eISSN: 09748369

Effects of chronic administration of efavirenz on DNA of the intracranial visual relay centers of adult Wistar rats

Biology and Medicine

JO Adjene, PS Igbigbi

Accepted: 16th Mar 2012, Published: 6th Apr 2012

Volume 4, Issue 1,Page 20-26, 2012

www.biolmedonline.com

Effects of chronic administration of efavirenz on DNA of the intracranial visual relay centers of adult Wistar rats

JO Adjene*, PS Igbigbi
Department of Anatomy and Cell Biology, Faculty of Basic Medical Sciences, College Of Health Sciences, Delta State University, Abraka, Delta State, Nigeria.

***Corresponding Author:** joadjene@yahoo.com, pigbigbi@yahoo.com

Abstract
The effects of chronic administration of efavirenz commonly used as part of Highly Active Antiretroviral Therapy (HAART) for the treatment of Human Immunodeficiency Virus (HIV) type-1 therapy on the DNA of the intracranial visual relay centre namely the superior colliculus and lateral geniculate body of adult Wistar rats were carefully studied. The rats of both sexes (n=20), with an average weight of 200g were randomly assigned into treatment (n=10) and control (n=10) groups. The rats in the treatment group received 600mg/70kg bogy weight of efavirenz dissolved in distilled water daily for 30 days through the orogastric tube. The control group received equal volume of distilled water daily for 30 days through the same route. The rats were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and given water liberally. The rats were sacrificed by cervical dislocation method on the thirty-first day of the experiment. The superior colliculus and lateral geniculate body were carefully dissected out and quickly fixed in 10% formal saline for histochemical study. The histochemical findings indicated that the treatment sections of the superior colliculus and lateral geniculate body showed less intense staining and appeared pachychromatic. The stained neurons and glia cells were few as compared to the control sections. There were observations of neuronal enlargement in the superior colliculus and lateral geniculate body of the treated sections. The superior colliculus of the treated rats showed evidence of hypertrophy and microcytic changes in the darkly stained DNA positive granules, while the treated section of the lateral geniculate body showed positively stained DNA granules of various sizes and shapes, with an indication of hypertrophy and microcytic changes as compared to the control group. Chronic administration of efavirenz may therefore have an adverse effect on the DNA of the superior colliculus and lateral geniculate body of adult Wistar rats. It is recommended that further studies aimed at corroborating these observations be carried out.

Keywords: Efavirenz; DNA; Superior colliculus; Lateral geniculate body; Wistar rats.

Introduction

Efavirenz is an antiretroviral drug that belongs to the class of drugs called non-nucleoside reverse transcriptase inhibitor (NNRTI) used as part of highly active antiretroviral therapy (HAART) for the treatment of HIV type-1 (AHFS, 2007). Efavirenz has been found to be effective in many combination regimes for the treatment of HIV infection, both in previously untreated and in treated individuals. It has been combined successfully with nucleoside consisting of lamivudine or emtricitabine plus abacavir, didanosine, stavidine, tenofovir or zidovudine to achieve virologic suppression in a high percentage of recipients (Staszewski *et al*., 1999; Gulick *et al.*, 2006). Most antiviral agents do not efficiently penetrate the Blood Brain Barrier (BBB) or are actively transported out of the central nervous system (Schranger and D'Souza, 1998). Even after antiviral treatment that successfully controls virus in the treatment compartments, the central nervous system may suffer continuing damage induced by HIV infection (Fox *et al*., 2000). Efavirenz may be taken once a day without regards to meal and it can penetrate the central nervous system and spinal fluids (AIDS INFONET, 2007; Puzantian, 2002).

Some adverse effect in the central nervous system has been commonly associated with efavirenz (Ruiz *et al.,* 1999). The most common central nervous system effects include confusion, insomnia, abnormal vivid dreams, dizziness and headache. Efavirenz has emerged as cornerstone of HAART regimens. The side effect profile of the drug is generally regarded as satisfactory. However, there are conflicting study results in the medical literature as well as conflicting studies from patients and physicians regarding the neuropsychiatric problems associated with efavirenz (Baker, 2006). Lipodystrophy, moderate or severe pain, abnormal vision, arthralgia. asthenia, dyspnea, gynecomastia, myalgia, myopathy and tinnitus have been reported concerning efavirenz (AHFS, 2007).

