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## Hematologic and Hepatic Enzyme Alterations Associated with Acute Administration of Antiretroviral Drugs

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### ABSTRACT

The aim of this study was to assess the effects of short-term administration of some antiretroviral drugs on hematological and hepatic parameters in albino rats. The rats were treated with 0.43, 0.43, 0.27 and 0.21 mg kg<sup>-1</sup> of Efavirenz, Abacavir, SNP 40 and Lamivudine respectively, for seven days. The control group received normal saline. On the eighth day, the rats were sacrificed; blood and liver were collected for White Blood Cell Count (WBC), Packed Cell Volume (PCV), Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) analysis. All the drugs showed significant increase ( $p < 0.05$ ) in %WBC (43.0, 42.6, 52.3 and 37.4%) for Efavirenz, Abacavir, SNP 40 and Lamivudine respectively. Abacavir, SNP 40 and Lamivudine significantly decreased ( $p < 0.05$ ) ALP by 66.67, 84.75 and 56.24%, respectively while Efavirenz and Abacavir caused significant increase and decrease ( $p < 0.05$ ) in AST by 9.09 and 16.36% respectively. Efavirenz, Abacavir and Lamivudine caused significant increase ( $p < 0.05$ ) in ALT by 321.6, 497.6 and 177%, respectively. The drugs significantly increased the immunity of the animals and Efavirenz, Lamivudine and Abacavir caused hepatic damage. The clinical implication of our findings is that hepatoprotective agents should be included in the treatment regimen when administering antiretroviral drugs such as Efavirenz, Lamivudine and Abacavir.

**Key words:** Antiretroviral drugs, immunity, hepatic, hematologic, therapy, acute administration

### INTRODUCTION

Several diseases, including infection with the human immunodeficiency virus (HIV), can alter hepatic enzymes activities (Whitehead *et al.*, 1999; Giannini *et al.*, 2005). Antiretroviral drugs (ARVDs) are drugs used for the treatment of infection by retroviruses primarily HIV. ARVDs are of different classes and act at different stages in the progression of HIV infection (WHO, 2003). Combination of antiretroviral drugs is known as Highly Active Antiretroviral Therapy (HAART) (Kovacs *et al.*, 1989).

The advent of antiretroviral therapy (ART) has made an impressive impact in the management of HIV infection, suppression of viral replication, reconstitution of immunological competence and reducing the morbidity and mortality associated with AIDS (Palella *et al.*, 1998; Detels *et al.*, 1998; Hogg *et al.*, 1999). Highly Active Antiretroviral Therapy (HAART) that consists of Protease Inhibitors (PIs) such as Indinavir or Nucleoside Analogues of Reverse Transcriptase

Inhibitors (NNRTIs) such as Efavirenz and Nevirapine in combination with Nucleoside Reverse Transcriptase Inhibitors (NRTIs) such as Lamivudine and Stavudine has led to drastic improvements in the prognosis of HIV infected/AIDS patients (Carpenter *et al.*, 1998; Carr and Cooper, 2000). Of the drugs used to treat HIV infection, PIs and NNRTIs have proved effective, given either alone or in combination, as part of highly active antiretroviral therapy (HAART) (Piscitelli and Gallicano, 2001). Medications involving a combination of ARVDs are encouraged in order to have a long lasting effect (Graham *et al.*, 1992; Hogg *et al.*, 1998).

The benefits of antiretroviral drugs are compromised by numerous side effects, adverse clinical events and toxicities. All antiretroviral drugs can have both short-term and long-term adverse events. The risk of specific side effects varies from drug to drug, from drug class to drug class and from patient to patient. Some of the clinical events include AIDS-related insulin resistance, lipodystrophy syndrome, gastrointestinal symptoms, hyperglycaemia, (Schambelan *et al.*, 2002; Anonymous, 2003; Montessori *et al.*, 2004; D'Arminio-Monforte *et al.*, 2000; Lucas *et al.*, 1999).

