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ADVERSE EFFECTS OF ZIDOVUDINE ON RAT'S VISION: ANTIOXIDANT AND ENZYMOLOGY PERSPECTIVES

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ABSTRACT

Background: The biochemical effects of chronic administration of Zidovudine on intracranial auditory relay centre of adult Wistar rats has been studied and reported. The objective of this related study is to investigate how the oxidative stress and antioxidant enzymes biomarkers in the tissues of intracranial visual relay centre of zidovudine-treated group compare with non-treated group. **Materials and Methods:** As in the study on auditory functions, the rats of both sexes (N=40), with an average weight of 200g were randomly assigned into treatment (n_1=20) and control (n_2=20) groups. The rats in the treatment group received 300mg/70kg (0.857mg/200g) body weight of zidovudine being the dosages required twice daily dissolved in distilled water daily and given for thirty days through orogastric tube administration While the control rats received equal volume of distilled water through the same route and for the same period. Catalase, glucose-6-phosphate dehydrogenase, glutathione peroxidase, lactate dehydrogenase and superoxide dismutase; as well as malondialdehyde were tested in the superior colliculus and lateral geniculate body tissues. **Results:** Malondialdehyde as a non-enzyme biomarker of oxidative stress as well as glutathione peroxidase and superoxide dismutase activities are higher on the treated superior colliculus, but lower in lateral geniculate body, with statistical significant ($P<0.05$) difference observed. **Conclusion:** The effects of zidovudine on antioxidant enzymes and oxidative stress in superior colliculus and lateral geniculate body of adult Wistar rats are discordant. Further studies are required to investigate whether enzymology and oxidative stress tests could be useful to ascertain zidovudine efficacy and toxicity on visual relay centers.

Keywords: lateral geniculate body, biochemical effects, superior colliculus, vision, Zidovudine

INTRODUCTION

Zidovudine (ZDV) is a type of medicine called a nucleoside reverse transcriptase inhibitor (NRTI). It was the first drug approved for AIDS;[1] and works by disrupting the HIV life cycle.[1,2] As one of highly active antiretroviral therapy (HAART) agents, zidovudine is a cytotoxic DNA chain terminator.[2] has been found to be effective in many combination regimens for the treatment of HIV infection.[3; 4] However, the use of zidovudine is not without some adverse toxicity concerns.[2-6] At some point, the debate on safety of zidovudine was whether it causes AIDS or not.[7; 8] Meanwhile, there is speculation that vision impairment may be a complication.[9; 10]

The lateral geniculate body (LGB) and superior colliculus (SC) constitutes the intracranial visual relay centers.[11; 12] LGB participates in the regulation of circadian function the hypothalamus,[11] while SC controls and regulates many movements of the eye and has a critical role in vision. It has been reported that the SC are involved in a somatosensory motor feedback loop that monitors the force of the active muscles together with the spatial position of the limb that is required for proper interaction with an object.[12] Fundamentals of multisensory integration include the neurons of the SC, which seems to play some spatio-temporal filter properties that are closely similar to those of the retina as well as those of their inputs from the cortical visual motion detector areas, suggesting their common role in motion analysis and related behavioral actions.[13]

HYPOTHESIS AND OBJECTIVE

We know that circadian rhythm is a function of rate of biochemical activities underlying physiology and driven by cellular energy demand, which in turn relates to antioxidant metabolism.[14] Therefore, given the pieces of knowledge that (i) LGB regulates circadian function the hypothalamus,[11] (ii) SC has a role in vision analysis,[13] and (iii) zidovudine may cause vision impairment;[9; 10] it is hypothesized that antioxidant enzyme activities and oxidative stress may increase in intracranial visual relay tissues of zidovudine treated group relative to non-treated group. The objective of this is to investigate whether oxidative stress indicated by malondialdehyde and antioxidant enzymes are increased in LGB and/or SC of zidovudine treatment compared to non-treated group.

MATERIALS AND METHODS

Research setting: This study was carried out as part of series of investigations, by Adjene and Igbigbi, on the effects of chronic administration of certain food, herbal or therapeutic agents on the brain.[15-20] This following protocol was employed in studying the biochemical effects of chronic administration of Zidovudine on auditory functions.[21]

Animals care and Ethics: The Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria granted approval before the commencement of the work. Forty adult wistar rats of both sexes with average weight of about 200g were randomly assigned into two groups: control (n_1=20) and treatment (n_2=20). The rats were obtained and maintained in the Animal Holding of the Department of Anatomy and cell Biology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Nigeria. The rats were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and given water liberally. ZDV was obtained from the President Emergency Plan for AIDS Relief (PEPFAR) Unit, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. This was employed in studying the biochemical effects of chronic administration of Zidovudine on auditory functions.[21]

Drug administration: The rats in the treatment group received 300mg/70kg (0.857mg/200g) body weight of zidovudine being the dosages required twice daily. The drug was dissolved in distilled water and administered twice daily for thirty days through the orogastric tube while the control rats received equal volume of distilled water through the same route and for the same period. this was employed in studying the biochemical effects of chronic administration of Zidovudine on auditory functions.[21]

Dissection of the brain, superior colliculus and lateral geniculate body: The rats in both groups were sacrificed by cervical dislocation and the skull was quickly opened with the aid of a pair of bone forceps to expose the brain. The superior colliculi and lateral geniculate bodies were carefully dissected out, weighed using the Mettler Toledo weighing balance and the biochemical techniques of the various assays were carried out.

