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Assessment of Cardiac Function Parameters of Rats in First and Second Line Fixed Dose of Anti-Retroviral Drugs Versus First Line with Switch to Second Line Combination

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Abstracts

The use of combined antiretroviral drugs may have a negative consequence on the clinical outcome of HIV-infected individuals resulting in metabolic complications. This study investigated the effect of first and second line fixed-dose combination (FDC) antiretroviral drugs on cardiac parameters in Wistar rats. Thirty-five (35) male Wistar rats (Raths Novegicus) were divided into seven (7) experimental groups (A, B1, B2, C1, C2, D1 and D2). Group A received normal rat pellet and clean water. Group B1 received 17.14 mg/kgbwt/24h of fixed-dose EFV/3TC/TDF as first line regimen for 15 days, while Group B2 received same regimen for 30 days. Group C1 received 6.43 mg/kgbwt/12h of fixed-dose 3TC/ZDVt3.57 mg/kgbwt/12h of LPV/r as second line regimen for fifteen (15) days, while Group C2 received same regimen for 30 days. Group D1 received first line regime for 30 days then switched to second line regimen for 15 days (a total of 45 days), while Group D2 received first line regime for 30 days, then switched to second line regimen for another 30 days (a total of 60 days). First and second line regimens showed significant ($P<0.05$) increase in serum level of markers of cardiac function in treated groups when compared with the control. From the study findings, First and second line FDC antiretroviral drugs exerted toxic effect on cardiac function in rats; however repeated dose at long term use may be tolerated.

Keywords: HIV/AIDS, Antiretroviral drugs, Cardiac markers, Fixed-dose.

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INTRODUCTION

The earliest well documented case of human immunodeficiency virus (HIV) in human dates to 1959 (30), but the vast of infections occurring outside sub Saharan African, can be traced back to a single unknown individual who got infected with HIV in Haiti, Northern America, and then brought the infection to other parts of united states in 1969 (12). The epidemic then rapidly spread among high-risk groups, initially. Sexually promiscuous gay men. By 1978, the prevalence of HIV among gay male residents of New York and San Francisco was estimated at 5% (United State Centre for Disease Control, US CDC, 1982), suggesting that several thousand individuals in the country had been infected by then.

HIV has been reduced due to the introduction of antiretroviral (ARV) drugs and this significantly increased the life expectancy among HIV-infected patients (28). Anti-retroviral drugs are of different classes including; the nucleoside and nucleotide reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), chemokine receptor (CCR5) antagonist, integrase strand transfer inhibitors (INSTIs) and post-attachment inhibitors (PAIs). These drugs act by inhibiting HIV replication at different stages in the HIV life cycle (15, 21).

Antiretroviral drugs have been modified to a fixed-dose combination (FDC) generally referred to as combination Anti-Retroviral Therapy (CART) or Highly Active Antiretroviral Therapy (HAART) due to advent of drug expansion programs by pharmaceutical companies, research institutions and state agencies; and are currently used in the treatment of HIV infection. FDC antiretroviral drugs include the combination of three different types of highly effective antiretroviral drugs, including NRTIs, NNRTIs and a drug from PIs or INSTLs class (29). This combination therapy has brought about a marked improvement in the prognosis of HIV disease (21). It is considered the most effective treatment for individuals with HIV infection (19). Treatment with FDC antiretroviral drugs 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 (11).

Cardiac biomarkers are substances that are released into the blood when the heart is stressed, injured or damaged. Measurements of these biomarkers are very useful in diagnosing diseases of the heart. Advanced age and use of tobacco as well as combination ARV drugs have been shown to be associated with greater risk of myocardial infarction (6). A prospective observational cohort study, reported an increased incidence of myocardial infarction and angina in HIV-positive patients taking antiretroviral drugs of the PIs class (10). These findings suggest a combined effect of HIV infection and ARV drugs on overall cardiovascular disease (CVD) risk. Drug toxicity can cause clinically significant myocardial dysfunction; drugs specific to the HIV/AIDS patients that can cause heart failure including AZT, interferon alpha, foscarnet, doxorubicin, pentamidine and amphotericin B (6).

