SMU
Sikkim Manipal University
Manipal
INSPIRED BY LIFE
SMU Medical Journal
ISSN : 2349 – 1604 (Volume – 2, No. 1, January 2015) Research article

Effect of Selected Antiretroviral Drugs on Malondialdehyde (MDA) and Catalase Levels in Healthy Rat Tissues

Kayode A. A. A* and **Kayode** O. T
Department of Chemical Sciences, College of Natural and Applied Sciences, Bells University of Technology, Km 8 Idiroko Road, P.M.B 1015, Ota Ogun State, Nigeria.

*Corresponding Author:
bolakayot@gmail.com, +2348038272670.

Manuscript received: 23.10.2014
Manuscript accepted: 26.11.2014

Abstract

The study assessed the effect of selected antiretroviral drugs used in the management of HIV/AIDS on the oxidative stress marker malondialdehyde (as TBARs) and the antioxidant enzyme, Catalase, of the liver and kidney tissues of healthy albino rats. 0.43, 0.43, 0.27, and 0.21mg/kg of Efavirenz, Abacavir, SNP 40 and Lamivudine respectively, were orally administered to four different groups of albino rats for seven days. The control group received normal saline. On the eighth day, the rats were sacrificed and the liver and kidney tissues were collected for Lipid peroxidation and Catalase activity analysis. Efavirenz and Lamivudine caused significant decrease ($P<0.05$) in lipid peroxidation in the liver while the reverse was obtained for Abacavir and SNP 40. SNP 40 caused a significant decrease ($P<0.05$) in lipid peroxidation in the kidney while the other drugs did not result in a significant change ($p>0.05$) compared to the

control. All the drugs caused significant increase (P<0.05) in Catalase activity in the liver and a significant decrease (P<0.05) in Catalase activity in the kidney. Taken together, the present observation suggests that the effects of antiretroviral drugs on oxidative stress markers (such as MDA) and on antioxidant enzymes (such as catalase) in healthy as well as in HIV infected humans (by way of extrapolation) may vary from drug to drug and from organ to organ. We therefore advocate for extensive clinical research to investigate the influence of antiretroviral drugs on antioxidants enzymes in HIV and HIV/AIDS patients.

Keywords: antiretroviral drugs, lipid peroxidation, liver, kidney, catalase.

Introduction

The acquired immunodeficiency syndrome (AIDS) is a fatal illness caused by a retrovirus known as the human immunodeficiency virus (HIV) that breaks down the body's immune system that infects CD4+ cells initially and progressively leads to AIDS [1]. The use of antiretroviral therapies (ART) is recommended worldwide for the management of HIV/AIDS. The World Health Organization (WHO) currently recommends first-line therapy with two nucleoside reverse transcriptase inhibitors (NRTIs) such as lamivudine and one non-nucleoside reverse transcriptase inhibitors (NNRTI) such as efavirenz [2].

Oxidative stress (when free radical generation exceeds antioxidant defense) occurs in many human diseases and makes significant contributions to their pathogenesis [3]. Free radicals are compounds possessing an unpaired electron, which renders them highly reactive and capable of causing oxidative damage to all the major macro-molecules in cells, including lipids, proteins and nucleic acids. A major family of free radicals is the reactive oxygen species, derived metabolically from molecular oxygen via super oxide anions. Oxidative attack on proteins results in the formation of protein carbonyls often with the loss of functionality of the parent protein [4].

Polyunsaturated fatty acids, which are major components of cell membranes, can also undergo free radical attack, producing lipid peroxidation products like malondialdehyde (MDA) and 4-hydroxynonenal. Under normal circumstances, the body is protected from such damage by a careful balance between pro-oxidants and antioxidants. In extra vascular spaces, the sulphydryls group of plasma proteins, including plasma albumin serve as antioxidants with enzymes and

Scavenging chemicals such as Vitamin C and vitamin E also having antioxidant activities [5].

Catalases are enzymes that catalyze the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor [6]. This protein is localized in peroxisomes in most eukaryotic cells. Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Here, its cofactor is oxidized by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate [7].

Treatment with antiretroviral drugs (ARD) and Highly Active Retroviral Therapies (HAART) may result in biochemical and physiological changes e.g. oxidative damage resulting from the presence of free radicals or the absence of antioxidants [8].

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. The drugs were obtained from PEPFAR, University Teaching Hospital (UCH), Ibadan, Nigeria.