The most common and troublesome toxicities of NRTIs and NNRTIs are especially hepatotoxicity (Reisler *et al.*, 2001; Schambelan *et al.*, 2002; Sulkowski *et al.*, 2002). Virtually every licensed antiretroviral medication has been associated with liver enzyme elevations (Abrescia *et al.*, 2005; Soriano *et al.*, 2008). Liver toxicity may also occur as a consequence of mitochondrial damage in patients receiving nucleoside analogues, particularly zidovudine, stavudine or didanosine (Verucchi *et al.*, 2004; Walker *et al.*, 2004).

Nucleoside analogs can occasionally cause myopathy, cardiomyopathy, pancreatitis, peripheral neuropathy and microvesicular steatosis of the liver, with lactic acidosis and/or liver failure (Fromenty *et al.*, 1995; Lewis and Dalakas, 1995; Brinkman *et al.*, 1998). These adverse effects have been mainly ascribed to drug-induced impairment of mitochondrial DNA (mtDNA) replication, causing mtDNA depletion, impaired oxidative phosphorylation and ATP deficiency (Fromenty *et al.*, 1995; Brinkman *et al.*, 1998).

It has been reported that Red blood cells and White blood cells indices are affected by age, sex, diet, malnutrition, co-infection and the consumption of medications or illicit drugs intake (Beers *et al.*, 2006; Mukiibi *et al.*, 1995; Miller *et al.*, 2006; Quinto *et al.*, 2006; Modjarrad *et al.*, 2008). Hematological and biochemical measurements can also be useful for clinical monitoring of HIV-infected individuals when viral load testing and CD4+ cell count monitoring are not readily available (Chen *et al.*, 2007). Effective antiretroviral therapy (ART) consistently results in sustained suppression of HIV ribonucleic acid (RNA) replication and gradual increase in white blood cell counts, sometimes to normal level (Lorig *et al.*, 1996).

Antiretroviral therapy and nutrition have been observed to cause an increased platelet counts at values greater than erythrocyte sedimentation rate and packed cell volume in HIV infected patients on antiretroviral therapy (Chukwurah *et al.*, 2007). This study was undertaken to investigate the possible hematologic and hepatic enzyme alterations that occurs in short-term use of these antiretroviral drugs: (Efavirenz, Abacavir, Lamivudine and SNP 40 (a combination of stavudine, lamivudine and nevirapine).

## **MATERIALS AND METHODS**

**Antiretroviral drugs:** Four antiretroviral drugs namely efavirenz, abacavir, lamivudine and SNP 40 (stavudine, lamivudine and nevirapine) were used during the course of this study between November, 2009 and April, 2010. The drugs were obtained from PEPFAR, University College Hospital, Ibadan, Nigeria.

**Animals and treatment:** Thirty albino rats, purchased from the animal house, Biochemistry Unit, Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria were housed and allowed to acclimatized for one week in animal house of the Biochemistry Unit, Department of Chemical Sciences, Bells University of Technology, Ota, Ogun state, Nigeria. They were fed with normal rat chow purchased from Agro Vet Ventures, Ota, Ogun State, Nigeria and water *ad libitum*. The rats were randomly divided into five groups and kept in wooden cages with wooden shaven beddings in a well ventilated room. All experimental groups shared the same environmental conditions. Group 1 served as the control and rats were treated with physiological saline. Rats in groups 2, 3, 4 and 5 were, respectively treated with efavirenz ( $0.43 \text{ mg kg}^{-1}$ ), abacavir ( $0.43 \text{ mg kg}^{-1}$ ), SNP 40 ( $0.27 \text{ mg kg}^{-1}$ ) and Lamivudine ( $0.21 \text{ mg kg}^{-1}$ ). The drugs were administered orally for seven consecutive days. Animals were sacrificed twelve hours after the last treatment. Blood samples were collected into heparinized bottles and later centrifuged (Uniscope SM112) at  $3000 \text{ g}$  for 30 min. Hematological analyses Packed Cell Volume (PCV) and total White Blood Cell Count (WBC) were carried out according to the methods described by Dacie and Lewis (1991).

