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Original Research Article

Effect of vitamin E on testosterone and dihydrotestosterone concentrations in Wistar rats administered artemether/lumefantrine and ciprofloxacin

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Abstract

Purpose: *To investigate the effect of vitamin E on testosterone and dihydrotestosterone concentrations in Wistar rats administered artemether/lumefantrine and ciprofloxacin.*
Methods: *Thirty-five albino Wistar rats (average weight of 200 g) were randomly divided into 7 groups of 5 rats per group. Group 1 was the normal control, while Groups 2 and 3 were administered 8 mg/kg artemether/lumefantrine and 7.14 mg/kg ciprofloxacin, respectively. Group 4 received artemether/lumefantrine and ciprofloxacin (AL-CIPRO) concomitantly, while Groups 5, 6 and 7 received Vitamin E in addition to AL, CIPRO and AL-CIPRO combinations, respectively. Plasma testosterone and dihydrotestosterone concentrations and testicular weight were determined.*
Results: *The concentrations of testosterone and dihydrotestosterone decreased non-significantly ($p > 0.05$) following separate administration of AL and CIPRO but significantly ($p < 0.05$) with concomitant administration compared with control. Vitamin E, in combination with the drugs, significantly ($p < 0.05$) increased testosterone and dihydrotestosterone concentrations compared to groups without vitamin E. Testicular weight exhibited the same pattern of result.*
Conclusion: *Artemether/lumefantrine and ciprofloxacin administration, singly and in combination, may induce reproductive toxicity, which could be due to free radicals generated from their metabolism. Vitamin E co-administration with the drugs (AL, CIPRO and AL-CIPRO) demonstrates an ameliorative effect against toxicity induced by the drugs, which may be attributed to its free radical scavenging ability.*

Keywords: *Vitamin E, Artemether/lumefantrine, Ciprofloxacin, Testosterone, Dihydrotestosterone*

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INTRODUCTION

Malaria is a parasitic disease with an annual occurrence of 300 - 660 million cases and mortality of almost a million. Over 2 billion people risk being infected with malaria especially pregnant women and children younger than 5 years [1]. The malaria parasite, *Plasmodium falciparum*, causes clinical disease through the lysis of infected red blood cells [1]. The primary rationale for malarial treatment is total clearance of *Plasmodium* parasites from the bloodstream using antimalarial drugs. The drugs act on schizonts, especially liver schizonts, and prevent

the advent of severe or chronic malaria and malaria-related anaemia, or death.

It is a common practice in Nigeria to co-administer antimalarial agents and antibiotics during malaria treatment. Ciprofloxacin is one of the habitually administered antibiotics during such drug combinations. Ciprofloxacin is a broad-spectrum fluoroquinolone antibacterial agent used in the treatment of bacterial infections such as *Salmonella typhi*. The drug has also been reported to exhibit anti-parasitic activity against *plasmodium* strains by inhibiting DNA replication in plasmodial mitochondria and genome of apicoplast [2].

Manifestation of reproductive toxicity has been documented following treatments with ciprofloxacin and artemisinin (and its derivatives) in Wistar rats and rabbits [3]. Artesunate has been associated with malformation of skeletal and cardiovascular tissues in rats and rabbits, coupled with fetal loss after implantation [3]. Reduced testicular function, low sperm production, count and motility have been reported in rats following ciprofloxacin administration. [4]. Furthermore, reports have shown that artemether/lumefantrine and ciprofloxacin, singly and in combination, induce cardiotoxicity [5] and hepato-nephrotoxicity [6] in rats.

Testosterone an androgen synthesized by Leydig cells in the testes regulates several functions in men, including the production of sperm. The 5-alpha reduction of testosterone yields dihydrotestosterone [7]. The crucial role of testosterone and dihydrotestosterone in male fertility and the effect of antimalarial drugs and antibiotic agents on these parameters necessitated this study.