Animals and Treatment

Thirty albino rats, purchased from the animal house, Biochemistry and Molecular Biology Unit, Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria were housed and allowed to acclimatized for one week in the 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. Groups 5 served as the control and were treated with physiological saline. Rats in groups 1, 2, 3, and 4 were respectively treated with efavirenz (0.43 mg /kg), abacavir (0.43 mg/kg), SNP 40 (0.27 mg/kg) and lamivudine (0.21mg/kg). The

drugs were administered orally for seven consecutive days. Animals were sacrificed twelve hours after the last treatment. All necessary ethical approval as regards animal use and treatment were obtained from the University.

Lipid peroxidation: Lipid peroxidation in post mitochondrial fraction was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) method described by Varshney and Kale [9].

Catalase: Microsomal catalase activity was determined according to the method described by Sinha [10].

Statistical Analysis

All data are expressed as Mean±SEM (standard error of mean). Significant differences were tested using the student t-test. Values of $p<0.05$ were considered statistically significant [11, 12].

Results and Discussion

Efavirenz: A significant ($p<0.05$) decrease in the level of lipid peroxidation in the liver was observed but it has no significant ($p>0.05$) effect on lipid peroxidation level in the kidney.

Abacavir: It shows a significant ($p<0.05$) increase in the level of lipid peroxidation in the liver whereas a reversal was the case in the kidneys of experimental rats.

SNP 40: This antiretroviral drugs resulted in a significant ($p<0.05$) elevation of lipid peroxidation level in the liver but shows no significant change ($p>0.05$) in the level of lipid peroxidation in the kidney.

Lamivudine: The acute administration of lamivudine brought about a significant ($p<0.05$) decrease in lipid peroxidation level in the liver but has no significant ($p>0.05$) effect on the level in the kidney.

All the antiretroviral drugs administered caused significant increase ($P<0.05$) in Catalase activity in the liver and a significant decrease ($P<0.05$) in Catalase activity in the kidney. These results are illustrated graphically in the figure-1 and figure-2 below.

The present study was designed to investigate the effect of selected antiretroviral drugs on oxidative stress markers when administered in the absence of the Human Immunodeficiency

Virus (HIV). Since studies have shown that HIV infection is accompanied with a progression in the oxidative stress process [1, 8, 13, 14, 15,] any antiretroviral drug whose administration potentially result in oxidative stress in non-infected animals would only worsen the oxidative damage in HIV- infected animals [16].

Antiretroviral drugs may induce (i) an increase in oxidant generation, (ii) a decrease in anti-oxidant protection, or (iii) a failure to repair oxidative damage. Oxidative stress-mediated cell damage occurs, in part, via reactive oxygen species (ROS). ROS include molecules like hydrogen peroxide; ions like the hypochlorite ion; radicals like the hydroxyl radical; and the super oxide anion which is both ion and radical. Radicals (also called "free radicals") are a cluster of atoms that contain an unpaired electron in their outermost orbit of electrons. This is an extremely unstable configuration, and radicals quickly react with other molecules or radicals to achieve the stable configuration. Once formed, ROS participate in a number of reactions, yielding additional free radicals such as hydrogen peroxide, peroxynitrite, or hypochlorous acid [17, 18]. The free radicals in HIV infection could result also from non-enzymatic protein oxidation and the subsequent oxidative degradation of glycated proteins.

We noticed that, increase in oxidative stress marker (MDA) in the liver was brought about by only the drug abacavir, which has been reported to cause a drug –induced hepatotoxicity [19, 20,21]. Efavirenz and lamivudine have also been reported to be hepatotoxic [19,20,21] .The fact that they did not result in any increase in lipid peroxidation level when compared to the control group does not invalidate the above claims but could mean that the drugs probably cause hepatic damage through other mechanism(s) other than by oxidative stress. SNP 40 shows a significant elevation in the lipid peroxidation level in the liver thereby suggesting an initiation in the oxidative stress process. However, our previous findings suggest that SNP 40 may not cause significant hepatic damage in short term administration. This then means that either the oxidative stress process initiated by the drug was reversible or the time was too short to get an observable hepatic damage. We should also bear in mind that SNP 40 being a combined retroviral therapy was designed to achieve both increased efficacy and reduced side-effects [22, 23, 24]. However, there is need for further work to really clarify and substantiate these findings, especially those involving

long term administration of the drug.