**Liver function test:** Alkaline phosphatase, Alanine aminotransferase and aspartate aminotransferase activities were assayed according to the methods described by Reitman and Frankel (1957).

**Alkaline phosphatase:** This method is based on the principle that alkaline phosphatase hydrolyses a colourless substrate of phenolphthalein that results in phosphoric acid and phenolphthalein at alkaline pH values. The pinkish coloured product is measured using a spectrophotometer at 405 nm.

**Alanine aminotransferase:** This method involves the monitoring of the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyl hydrazine.

**Aspartate aminotransferase:** The principle of the method used involved monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl hydrazine.

**Statistical analysis:** All data are expressed as Mean $\pm$ SEM (standard error of mean). Significant differences were tested using the student t-test. Values of  $p < 0.05$  were considered statistically significant (Zar, 1984; Bamgboye, 2002).

## RESULTS

Acute administration of the four drugs caused a significant percentage increase (43.0, 42.6, 52.3 and 37.4% for efavirenz, abacavir, SNP 40 and Lamivudine) respectively in the white blood cell count when compared to the control. However, the antiretroviral drugs elicited an overall significant ( $p < 0.05$ ) decrease in the packed cell volume over controls as shown in Table 1.

It was observed that there was significant ( $p < 0.05$ ) decrease in the percentage white blood cell count for the other drugs (efavirenz, abacavir and Lamivudine) compared to the SNP 40 as depicted in Table 1. While for percentage packed cell volume, there was significant ( $p < 0.05$ ) decrease in group treated with efavirenz as compared to group treated with SNP 40 whereas for abacavir and lamivudine there was No Significant (NS) decrease in percentage packed cell volume compared to SNP 40.

Table 1: Effect of acute administration of Efavirenz, Abacavir, SNP 40 and Lamivudine on hematological indices in rats

Drug treatments	WBC count (%)	PCV (%)
Efavirenz	43.0±4.3*( <sup>○</sup> )	45.9±4.6*( <sup>○</sup> )
Abacavir	42.6±3.5*( <sup>○</sup> )	54.7±10.3*
SNP 40	52.3±8.5*	62.4±17.7*
Lamivudine (0.27 mg kg <sup>-1</sup> )	37.4±8.0*( <sup>○</sup> )	51.4±11.6*
Control	13.8±2.1	87.1±8.9

Values are Mean±SD for 6 rats in each group. \*, (<sup>○</sup>)Indicates significant difference (p<0.05), \*Indicates a significant difference when compared to the control group while (<sup>○</sup>)Indicates a significant difference when compared to the SNP 40 group. WBC: White Blood Cell count, PCV: Packed Cell Volume

Table 2: Effect of antiretroviral drug treatment on rat hepatic enzyme activities

Drug treatments	ALP (U L <sup>-1</sup> )	AST (U L <sup>-1</sup> )	ALT (U L <sup>-1</sup> )
Efavirenz	14.11±1.92( <sup>○</sup> )	262.00±17.89*( <sup>○</sup> )	5.27±1.21*( <sup>○</sup> )
Abacavir	5.52±1.59*( <sup>○</sup> )	184.00±13.42*	7.47±0.31*( <sup>○</sup> )
SNP 40	2.45±0.53*	203.33±20.66	1.57±0.06
Lamivudine	6.75±2.96*( <sup>○</sup> )	200.00±20.00	3.47±0.38*( <sup>○</sup> )
Control	16.56±3.32	220.00±20.00	1.25±0.78

Values are Mean±SD for 6 rats in each group. \*, (<sup>○</sup>)Indicates significant difference (p< 0.05). \*Indicates a significant difference when compared to the control group while (<sup>○</sup>)Indicates a significant difference when compared to the SNP 40 group. ALP = Alkaline Phosphatase, AST: Aspartate aminotransferase, ALT: Alanine aminoaransferase, (U L<sup>-1</sup>): Units per litre

The results obtained for liver enzymes activities for all the drug treatment groups are illustrated in Table 2.

