EFFECT OF LAMIVUDINE ON SELECTED HEPATIC ENZYMES (ALANINE AMINOTRANSFERASE, ALKALINE PHOSPHATASE, ASPARTATE AMINOTRANSFERASE) IN ALBINO RATS

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Abstract: Occurrence of hepatic diseases being one of the major sources of death associated with HIV people equivalent topotent antiretroviral drugs. This study evaluates the effects of Lamivudine on selected liver enzymes (aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP)) in Wistar rats. Fifty two (52) Wistar rats were selected randomly into groups of four (4); group 1 received distilled water, group 2 to 4 were administered with 25 75 and 100% in mg/kg body weight of Lamivudine. Thevarious experimental groups were pre-disposed to the same conditions. Drugs was orally administered daily although 33 days. Animals were sacrificed in batches includes; eleven (11) days of exposure, twenty two (22) days of exposure and lastly, thirty three (33) days of exposure. Whole Blood samples were collected into sample bottles for hepatic function test. The result showed that, the effect is concentration and time dependent. The significant difference ($P<0.05$) showed reduction in ALT, AST and ALP at lower concentration (25%) in days 11, 22 and 33 (21.75±14.52, 102.75±68.59 and 112.75±75.17), (31.25±4.27, 121.25±3.40 and 167.50±5.97) and (23.00±15.43, 92.75±61.89 and 125.00±83.53) when compared with the control group (29.50±3.54, 109.00±2.83 and 138.00±12.73). While at medium and highest concentrations showed significant increased in days 11, 22 and 33 when compared with the control group. In conclusion, treatment with increased and prolonged doses of Lamivudine may result to hepaticinjuryas observed from the drastic increase liver function test parameters.

Keywords: Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase lamivudine, hepatic enzymes

Introduction

Human Immunodeficiency Virus (HIV) being a family of lentivirus that triggered Acquired Immunodeficiency Syndrome (AIDS) (Weiss, 1993; Doueke *et al.*, 2009). In Sub-Saharan Africa, HIV/AIDS is a worst epidemic to the occupant (Akinsete, 2002; Onwuliri *et al.*, 2003). Nigeria estimated approximately 3.6 percent individual living with AIDS and HIV. Though HIV incidence is greatly reduced in Nigeria compared to several African nations including Zambia and South Africa, Nigeria population sizes about 204.9 million translated that at the end of 2009, the possibility that an anticipated of 3.3 million populace with HIV will be infected (UNAIDS, 2010; UNDP, 2011). About 220,000 individuals died in Nigeria from AIDS in 2009. In 2010 sum total of life anticipation was 52 years (UNDP 2011). ART First-line is currently getting to above 3 million with countries living with middle or low income (WHO, 2008).

Antiretroviral therapy-related to hepatic injury (ARHI) can be classified by an increase in serum liver enzymes like; alanine aminotransferase typically better than aspartate aminotransferase. Also, it is grouped as one of the major basis of treatment deferment in HIV patients (Nunez *et al*., 2006). Its prophylaxis and management is thus vital in HIV-infected subjects placed on extremely potent antiretroviral therapy (HAART) (Palella *et al*., 2006). The occurrence of an extensive inconsistency in the requirement used clinically to classify the level of hepatotoxicity associated with antiretroviral agents cannot be overemphasized. Certain studies exploit ALT indexes as minimum twice the greater limits of standard (Hernandez *et al*., 2001) while some were in use as complete threshold (e.g., >100 IU/ml), despite the standard of hepatic function tests (den Brinker *et al*., 2000). Numerous problems arise such as; balance involving toxicity and cost are the mainexisting challenges in resource-limit scenery. Since the limited accessibility, expediency and cost of allocated-dose mixture, majority of the patients obtain first-line treatment, including lamivudine, zidovudine, stavudine and nevirapine. There are nucleoside forms of reverse transcriptase blockades. Combination of two drugs makes it active with persistent effect when compared to a single drug regime, which enhancesthe reduction of pill plight via supporting compliance with antiretroviral drug treatment. Zidovudine and Lamivudine are act via reverse transcriptase blockade inhibiting the enzyme action, reverse transcriptase are requisite to virus reproduction. It decreases the body viral load by elevating CD4+ cell level. Surveys shown that increase in hepatic injury prevalence of in HAART-managed patients by recognized life menacing hepatotoxic actions with liver diseases end-point linked with patients prone to antiretroviral (Spengler *et al*., 2002).

