). The hormonal control of mammalian spermatogenesis has been widely discussed (Steinberger, 1971; Matsumoto, 1989). Testicular chloroquine level in animals has been reported in literature (Grundmann et al., 1970). Adeeko et al. (1993) reported a higher concentration of chloroquine in the testes of pre-pubertal male Wistar rats than in adult rats. They suggested this may be as a result of the presence of more DNA in the pre-pubertal testes to which chloroquine has been shown to bind (Washington et al., 1973).

The observations in some of these studies suggest that the short term use of quinine and chloroquine even at the therapeutic doses for treating malaria in adult humans should be expected to exert toxic effects on the male reproductive system which could result in some form of anti-fertility effects in adult men. There is, however, no evidence from clinical studies or pharmacovigilance reports, suggesting the occurrence of this adverse effect in adult men treated with standard doses of these anti-malarial alkaloids. There is, therefore, a need to undertake an investigation into the toxic effects of these drugs on the reproductive system in adult men to enable us determine whether these effects being suggested by *in vitro* and *in vivo* (rats and mice) studies will be reproducible . This study seeks to determine the in vivo effects of the anti-malarial alkaloids (chloroquine and quinine) on the sperm properties and blood levels of the reproductive hormones of adult men.

MATERIALS AND METHODS

Recruitment of research subjects

The study was carried out in Abraka, Delta state, Nigeria. Twenty healthy adult male volunteers were recruited from the student and staff population of Delta state university, Abraka campus. Approval for the study was given by the Delta State University, College of Health Sciences Research and Ethics committee and all the participants were thoroughly educated about the aim of the research and their expected roles as subjects before a written informed consent was obtained from each one of them at the commencement of the experiment. The subjects were thereafter allotted to any of groups A, B or C with 5 persons each in groups A and B and 10 persons in group C.

Procurement of the drugs

All the drugs were procured from Rio pharmacy, a certified pharmacy store, in Abraka. The chloroquine tablets that contained 250 mg chloroquine phosphate were manufactured by DANA Pharmaceuticals Limited with batch number QT7122. The quinine tablets were also manufactured by DANA pharmaceuticals Limited, Nigeria.

Dosing schedules

Group A subjects were allergic to chloroquine and so had 600 mg of quinine tablets 8 hourly for one week. Group B subjects were

Table 1. Effect of treatment with anti-malarial alkaloids on mean blood level of follicle stimulating hormone (FSH; mIU/ml).

Specimen collection time	Quinine (A)	Chloroquine (B)	Control (C)
Pre-treatment	3.29 ± 0.09	5.1 ± 0.70	3.5 ± 0.25
Post- treatment	3.16 ± 0.16	4.92 ± 0.32	3.8 ± 0.20
65th Day	3.08 ± 0.18	5.06 ± 0.19	3.3 ± 0.30

Table 2. Effect of treatment with anti-malarial alkaloids on mean blood level of leutinizing hormone (LH; mIU/ml).

Specimen collection time	Quinine	Chloroquine	Control
Pre-treatment	5.00 ± 0.40	7.06 ± 0.18	6.20 ± 0.30
Post- treatment	7.64 ± 0.71	7.30 ± 0.30	5.8 ± 0.20
65th Day	6.90 ± 1.1	7.24 ± 0.18	6.00 ± 0.10

tolerant to chloroquine and so had the adult dose of chloroquine; 4 tablets daily for two days and 2 tablets daily for a day. Group C subjects had neither of each drug in the study period and served as control. During the study period subjects requested not to self medicate when they fall ill and detailed records of other drugs that unavoidably had to be ingested were kept and analysed to exclude any possible confounding variable arising from such.

Collection of the specimens

Venous blood (5 ml) was collected by cubital venepuncture and semen collected by masturbation from each of the subjects. Group C subjects were further sub-grouped into C1 and C2 composed of 5 persons each and specimen collection from C1 was patterned after group A subjects while C2 was patterned after group B subjects. Time sequence of collection of specimens was:

I. Pre-treatment: Before commencement of actual dosing of subjects with the anti-malarial alkaloids (drugs).
II. Post-treatment: At the completion of the stipulated dosage regimen for each group of subjects. This was by the 4th day for chloroquine treated subjects and day eight for the quinine treated subjects.
III. 65th day from the commencement of dosing. 64 days being assumed as the duration of one germinal cycle during spermatogenesis (Heller and Clemont, 1963)

Hormone assay

The blood specimens from the subjects were collected into heparinized bottles and were immediately centrifuged to separate the sera from the cells. The sera were labeled and then stored in the freezer until they were analysed. Testosterone, follicle stimulating hormone and luteinizing hormones were assayed using the enzyme immunoassay methods (2004).