The superior colliculus and lateral geniculate body constitutes the intracranial visual relay centers. The lateral geniculate body in mammals is considered as part of the thalamic nuclei for processing visual information (Altman and Bayer, 1981). In rats, the lateral geniculate body receives input from the geniculate leaflet, which participates in the regulation of circadian function through its projection to the circadian pacemaker of the hypothalamus (Moore and Card, 1984). The superior colliculus is concerned with ocular movement. Such movements can result from stimulation of a wide area in the pretectal and tegmental regions of the brain. The superior colliculus controls and regulates many movements of the eye and head. It acts as an integrative center subserving visual perception. Thus, it also has a role in certain aspects of vision. Its major role is to co-ordinate responses evoked by a variety of sensory signals with behavioral movements that directs the head, eyes and ear towards the environmental stimulus. Thus, the superior colliculus has a critical role in visual localization, orientation tracking movements, accommodation and papillary reflex. Its superficial layers are concerned with vision (Reczkowski and Diamond, 1978), and its deep layer has been implicated in eye movements and somesthetic input (Altman and Bayer, 1981).

It has been observed in monkey that the neurons in the superior colliculus 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 (Nagy *et al*., 2006). Multisensory depression is a fundamental index of multisensory integration in the neurons of the superior colliculus. Nitregic interneurons play a role in refining the cortico-collicular projection patterns that are believed to be essential for superior colliculus output neurons. It is engage in multisensory integration and to support normal orientation responses to cross modal stimuli (Stein *et al.,* 2009). The loss of these cortical influences permits visual orientation behavior in the presence of a normal disruptive auditory stimulus (Jiang and Stein, 2003).

The superior colliculus neurons play some spatial- temporal filter properties that are closely similar to those of their 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 (Waleszczyk *et al.,* 2007).

Cortical structures such as the medial and lateral geniculate bodies, inferior and superior colliculi have higher glucose utilization than other structures (Siesjo, 1978). There is a correlation between functional activity and metabolic rate such as in the visual and auditory system (Siesjo, 1978). Since efavirenz crosses the BBB, it is relevant to investigate its histochemical effect on the superior colliculi and lateral geniculate body. It is probable that the adverse effects of efavirenz on dizziness and headache may be due to direct effect of efavirenz on the superior colliculus and lateral geniculate body. Neuronal and glia cells showed the presence of DNA, which are the transmitters of genetic information. The DNA is also involved in protein synthesis where information stored in them is transferred to RNA. Feulgen reaction specifically stained nuclear chromatin; the aldehyde liberated during hydrolysis is from the deoxypentose but not from ribose. The integrity of nuclear DNA is one of the most extensively used biochemical markers for cell death (Cohen and Yielding, 1965). This present study was to elucidate the biochemical effects of chronic administration of efavirenz on the DNA of the intracranial visual relay center of adult Wistar rats.

Materials and Methods

Animals: The School of Basic Medical Sciences, University of Benin granted approval before the work began. Twenty adult Wistar rats of both sexes with average weight of 200g were randomly assigned into two groups; control (n=10) and treatment (n=10). The rats were obtained and maintained in the Animal Holding of the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Nigeria. They were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and given water liberally. Efavirenz was obtained from the President Emergency Plan for AIDS Relief (PEPFAR) Unit, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria.

