The Impact of Zidovudine (An Antiretroviral Drug) on Some Serum and Erythrocyte Biochemical Parameters in Wistar Albino Rats

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Abstract: ***Zidovudine (AZT) is a nucleoside analog, a reverse transcriptase inhibitor (NRTI) and a type of antiretroviral drug used for the treatment of HIV/AIDS. The administration of zidovudine to the wistar albino rats showed an increase in erythrocyte fragility as can be seen from figure 1. There were significant ($p<0.05$) increases in serum Aspartate aminotranseferase (AST) activity, and Glutathione-S- transeferase (GST) activity, The result also showed non-significant ($p>0.05$) decrease in serum ALP activity, significant ($p<0.05$) decrease in Fe^{2+}/Fe^{3+} ratio and NADH methaemoglobin reductase activity. Findings from this study have revealed that zidovudine is hepatotoxic, increases the concentraton of ferric iron in the body thus imparing oxygen transport and also affects the erythrocyte membrane proteins adversely.***

Keywords: zidovudine, nucleoside analog, reverse transcriptase, inhibitor, antiretroviral

1. Introduction

HIV (Human Immune deficiency virus) is a lent virus (a member of the retrovirus family) that causes Acquired Immunodeficiency Syndrome (AIDS). It is a condition in humans in which the immune system begins to fail, leading to life-threatening opportunistic infections. (Douek et al., 2009). From its discovery in 1981-2006, AIDS has killed more than 25 million people worldwide (Levin et al., 2010). HIV infects about 0.6% of the world's population. (Greener.,2002)). According to current estimates, HIV is set to infect 90million people in Africa resulting in a minimum estimate of 18 million orphans (Morgan et al., 2002). HIV infects primarily vital cells in the human immune system such as helper T cell (specifically CD4+ T cells), macrophages and dendritic cells. HIV infection leads to low levels of CD4+ T cells, When CD4+ T cell members decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections. Most untreated people infected with HIV eventually develop AIDS. These individuals die mostly from opportunistic infections or malignancies associated with the progressive failure of the immune system (Klein and Abigail. 2010).

Antiretroviral drugs are medications for the treatment of infection by retroviruses, primarily HIV (Botes, 2003). Antiretroviral treatment reduces both the mortality rate and the morbidity of HIV infection and increases the life expectancy of infected people even after HIV has progressed to diagnosable AIDS (Kitahata et al., 2009). Although ART is not a cure for HIV/AIDS, it can significantly prolong and improve the lives of HIV-infected people (Hicks et al., 2003). Antiretrovirals slow down the production of HIV and give the body a chance to build up its CD4+ cell count which in turn helps the body fight against opportunistic infections (Henry ., 2000)

Zidovune (INN) or azidothymidine (AZT) is a nucleoside analog, reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug used for the treatment of HIV and AIDS. It is an analog of thymidine (Izzedine et al., 2001). Zidovudine was the first approved treatment for HIV sold under the names Retrovir and Retrovis (Broder., 2009). The cellular DNA polymerase used by mitochondria to replicate is more sensitive to the inhibitory effects of Zidovudine accounting for its toxic effects on cardiac and skeletal muscle, causing myositis (Cazzaline et al., 2001). Common side effects of Zidovudine include: nausea, headache, changes in body fat and discoloration of fingernails and toenails. More severe side effects include: anemia and bone marrow suppression which can be overcome using erythropoietin or darbepoetin treatments respectively (Brinkman et al., 1998).The purpose of this work therefore is to ascertain the possible side effects of zidovudine on the liver, erythrocyte membrane elasticity and oxygen transport in the body.

2. Materials and Methods

2.1 Chemicals

Except otherwise stated, all the chemicals used were of analytical grade and were manufactured by Randox chemical laboratories and BDH chemicals limited, both in England.

2.2 Experimental Animals

A total of fourty-five albino rats (*Rattus norvergicus*), average weight of 150g were obtained from the Animal house of the Department of Biochemistry, University of Port-Harcourt, Port-Harcourt Nigeria, and were maintained under standard housing conditions(temperature: 23.8° C;photoperiod:12h natural light and 12h dark;humidity:52-56%).The animals were fed rat pellets(Bendel Feeds and Flour mill Ltd, Ewu,

Nigeria) and were exposed to clean tap water throughout the period of the study.