Drug related to hepatotoxicity generated economic stress toward prepared strained health budgets, because subsequent visits and admissions to hospitals are frequently obligatory for suitable patient management and treatment (Nunez *et al*., 2006). In conclusion, antiretroviral medications impede the maintenance of HIV control. Cruelty of certain agents ranges from lack of symptoms associated with liver degradation and possible effectthatvary from impulsive liver steatosis and necrosis (Clark *et al*., 2002). This study evaluates the effects of Lamivudine on selected liver enzymes (aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in Wistar rats. To avert the possible adverse effect of anti-retroviral associated with the liver.

Materials and Methods

Antiretroviral drug of choice (Lamivudine with a brand name lavudine from Evans Pharmaceuticals) used was obtained from standard pharmaceutical store situatedin University of Port Harcourt Teaching Hospital, Rivers State, Nigeria. All reagents used in this study were of analytical grade. Equipment used includes; Centrifuge (Universal 320, Hettic Zentrifugen Germany), refrigerator (Frestech), colorimeter (Jenway 6051 colorimeter; UK), weighing balance (Mettle Toledo AB 204, Switzerland), Spectrophotometer (Beckman Coulter, DU 520 General Purpose UV/Visual), water bath (UNISCOPE-Sn801A Surgifriend Medicals, England).

The experiment animals

Fifty two (52) albino rats were obtained from the animal house of University of Nigeria Enugu Campus (UNEC). The animals were acclimatized for two week in the Biochemistry Animal House of University of Port Harcourt. They were fed with standard rat pellets and water *ad libitum*. The animals

were housed in a conducive plastic cage with wood shaven beddings.

Experimental procedure

Fifty two (52) albino rats weighed (180 – 200 g) were randomly selected into groups of four; group 1, 2, 3 and 4. Group 1 is the control group (n=13 of 4 per subgroup) received distilled water. Group 2, 3 and 4 were treated orally with three various concentration of Lamivudine from 150 mg/kg (0.1 mg/ml of 25%, 0.3 mg/ml of 75%, and 0.4 mg/ml of 100%). Animals were anesthetically sacrificed using chloroformin days 11, 22 and 33 after the last treatment across the groups.

Drug administration

0.5 ml of 100% (0.4 mg/ml) Lamivudine solution was orally administered diverse concentrations across the groups using oral gastric tube via gavaging. Administration was orally done once daily.

Collection of blood and plasma preparation

Eleven, twenty two and thirty three days following the last exposure of Lamivudine regime, animals were anaesthezide via chloroform. Blood samples collected through abdominal aortal vein were transferred into anticoagulant sample bottles. The collected blood samples were spun at 5000 rpm using centrifuge to obtain theplasma.

Method of analysis

a. The designated method of assay of Plasma alkaline phosphatase activity was based on colorimetric method by Rec (1972).
b. The designated method of assay of Plasma aspartate aminotransferease activity was based on colorimetric method by Reitman and Franke (1957).
c. The designated method of assay of Plasma alanine aminotransferease activity was based on colorimetric method by Schmidst and Schmidst (1963).

Statistical analysis

The values obtained were analyzed using Graph pad prism version 6. Data was presented as mean ± S.E.M, and statistical significance between treated and control groups were calculated using One way ANOVA, followed by Dunnett's test where $p<0.05$ was considered statistically significant.