Biochemical analyses: The different samples (superior colliculi and lateral geniculate bodies) from the experimental animals were dissected out, homogenized in a mortar and pestle with a pinch of acid

washed sand and a total of 5mls of normal saline (0.9%) added sequentially during the homogenization process. The homogenates were centrifuged at 3500rpm for 5 minutes with the aid of a centrifuge. The clear supernatants were collected using a micropipette and transferred into an empty specimen container and refrigerated till needed for the assays.[21] All biochemical analyses followed the protocols previously published.[17; 18] For instance, the superoxide dismutase (SOD) activities in these tissues were determined by the method of Misra and Fridovich,[22] while malonyldialdehyde (MDA) was estimated by the thiobarbituric acid reactive species (TBARS).[21]

Statistical analysis: The results were calculated using mean and standard error of means (SEM) respectively. The results from the various assays were analyzed using one way analysis of variance (ANOVA) and *'P < 0.05'* was taken as cut-off point for a significant level.

RESULTS

The observations of effects of zidovudine on the oxidative stress parameters on the SC and LGB are presented below (Table 1). MDA as a non-enzyme biomarker of oxidative stress as well as catalase and glutathione peroxidase (GPx) activities are higher on the treated SC, but lower in LGB, with statistical significant ($P<0.05$) difference observed.

Table 1: Mean levels of parameters in treated and control groups

Test	Brain tissue	Superior colliculus	Lateral geniculate body
SOD Units/L	**Control**	91.00 ± 6.00	45.00 ± 6.00
	ZDV	91.00 ± 5.00	*10.50 ± 4.00
LDH Units/L	**Control**	9.68 ± 0.003	9.68 ± 0.005
	ZDV	**19.37 ± 0.004	**193.66 ± 0.004
GPx Units/L	**Control**	3.95 ± 0.02	3.96 ± 0.02
	ZDV	**4.23 ± 0.8	*2.58 ± 1.00
G6PDH nm/min	**Control**	19.23 ± 4.00	17.63 ± 6.00
	ZDV	**137.87 ± 3.00	**110.57 ± 2.00
Catalase Units/L	**Control**	607.02 ± 3.00	620.04 ± 10.00

	ZDV	**616.67 ± 4.00	*504.81 ± 13.00
MDA x10^{-5} Units/L	**Control**	4.16 ± 0.05	15.06 ± 0.1
	ZDV	*4.36 ± 0.04	*5.5 ± 0.19

*Statistically significant (P < 0.05) decrease in ZDV-treated group compared to control

**Statistically significant (P < 0.05) increase in ZDV-treated group compared to control

The result of the SOD activities showed a significant (P<0.05) decrease in the LGB and no significant difference in the tested SC as compared to their corresponding control. Lactose dehydrogenase (LDH) activities showed a significant (P<0.05) increased in the ZDV tested LGB and SC as compared to the control group. The result of the GP_x activities showed a significant (P<0.05) increase in the ZDV tested SC as compared to the control group, while the GP_x in the ZDV tested LGB had a significant (P<0.05) decreased activities as compared to the control group. Glucose-6-phosphate dehydrogenase (G-6-PDH) activities revealed a significant (P<0.05) increased in the AZT tested LGB and SC as compared to the control group in this experiment. The catalase activity of the ZDV tested LGB was significantly (P<0.05) decrease as compared to the control group. While the catalase activity of the DVT tested SC was significantly (P<0.05) increased as compared to the control group. MDA activities showed a significant (P<0.05) decreased in the ZDV tested LGB as compared to the control group. There was a significant (P<0.05) increased in the ZDV tested SC as compared to the control group.

DISCUSSION

NRTI agents are known to cause toxicities to numerous tissues, including the liver and vascular endothelial cells. ZDV significantly oxidizes glutathione redox potential, increase total cellular and mitochondrial-specific superoxide, and decrease mitochondrial membrane potential; as well as increase lactate release, and apoptosis.[23] It is known that one of the mechanisms of DNA damage and mitochondrial dysfunction is by inhibition of glutathione metabolism.[24] It is also known that vitamin C is able to attenuate zidovudine-induced oxidative stress;[25; 26] that is, bearing in mind that the antioxidant function of ascorbic acid is by its capacity to recycle glutathione. However, much is yet to be done in terms of determining the impact of ZDV on antioxidant enzymes.