2.3 Drug Preparation

The drug was crushed into powder using a clean mortar and pestle, the crushed powder was then mixed with distilled water into different concentrations. The concentrations were calculated from the normal dosage in a 70kg man i.e. 300mg every 12 hours.

2.4 Animal grouping

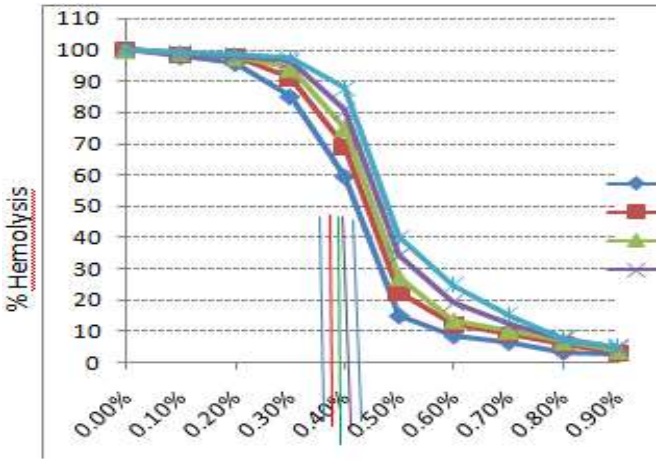
Forty-five healthy wistar albino rats were grouped into five (A-E) of nine rats each. Rats in group A (control) received no drugs at all. Those in groups B-E were administered 1.0ml of 0.20, 0.40, 0.60 and 0.80mg/100g body weights of zidovudine respectively. The drug was administered twice daily throughout the duration of the experiment (21 days). *Blood Sample collection and analysis of haematological parameters.*

Three (3) rats were sacrificed from each group on the 7th, 14th and 21st day in the course of the experiment. The animals were anesthetized by exposing them to chloroform vapour. Through direct scannulation of the heart, using a sterile syringe, blood samples were collected from the heart directly in heparinized lithium bottles and analysed for the following biochemical indices: Aspartate transaminase activity, (Doumas and Briggs.,1969),Glutathione-S transeferase activity (Habig et al 1974,Anosike et al 1991).,and Alkaline phosphatase activitiy (Bessey et al 1964).The red blood cell was analysed for erythrocyte osmotic fragility(Dacie et al., 2005), erythrocyte NADH methaemoglobin reductase activity (Tietz,1976) and Fe^{2+}/Fe^{3+} ratio (Davidson and Henry.,1974).

2.5 Statistical Analysis

Data was analysed by the use of a one way analysis of variance (ANOVA). Means were compared by the Duncan's (1957) multiple range test and significance was accepted at 95% confidence level.(p=0.05)

3. Results



Mean corpuscular fragility from the graph= A---0.42, B---0.46, C-- -0.47, D--- 0.49, E--- 0.51

Figure 1: Osmotic fragility curve showing the effect of zidovudine on the erythrocyte fragility of the wistar albino rats

Table 1: Effects of different concentrations of zidovudine on some serum enzyme activities as well as on Fe^{2+}/Fe^{3+} ratio.

Doses (mg/100g) body weight	GST activity (U/ml)	ALP activity (U/I)	AST activity (U/I)	NADH metHb reductase (iu/L)	Redox ratio
Control	1.59 ± .001^a	488.2 ± 51^c	55.2 ± 1.80^a	03.0 ± 0.0^a	2.00 ± 0.0^a
0.20	1.64 ± .040^a	437.0 ± 55^b	62.2 ± 3.46^b	1.7 ± 0.1^b	1.42 ± 0.1^c
0.40	1.68 ± .127^a	413.0 ± 53^b	62.4 ± 2.19^b	1.3 ± 0.2^c	1.47 ± 0.0^c
0.60	1.71 ± .025^a	428.0 ± 53^b	60.3 ± 2.45^b	1.0 ± 0.0^c	0.98 ± 0.1^d
0.80	1.85 ± .060^a	403.7 ± 53^b	65.2 ± 2.61^c	0.9 ± 0.1^c	1.57 ± 0.0^b

Values represent means ±SEM of triplicate determinations. Means with the same superscript along the same row are not significantly different at 95% confidence level (P<0.05).