Results and Discussion

Results shown from Tables 1, 2 and 3 exhibited the plasma level of alanine aminotransferease (ALT) with significant decrease in the lowest concentration ($p<0.05$) (21.75±14.52; 31.25±4.27 and 23.00±15.43) with an increase in the medium and highest concentration (31.25±1.89 and 36.75±1.71; 33.00±0.82 and 37.50±1.91; 33.00±0.83 and 42.50±2.08) of lamivudine treatment for 11, 22 and 33 days when compared with the control (29.50±3.54), this concurred with the work of Allston (1993). The graded concentrations showed an increase in Plasma aspartate aminotransferease activity was experiential across groups 2, 3 and 4(102.75±68.59, 154.25±4.35 and 168.75±6.99; 121.25±3.40, 134.50±3.00 and 139.00±1.83; 92.75±61.89. 141.75±1.71 and 151.00±2.59) lamivudine treatment for 11, 22 and 33 days when compared with the control (109.00±2.83). Plasma alkaline phosphatese (ALP) activity in Table 1, 2 and 3 increased significantly ($p<0.05$) across the treatment groups in days 11, 22 and 33 (112.75±75.17, 154.25±5.56 and 170.75±10.31; 167.50±5.97, 193.00±4.55 and 205.00±8.51; 125.00±83.53, 193.75±6.13 and 218.75±16.36) when compared with the control (138.00±12.73), this is in line with the report of Burke (2002). This driftwas found in in days 11, 22 and 33. Alkaline phosphatase (ALP) activity is inparallel pattern as alanine aminotransferease (ALT) which showed a significant increase across the treated groups specifically at highest concentration of lamivudine involved in the period of exposure compared with control group. Highest activity of the treatment was observed across the treatment days and decreased with the lowest treatment groups to with concentration dependent manner.

Table 1: Effects of anti-retroviral drug on liver function test after 11 days of exposure

Groups	Conc. (mg/ml)	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	DW	29.50±3.54^a	109.00±2.83^a	138.00±12.73^a
Lamivudine	0.1	21.75±14.52^b	102.75±68.59^b	112.75±75.17^b
Lamivudine	0.3	31.25±1.89^a	154.25±4.35^c	154.25±5.56^b
Lamivudine	0.4	36.75±1.71^c	168.75±6.99^c	170.75±10.31^c

The values were presented in Mean±SEM); Superscript 'a' represents significant difference when compared with the control value at $p< 0.05$

Table 2: Effects of anti-retroviral drug on liver function test after 22 days of exposure

Groups	Conc. (mg/ml)	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	DW	29.50±3.54^a	109.00±2.83^a	138.00±12.73^a
Lamivudine	0.1	31.25±4.27^a	121.25±3.40^b	167.50±5.97^b
Lamivudine	0.3	33.00±0.82^a	134.50±3.00^c	193.00±4.55^c
Lamivudine	0.4	37.50±1.91^b	139.00±1.83^c	205.00±8.51^c

The values were presented in Mean±SEM); Superscript 'a' represents significant difference when compared with the control value at $p< 0.05$

Table 3: Effects of anti-retroviral drug on liver function test after 33 days of exposure

Groups	Conc. (mg/ml)	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	DW	29.50±3.54^a	109.00±2.83^a	138.00±12.73^a
Lamivudine	0.1	23.00±15.43^a	92.75±61.89^a	125.00±83.53^a
Lamivudine	0.3	33.00±0.83^a	141.75±1.71^b	193.75±6.13^b
Lamivudine	0.4	42.50±2.08^b	151.00±2.59^b	218.75±16.36^b

The values were presented in Mean±SEM); Superscript 'a' represents significant difference when compared with the control value at $p< 0.05$