EXPERIMENTAL

Drugs

Artemeter/lumefantrine (Novartis Pharmaceutical Corporation, Switzerland), Ciprofloxacin (Micro Labs Limited, India), and vitamin E (Mega Life Sciences Public Company Limited, Thailand) were purchased from a reputable Pharmacy in Uyo metropolis, Uyo, Nigeria.

Animals and study design

Thirty-five (35) male Wistar rats (average weight of 200 g) were obtained from the animal house, Department of Pharmacology and Toxicology, University of Uyo, Uyo. The animals were housed and handled under standard laboratory conditions following international guidelines by National Research Council [8]. The Animals were randomly divided into seven groups of five animals each. Group 1 (Normal Control) received 1 mL distilled water. Groups 2 – 4 received therapeutic doses of AL (8 mg/kg), CIPRO (7.14 mg/kg) and both drugs (AL 8 mg/kg and CIPRO 7.14 mg/kg), respectively. On the other hand, Groups 5 – 7 received AL and Vit E (8 mg/kg; 8.5 IU/kg), CIPRO and Vit E (7.14 mg/kg and 8.57 IU/kg, respectively), and the combination of both drugs and Vit E (8 mg/kg of AL, 7.14 mg/kg of CIPRO and 8.57 IU/kg of vit E), respectively.

Drug preparation and administration

Four tablets of AL (20 g artemether and 120 g lumefantrine per tablet) and one tablet of ciprofloxacin USP (500 mg) were pulverized separately using mortar and pestle. The powdered samples were dissolved in water to obtain a separate 100 mL stock solution. Two soft gel capsules of vitamin E containing 800 IU were aspirated using a 1 mL syringe and reconstituted in 100 mL of corn oil. Therapeutic doses of all the drugs were administered using a 2 mL syringe through oral gavage based on the body weight of the animals. Administration of AL was for three days, while CIPRO and Vit E were administered for 5 days.

Collection samples

The animals were euthanized with chloroform inhalation and sacrificed. Blood was collected into an anticoagulant-containing sample bottles through cardiac puncture. Plasma was obtained by centrifugation of the whole blood for 15 minutes at 3500 rpm and used to assay for testosterone and dihydrotestosterone concentrations. The left and right testes of the animals were excised and weighed using a digital mini-scale.

Assay of testosterone and dihydrotestosterone concentrations

Testosterone concentration was assayed using a testosterone ELISA kit (Calbiotech Inc. Cordell Ct., El CA 92020) while DHT concentration was estimated using a rat-dihydrotestosterone ELISA kit (Bioassay Technology Laboratory) as described by the manufacturers.

Statistical analysis

Statistical Packages for the Social Sciences (SPSS) version 25 was used for data analysis. One-way analysis of variance (ANOVA) and Newman Keuls Multiple Comparison Test were

used for data analysis. The results, presented as mean ± standard deviation (SD), were considered significant at $p < 0.05$.

RESULTS

Plasma concentrations of testosterone and dihydrotestosterone

Table 1 and Table 2 present the results of plasma concentration of testosterone, DHT and testicular weight of albino rats administered AL, CIPRO and vitamin E. Significant ($p < 0.05$) decrease in the concentration of testosterone in Group 4 relative to the control group was observed. Testosterone concentration significantly increased in groups 5, 6 and 7 that received vitamin E in addition to AL and CIPRO relative to Groups 2, 3 and 4, respectively. Concentration of DHT significantly ($p < 0.05$) decreased in Group 4 when compared with control but significantly ($p < 0.05$) increased in Groups 5, 6 and 7 when compared with Groups 2, 3 and 4, respectively. The weight of the left and right testes recorded the same pattern.