The results of the effect of the antiretroviral drugs on the lipid peroxidation level in the kidney suggest that in short term oral administration , these drugs may not be involved in the initiation or on set of drug –induced nephrotoxicity following oxidative stress. Even in HIV-infected individuals, drug- related renal toxicity from the use of antiretroviral drugs are very few and only those with the history of kidney failure or kidney diseases have been reported to have problems metabolizing antiretroviral drugs. Most antiretroviral agents are relatively free of renal toxicity [25, 26, 27, 28, 29]. Our results further indicate that the antiretroviral drugs increased catalase activities in the liver. This could be due to the response of liver to an increased production of reactive oxygen species or probably an induction of the enzyme by the drugs and/or their metabolites. The observed overall decreased of the catalase activities in the kidney suggest that the organ may not be able to protect itself from cellular damage (especially those caused by hydrogen peroxide) in the case of hydrogen peroxide and hydroxyl radical – induced oxidative stress. It is generally accepted that hydrogen peroxide can be detoxified by catalase, which removes it when present at high concentration and glutathione peroxidase, which destroys it when present at a steady state [30].

Conclusion

Taken together, the present observation suggests that the effects of antiretroviral drugs on oxidative stress markers (such as MDA) and on antioxidant enzymes (such as catalase) in healthy as well as in HIV infected humans (by way of extrapolation) may vary from drug to drug and from organ to organ. We therefore advocate for extensive clinical research to investigate the influence of antiretroviral drugs on antioxidants enzymes in HIV and HIV/AIDS patients.

Acknowledgement

We appreciate the help and support we received during this study from our students and staff of the Chemical Sciences Department, the staff of the Health Center and the Central Research Laboratory, Bells University of Technology, Ota, Ogun State, Nigeria.

References

1. Pasupathi P, Ramchandran T, Sindhu P J, Saranavan, G and Bakthavathsalam G., Enhanced Oxidative Stress Markers and Antioxidant Imbalance in HIV Infection and AIDS Patients, *Journal of Scientific Research* ; Vol. 1, No 2, (2009)
2. WHO. Scaling up Retroviral Therapy in Resource Limited Setting. *World Health Organization* (2003); Rome
3. Dasgupta A, Malhotra D, Levy H, Marcadis D, Blackwell W and Johnston D., Decreased total antioxidant capacity but normal lipid hydroperoxide concentrations in sera of critically ill patients, *Life Sci.*, 60 (4-5), 335-40 (1997)
4. Zoccali C, Mallamaci F and Tripepi G., AGE's and carbonyl stress: potential pathogenic factors of long-term ureamic complications**,** *Nephrol Dial Transplant*., 15(suppl 2)**:**S7-10.) (2000)
5. Halliwell B., Reactive oxygen species in living systems: source, Biochemistry and role in human disease, *American Journal of Medicine*., (suppl 3C)**,** S114-122 (1991)
6. Chelikani P, Fita I and Loewen P., Diversity of structures and properties among catalase, *Cell Mol Life Sci*.; 61 (2), 192-208 (2004)
7. Hiner A, Raven E, Thorneley R, Garcia-Canovas F and Rodrigues L., The mechanisms of compound formation in heme peroxidases, *J Inorg Biochem*., 91 (1), 27-34 (2002)
8. Judith L, Ngondi, Julius Oben, David Musoro Forkah, Lucein Honore Etame and Dora Mbanya., The effect of different combination therapies on oxidative stress markers in HIV infected patients in Cameroon, *AIDS Research and Therapy*., 3:19 (2006)
9. Varshney, R and Kale, R. K., Effects of Calmodulin Antagonist on Radiation Induced Lipid Peroxidation in Microsomes, *Int. J. Rad. Biol.*, 58:733-743 (1990)
10. Sinha, A. K., Enzymic synthesis of saccharopine-14C, *Anal. Biochem.*,; 43:468-471 *(*1997)
11. Zar, J.H: Biostatistical Analysis. Prentice-Hall, USA.; pp: 620 (1984)
12. Bamgboye, E. A., A companion of Medical Statistics. Fobam Publishers, Ibadan, (2002). ISBN: 9780566619

