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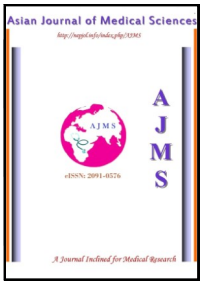

Hematologic Toxicity of Antiretroviral Drug, Zidolam (zidovudine and lamivudine) in Adult Wistar Rats

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Abstract

Objective: Zidolam is an highly active antiretroviral combination therapy (HAART) for the treatment of HIV infection. Efforts in this study intend to buttress evidence of hemato-toxicity associated with administration of HAARTs by using Zidolam.

Material & Methods: 30 albino rats with body weight (bwt) of 150 – 230 gm were used for the 2-phase study. Solution of the drug in sterile water was administered via oral cannula to the 2 groups of 10 rats (5 males and 5 females) each at daily dose of 1.29mg/100gm bwt respectively for 21 days during phase I. Phase II was a recovery study involving 10 rats (5 males and 5 females) exposed to dose regimen as in phase I, and sacrificed after 21-days withdrawal of treatment. The control group of 10 animals (5 males and 5 females) was given sterile water ad-libitum. Blood samples were collected by cardio-puncture from the rats for hematology at the end of each phase.

Results: Zidolam caused significant reduction ($P < 0.05$) in the hematological parameters of the animals. Discontinuation of the drug use caused gradual restoration of the parameters in the recovery group.

Conclusion: The results suggested that Zidolam could induce anemia and leucopenia in the treated animals. This supports the reason it is used with caution in anemic and leucopenic patients with HIV infection and perhaps reason for pre-hematological screening before treatment with zidolam.

Key Words: Zidolam; hematology; wistar rats; Highly active antiretroviral combination therapy (HAART)

1. Introduction

Human Immunodeficiency Virus is an RNA retrovirus. It is the infectious agent that causes acquired immunodeficiency 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]

AIDS is now a pandemic and sub-Saharan Africa has been described at the worst hit region by AIDS1, Nigeria having the second largest population. [2,3] UNAIDS estimates in year 2007 suggested that 33.2 million people worldwide had AIDS, it caused death in 2.1 million people (including 330,000 children) and 76% of those deaths occurred in sub-Saharan Africa.[4]

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The introduction of Highly Active Antiretroviral Therapy (HAART), a cocktail of nucleoside and non-nucleoside analogues capable of inhibiting reverse transcriptases and proteases, in industrialized countries during the mid-1990s led to well documented reductions in the risk of AIDS-defining illness, AIDS related mortality and hence, improve the quality of life of people living with HIV/AIDS.[5-7]

With AIDS becoming a global emergency, treatment using antiretroviral therapy became the most effective health care intervention.[7] However, it has been documented up to 25% of patients discontinue their initial HAART regimen because of treatment failure (inability to suppress HIV viral replication to below the current limit of detection, 50 copies/mL), toxic effects or noncompliance within the first 8 months of therapy.[8,9] One of such HAART is Zidolam, also known as combivir, consisting of zidovudine (AZT) and lamivudine (3TC).They belong to a class of antiretrovirals

known as nucleoside analogue reverse transcriptase inhibitors (NRTIs) which act by inhibiting reverse transcription by incorporating into the newly synthesized viral DNA and preventing its further elongation. The NRTIs are the backbone of the HAART.[10] Combining Zidovudine and Lamivudine has been shown to have synergic antiretroviral effect.[13]

Despite the therapeutic effect of reducing HIV-1 viral load and increasing CD4 cell count, antiretroviral therapy (including Zidolam) have a wide range of adverse effects on the human body that have been previously studied. The etiology of this effects are thought to be complex.[10, 11]

Common (greater than 1 in every 100 patients treated) but mild adverse effects occurring early in most antiretroviral regimens include, gastrointestinal effects such as bloating, nausea, vomiting, and diarrhea which may be transient, disappearing within a few weeks even if treatment is continued or may persist throughout therapy. Others may include fever, rash (red, raised or itchy), increase in certain liver enzymes, joint pain, muscle pain, dizziness, cough, nasal symptoms, tiredness, insomnia and hair loss.[10,12] Cases of pancreatitis, thrombocytopenia, dyspnoea, nail or skin pigmentation have been recorded but are either uncommon or rare.[12]

Hematologic toxicity including neutropenia and anemia particularly in patients with advanced HIV-1 disease have been associated with the use of zidovudine, one of the components of Zidolam.[13,10,14] Zidolam causes an onset of anaemia between 2 – 4 weeks of administration.[16] Symptomatic myopathy occurred in about 17% of people with prolonged use of zidovudine in another study.[13] Greater risk of Lactic acidosis and hepatomegaly with steatosis (linked to mitochondrial toxicity), including fatal cases, have been reported with the use of nucleoside analogues including zidovudine.[13,14] Others include insulin resistance leading to hyperglycemia and lipodystrophy.[10,14]

Acute exacerbations of hepatitis B have been reported in patients who are co-infected with hepatitis B virus (HBV) and human immunodeficiency virus (HIV-1) and have discontinued lamivudine, a component of Zidolam.[13]

This research investigates the relationship between anti retroviral therapy and hematologic parameters (PCV,RBC, Hb, WBC) in mammalian models (adult albino rats).

