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Gonadotoxicity Evaluation of Oral Administration of Zidolam in Male Albino Rats

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Abstract

Aim. Zidolam is an antiretroviral combination therapy consisting of zidovudine and lamivudine for the treatment of Human Immunodeficiency Virus (HIV) infection. The objective of this research is to investigate the relationship between oral administration of zidolam and fertility in adult male albino rats

Material and Methods. Fifteen male albino rats with body weight (bwt) of 150 – 220 gm were used for the 2-phase study. Solution of the drug in sterile water was administered via oral cannula to 5 male rats each at daily dose of 1.29 mg/100 gm bwt respectively for 21 days during phase I. Phase II was a recovery study involving 5 male rats exposed to dose regimen as in phase I, and sacrificed after 21-day withdrawal of treatment. The control group of 5 male rats was given sterile water ad-libitum during the period.

Results. Zidolam caused significant decrease ($P< 0.05$) in the progressive sperm motility, sperm count, testosterone and viability of the animals while there was no significant change in the pH of the semen and all these factors may impair fertility. Discontinuation of the drug use caused restoration of the depressed values in the recovery group. The results suggested that Zidolam could induce reversible changes in all the parameters under investigation in the treated animals.

Conclusion. In conclusion, our findings demonstrated that the use of antiretroviral drugs could have deleterious effects on spermatogenesis hence cause sperm alterations which can lead to infertility even in normal subjects as the wistar rats used for the experiment were HIV –free.

Introduction

Human Immunodeficiency Virus is an RNA retrovirus, infectious agent that causes Acquired Immuno Deficiency Syndrome (AIDS), a disease that leaves a person vulnerable to life-threatening infections. Scientists have identified two types of this virus. HIV-1 is the primary cause of AIDS worldwide. HIV-2 is found mostly in West Africa [1]. The first report of cases of Acquired Immune Deficiency Syndrome (AIDS) appeared in the Morbidity and Morbidity Report (1981) in the USA. Twenty five years later, AIDS had become a worldwide epidemic infecting more than 65 million people of which 25 million have died [2]. Of the people infected with the disease, 44.2 million (68%) are in Sub- Saharan Africa, making it the region with the highest overall AIDS prevalence rate in the general adult (15 – 49 years) population [3]. Since the early 1990s, it has been clear that HIV would help undermine development in countries badly affected by the virus [1]. These effects are becoming increasingly visible in the hardest-hit region of all, sub-Saharan Africa, where HIV is now deadlier than war itself: in 1998, 200 000 Africans died in war but more than 2 million died of AIDS. AIDS has become a full-blown

development crisis [1]. HIV/AIDS is chronic lifelong disease with no known cure, and therefore, people living with HIV (PLHIV) have to be followed medically for the rest of their lives [4, 5]. The core component of treatment and care of PLHIV is provision of antiretroviral treatment (ART). Optimal ART increases the length and quality of life of HIV-infected patients, and reduces the onward transmission of the virus. Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily_HIV. When several such drugs, typically three or four, are taken in combination, the approach is known as highly active antiretroviral therapy, or HAART [6]. Not much work has been done on antiretroviral (ARV) therapy in Nigeria until recently.

This can be attributed to the poor coordination efforts in the fight against HIV [7]. In 1984, it became clear that HIV was the known cause of AIDS. Not quite long after this knowledge, drugs that could devastate the virus were placed on clinical trials [7].

Recent advances in the treatment of HIV-1 infection involving co- administration of reverse transcriptase and protease inhibitors to achieve near-complete suppression of HIV-RNA concentrations have led to considerable improvements in life expectancy of infected individuals [8]. HAART, i.e., the use of aggressive combination antiretroviral regimens consisting of reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs), has become the standard of care [9, 10]. Zidolam is an antiretroviral drug belonging to the antiretroviral group called nucleoside analogue reverse transcriptase inhibitors (NRTIs). It is used in antiretroviral combination therapy for the treatment of HIV infection. It reduces the amount of HIV in the body, and keeps it at a low level. It also increases CD4 cell counts. CD4 cells are a type of white blood cells that plays an important role in maintaining a healthy immune system to help fight infection [11].

The increasing reports of adverse clinical events and toxicities have diminished the enthusiasm generated by HAART. Some of the clinical events include AIDS – related insulin resistance, lipodystropy syndrome, gastrointestinal symptoms and hyperglycaemia, which is observed in 30 – 80% of patients who are well controlled with HAART [12].

The above toxicities coupled with limited knowledge of HAART have generated confusion and loss of confidence amongst the population in Africa which may militate against the acceptance and compliance to these drugs [12]. Local studies on the safety of these drugs are few hence this study aimed to assess the effects of Zidolam on male reproductive system in albino rats.

Materials and Methods

Animals

Approval for the use of the Wistar rats was sought from the Committee on the use of Animals, Department of Physiology, Olabisi Onabanjo University, Nigeria.

Fifteen male adult wistar strain rats weighing between 150 gm and 220 gm were obtained from our departmental animal house and were housed five animals per cage at room temperature where they were acclimatized for a period of seven days. The animals were fed with standard rat pellets (Ladokun Feeds Nig. Ltd.) and water ad libitum.

Experimental procedure

The study was divided into two phases involving the use of drug and sampling in the first phase, as well as drug administration, recovery period followed by sample collection in the second phase.Zidolam was obtained from General Hospital, Ijebu Igbo, Nigeria. The drug was administered orally at therapeutic (T) dose of 1.29 mg per 100 gm body weight (bwt) to each rat daily for 21 days.