Levels of some enzymes and protein referred to as cardiac biomarkers that are linked to the injury of the heart are measured to detect CVD. These include the enzymes, creatinine kinase (CK) and lactate dehydrogenase; and the proteins, troponin I (TnI) and troponin T (TnT). Rinse in one or more of these cardiac biomarkers are associated with heart injury (9). This study assessed cardiac parameters of rats in first and second line fixed doses of anti-retroviral drugs versus first line with switch to second line combination.

MATERIALS AND METHODS

Materials

Chemicals and Reagents

The assay kit for the determination of activities of lactate dehydrogenase (LDH), creatinine kinase (CK), were obtained from Fortrees Diagnostic Limited, Unit 2C Antrim Technology Park, Antrim, BT41 1QS, United Kingdom.

Experimental Animals

Experimental Animals (male albino Wistar rats) used for this study were purchased from the Animal House, faculty of Basic Medical Science, University of Uyo. The animals were kept in standard plastic cages and housed in a good atmospheric condition under a 12-hour day/light cycle. They were allowed free access to rat pellet and clean water *adlibilum.* The feeding lasted for a period of one month to get the desired weight of 200 g and above. During this period, the rats got acclimatized to the environment prior to the commencement of the experiment. Body weight of the animals was taken at baseline and weekly throughout the experimental period.

Drug Sample

The following fixed-dose combination (FDC) antiretroviral drugs (first and second line regimens) manufactured by Mylan Laboratories Limited, India were obtained from University of Uyo Teaching Hospital (UUTH) for the study.

i. First line Regimen: FDC of TDF/3TC3EFV (Symfi® or Telura®) containing two (2) NRTIs [Tenofovir Diisoproxil Fumarate (TDF)/Lamivudine (3TC)] and one (1) NNRTI [Efavirenz (EFV)] in one table. Thus, a single dose of TDF/3TC/EFV contains 300mg of TDF, 300mg of 3TC and 600mg of EFV.

ii. Second Line Regimen: FDC of 3TC/ZDV (Combivir®) containing two (2) NRTI [Lamivudine (3TC)/Zidovudine (ZDV] in one table, co-administered with boosted Lopinavir (LPV/r) (Kaletra®). A single dose of 3TC/ZDV contains 150mg of 3TC and 300mg of ZDV, while a dose of LPV/r is made up of 200 mg of LPV co-formulated with 50 mg of ritonavir (r).

Experimental design

A total of thirty-five (35) male albino rats (*Rattus novegicus*) of the Wistar strain weighing between two hundred (200) and two hundred and fifty (250) grams were used in the study. The rats were divided into four groups (A, B, C and D). Group A which had five (5) rats served as control. Groups B, C, and D had ten (10) rats each; they were sub-divided into B_1, B_2, C_1, C_1, D_1 and D_2. This gave a total of seven (7) experimental groups of five (5) animals each. The cages were labeled accordingly and drug administration carried out as follows:

S/N	Groups	Specification
I	A	Normal animal fed with rat pellets and distilled water, received no treatment.
II	B_1	Received 17.14mg/kg/bwt/24h of fixed-dose 3TC/TDF/EFV as first line regimen for fifteen (15) days.
III	B_2	Received 17.14mg/kg/bwt/24h of fixed-dose 3TC/TDF/EFV as first line regimen for thirty (30) days.
IV	C_1	Received 6.43mg/kg/bwt/12h of fixed-dose 3TC/ZDV + 3.57mg/kg/bwt/12h of LPV/r as second line regimen for fifteen (15) days.
V	C_2	Received 6.43mg/kg/bwt/12h of fixed-dose 3TC/ZDV + 3.57mg/kg/bwt/12h of LPV/r as second line regimen for thirty (30) days.
VI	D_1	Received 17.14mg/kg/bwt/24h of fixed-dose 3TC/TDF/EFV as first line

		regimen for thirty (30)) days, then switched to 6.43mg/kg/bwt/12h of fixed-dose 3TC/ZDV + 3.57mg/kg/bwt/12h of LPV/r as second line regimen for fifteen (15) days (a total of 45 days).
VII	D_2	Received 17.14mg/kg/bwt/24h of fixed-dose 3TC/TDF/EFV as first line regimen for thirty (30) days, then switch to 6.43mg/kg/bwt/12h of fixed-dose 3TC/ZDV + 3.57mg/kg/bwt/12h of LPV/r as second line regimen for another thirty (30) days (a total of 60 days). Note: Bw= Body weight