Table 1: Plasma concentrations of testosterone and dihydrotestosterone in male Wistar rats after concomitant administration of artemether/lumefantrine and ciprofloxacin

Group	Testosterone (ng/mL)	Dihydrotestosterone (DHT) (ng/L)	Dihydrotestosterone/ Testosterone
1 (Normal control)	1.99±0.04	0.91±0.09	0.46
2 (AL)	1.33±0.10	0.89±0.06	0.67
3 (CIPRO)	1.35±0.10	0.77±0.08	0.57
4 (AL+CIPRO)	0.94±0.05[a]	0.58±0.06[a]	0.62
5 (AL+vit E)	1.68±0.11[b]	0.93±0.04[b]	0.55
6 (CIPRO+vit E)	1.56±0.11[c]	0.76±0.06[c]	0.51
7 (AL+CIPRO+vit E)	1.43±0.06[d]	0.82±0.18[d]	0.57

Significant differences ($p < 0.05$) between groups are indicated as superscript and defined thus: a = Test groups are compared with control; b = Group 5 is compared to Group 2; c = Group 7 is compared to Group 3; d = Group 7 is compared to Group 4 ($p < 0.05$). AL = artemether/lumefantrine; CIPRO = ciprofloxacin; vit E = Vitamin E

Table 2: Testicular weight of Wistar rats after concomitant administration of artemether/lumefantrine and ciprofloxacin

Group	Right testis (g)	Left testis (g)
1 (Normal control)	1.38±0.22	1.38±0.30
2 (AL)	0.96±0.35	0.90±0.29
3 (CIPRO)	1.02±0.16	1.06±0.18
4 (AL+CIPRO)	0.60±0.20[a]	0.66±0.29[a]
5 (AL+vit E)	1.10±0.10	1.16±0.09
6 (CIPRO+vit E)	1.08±0.44	1.14±0.40
7 (AL+CIPRO+vit E)	0.86±0.34	0.88±0.24

Significant differences ($p < 0.05$) between groups are indicated as superscript and defined thus: a = Test groups are compared with control; b = Group 5 is compared to Group 2; c = Group 7 is compared to Group 3; d = Group 7 is compared to Group 4 ($p < 0.05$). n = 5; AL = artemether/lumefantrine; CIPRO = ciprofloxacin; vit E = Vitamin E

DISCUSSION

Malaria infection is a tropical disease. The treatment regimen involves the use of antimalarial drugs, especially WHO-accepted artemisinin-based combination therapy. Due to the co-existence of malaria with other parasitic infections, the disease is often treated in combination with antibiotic agents, with or without a confirmatory test, especially in Nigeria. These medications are reported to be associated with some toxicities, including reproductive toxicity [4]. The concentrations of testosterone and dihydrotestosterone are useful tools in evaluating the effect of a drug on reproductive function. The hormone that causes male sexual traits to develop is testosterone, an androgen that is mostly produced in the Leydig cells of the testicles. Testosterone in males' controls a variety of processes, such as sperm and red blood cell synthesis, muscle size and strength, bone mass, fat distribution and sex drive [9].

In this study, there was a decrease in testosterone concentration in animals administered AL and CIPRO singly and AL-CIPRO combination compared with normal

control. This observation agrees with Raji *et al*. [10] who reported a significant decrease in concentration of testosterone when artemether was given to male Wistar rats. Studies also report that administration of Artemisinin-based combination treatments (ACTs) results in decrease in serum testosterone levels [11]. Antibiotics, especially fluoroquinolones, have also been reported to cause a reduction in testosterone concentration [12]. The observed reduction in serum testosterone concentration in this study may suggest a possible inducement of reproductive toxicity following antimalarial and antibiotic co-administration, which could be through generation of free radicals during their metabolism. Most antimalarial agents have also been reported to induce toxicity to male gonads [10].

Co-administration of vitamin E along with the drugs increased testosterone concentration significantly, demonstrating antioxidant property of vitamin E against free radical-induced toxicity. Vitamin E is known to exert antioxidant effect by limiting the rate of lipid peroxidation caused by free radicals in tissues. It protects spermatozoa by maintaining stability of polyunsaturated fatty acids in cell membranes [13]. In this study, vitamin E may have a protective function on the Leydig cells of the testes against free radicals generated from artemether/lumefantrine and ciprofloxacin metabolism, hence improving the synthesis of testosterone.