Drug administration: The rats in the treatment group received the recommended dosage of 600mg/70kg body weight of efavirenz dissolved in distilled water for thirty days through orogastric tube administration while the control rats received equal volume of distilled water

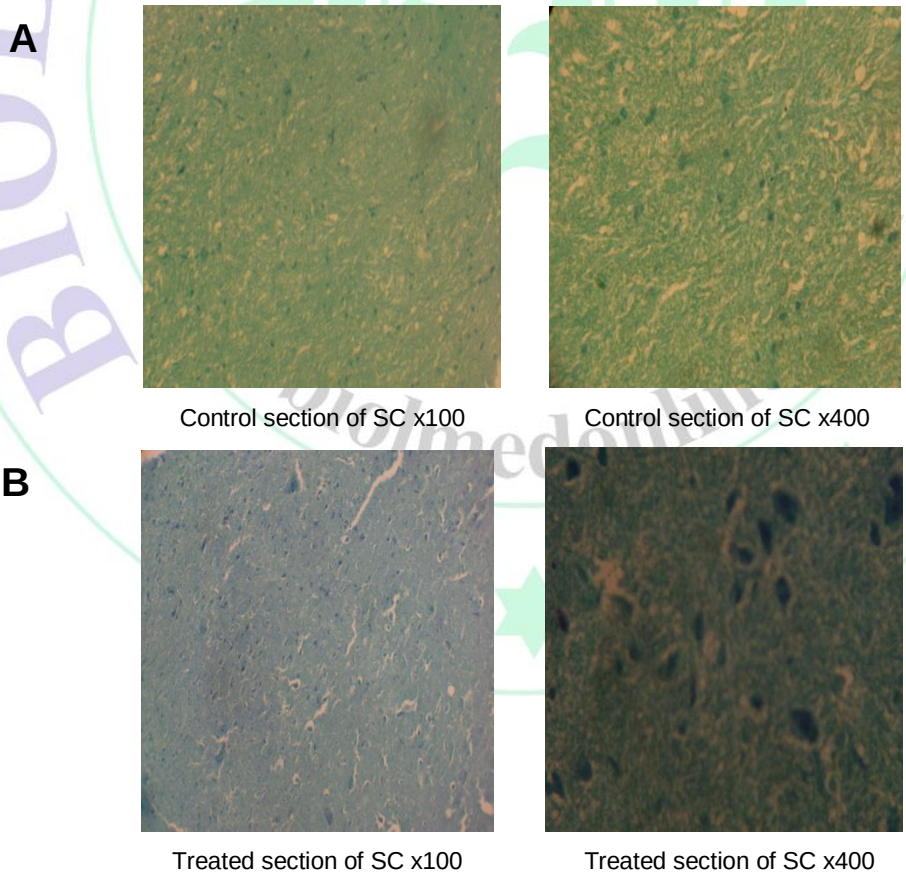
through the same route and for the same period. The rats were sacrificed by cervical dislocation on the thirty-first day of the experiment. The skulls were opened using bone forceps to expose the brain of the rats and the superior colliculus and lateral geniculate body were quickly dissected out and fixed in 10% formal saline for DNA staining techniques.

Histochemical study: The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 6 microns thick were obtained using a rotatory microtome. The deparaffused sections were stained routinely for DNA using Feulgen and Rosenbach (1924) staining procedures. The sections were then rinsed in distilled water, dehydrated through ascending grades of alcohol, cleared in xylene and mounted in DPX for DNA observation. The photomicrographs of the desired results were obtained using research photographic microscope in the Department of Anatomy, School of Basic Medical Sciences, University of Benin, Nigeria.

Results

The control sections of the superior colliculus and lateral geniculate body stained deep magenta color. The stained nuclei were numerous and did not appear enlarged. The nuclei were distinct and widely distributed (plates 1A and 2A).

The treatment sections of the superior colliculus and lateral geniculate body showed less intense staining and appearing pachychromatic. The stained neurons and glia were few when compared to the control sections. There were observations of neuronal enlargement in both the superior colliculus and lateral geniculate body of the treated sections (plates 1B and 2B). The treated section of superior colliculus of the rats showed evidence of hypertrophy and microcytic changes in the darkly stained DNA positive granules (plate 1B), while the treated section of the lateral geniculate body of the rats showed positively stained DNA granules of various sizes and shapes, with an indication of hypertrophy and microcytic changes (plate 2B).