Method of conducting sperm analysis

The technique for the collection of spermatozoa from the subjects and the conduction of sperm count, estimation of percentage forward motility and abnormal morphology were as described by Cheesbrough (1984).

Statistical analysis

Results were expressed as mean ± SEM or percentages. Statistical significance of the results was done with single Factor ANOVA test using the Microsoft Excel 2003 statistical software. On the one hand, pre-treatment effects were compared with those of post-treatment and the 65th day before eventually comparing the effects of quinine and chloroquine with those of their respective controls. Level of statistical significance were fixed at $P<0.05$.

RESULTS AND DISCUSSION

The mean blood level of follicle stimulating hormone in both treated and control groups ranged between 3.08 and 5.10 mIU/ml (Normal: 1 - 7 mIU/ml). The mean blood level of leutinizing hormone in treated and control groups ranged from 5.00 to 7.64 mIU/ml (Normal: 1 - 7 mIU/ml). The mean blood level of testosterone ranged from 4.70-6.16 ng/ml (normal: 3-10 ng/ml). The mean percentage active forward motility in treated and control subjects ranged from 63-90% (normal >50%). The mean sperm count or density in treated and control subjects ranged from 57 10^6/mL to 82 x 10^6/mL (normal > 20 x10^6). The average percentage abnormal sperm morphology ranged from 0.2 - 2.2% (< 20% is compatible with normal fertility).

The results at first glance appear to suggest that treatment of adult men with standard anti-malarial doses of chloroquine and quinine produces a lowering of the post-treatment and 65th day mean values of percentage forward sperm motility (Table 4), sperm count (Table 5) and blood testosterone levels (Table 3) when compared with the pre-treatment mean values. On the contrary, post-treatment and 65th day mean values of percentage abnormal sperm morphology (Table 6), blood follicle stimulating hormone (Table 1) and leutinizing hormone levels (Table 2) were higher than the pre-treatment mean values. However, none of the post-treatment or 65th day means of these variables were distantly removed from their respective pre-treatment and control means. Also,

Table 3. Effect of treatment with anti-malarial alkaloids on the mean blood level of testosterone (ng/ml).

Specimen collection time	Quinine	Chloroquine	Control
Pre-treatment	5.38 ± 1.02	5.22 ± 0.37	5.50 ± 0.25
Post- treatment	5.46 ± 0.37	4.94 ± 0.39	5.56 ± 0.30
65th Day	6.16 ± 0.63	4.70 ± 0.42	5.00 ± 0.15

Table 4. Effect of treatment with anti-malarial alkaloids on the percentage forward sperm motility.

Specimen collection time	Quinine	Chloroquine	Control
Pre-treatment	80%	80%	90%
Post- treatment	63%	62%	95%
65th Day	65%	65%	80%

Table 5. Effect of treatment with anti-malarial alkaloids on sperm count (x10^6/ml).

Specimen collection time	Quinine	Chloroquine	Control
Pre-treatment	82 ± 12.80	77 ± 7.0	80 ± 7.0
Post- treatment	67 ± 3.74	63 ± 4.9	65 ± 3.2
65th Day	57 ± 8.0	79 ± 8.9	80 ± 5.3

Table 6. Effect of treatment with anti-malarial alkaloids on percentage abnormal epididymal sperm morphology.

Specimen collection time	Quinine	Chloroquine	Control
Pre-treatment	2.2%	1.8%	0.5%
Post- treatment	2.5%	2.0%	0.2%
65th Day	2.0%	1.2%	0.4%

Table 7. Results (P-values) of statistical analysis with single factor ANOVA test.

Comparison	FM	SC	AM	FSH	LH	Testosterone
Quinine vs Control	0.0521	0.5123	*0.0231	0.0865	0.9165	0.0696
Quinine pre-tx vs post-tx	0.1412	0.8678	*0.0111	0.1216	0.8329	0.1446
Quinine pre-tx vs 65th day	0.2999	0.4893	0.1977	0.2764	0.5508	0.2261
Chloroquine vs Control	0.0529	0.4893	0.1208	*0.0007	0.5589	0.1606
Chloroquine pre-tx vs post-tx	0.1480	0.8301	*0.0105	*0.0161	*0.0369	0.0881
Chloroquine pre-tx vs 65th day	0.2999	0.1835	0.4597	0.1216	0.8219	0.5058