Dihydrotestosterone is synthesized in the testes and other tissues through the reduction of testosterone by the enzyme 5α-reductase. It performs several functions including influencing the secretion of gonadotrophin in men and preventing erectile malfunction in rats. Higher levels of DHT have been reported to improve cardiovascular functions in men [14]. There was a decrease in DHT concentration following single and concomitant administration of AL and CIPRO. Dihydrotestosterone is the active form of testosterone in tissues, hence its reduction in concentration following administration of the drugs is a result of reduced testosterone concentration observed. Gustafsson *et al*. [15] reported that reduced levels of DHT in men may be associated with a higher risk of prostate cancer. The significant reduction in DHT levels observed in this study may support erectile dysfunction and reproductive impairment as well as a reduction in the level of spermatogenesis. Previous studies [14] also report a positive correlation between the concentration of testosterone and dihydrotestosterone in men.

Vitamin E co-administered with the AL and CIPRO in this study increases concentration of dihydrotestosterone countering the effect of drug-induced toxicity. Artemether/lumefantrine has been reported to induce degenerative damage in the testes of albino rats and is capable of causing infertility [16]. Such degenerative changes could affect the synthesis of testosterone and dihydrotestosterone as evident in the reduced levels of these hormones in the present study. Vitamin E exhibited a protective effect against the drug-induced testicular damage as demonstrated in the improved concentrations of testosterone and dihydrotestosterone observed in this study.

Furthermore, dihydrotestosterone/testosterone ratio is often determined in clinical practice to assess deficiency of 5α reductase, the enzyme that catalyzes the conversion of testosterone to DHT [17]. The DHT/Testosterone ratio in this study was observed to increase marginally following AL and CIPRO administration when compared to control group. Concomitantly administration of vitamin E with the drugs showed no significant change in the ratio in comparison with normal control. Increased levels of this ratio as well as DHT levels have been associated with prostate cancer with several other biological effects while slightly elevated concentration has been reported to be of no clinical concerns [14].

Also, changes in organ weight have been widely used in the assessment of tissue-specific drug toxicity in general and changes in testicular weight is an important indicator in predicting the risk of reproductive toxicity [17]. The present study demonstrates a significant decrease in testicular weight in all the test groups in comparison with normal control which may be an indication of drug toxicity. The reduced testicular weight may be due to damage to the cellular structure of the testes, which has been widely reported to be associated with artemether/lumefantrine toxicity [16]. Vitamin E, co-administered with AL and CIPRO in Groups 5, 6 and 7, respectively, improved observed toxicity induced by AL and CIPRO. This observation corroborates reports that vitamin E improves morphometric parameters of reproductive organs in male rats [18].

CONCLUSION

The study demonstrates that artemether/lumefantrine and ciprofloxacin administration may be associated with reproductive toxicity in males, as shown in the reduced concentrations of testosterone and

dihydrotestosterone. Vitamin E exhibits a protective effect by increasing the concentrations of testosterone and dihydrotestosterone and ameliorating possible reproductive toxicity. This may be attributed to the antioxidant activity of vitamin E against free radicals generated by both drugs.

DECLARATIONS

Acknowledgement/Funding

None.

Ethical approval

Ethical approval for the study was granted by University of Uyo Health Research Ethics Committee (ref no. UU/CHS/IHREC/VOL.1/92).

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jessie Ndem conceived and designed the study, Jessie Ndem, Utibe Bassey, Anthony Uwah and Ubong-Isaac Anwana, Uduak Luke executed the work and collected the data under the supervision of Jessie Ndem. Utibe Bassey and Jessie Ndem analyzed the data. Jessie Ndem and Utibe Bassey wrote the manuscript. All the authors read, corrected and approved the manuscript for publication.

Availability of data and materials

The datasets used/analyzed during the current study are available from the first or corresponding author upon reasonable request.

Use of Artificial Intelligence/large language models

None.

Use of research reporting tool

None.

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