Preparation of Stock Solution

Drugs used in the study were all presented in tablet form. Therapeutic dosage of the drugs for human adult weighing seventy (70) kg were 1200 mg of fixed-dose EFV/3TC/TDF; 450 mg of fixed-dose 3TC/ZDV and 250 mg of LPV/r respectively. To obtain the corresponding therapeutic dosage for the rat models one tablet each of 3TC/TDF/EFV (1200 mg) and 3TC/ZDV (450 mg) were crushed with pestle and mortar, dissolved in 100ml of distilled water to get stock solution of concentration of 12 mg/ml and 4.5 mg/ml respectively. Equally, two tables of LPV/r (500 mg of 250 mg each) were crushed and dissolved in 100ml of distilled water to give a concentration of 5.0 mg/ml. required dosage for each of the rats were calculated based on the body weight then measured as aliquot and administered to the animals through oral intubation.

Collection of Blood Sample, Preparation of sera and Tissue Sample

At the end of administration period (15, 30, 45 and 60 days), the experimental animals were fasted overnight and anaesthetized by dropping each in a transparent glass jar saturated with chloroform fumes. Blood sample was collected from each animal by cardiac puncture after dissection using sterile needles and syringes into a labeled sample bottle. Sample from each animal were emptied into plain sample bottles and centrifuged at 3000 rpm for 10 minutes using a bench top centrifuge. The serum collected was preserved in the refrigerator for biochemical analysis which was carried out promptly.

Assessment of cardiac function markers

Cardiac markers assessed in this study were Lactate Dehydrogenase (LDH) and Creatinine Kinase (CK).

Serum lactate dehydrogenase (LDH) activity

Lactate Dehydrogenase (LDH) was determined based on enzymatic rate method described by Wacker (1956). Lactate dehydrogenase (LDH) catalyzes the oxidation of lactate to pyruvate in the presence NAD, which is subsequently reduce to NADH.

$$\text{Lactic acid} + NAD^+ \xrightarrow{\text{LDH}} \text{Pyruvate} + NADH + H^+$$

The rate of NAD reduction and NADH formation measured at 340 nm is directly proportional to serum LDH activity. Lactate dehydrogenase measurements are used in the diagnosis and treatment of cardiac diseases, li9ver diseases and tumors of the lung or kidney.

Serum creatinine kinase (CK) activity

Serum Creatinine Kinas (CK) was assayed using Rosalki (1977) technique. The technique is based on enzyme coupled reactions which optimizes the reaction by reactivation of CK activity with an enzyme reactivator. Creatinine kinase (CK), present in the sample, catalyzes the transfer of a high energy phosphate group from creatinine phosphate to adenosine triphosphate (ATP). The ATP produced in this reaction is subsequently used to phosphorylate glucose to produce glucose-6 phosphate (G-6-P) in the presence of hexokinase. G-6-P is then oxidized by glucose-6-phosphate dehydrogenase (G-6-PDH) with the concomitant reduction of nicotinamide adenine dinucleotide phosphate (NADP) to nicotinamide

adenine dinucleotide phosphate reduce (NADPH).

$$\text{Creatinine Phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{Creatinine} + \text{ATP}$$

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{G6PH}} \text{ADP} + \text{Glucose-6-phosphate}$$

$$\text{G-6-P} + \text{NADP} \xrightarrow{\text{HK}} \text{6-Phosphogluconate} + \text{NADPH} + \text{H}^{+}$$

The rate of formation of NADPH is observed at 340 nm and is proportional to the activity of CK in the sample. These reactions occur in the presence of N-acetyl-L-cysteine (NAC) which is present as an enzyme reactivator.