*P < 0.05 (Significant).
FM = Forward sperm motility, SC = Sperm count; AM = Abnormal Morphology; LH = Leutinizing Hormone; tx = treatment.

all the results were observed to lie within the normal ranges for their respective methods of analysis. Statistical analysis using single factor ANOVA test to compare pre-treatment effects with those of post-treatment and 65th day and also to compare effects due to chloroquine and quinine treatment with those of control groups (C1 and C2) who had neither of these medications (Table 7), suggest that quinine and chloroquine treatment did not have any significant effect on percentage forward sperm motility, sperm count and blood level of testosterone (all

P values < 0.05). Quinine treatment appears to significantly affect percentage abnormal sperm morphology when treated was compared with control (P = 0.0231) and when pre-treatment and post-treatment effects were compared (P = 0.0111). Treatment with chloroquine significantly affected percentage abnormal sperm morphology only when pre-treatment and post-treatment effects were compared with each other (P = 0.0105). Similarly, chloroquine treatment also significantly affected blood level of FSH when means of treated groups were compared with controls (P = 0.0007) as well as pre-treatment and post-treatment compared with each other (P = 0.0161). Blood level of LH was only significantly affected by chloroquine treatment when pre-treatment effects were compared with post-treatment effects (P=0.0369).

The sperm analysis reference values (WHO, 1999) stipulated that a sperm concentration of 20 x 10^6 sperm/ml or more, percentage normal sperm morphology not less than 30% and percentage forward motility of 50% or more within 60 min of ejaculation are compatible with fertility. Kruger et al. (1995) described even a stricter criterion, validated by the 4th WHO manual, where less than 14% normal morphology would be indicative of the need for assisted conception. The observation that none of these criteria was breached in either the treated or control subjects would suggest that it is unlikely that the short-term use of chloroquine and quinine to treat episodes of malaria significantly alter the fertility status of the users. Of the assessed variables used to define sperm properties in this study, percentage abnormal morphology seem to be more susceptible to the effects of these anti-malarial alkaloids taking into consideration the existence of statistical significance. However, Larry and Stunct (1991) reported that morphology results could be affected by staining techniques, subjectivity of observation and the definition of sperm malformation.

According to the World Health Organization (1998), malaria causes morbidity in up to 500 million of the world's population annually. It, therefore, follows that quite a significant percentage of the global population use anti-malarial drugs, of which chloroquine and quinine are still widely popular (Olatunde, 1980; Bloland et al., 1993; Solako et al., 1990). Contrary to expectations arising from these agents ability to significantly alter fertility suggested in disparate *in vitro* and *in vivo* animal studies, there are no pharmacovigilance or clinical reports suggesting that malarial patients treated with these agents became transiently or permanently sub-fertile or infertile. Steinberger (1971) and Matsumoto (1989) demonstrated, in previous experiments, that the formation of A and B spermatogonia, formation of primary spermatocytes and progression of the meiotic prophase do not require neither gonadotropic nor gonadal hormones, while the process of reduction division is under control of testosterone. This seems to be collaborated by the report by Abayomi et al. (1992) that chloroquine treatment of rats ultimately would lead to infertility by resulting in late germ cell developmental arrest with depletion of sperma-tids. Also the elimination of Leydig cells after 16 weeks injection of rats with chloroquine (Ashiru et al., 1991) would be expected to markedly affect spermatogenesis by reducing the blood testosterone levels. The normal physiological endocrine response to reduction in primary spermatogenesis is a reactive elevation in the blood levels of the gonadotropins.

Although following short term administration of quinine and chloroquine the mean pre-treatment blood levels of testosterone were lower than post-treatment and 65th day levels, this effect was however not statistically significant and all the means were within the limits of normal ranges. The expected rise in the gonadotropins were somewhat reflected in the results but their mean blood levels remained essentially within the ambits of normal even though the subjection of this aspect of the result to statistical test of significance were occasionally positive.

Conclusion

This study suggest that contrary to suggestions by *in vitro* and *in vivo* animal studies, the ingestion of standard adult doses of anti-malarial alkaloids (quinine and chloroquine) by adult men does not significantly affect percentage forward sperm motility, sperm count and blood levels of testosterone. Percentage abnormal sperm morphology and blood levels of the gonadotrophins appear to be more susceptible to treatment with these agents. The range of values obtained as means were, however, all within the normal limits of for the method of analysis used in the assessments of all the variables. We conclude from this study that the proposition based on the findings in studies in non-human subjects that short-term use of these agents to treat malaria have significant anti-spermatogenic and possible anti-fertility or contraceptive effect in humans has not been collaborated.

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