13. Allard JP, Aghdassi E, Chau J, Tam C, Kovacs CM, Salit IE and Wallmsley SL., Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects, *AIDS*., **12**, 1653-1659 (1998)
14. Papadopulos-Eleopulos E, Hedland-Thomas B, Causer D, et al., Changes in thiols and glutamate as consequences of simian immunodeficiency virus infection, *Lancet*; **11**, 1013 (1991)
15. Eck HP, Gmunder H, Hartmann M, et al., Low concentrations of acid soluble thiol (cysteine) in the blood plasma of HIV-1-infected patients, *Biol Chem Hoppe-Selyer*; **370**, 101-108 (1989)
16. AntonellaMandas, Eugenio Luigi Iorio, Maria Gabriella Congiu, Cinzia Balestrieri, AntonelloMereu,1 Daniela Cau,1 Sandra Dess`ı, and Nicoletta Curreli., Oxidative Imbalance in HIV-1 Infected Patients Treated with Antiretroviral Therapy, *Journal of Biomedicine and Biotechnology*; Volume 2009, Article ID 749575, 7 pages *(*2009)
17. Maytin M, Leopold J and Loscalzo J., Oxidant stress in the vasculature, *Curr Atheroscler Rep*., **1**, 156-164 (1999)
18. Bo Jiang, Valeria Y. Hebert, Yuchi Li, J. Michael Mathis, J. Steven Alexander and Tammy R. Dugas., HIV antiretroviral drug combination induces endothelial mitochondrial dysfunction and reactive oxygen species production, but not apoptosis, *Toxicology and Applied Pharmacology*; Volume 224, Issue 1, Pages 60-71 (2007)
19. Kayode A. A. A, Kayode O. T, Aroyeun O. A and Stephen M. C.., Hematologic and Hepatic Enzyme Alterations Associated with Acute Administration of antiretroviral Drugs, *J. Pharmacol. Toxicol*., 6 (3) 293-302 (2011)
20. Umar, R.A., S.W. Hassan, M.J. Ladan, I.K. Matazu and B. Shehu et al., Adverse hepatic effects associated with administration of antiretroviral drugs (nevirapine, lamivudine and stavudine) to albino rats, Implication for management of patients with HIV/AIDS, *Asian J. Biochem*., 3, 19-25 (2008)
21. Nicholas, K. and D. Dieterich., Hepatotoxicity of antiretroviral therapy. *AIDS Re.,* 5, 36-43 (2003)

22. Hogg, R.S., B. Yip, C. Kully, K.J.P. Craib, M.V. O Shaughnessy, M.T. Schechter and J.S. Montaner., Improved survival among HIV-infected patients after initiation of triple drug antiretroviral regimens, *Can. Med. Assoc. J*., 160: 659-665 (1999)
23. Carr, A. and D.A. Cooper., Adverse effects of antiretroviral therapy, *Lancet* 356, 1423-1430 (2000)
24. Piscitelli, S.C. and Gallicano K.D., Interactions among drugs for HIV and opportunistic infections, *N. Engl. J. Med*., 344, 984-996 (2001)
25. Jeffrey S. Berns and Nishaminy Kasbekar: Highly Active Antiretroviral Therapy and the Kidney: An Update on Antiretroviral Medications for Nephrologists. *Clin J Am Soc Nephrol*; 1, 117–129 (2006)
26. Franceschini N, Napravnik S, Eron JJ Jr, Szczech LA, Finn WF., Incidence and etiology of acute renal failure among ambulatory HIV-infected patients, *Kidney Int*., 67, 1526–1531(2005)
27. Izzedine H, Launay-Vacher V, and Deray G: Antiviral drug induced nephrotoxicity. *Am J Kidney Dis*; 45, 804–817 (2005)
28. Daugas E, Rougier JP, Hill G: HAART-related nephropathies in HIV-infected patients. Kidney Int; 67, 393–403 (2005)
29. Berns JS, Cohen RM and Rudnick MR., Antiviral agents, In: Clinical Nephrotoxins. Renal Injury from Drugs and Therapeutics, 2nd Ed., edited by DeBroe ME, Porter GA, Bennett
    WM, Verpooten GA, Dordrecht, Kluwer Academic Publishers, pp 249–263 (2003)
30. Casado, A., Dela Torre, R., Lopez-Fernandez, M., Carrascosa, D., Casado, M.C. &Ramirez, V. Superoxide dismutase and catalase blood vessels in patients with malignant diseases. Cancer Lett., 91, 19-23.