2. Material and Methods

2.1. Animals

Thirty adult wistar strain rats of both sexes (15 females and 15 males) weighing between 150gm and 220gm 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.

2.2. 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 100gm body weight 20 respectively to the rats daily for 21 days.

Phase 1 entails the use of 10 rats (5 males and 5 females). Each rat in the group was treated with 1.29mg/100gm body weight of Zidolam daily for 21 days.

Phase 2 was a recovery study involving ten rats (5 males and 5 females). The animals were given 1.29mg/100gm body weight of Zidolam each for twenty one days and allowed to recover from the treatment for another twenty one days.

There was a group of 10 rats (5 males and 5 females) given sterile water throughout the study, and these serve as the control (C) in both phases.

2.3. Analytical procedure

The rats were weighed prior to treatment and at the end of each phase to obtain differential weight gains (if any).

Determination of Hematological parameters: Blood sample was collected via cardio-puncture for analyses. Hematology was done according to standard methods. [21]

2.4. Statistics

All calculations were done using the SPSS-V15 statistical software package 22 for analysis of the data. The data were presented as Means ± Standard deviation (SD), and statistical analysis carried out using the Student's t-test and ANOVA. Differences were considered to be of statistical significance at an error probability of less than 0.05 ($P<0.05$).

3. Results

Effect of zidolam on body weight: There was no significant change in body weights of Zidolam treated rats when compared with the controls (data not shown). This trend was also observed in the recovery group. Effect of zidolam on hematological parameters: There was significant reduction ($p < 0.05$) in all the hematological parameters under investigation after the administration of 1.29 mg/100gm bwt of Zidolam daily for 21 days (table-1).

Table-1: Effects of Zidolam on hematological parameters

Groups	PCV (%)	Hb (x 10g/L)	WBC (10^9/L)	RBC (10^{12}/L)
Control group male	43.60 ± 6.43	14.34± 0.88	5.46± 1.44	5.06± 0.71
Control group female	43.80 ± 2.59	13.44± 1.37	4.72± 1.11	4.40± 1.42
Test group male	41.20± 2.07*	8.08± 0.69*	3.58 ± 1.01*	4.20± 0.54*
Test group female	41.00 ± 4.16*	6.84 ± 1.10*	3.26 ± 0.59*	4.15 ± 0.21*
Recovery group male	41.60± 2.77*	11.54± 1.71*	3.80± 0.25*	4.94± 0.37*
Recovery group female	41.40± 1.58*	10.46 ± 2.01*	3.66± 0.19*	4. 42 ± 0.93*

*Values are significantly lower at $p < 0.05$

Drug withdrawal during recovery phase resulted in gradual restoration of the hematological values as noticed in table 1.

4. Discussion

In this study the effect of the antiretroviral drug, Zidolam on the hematological parameters of experimental albino rats were examined. This investigation demonstrated that administration of Zidolam to rats significantly ($P<0.05$) reduced the measured hematological parameters of the animals. It also demonstrated that the noticeable effect is reversible as observed in the recovery group (Table I). This could be the basis of stopping medication or dosage adjustment in patient adversely affected by zidolam's toxicity.[15]

Previous study showed the toxic effects of antiretroviral drugs lead to anemia and neutropenia . These could be as a result of these drugs interfering with the progenitor cells of the bone marrow leading to suppression of their activity.[10,12-14]

The onset of anaemia occurred within the 21 day period of the study, corresponding to onset of anemia noticed upon administration of Zidolam within 2-4 weeks described in previous studies.[15,16] Lower base line hemoglobin is also noticed and it could be attributed to the drug induced bone marrow suppression of progenitor cells common with Zidovudine.[10,15] Treatment by erythropoietin therapy is possible but expensive.[15] Administration of Zidovudine to HIV-1 patients should be done with caution as most infected patients are anaemic even before treatment.[10,15] Hence it is suggested that if possible zidovudine should be substituted for other nucleoside analogues e. g. stavudine, didanosin or tenofovir. in patients with severe anaemia (Hb < 10mg/dL). Reduction in zidovudine dosage is possible but on a long term it causes drug resistance and treatment failure. [16]

Lamivudine is not seriously implicated in hematotoxicity of zidolam as suggested by Umar et al.[17] However rare cases of neutropenia has been observed.[14]

The Hemoblogbin concentration in the female rat test group is lower than that of the male suggesting that female are at higher risk to develop anemia. This observation is compatible with earlier studies done by AIDSinfo as well as in National AIDS Manual.[14,16] Macrocytosis has also been described with the entire class of NRTIs and it is a reflection of bone marrow toxicity.[18]

Neutropenia is equally common in the advanced stages of HIV and is often caused by concomitant myelosuppression from drug therapy.[12]

Another study by Cuttelod M et al attributes decrease in number of all blood cells (severe pancytopenia) to hemophagocytic syndrome.[19]

5. Conclusion

This study indicated that Zidolam induced anemia and caused leucopenia hence the need to include drugs that could boost the hematological profile in the medications of patients undergoing antiretroviral therapy. The study encourages estimation of baseline hematological parameters, repeated testing and serial monitoring so as to justify patients to be excluded or treated with zidolam.

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