Phase 1 entails the use of 5 male rats. Each rat in the group was treated with 1.29 mg/100 gm bwt of Zidolam daily for 21 days.

Phase 2 was a recovery study involving five male rats. The animals were given 1.29 mg/100 gm bwt of Zidolam each for twenty one days and allowed to recover from the treatment for another twenty one days.

There was a group of 5 male rats given sterile water throughout the study, and these serve as the control (C) in both phases.

Analytical procedure

The rats were weighed prior to treatment and at the end of each phase to obtain differential weight gains (if any). The animals were anaesthetized with di-ethyl ether after which they were sacrificed by exsanguination.

Determination of serum testosterone: Serum obtained from the blood collected via cardiac puncture

was used to measure the level of testosterone using the tube-based Enzyme Immuno-Assay (EIA) technique. The kit was manufactured in the United Kingdom by Immunometrics (UK) Ltd. [14].

Determination of sperm motility: A simple classification system proposed by the World Health Organization [15], which provides the best possible assessment of sperm motility, was used.

Determination of sperm count: The new improved Naubauers counting chamber was used in the determination of sperm count. Drops of semen were placed in the chamber and a cover slip was applied. This was placed under light microscope and spermatozoa counted on each of the five squares.

Sperm Viability: was done using the Eosin/Nigrosin stain. Semen was squeezed into a slide and two drops of the stain was added. A thick smear was made and then dried. The resulting stain was studied under the microscope using *40 objective. The live, motile sperm cells were unstained while the non-motile, dead sperm cells were stained.

Sperm pH: the pH was determined by using narrow range litmus paper.

Statistics

All calculations were done using the SPSS-V15 statistical software package (Norusis) [16] for analysis of the data. The data were presented as Means ± Standard deviation (SD), and statistical analysis carried out using ANOVA. Differences were considered to be of statistical significance at an error probability of less than 0.05 (P<0.05)

Results

Effect of Zidolam on body weight: There was no significant change in body weights of Zidolam treated rats when compared with the control. (Data not shown).

Table 1: Effect of Zidolam on sperm parameters after 21 days treatments and 21 days of recovery.

Groups	Sperm Motility (%)	Spermcount (10^6/mL)	Viability (%)	pH
Control	86.00 ± 4.18	82.20 ± 5.26	89.00 ± 2.24	7.60 ± 0.16
Test	72.00 ± 5.70*	48.60 ± 7.40*	68.00 ± 5.70*	7.50 ± 0.22
Recovery	80.00 ± 3.54*	68.80 ± 12.24*	80.00 ± 3.54*	7.78 ± 0.29

*P < 0.05 (p is significant at p < 0.05). The test and recovery groups were compared against the control.

Effect of Zidolam on Sperm parameters: The results are shown in Table 1.

Effect of Zidolam on sperm parameters (sperm counts, viability, motility and pH): Administration of Zidolam at 1.29 mg/100 gm of body weight/day for 21 days significantly reduced (p<0.05) the progressive sperm motility, sperm count and viability when compared with the control. There was no significant change in values of pH of the treated rats' semen when compared with the control. Drug withdrawal resulted in gradual restoration of sperm parameters in the male rats.

Table 2: Effect of Zidolam on serum testosterone levels.

Groups	Testosterone (nmol/L)
Control	2.45 ± 0.05
Test	1.09 ± 0.04*
Recovery	1.55 ± 0.03*

*P < 0.05 (p is significant at p < 0.05). The test and recovery groups were compared against the control.

Effect of Zidolam on serum testosterone level: Serum testosterone level of Zidolam in treated rats significantly was reduced (p<0.05) when compared to the control. However, there was an appreciable increase in serum testosterone levels of rats in recovery group (Table 2).

Discussion

In this study, the effects of Zidolam (an antiretroviral drug consisting of zidovudine and lamivudine) on some seminal parameters were examined. We demonstrated a statistically significant reduction (p < 0.05) in the percentage of progressively motile spermatozoa, sperm count value, serum testosterone level and sperm viability during treatment with Zidolam for 21 days. There was no significant change in semenmn PH during the course of treatment.

There are several possible explanations for the observed decrease in progressively motile spermatozoa. Mitochondria are abundant in spermatozoa and provide adenosine triphosphate, necessary to maintain progressive motility [17].

Decreased motility may be due to changes in spermatozoa metabolism due to antiretroviral therapy (ART). Various studies have demonstrated a relationship between mitochondria and sperm motility [18-20, 21]. As several ARTs have mitochondrial toxicity [22, 23], the observed changes in motility could be due to the ART.

Antiretroviral drugs are currently implicated in various metabolic and endocrine dysfunctions [24] that could in turn also affect testis, reproductive tract and gamete functions hence leading to significant decrease observed in sperm count, serum testosterone and viability in this current research. Dysfunction of the prostate and seminal vesicles, which are responsible for about 90–95% of the ejaculate volume and semen pH, could be due to the effects of ART drugs present in the genital tract [25-27].

In conclusion, our findings demonstrated that use of antiretroviral drugs could have deleterious effects on spermatogenesis hence cause sperm alterations which can lead to infertility even in normal subjects as the wistar rats used for the experiment were HIV –free.

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