This study has investigated whether oxidative stress indicated by malondialdehyde and

antioxidant enzymes is increased in LGB and/or SC of zidovudine treatment compared to non-treated group. We report increases in antioxidant enzyme activities and oxidative stress levels associated with zidovudine treatment, especially in the SC tissue. All enzymes presented with increased activities in the treated tissues, though SOD, GPx and catalase showed confounding variations. Given that the tissues studied constitute the intracranial visual relay centre, it is inferred (but not conclude) that there is a potential of zidovudine to impair vision function.

It may be pertinent to point out that observable relationship between antioxidant enzymes is dependent on type of specimen. It has been observed the catalase activity was directly associated with plasma GPx but indirectly with RBC GPx level.[27] In this study, what *seems to be confounding* observation is that catalase, Gpx and SOD activities as well as MDA levels are significantly lower in the ZDV-treated LGB, but higher in the ZDV-treated SC compared to their respective controls. It *seems to be confounding* because the decrease in levels could mean antioxidant effect of ZDV, whereas the increased levels could oppositely be interpreted as the pro-oxidant properties of the agent.

Ideally**,** a rise in SOD activity should neutralize oxidative stress. This means that MDA should be reduced, especially in treatment group. This assumes that the oxidative stress is due to a manageable level of superoxide anion radical. However, there are occasions where SOD activity cannot cope with increased ROS, and there is the possibility whereby SOD will remain active until all necessary co-factors will be available; and the physiological system adjusts in a complex fashion. For instance, catalase and SOD activities may increased in direct proportion to sulfide tolerance in thiobios and to ambient oxygen concentration, but inversely proportional to light sensitivity.[28] The results from this study do not demonstrate a corroborative or direct or inverse relationship between catalase and SOD in all tissues. Instead; the results tend to indicate oxidative stress in SC, but not in the LGB.

Based on the observations and possible underlying explanations, it seems that zidovudine has antioxidant properties with a paradoxical pro-oxidant effect, and that the effects could vary in different tissues. This is quite contrary to current knowledge. Generally, antiretroviral agents have been speculated to induce oxidative stress,[29] while antioxidants have been indicated to alleviated such adverse effect in zidovudine therapy.[26; 30] A major limitation in this study is that no vision impairment was observed in any of the treated Wistar rats. Therefore, further studies are required, especially looking at the effects of zidovudine on antioxidant enzymes and oxidative

damage in tissues of intracranial visual relay centre.

CONCLUSION

This study is unable to conclude that zidovudine may cause vision impairment. It presents observation of the effect of zidovudine on antioxidant enzymes and product of oxidation. There is a potential phase of reduction in MDA generation with maintained increase in GPx levels, which portends that the speculated pro-oxidant effect of zidovudine may be limited.

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Contribution of authors

Adjene and Igbigbi conceptualized the study. Adjene did the laboratory work. Nwose interpreted to results.

REFERENCES

1. Acosta EP, Page LM and Fletcher CV. Clinical pharmacokinetics of zidovudine. An update. Clin Pharmacokinet 1996;30(4):251-62.
2. Duesberg PH. The role of drugs in the origin of aids. Biomed Pharmacother 1992;46(1):3-15.
3. Gulick RM, Ribaudo HJ, Shikuma CM, Lalama C, Schackman BR, Meyer Wr et al. Three- vs four-drug antiretroviral regimens for the initial treatment of hiv-1 infection: A randomized controlled trial. JAMA 2006;296(7):769-81.
4. Panel on Antiretroviral Guidelines for Adults and Adolescents Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Available at: http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf. Accessed 19th Dec 2013.
5. Campbell TB, Smeaton LM, Kumarasamy N, Flanigan T, Klingman KL, Firnhaber C et al. Efficacy and safety of three antiretroviral regimens for initial treatment of hiv-1: A randomized clinical trial in diverse multinational settings. PLoS Med 2012;9(8):e1001290.
6. Richmann DD. Azt resistance in isolates of hiv. Immunodefic Rev 1991;2(4):315-18.
7. Giraldo R. Anti-retrovirals can cause aids [Web page]. Virusmyth 2000. Available at: http://www.virusmyth.com/aids/hiv/rgretrovirals.htm. Accessed 19th Dec 2013.