**Efavirenz:** Acute administration was associated with significantly (p<0.05) elevated AST (9.09%) and ALT (321.6%) levels when compared to the control. However, there was a significant (p<0.05) decrease in ALP activities.

**Abacavir:** The acute administration of abacavir caused a significant (p<0.05) decrease in the activities of aspartate aminotransferase (AST) and alkaline phosphatase (ALP). Whereas the levels of alanine aminotransferase (ALT) were significantly (p<0.05) elevated as compared to the control.

**Lamivudine:** This antiretroviral drug resulted in significantly (p<0.05) elevated levels of ALT and lowered levels of ALP but caused no significant change in AST upon acute administration.

**SNP 40:** SNP 40 acute administration brought about a significant (p<0.05) decrease in the levels of ALP but did not result in any significant change in the levels of AST and ALT when compared with the control.

Table 2 also shows the comparison of the result for liver enzymes activities obtained between SNP 40 and the other three drugs. There was significant (p<0.05) increase in the activities of ALP in the groups treated with efavirenz, abacavir and lamivudine as compared with the group treated with SNP 40.

It was observed that there was significant (p<0.05) increase in AST levels in group administered with efavirenz as compared with SNP 40. There was no significant change in the levels of AST in groups treated with abacavir and lamivudine as compared with the group administered with SNP 40.

Also there was a significant ( $p < 0.05$ ) increase in the level of ALT in groups treated with efavirenz, abacavir and lamivudine as compared with the group treated with SNP 40.

## DISCUSSION

The hematological parameter is a predictive index for evaluating the level of immune system of the body. Significant increase in WBC has been attributed to effect of using antiretroviral drugs (Amegor *et al.*, 2009). All the drugs significantly increased the percentage WBC and showed a decrease in percentage of PCV when compared to the control. This agrees with the observation of Chukwurah *et al.* (2007). The reported significant increases observed in the WBC count during this study are indication of the ability of antiretroviral drugs to boost the immune system and reduce the risk of an opportunistic infection (Baker *et al.*, 2007; Amegor *et al.*, 2009; Wei *et al.*, 1995).

Hepatotoxicity is associated with all antiretroviral drugs currently used. A significant increase in plasma aminotransferase concentrations has been observed in 1-9% of patients in registration trials with different protease inhibitors (PIs) (Sulkowski *et al.*, 2004). In most cohort studies, higher rates of liver toxicity have been found, especially in hepatitis B virus (HBV) - or hepatitis C virus (HCV) - coinfecting patients (Sulkowski, 2004; Bonnet *et al.*, 2002; Saves *et al.*, 1999; Saves *et al.*, 2000; Den Brinker *et al.*, 2000; De Luca *et al.*, 2002; Aceti *et al.*, 2002; Wit *et al.*, 2002).

Injury to the liver, whether acute or chronic, eventually results in an increase in serum concentrations of aminotransferase (Dufour *et al.*, 2000; Lee 2003; Speech and Liehr, 1983; Lott and Wolf, 1986). AST and ALT are enzymes that catalyze the transfer of  $\alpha$ -amino groups from aspartate and alanine to the  $\alpha$ -keto group of ketoglutaric acid to generate oxaloacetic and pyruvic acids respectively, which are important contributors to the citric acid cycle. Both enzymes require pyridoxal-5'-phosphate (vitamin B<sub>6</sub>) in order to carry out this reaction, although the effect of pyridoxal-5'-phosphate deficiency is greater on ALT activity than on that of AST (Dufour *et al.*, 2000, Lee, 2003). Both aminotransferases are highly concentrated in the liver. An increase in ALT serum levels is more specific for liver damage (Wroblewski, 1958; Rej, 1989). Increases in activities of ALT and AST directly reflect a major permeability problem or cell rupture (Benjamin, 1978). AST exhibits high activity in cytoplasm, mitochondrion and microsomes of liver, heart, kidney and brain (Benjamin, 1978). ALT is principally found in the cytosol of hepatocytes. Elevated ALT levels are associated with acute liver and cholestatic disease (Wolf, 2003). ALP is a marker enzyme for plasma membrane and endoplasmic reticulum (Wright and Plummer, 1974).