Statistical analysis

Data were analyzed using SPSS statistical software package version 20.0 and results expressed as mean ± standard error of mean (SEM). Analysis of Variance (ANOVA) and Least Significant Difference (LSD) multiple post hoc comparison tests were carried out on the data and Mean difference between groups were considered statistically significant at $p<0.05$.

Ethical consideration

Prior to the commencement of this study approval was sought and granted by Faculty of Basic Medical Sciences Ethical Committee, University of Uyo, Nigeria.

RESULTS AND DISSCUSIONS

Results

Table 1 shows the mean $^{+}$ SD of activities of lactate dehydrogenase (LDH) and creatine kinase (CK) of male albino Wistar rats treated with first and second line FDC antiretroviral drugs. Observed from the result was statistically significant ($P<0.05$) increase in serum LDH in all the treated Groups when compared with control group; but Groups C_2 and D_2 showed reduced activity of the enzyme when prepared with Group B_2 and C_1 Same was observed in the enzyme activity in Group D_1 and D_2 when compared with Group C_2. Serum activity of CK showed significant ($P<0.05$) increase with the high activity in Groups C_2, D_1 and D_2 compared to Group A (control) and with Group B_1 and C_1.

Table 1: Effect of treatment with first and second line FDC antiretroviral drugs (17.14mg/kgbwt/24h of EFV/3TC/TDF and 6.43mg/kgbwt/12h of 3TC/ZDV + 3.57mg/kgbwt/12h of LPV/r) on cardiac function in male albino Wistar rats

GROUPS (n=5) (U/L)	LDH (U/L)	CK
A (control)	202.67 + 13.54	9.57+ 0.93
B_1 (1^{st} Line) 15 days	336.33 + 6.36^{a}	12.29 + 2.32
B_2 (1^{st} Line) 30 days	366.75 + 29.99^{a}	14.43 + 1.33
C_1 (2^{nd} Line) 15 days	374.50 + 1.50^{a}	10.69 + 2.35
C_2 (2^{nd} Line) 30 days	345.67 + 17.36^{abcd}	23.59 + 2.65^{abcd}
D_1 (B_2 to C_1) 45 days	309.67 + 31.94^{ac}	22.99 + 2.02^{abcd}
D_2 (B_2 to C_2) 60 days	294.67 + 22.81^{acdc}	26.23 + 2.57^{abcd}

Values are presented as Mean + Standard Error of Mean (SEM).

Source: Computed by the researcher from raw data of biochemical analysis (2019).

Legends: LDH = Lactate Dehydrogenase; CK = Creatinine Kinase; ^{a} = significantly different when compared to Group A ($p<0.05$); ^{b} = significant different when compared to Group B_1 ($p<0.05$); ^{c} = significant different when compared to Group B_2 ($p<0.05$); ^{d} = significant different when compared to

Group C_1 ($p<0.05$); [e] significant different when compared to Group C_2 ($p<0.05$); n = number of animals per group.

Discussion

The main role of the heart is to pump oxygen-rich blood to every cells of the body. The blood vessels, a network of interconnecting arteries, arterioles, capillaries, and veins provide the pathway in which blood travel. The pumping of the heart is a function of the cardiac muscle. Cardiovascular disease (CVD) is a general term used to describe medical conditions that affect the heart and blood vessels (27). Such conditions among others include coronary artery diseases (CAD) such as angina, myocardial infarction (commonly known as heart attack), high blood pressure, atherosclerosis (hardening of arteries), heart failure and strokes. Use of ARVA drugs by HIV-infected individuals have markedly improved the quality of life and prognosis of the affected patients, however, studies have shown that certain antiretroviral drugs can induce heart disease.