Alanine aminotransferease increased with concentration dependent in the liver with less amount in the kidney, skeletal muscle, heart, spleen, lung and pancreas (Penttila *et al*., 1975). An increase in the levels of ALT generally surmount to hepatic diseases in relation with several extent of liver necrosis includes; viral, carcinoma, toxic hepatitis, obstructive jaundice and cirrhosis. Usually, ALT is typically elevated than AST in acute associated toxic or viral hepatitis, while for several patients prone to chronic liver disease, the level of ALT generally arelesser than the level of AST. Increases in the level of ALT exhibited a widespread muscle and trauma disorders, circulatory malfunction linked with shock, myocardial infarction, hypoxia and hemolytic disorder (Penttila *et al*., 1975; Allston, 1993). An increase in the activities of the implicated enzymes elicits cell destruction which resulted from numerous mechanisms. These comprises of metabolic host- arbitrated damage, mitochondrion toxicity, hypersensitivity response and immune build-up. All nucleoside analogues found deterious with hepatotoxicity having several rampant with zalcitabine, stavudine and didanosine. These drugs showed greater affinity for polymerase-gamma in mitochondrial and presented increased toxicity rate than zidovudine, lamivudine, abacavir and tenofovir. This agents belongs to the class (NRTIs) selectively DNA polymerases-γ inhibitors from host cells and possiblyinfluence mitochondrial or nuclear DNA. DNA polymerase-γ found in mitochondrion DNA (mtDNA) reproduced via targeted NRTIs with successive mtDNA removals and resultant dearth in mtDNA preset enzymes in

mitochondrial respiratory sequence. Oxidative phosphorylation blight in mitochondrial linked with energy production deficits (ATP), intracellular adipose accumulation upstream in Krebs cycle with oxidative phosphorylation associated with lactate synthesis via anaerobic respiration. In clinical intensity, mitochondrial deficits is liable for undesirable effects like severe lactic acidosis, fat reorganization syndrome grouped and hepatic steatosis under total NRTI - induced mitochondrial cythopathy (Kakuda, 2000; Cote *et al*., 2002; Vittecoo *et al*., 2002). Mitochondrial spectrum of toxicity in NRTI drugs varies from unclear symptoms to syndrome of lactic acidosis with fulminate liver failure (de Mendoza *et al*., 2004; Coghlan, *et al*., 2001). Zidovudine is concerned with lethal hepatic failure cases in severe AIDS patients.

Patients with micro vesicular steatosis and substantial hepatomegaly which developed to fulminant liver failure (Frieman *et al*., 1993). Zidovudine can as well stimulate a commonly hypertrophy, glycogen build-up, parenchymal inflammation and lipidosis. Generally, elevation in ALT and AST with slighter increases of ALP enhances hepatic cell necrosis, with reverse peaks to cholestasis (Burke, 2002). An increased proportion of ALT to ALP is an indication to diagnosed acute liver necrosis. Other exceptions includes acute alcoholic hepatitis, ALT and AST (mainly ALT) are generally lesser than 10 times the standard in rising cholangitis triggered by choledocholithiasis, ALT and AST also promote greater level than ALP (Burke, 2002). The result proposed absence hepatocellular necrosis, since ALT and ALP increase triggered cholestasis. In conclusion, antiretroviral regimen with lamivudine are associated with liver enzymes, conversely liver function test required check twice a month for the period of starting treatment regimen so as toverifyonset of hepatic toxicity for healthy liver at initiation of treatment.

Conclusion

In conclusion, antiretroviral regimen with lamivudine are associated with liver enzymes, conversely liver function test required check twice a month for the period of starting treatment regimen so as to verify onset of hepatic toxicity for healthy liver at initiation of treatment.

Conflict of Interest

Authors declare that there is no conflict of interest reported in this work.

References

Akinsete I 2002. HIV/AIDS: The Nigerian and Global situation analysis. National Action Committee on AIDS (NACA) Abuja, Nigeria.

Allston CA 1993. Non protein nitrogenous compounds and renal functions clinical chemistry concepts and applications. Anderson SC, Cockrayne S eds (WB Saunders Philadelphia USA), p. 369.

Burke MD 1975. Liver function. *Human Pathology*, 6: 273–86.

Clark S, Creighton S, Portmann B, Taylor C, Wendon J & Cramp M 2002. Acute liver failure associated with antiretroviral treatment for HIV: A report of six cases. *J. Hep.*, 36: 295-301.

Coghlan M, Sommadossi J, Jhala N, Many W, Saag M & Johnson V 2001. Symptomatic lactic acidosis in hospitalized antiretroviral-treated patients with HIV infection: A report of 12 cases. *Cl. Infect. Dis.*, 33: 1914-1921.