8. Duesberg PH. Can epidemiology determine whether drugs or hiv cause aids? *AIDS-Forschung (AIFO)* 1993;12(627-35.
9. Lalonde RG, Deschênes JG and Seamone C. Zidovudine-induced macular edema. Ann Intern Med 1991;114(4):297-98.
10. Geier SA, Held M, Bogner JR, Kronawitter U, Berninger T, Klauss V et al. Impairment of tritan colour vision after initiation of treatment with zidovudine in patients with hiv disease or aids. Br J Ophthalmol 1993;77(5):315-16.
11. Moore RY and Card JP. Intergeniculate leaflet: An anatomically and functionally distinct subdivision of the lateral geniculate complex. J Comp Neurol 1994;344(3):403-30.
12. Nagy A, Kruse W, Rottmann S, Dannenberg S and Hoffmann K-P. Somatosensory-motor neuronal activity in the superior colliculus of the primate. Neuron 2006;52(3):525-34.
13. Waleszczyk WJ, Nagy A, Wypych M, Berényi A, Paróczy Z, Eördegh G et al. Spectral receptive field properties of neurons in the feline superior colliculus. Exp Brain Res 2007;181(1):87-98.
14. Richards RS, Nwose EU and Bwititi P. Biochemical basis of circadian rhythms and diseases: With emphasis on post-traumatic stress disorder. Med Hypotheses 2011;77(4):605-09.
15. Kendabie KO, Adjene JO. Histological studies of the effects of red pepper on the stomach of adult wistar rats. Rev Electron Biomed / Electron J Biomed 2007; 3: 13-17.
16. Adjene JO, Adenowo TK. Histological studies of the effect of chronic administration of Chloroquine on the inferior colliculus of adult Wistar rat. JMBR 2005; 4: 83-87.
17. Adjene JO, Avbunudiogba JA, Awhin PE and Igbigbi PS. Biochemical effects of chronic administration of efavirenz on the intracranial auditory relay centers of adult wistar rats. Genomic Medicine, Biomarkers, and Health Sciences 2012;4(3):85-89.
18. Adjene JO, Avbunudiogba JA and Igbigbi PS. Oxidative stress induced by chronic administration of efavirenz on the intracranial visual relay centers of adult wistar rats. Biology and Medicine 2011;3(5):16-24.
19. Adjene JO, Caxton-Martins AE. Some histological effect of chronic administration of Chloroquine on the medial geniculate body of adult wistar rat. Afr J Med Med Sci 2006; 35(2):131-135
20. Adjene JO, Igbigbi PS, Nwose EU. Histological effects of chronic administration of efavirenz on lateral geniculate body of adult Wistar rats. North Am J Med Sci 2010; 2(1):1-4
21. Igbigbi PS, Adjene JO, Nwose EU. Effects of zidovudine on auditory functions:

enzymology and oxidative stress perspectives. Diag Ther Stud 2013; 2(4):76-82.

22. Misra HP and Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247(10):3170-75.
23. Kline E, Bassit L, Hernandez-Santiago B, Detorio M, Liang B, Kleinhenz D et al. Long-term exposure to azt, but not d4t, increases endothelial cell oxidative stress and mitochondrial dysfunction. Cardiovasc Toxicol 2009;9(1):1-12.
24. Mattson DM, Ahmad IM, Dayal D, Parsons AD, Aykin-Burns N, Li L et al. Cisplatin combined with zidovudine enhances cytotoxicity and oxidative stress in human head and neck cancer cells via a thiol-dependent mechanism. Free Radic Biol Med 2009;46(2):232-37.
25. Ngondi J, Oben J, Forkah D, Etame L and Mbanya D. The effect of different combination therapies on oxidative stress markers in hiv infected patients in cameroon. AIDS Res Ther 2006;3(1):19.
26. Papparella I, Ceolotto G, Berto L, Cavalli M, Bova S, Cargnelli G et al. Vitamin c prevents zidovudine-induced nad(p)h oxidase activation and hypertension in the rat. Cardiovasc Res 2007;73(2):432-38.
27. Crawford A, Fassett RG, Coombes JS, Kunde DA, Ahuja KDK, Robertson IK et al. Glutathione peroxidase, superoxide dismutase and catalase genotypes and activities and the progression of chronic kidney disease. Nephrol Dial Transplant 2011;26(9):2806-13.
28. Morrill AC, Powell EN, Bidigare RR and Shick JM. Adaptations to life in the sulfide system: A comparison of oxygen detoxifying enzymes in thiobiotic and oxybiotic meiofauna (and freshwater planarians). Journal of Comparative Physiology B 1988;158(3):335-44.
29. Jiang B, Khandelwal AR, Rogers LK, Hebert VY, Kleinedler JJ, Zavecz JH et al. Antiretrovirals induce endothelial dysfunction via an oxidant-dependent pathway and promote neointimal hyperplasia. Toxicol Sci 2010;117(2):524-36.
30. de la Asunción JG, Del Olmo ML, Gómez-Cambronero LG, Sastre J, Pallardó FV and Viña J. Azt induces oxidative damage to cardiac mitochondria: Protective effect of vitamins c and e. Life Sci 2004;76(1):47-56.