Control section of SC x100 Control section of SC x400

Treated section of SC x100 Treated section of SC x400

Plate 1: DNA in the superior colliculus (SC) (Feulgen method).

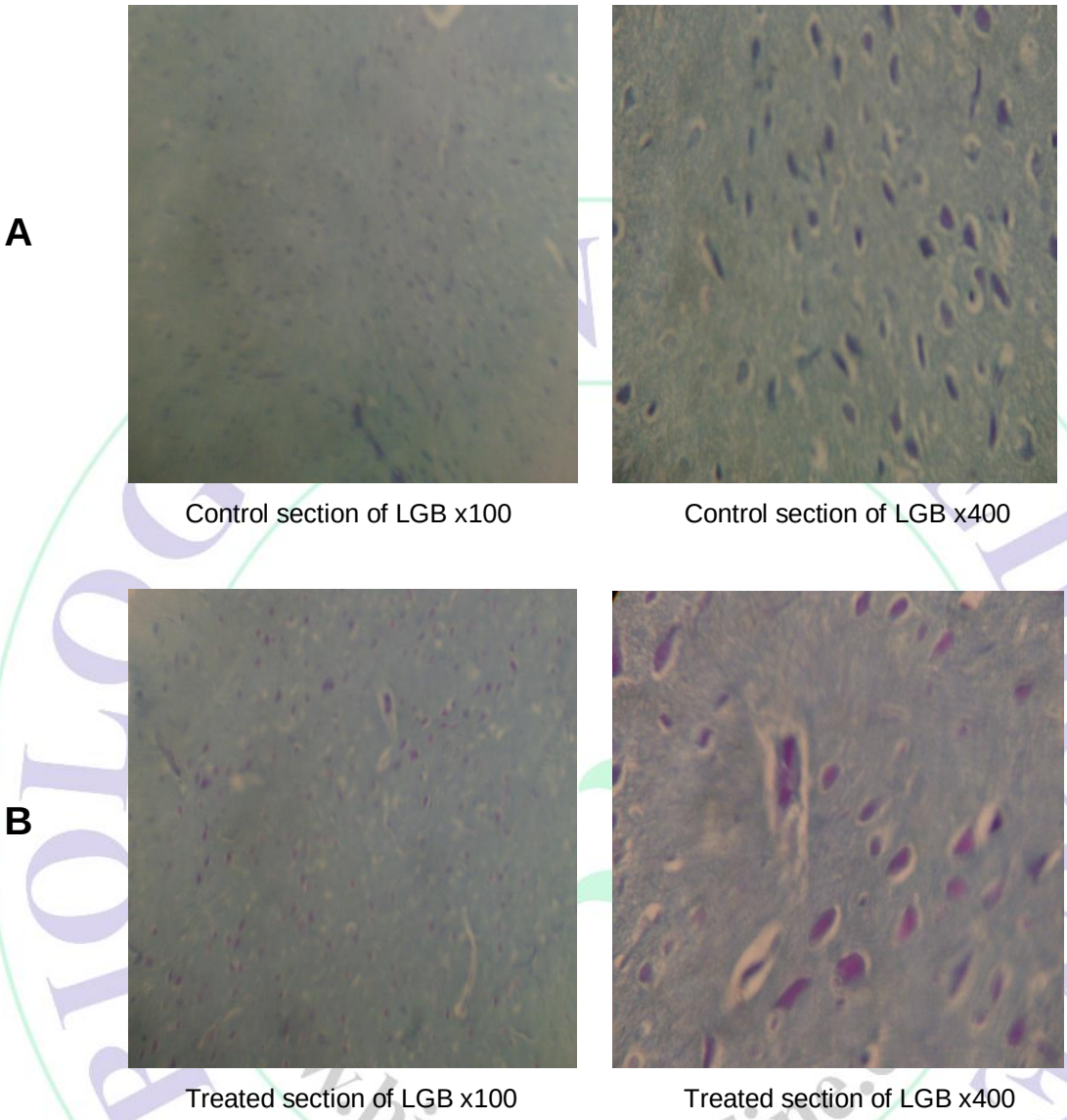


Plate 2: DNA in the lateral geniculate body (LGB) (Feulgen method).