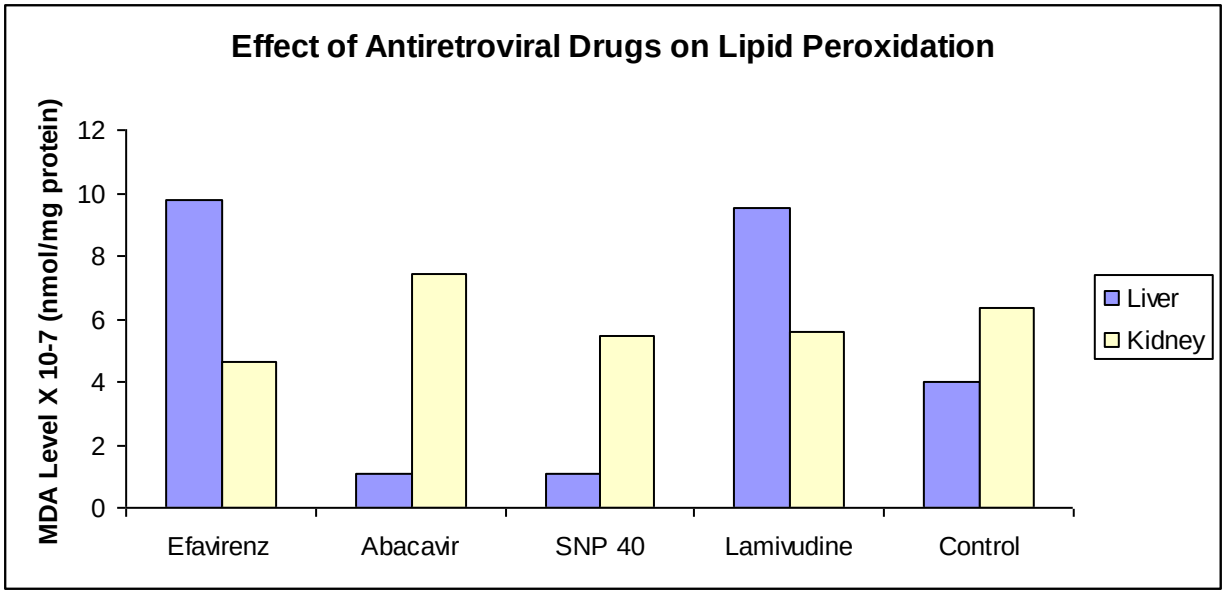

Figure- 1: Effect of Antiretroviral Drugs on Lipid Peroxidation

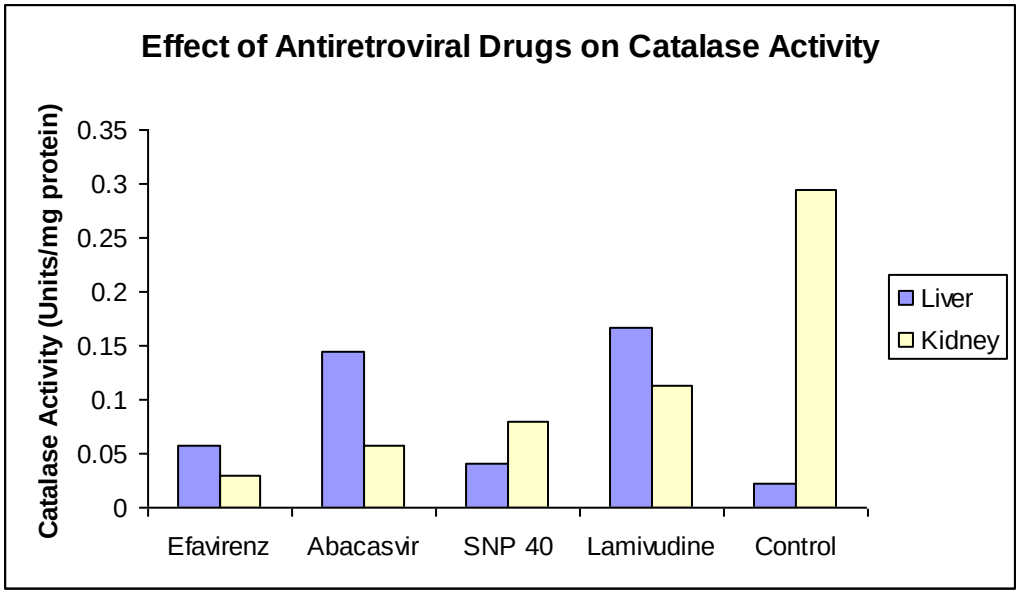

Figure- 2: Effect of Antiretroviral Drugs on Catalase Activity.

Authors Column

Kayode A. A. Abolanle is a lecturer in the Department of Chemical Sciences, Bells University of Technology, Ota, Ogun State, Nigeria. He holds a B. Sc (Biochemistry) from the University of Ilorin, Nigeria. His undergraduate dissertation was on "Effects of Nalidixic acid and Arteminisin on rats infected with *T-vivax*. He was awarded Masters of Science (M. Sc) degree in Biochemistry, University of Ibadan, Nigeria. His M. Sc work was on the Antiulcerogenic Activity of *Parquetina nigrescens*. His research focus is Isolation, Characterization and Mechanism of action of the Main Antiulcer Constituent of Nigerian Medicinal Plants. He has over eight (8) publications in reputable journals.

SMU Medical Journal, Volume – 2, No. – 1 , January, 2015, PP. 191 – 201.