As observed from the mean corpuscular fragility obtained from the graph, there was an increase in erythrocyte osmotic fragility of the animals from 0.42 for the control to 0.51 at 0.8mg of zidovudine/100g body weight. (Mean corpuscular fragility is the salt concentration at which 50% hemolysis occurs). Significant (P<0.05) increase in GST activity was also noticed with increasing drug concentrations from 0.40, to 0.80mg/100g body weight. There was also non-significant (P>0.05) decrease in serum ALP levels as the drug concentration increased from 0.20 to 0.80 as compared with the control. Significant (P<0.05) increase in serum AST activity was also observed. There were significant (P<0.05) decrease in Fe^{2+}/Fe^{3+} ratio when compared to the control values. There were significant (P<0.05) decrease in NADH methaemoglobin reductase activity when compared with the control. The increase was also observed to be dose dependent. Also significant increase in both Fe^{2+} and Fe^{3+} concentrations was noticed as compared to the control. From 2.00 to 2.76 for Fe^{2+} and from 1.00 to 1.76 for iron Fe^{3+}. At increasing concentrations of zidovudine.

0.42 0.51 0.46 0.49 0.47

4. Discussion

The change in the red blood cells osmotic fragility of animals given zidovudine may be due to chemically induced changes in the properties of the red blood cell membranes. Zidovudine may thus be involved in the modification of the physical condition of the proteins on the cell membrane which resulted to change in the permeability of the RBCs membrane. The influx of water into the cell leads to an increase in the hydrostatic pressure on the inner cell membrane that usually ends with hemolysis (Rabini *et al.,* 1997). Studies indicate that increase osmotic fragility could be associated with hemolytic anemia, especially hereditary spherocytosis and immune hemolytic states. Hereditary spherocytosis may be the result of an autosome dominant transmitted defect in red cell structural proteins. It is associated with a compensated or uncompensated hemolytic state, which is

relieved by splenectomy. It has been reported recently found in individuals who have related anemia and whose red blood cells show osmotic fragility (Kuchel *et al.,* 1997). Osmotic fragility is also increased in cases of malaria infestation.

The observed decrease in serum ALP activity may be linked to Pernicious anemia which results from vitamin B_6 malabsorbtion (www.drkaslow.com) .This shows that zidovudine may interfere with the cell's ability to absorb vitamin B_{12} which is essential to maintain normal haemoglobin levels. Zidovudine may thus induce pernicious anaemia. Also,serum alkaline phosphstase is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine. An Increased serum alkaline phosphatase activity may be due to;congestion or obstruction of the biliary tract,which may occur within the liver,in the ducts leading from the liver to the gall bladder,or in the duct leading from the gall bladder through the pancreas that empty into the duodenum(small intestine).(Murray et al 2006).Any of these organs,liver,gall bladder,pancrease,or duodenum may be involved in the heart or lung(myocardial or pulmonary infarctions).The subsequent increase in ALP could mean that zidovudine exerts some effects on the hepatobiliary system by probably blocking the hepatobiliary tract. The increase observed in AST activity may mean that zidovudine affects the liver negatively,It may also pose harm to the red blood cells, cardiac muscles, kidney and brain tissue.

Research has it that men with elevated aminotransferase levels had relatively high cholesterol levels. (Almo *et al*., 1994). Aminotransferase levels are highly correlated with the Ƴ-glutamyltransferase level, which has been reported to be associated with ischemic heart disease and stroke. (Gaze. 2007). Zidovudine may therefore induce blood pressure and increased fasting glucose as well as cause heart disease and stroke. Research has also shown that an elevated serum aminotransferase level may be an independent predictor of Intra cerebral haemorrhage (ICH). Men with elevated serum aminotransferase levels could be regarded as a high-risk group for ICH. They should therefore be assessed for other vascular risk factors and it is strongly recommended that they control those that are modifiable (Ghany *et al*., 2005).