Our results show that efavirenz, abacavir and lamivudine caused significant increase in hepatic enzymes activities. This is an indication that efavirenz, abacavir and lamivudine can result in hepatic damage and can also lead to an increase in hepatotoxicity in patients with liver diseases. This is consistent with the work done by Umar *et al.* (2008). However, we observed that abacavir and lamivudine appears to be less hepatotoxic than efavirenz, which conforms to reports that abacavir and lamivudine are less hepatotoxic (Nicholas and Dieterich, 2003). These two drugs are preferred for HIV/AIDS patients with high risk of hepatotoxicity especially those with hepatitis B or hepatitis C coinfection (Nicholas and Dieterich, 2003). As indicated by the results, only efavirenz is associated with significant activities of ALT and AST upon acute administration. Elevated activities of these enzymes indicate cell damage which might have resulted from several mechanisms; generation of toxic species, peroxidation of membranes etc. Present results are in agreement with reports by Sulkowski *et al.* (2002) on the toxicity of efavirenz.

The exact mechanisms by which efavirenz causes adverse hepatic events have not been elucidated, but Lee (2003) reports that drug-induced liver injury occurs via at least 6 mechanisms

involving various intracellular organelles, with consequent disruption of intracellular calcium homeostasis, decrease in ATP levels and finally the hepatocyte get inflamed and rupture. Other cells within the liver may be the target of drug-induced liver damage or modulate incipient reaction. For example, kupffer cells may activate cytokines which may amplify liver injury or macrophages may augment injury, produce fibrosis or granulomas (Lee, 2003).

Our study also suggests that lamivudine may not cause significant hepatic injury when used over a short period of time. This is in support of the reports by Nicholas and Dieterich (2003). In recent times, there have been no reports in literature as regards the possible toxic effect of SNP 40 when used for a short period of time. The results of our study show that SNP 40 may not cause significant hepatic damage in short term administration.

## **CONCLUSION**

Administration of drugs that are hepatotoxic to HIV/AIDS patients may have adverse effects. Also the lives of patients are put at risk. A damaged liver may fail to metabolize drugs and thus prolong their stay in circulation and cause further toxicity or drugs that need to be metabolized to active forms may not be so converted. There is high chance that a drug may be withdrawn due to its toxicity or the patient may fail to comply with the treatment regimen both of which may adversely affect the outcome of treatment.

In summary, our findings here suggest that treatment of rats with antiretroviral drugs *in vivo* (efavirenz, abacavir, SNP 40 and lamivudine) brought about an increase in the immunity of the body. Oral administration of Efavirenz, abacavir and lamivudine caused significant liver damage while SNP 40 did not significantly result in liver damage. Thus Efavirenz, abacavir and lamivudine must be used with caution and close monitoring of liver enzymes may be critical in preventing life threatening events. Inclusion of agents which are hepatoprotective in the treatment regimen should be seriously considered when administering antiretroviral drugs. However, more research work needs to be done to determine other effects of these antiretroviral drugs with the aim of reducing hepatotoxicity and thus extending the lifespan of people living with the disease and also to assess the specific mechanisms of the hepatic toxicity through histopathological investigations. Also further research work should be done to assess the probable long term effect of SNP 40 on liver enzyme functions.

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