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JO Adjene, PS Igbigbi
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Effects of chronic administration of efavirenz on DNA of the intracranial visual relay centers of adult Wistar rats

JO Adjene*, PS Igbibi
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, pigbibi@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 body 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, stavudine, 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, gynecomasia, myalgia, myopathy and tinnitus have been reported concerning efavirenz (AHFS, 2007).
The superior colliculus and lateral geniculate body constitute 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 coordinate 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 deparaffined 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).

**Plate 1:** DNA in the superior colliculus (SC) (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 microcystic 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 microcystic 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 microcystic 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.

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