Discussion

The treatment sections of the superior colliculus and lateral geniculate body showed less intense staining and appearing pachychromatic. The stained neurons and glia were few when compared to the control sections. There were observations of neuronal enlargement in both the superior colliculus and lateral geniculate body of the treated sections. The treated section of superior colliculus of the rats showed evidence of hypertrophy and microcytic changes in the darkly stained DNA positive granules, while the treated section of the lateral geniculate body of the rats showed positively stained DNA granules of various sizes and shapes, with an indication of hypertrophy and microcytic changes. Neurons and glia cells showed the presence of deoxyribonucleic acid, which are the transmitters of genetic information. The DNA is also involved in protein synthesis where information stored in them is transferred to ribonucleic acid. The neurons are differentiated from glia by the presence of a large sized nucleus, which stained more intensely. Feulgen

reaction specifically stained nuclear chromatin; the aldehyde liberated during hydrolysis is from deoxypentose but not from ribose. DNA is important for normal maintenance of cellular integrity. Chloroquine acts on cell sites by binding to DNA, RNA and proteins. This binding alters the biological and physiological characteristics of DNA helix (Cohen and Yielding, 1965). It has been reported that chronic administration of efavirenz in adult Wistar rats resulted in some cellular degenerative changes like sparse cellular population, pyknotic nuclei with some microcystic changes, autophagic vacuoles and vacuolations in the stroma of the treated superior colliculus and lateral geniculate body as compared to the control group (Adjene *et al.*, 2010; Adjene and Momah, 2010). In this study efavirenz administration was observed to affect the staining intensity of nuclei in the neurons and glia cells of the superior colliculus and lateral geniculate body. The cells that stained following efavirenz administration had enlarge darkly stained nuclei and some microcytic changes among others. The enlarged darkly stained DNA granules may indicate possible adverse effects of efavirenz on DNA integrity. Chloroquine has been reported to cause complete inhibition of DNA synthesis (Cohen and Yielding, 1965) and interferes with protein synthesis through the inhibition of DNA replication (Amenta *et al*., 1978; Seglen *et al.*, 1979; Crab *et al.,* 1980). It is probable that efavirenz interference in this experiment might be the probable cause of the hypertrophied DNA granules and microcytic changes observed in this experiment. Nuclei hypertrophy leads to cell death, which may underscore the reduction in cellular density and staining intensity following treatment with efavirenz. In this study, the toxic effect of efavirenz on DNA was revealed by the less staining intensity observed in the treatment group. These effects could obviously affect the integrity and competence of the intracranial visual relay centre in regard to their activities mediated in visual sensibilities.

Conclusion

In this experiment, the DNA staining revealed that efavirenz administration affects the staining intensity of nuclei in the neurons and glia cells of the superior colliculus and lateral geniculate body in the treated adult Wistar rats. The cells had enlarged, darkly stained DNA granules with some microcytic changes and characteristic vacuolations in the parenchyma of the superior colliculus and lateral geniculate body among others in the treated sections of the DNA as compared to the control.

Ethical Approval

The study was approved by the Animal Ethics Committee of the College Of Health Sciences, Delta State University, Abraka, Delta State, Nigeria.

Conflict of Interests

Authors have no conflicting interests.

References

Adjene JO, Igbigbi PS, Nwose EU, 2010. Histological effects of chronic administration of efavirenz lateral geniculate body of adult Wistar rats. North American Journal of Medical Sciences, 2: 1-4.

Adjene JO, Momah V, 2010. Histological effects of chronic administration of efavirenz on the superior colliculus of adult Wistar rats. Bioscience Research Communication, 22(6): 47-52.

AIDS INFONET, 2007. Efavirenz (Sustiva) Fact Sheet, 432.

Altman AS, Bayer CS, 1981. Time of origin of neurons of rat superior colliculus in relation to other components of the visual and visuomotor pathways. Experimental Brain Research, 42: 424-434.