Cote HC, Brumme ZL & Craib KJ 2002. Changes in mitochondrial DNA as a maker of nucleoside toxicity in HIV-infected patients. *New Eng. J. Med.*, 346: 811-820.

De Mendoza C, de Ronde A, Smolders K, Blanco F, Garcia-Benayas T & de Baar M 2004. Changes in mitochondrial DNA copy number in blood cells from HIV-infected patients undergoing antiretroviral therapy. *AIDS Res. Hum. Retro.*, 20: 271-273.

Den Brinker MW, Ferdinand WNM, Wertheim-van D, Pauline ME, Jurriaans S, Weel J, Van R, Pakker NG, Reiss P, Danner SA, Jan WGL & Joep MA 2000. Hepatitis B and C virus co-infection and the risk for hepatotoxicity of highly active antiretroviral therapy in HIV- infection. *AIDS*, 14: 2895-2902.

Doueke DC, Roederer M & Koup RA 2009. Emerging concepts in the immunopathogenesis of AIDS. *Annual Re. Med.*, 60: 471–84.

Freiman J, Helfert K, Hamrell M & Stein D 1993. Hepatomegaly with severe steatosis in HIV-seropositive patients. *AIDS*, 7: 379-385.

Hernandez L, Gilson I, Jacobson J, Affi A, Puetz T & Dindzans V 2001. Antiretroviral hepatotoxicity in HIV-infected patients. *Alim. Pharm. & Ther.*, 15: 1627-1632.

Kakuda TN 2000. Phamacology of nucleoside and nonnucleoside reverse transcriptase inhibitor-induced mitochondrial toxicity. *Cl. Ther.*, 22: 685-708.

Nunez MJ, Martin-Carbonero L, Moreno V, Valencia E, Garcia-Samaniego J & Gonzalez-Castillo J 2006. Impact of antiretroviral. *AIDS*, 22: 825-829.

Onwuliri VA, Kanki P, Umeh MM & Awari A 2003. Educating sex workers in Nigeria. 13th International Conf. AIDS STDS Africa, Nairobi, Sept. 21st – 26th 2003 No. 126598, p. 6.

Palella F, Baker R, Moorman A, Chmiel J, Wood K & Brooks J2006. Mortality in the highly active antiretroviral therapy era: Changing causes of death and disease in the HIV outpatient study. *J. Ac. Imm. Def. Syn.*, 43: 27-34.

Penttila IM, Jokela HA, Viitala AJ, Heikkinen E, Nummis & Pystynen P 1975. Activities of aspartate and alanine animotransterases and alkaline phosphatase in sera of healthy subjects. Scand. *J. Cl. Lab. Invest.*, 35: 275-284.

Rec GSCC 1972. Colorimetric method for serum alkaline phosphatase determination. *J. Cl. Bioch..*, 10(2): 182.

Reitman S& Frankel S 1957. A colourimetric method for the determination of serum glutamic-oxaloacetic and glutamic-pyruvic transaminase. *A. J. Cl. Path.*, 28: 56-61.

Schmidt E & Schmidt FW 1963. Enzyme. *Bio. Cl.*, 3: 1-7.

Spengler U, Lichterfeld M & Rockstroh JK 2002. Antiretroviral drug toxicity-therapy. *Reuters Health*, 12-18.

UNAIDS 2010. UNAIDS Report on the Global AIDS Epidemic.

UNDP 2011. Human Development Report 2011.

Vitecoo D, Jardel C & Barthelemy C 2002. Mitochondrial damage associated with long-term antiretroviral treatment: Associated alteration or causal disorder? *J. Acq. Imm. Def. Syn.*, 31: 299-308.

Weiss RA 1993. How does HIV cause AIDS? *Sc.*, 260(5112): 1273–1279.

World Health Organisation 2008. Towards Universal Access: Scaling up Priority HIV/AIDS interventions in the health sector: progress report. Available from http://www.who.int/hiv/mediacenter/2008/progresssreport/en/index.html.