An increase in serum GST levels was also observed as the drug concentration increased; the increase in GST activity noticed throughout the experiment may indicate that zidovudine has harmful effects on the liver. Zidovudine may cause injury to the hepatic system; its injurious effect is prorportional with the concentration administered as the result showed concentration dependent effect. This is in agreement with increase in AST activity observed earlier in this work. Increased GST activity indicates early hepatocyte injury. Alpha glutathione S-transferase (GST) is a superior marker of hepatocyte injury from toxicity; ischemia, and other liver injury. It is unique to hepatocytes, found in high concentrations, and is readily released in response to injury. It comprises 5% of the soluble protein of hepatocytes. Its rapid release into and removal from the circulation provides immediate information regarding liver status (Allocati *et al.,* 2009). GSTs are found in most tissues, most notably in the: brain pituitary, heart, lung, liver, kidney, adrenals, pancreas and gonads. Tissue specific expression of GST isoenzymes is indicative of varied biological regulation, which may account for organ-selective toxicity. Mouse studies have shown GST expression in kidney to be similar to that in the liver.

The significant increase in both Fe^{2+} and Fe^{3+} on administration of zidovudine may mean that zidovudine actually promotes iron accumulation and overload as revealed from literature. Zidovudine interferes with heme synthesis and, in an animal model, has been shown to increase iron acquisition. (Boelaert *et al*., 1996). In this study, ferritin levels were observed to be higher than the ferrous iron which may lead to poor oxygen binding of haemolgobin in patients administered zidovudine, as ferritin is a poor carrier of oxygen. Human with advance human immunodeficiency virus infection, present some evidence of iron accumulation (Goldin *et al*., 1993). Ferritin concentration increases with HIV disease progression. An increased iron store predisposes patients to certain microbial infections leading to decrease in iron level. This association may cause impairment of the immune system by HIV (Denomme., 2004). Iron and its binding protein have immune regulatory properties and shifting of immunoregulatory balances by iron excess or deficiency may produce severe deleterious physiological effects. Trace metal overload suppresses immune function and increase morbidity and mortality (Linberg *et al*., 1998). If the iron overload becomes severe, (usually when the amount of iron in the body exceeds 15 g) the condition is diagnosed as "hemochromatosis" (Bullen *et al*., 1991). Iron stored in the body becomes depleted and hemoglobin synthesis is inhibited. Iron is required for particular steps of the HIV replication life-cycle in cells. Increased bone marrow iron is associated with shortened survival and increased opportunistic infections (Nelson and Cox, 2000).

The decrease in NADH methaemoglobin reductase activity was observed to be dose dependent. NADH methaemoglobin reductase is an enzyme that is required to convert methaemoglobin to haemoglobin. This enzyme therefore ensures that methaemoglobin is maintained in very low concentrations and that iron exist in a more useful ferrous form (Fe^{2+}).By this act, methaemoglobin increases the oxygen binding capacity of haemoglobin makng it useful for respiration. The decrease observed in NADH methaemoglobin reductase in this work indicates that zidovudine reduces the oxygen binding capacity of haemoglobin by reducing the activity of NADH methaemoglobin reductase. Methaemoglobin is a derivative of haemoglobin in which the iron of the deoxygenated heme complex has been oxidized to the ferric form. Haemoglobin in this state is incapable of being oxygenated and consequently of no value for respiration. This is because methaemoglobin which is not accompanied by superoxide (O_2^-) renders haemoglobin incapable of binding normal triplet O_2 as it occurs in the air. It was thus assumed that iron remained as Fe (II) when oxygen gas was bound to it. Research has shown that in the normal erythrocyte, Methaemoglobin is continuosly being reduced by specific mechanisms so that

concentration at any given time is very small less than 1-2% of the total pigment. Cyanosis becomes apparent when more than 1.5g% of methaemoglobin appears in the blood. Some compounds that enhance methaemoglobin formation include: nitrites, sodium nitrite, sulphonamide drugs and aniline derivatives (Jaffe, 1959). The decrease in enzyme activity; NADH Methaemoglobin reductase indicates that the drug zidovudine may induce cyanosis.

5. Conclusion

The effect of increasing zidovudine concentrations lead to increasing osmotic fragility, increase in the activities of:aspartate transaminase, glutathione-S transeferase, and decrease in alkaline phosphatase activity, redox ratio and NADH methaemoglobin reductase. Thus zidovune, apart from inhibiting red blood cell production, may also exert other harmful effects on the cells considering its impact on the erythrocyte membrane, the liver and the methaemoglobin concentration, appropriate measures should be adhered to while prescribing this medicine to subjects considering these possible side effects. methaemoglobin reductase indicates that the drug zidovudine may induce cyanosis.

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