Amenta JS, Hilvko TJ, Mcbee AG, Shinozuka H, Brochner S, 1978. Specific inhibition by ammonium chloride of autophagy associated proteolysis in cultured fibroblast. Experimental Cell Research, 115: 357.

American Hospital Formulary Service (AHFS), 2007. Drug Information, 86: 694.

Baker R, 2006. Central nervous system toxicities and efavirenz. Available at www.hivandhepatitis.com

Cohen SN, Yielding KL, 1965. Actions of chloroquine. Proceedings of National Academy of Sciences, USA, 54: 521.

Crab DW, Jerslid RA, Mecure SA, Swartzentruber MS, Harvis RA., 1980. Inhibition of hepatocyte proteolysis and lactate in chloroquine. Archives of Biochemistry and Biophysics, 203(1): 49.

Feulgen R, Rossenbeck H, 1924. Mikroskopich chemisher Nachweisseiver nucleinsaqure von typus der thermonucleinsaure und die preparaten. Hoppe-Seylers. Zeitschrift fur Physikalische Chemie (Berlin), 135: 203.

Fox HS, Weed MR, Resindiz SH, Baig J, Horn FW, Dailey PJ, *et al.*, 2000. Antiviral treatment normalizes neuropsychological but not movement abnormalities in Simian immunodeficiency virus infected monkeys. Journal of Clinical Investigation, 106: 37-45.

Gulick RM, Ribaudo HJ, Shikuma CM, 2006. Three versus Four-Drug Antiretroviral regimens for the initial treatment of HIV-1 infection: A randomized controlled trial. Journal of American Medical Association, 296(7): 769-781.

Jiang W, Stein BE, 2003. Cortex controls multisensory depression in superior colliculus. Journal of Neurophysiology, 90: 2123-2135.

Moore RY, Card JP, 1984. Intergeniculate leaflet: An anatomically and functionally distinct subdivision of the lateral geniculate complex. Journal of Comparative Neurology, 344: 403-444.

Nagy A, Krusel W, Rottman S, Dannenberg S, Hoffmann K, 2006. Somatosensory-motor neuronal activity in the superior colliculus of the primate. European Journal of Neuroscience, 24(3): 917-924.

Puzantian T, 2002. Central nervous system adverse effect with efavirenz case report and review. Pharmacotherapy, 22(7): 930-933.

Reczkowski D, Diamond D, 1978. Cells of origin of several efficient pathways from the superior colliculus in *Galago senegalerisis*. Brain Research, 146: 351-357.

Ruiz NM, Bessen LJ, Manion DJ, 1999. Potential adverse experiences associated with efavirenz (sustiva in adults) for the efavirenz clinical development team. Presented at the 6th Conference on Retrovirus and Opportunistic Infections, Chicago, IL.

Schranger LK, D'Souza MP, 1998. Cellular and anatomical reservoirs of HIV-1 in patient receiving potent antiretroviral combination therapy. Journal of American Medical Association, 280: 67-71.

Seglen PO, Carinde B, Solhein AE, 1979. Inhibition of the lysosomal pathway of protein degradation in isolated rat hepatocytes by ammonia, methylamine chloroquine and leupelin. European Journal of Biochemistry, 94: 215-225.

Siesjo BK, 1978. Utilization of substrates by brain tissues. Brain energy metabolism. John Wiley and Sons, USA. 101-130.

Staszewski S, Miller V, Sabin C, Schlecht C, Gute P, Stamm S, *et al.*, 1999. Determinant of sustainable CD4 lymphocyte count increases in response to antiretroviral therapy. AIDS, 13: 951-956.

Stein BE, Stanford TR, Rowland B, McHaffie JG, Lavadas E, 2009. Plasticity and synergy in multisensory integration. Presented at the 10th International Multisensory Research for the City College of New York.

Waleszczyk WJ, Nagy A, Wypych M, Berenyi A, Paroczy Z, Eordegh G, *et al.*, 2007. Spectral receptive field properties of neurons in the feline superior colliculus. Experimental Brain Research, 181(1): 87-98.