There are evidences of ARV drug-mediated metabolic derangements (1) and its potential risk for cardiovascular diseases (CVD) in the long-term. ARV drug from PIS and NRTIs class have been associated with increased risk of myocardial infarction (7) and cardiovascular abnormalities (17) with many changes resembling coronary artery disease (13). Rats exposed to eight weeks treatment with drugs from PI class were found to display cardiac dysfunction (22). Other previous studies suggested that drugs from PI class are associated with endoplasmic reticulum (ER) stress response to be an important cellular signaling pathway of PIs-induced cardop-metabolic syndromes (18, 24). However, in a recent study conducted by Everson et al. (2018), it was reported that FDC containing tenofovir (TDF), emtricitabine (FTC) and efavirenz (EFV) did not have a negative effect on the cardiac function in rats.

Compelling evidence from animal models indicate that zidovudine (ZDV), and some drugs from NRTIs class have marked adverse effects on myocardial structure and function that are mediated by mitochondrial toxicity (14). Inhibition of mitochondrial DNA polymerase by ZDV, causes mitochondrial damage, and leads to focal myocardial necrosis (3). Hassan et al. (2009) earlier reported that elevations in CK, LDH and transaminases (Well-known indicators of necrotic damage) activities are common biochemical abnormalities associated with ZDV.

High activities of serum LDH and CK enzymes observed in this study is in consonant with these earlier studies and another recent case reported an HIV-positive patient on FDC of zidovudine (ZVD-300 mg), lamivudine (3TC-150 mg), and nevirapine (NPV-200 mg) showed elevated activities of CK, LDH and transaminases pain followed by intractable vomiting and breathlessness with significant weight loss. On withdrawal of the fixed-dose ZDV/3TC/NPV on course, a significant fall in activities of CK, LDH, transaminases as well as serum amylase and lipase were observed (25).

LDH plays an important role in the human body represented by the anaerobic conversion of pyruvic acid to lactic acid and vice versa under normal conditions. LDH is produced in human body in little amount with low monitory value. There are many factors responsible for increased LDH activity in blood stream. They include prolonged exercise, and some physiological disorder such as severity of preeclampsia, ascites, allergy and drugs (20). The presence of LDH in muscle plays a very important role for muscular tissues through its ability to convert muscular lactic acid into pyruvic acid, an essential step in producing cellular energy.

Moreover, LDH is not restricted to a specific type of muscle, it is found in various types of muscle, especially skeletal and cardiac muscles with a greater concentration in the myocardium. Thus, long term use of ARV drugs may result in an infarct in the myocardium. These muscles are also known to contain CK which may be released into the blood with greater levels as a result of various muscular abnormalities including cardiac and skeletal muscle necrosis. Therefore, in order to associate high activity of serum LDH with muscular disorder it is important to measure the CK level also. High activity of CK in the serum may be an indication of damage to CK-rich tissue, such as in myocardial infarction, myositis,

myocarditis and rhabdomyolysis (2); thus, CK is a better indicator of heart or muscular damage. Therefore, estimation of CK along with LDH serve as a suitable diagnostic marker for muscular damage such as cardiac manifestations associated with acute myocardial infarction and patient with prosthetic heart valves (4, 16).

Conclusion

Standard antiretroviral drugs recommended by WHO were used as preferred first and second line regimens respectively for management and treatment of HIV/AIDS in this study and these FDC antiretroviral drugs contain TDF/EFV/3TC and 3TC/ZDV + LPV/r. Findings from this study have revealed that the regimens exhibit toxic tendency on cardiac tissues of the tested animals. Nevertheless, switching from first to second line regimen did not expose the animals to toxic consequences of severe drug effect. Thus, the use of these combined regimens in HIV/AIDS management and treatment should be encouraged but cardiac markers of the recipients should be in check.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Permission and approval for animal studies were obtained from the Faculty of Basic Medical Sciences Ethical Committee, University of Uyo, Nigeria.

HUMAN AND ANIMAL RIGHTS

The care and use of animals in this study was in accordance with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH, 1996).

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

CONFLICT OF INTEREST

Authors declared no conflict of interest

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