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Original Communication

EFFECTS OF CHRONIC ADMINISTRATION OF EFAVIRENZ ON THE NISSL SUBSTANCES ON THE INTRACRANIAL VISUAL RELAY CENTRES OF ADULT WISTAR RATS

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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 nissl substance of the intracranial visual relay centres 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 histological study. The histological findings indicated that the treated sections of the superior colliculus and lateral geniculate body showed that the nissl substances were less intensely stained as compared to the control. The parenchyme was vacuolated, there were evidence of hypertrophy and more spaces between the axonal mesh around the sparsely distributed neurons as compared to the control group. The treated section of the superior colliculus showed neurons with faintly stained nissl substances of various sizes and with evidence of hypertrophy while that of the lateral geniculate body showed less intense and enlarge nissl substances with characterized microcytic changes. Chronic administration of efavirenz may have an adverse effect on the nissl substances 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, Nissl Substance, 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 human immunodeficiency virus (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 highly active antiretroviral therapy (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 centres. 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 behavioural 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). 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 behaviour in the presence of a normal disruptive auditory

stimulus (Jiang et al., 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 behavioural 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 blood brain barrier, it is relevant to investigate its histological 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 nissl substances of superior colliculus and lateral geniculate body.

Nissl substances had been reported to play key roles in cellular metabolism (Adams, 1965). Nissl bodies are nodal points in the endoplasmic reticulum, which permeate the cell body and dendrites. They are absent at the axon and axon hillock (Noback and Demarest, 1981). It had been reported that nissl body comprises of broadsheet of rough or granular endoplasmic reticulum, free ribosomes and ribosomes in clusters or rosette. These ribosomes had been found in large amounts in the cytoplasm of neurons as cytoplasmic RNA. Under the control of nuclear deoxyribonucleic acid (DNA), cytoplasmic RNA is concerned with protein synthesis. Alterations in nissl substances are characterized by a change of membrane configuration forming lamella bodies such as anulate lamellae as elaborated with electron microscope (Davis and Robertson, 1991). This present study was to elucidate the effects of chronic administration of efavirenz on the nissl substances of the intracranial visual relay centres of adult wistar rats.

MATERIALS AND METHODS

Animals care ethics

The School of Basic Medical Sciences, University of Benin grant approval before the commencement of the work. Twenty adult wistar rats of both sexes with average weight of 200g were randomly assigned into two groups; control (n=10) and test (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, Benin City, Edo State, Nigeria. They were fed with grower's mash obtained from Edo Feeds and Flour Mill Limited, Ewu, Edo State, Nigeria and given water liberally.

Drug administration

Efavirenz was obtained from the President Emergency Plan for AIDS Relief (PEPFAR) Unit, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. 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

colliculi and lateral geniculate bodies were quickly dissected out and fixed in 10% formal saline for nissl substance staining techniques.

Histological 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 nissl substance using thionin method of Paget and Eccleston (1960) procedures. The sections were then rinse in distilled water, dehydrated through ascending grades of alcohol, cleared in xylene and mounted in DPX for nissl substance 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, Benin City, Edo State, Nigeria.

RESULTS

The control group showed intensely stained nissl substances in the neurons of the superior colliculus and lateral geniculate body. No vacuolations were observed in the stroma of the superior colliculus and lateral geniculate body of the control group in the adult wistar rats (plates 1A and 2A).

The superior colliculus and lateral geniculate body of the tested rats showed that the nissl substances stained less intensely as compared to the constant intensity observe in the neurons of the control rats. Apart from the characteristics vacuolations of the parenchyma, there appeared more spaces between the axonal mesh around the sparsely distributed neurons in the tested rats. This is mostly prominent in the lateral geniculate bodies (plates 1B and 2B). The tested section of superior colliculus showed neurons with faintly stained nissl substances of various sizes with evidence of hypertrophy (Plate 1B), while the tested section of lateral geniculate body showed less intense and enlarge stained nissl substances with characterized microcytic changes (Plate 2B).

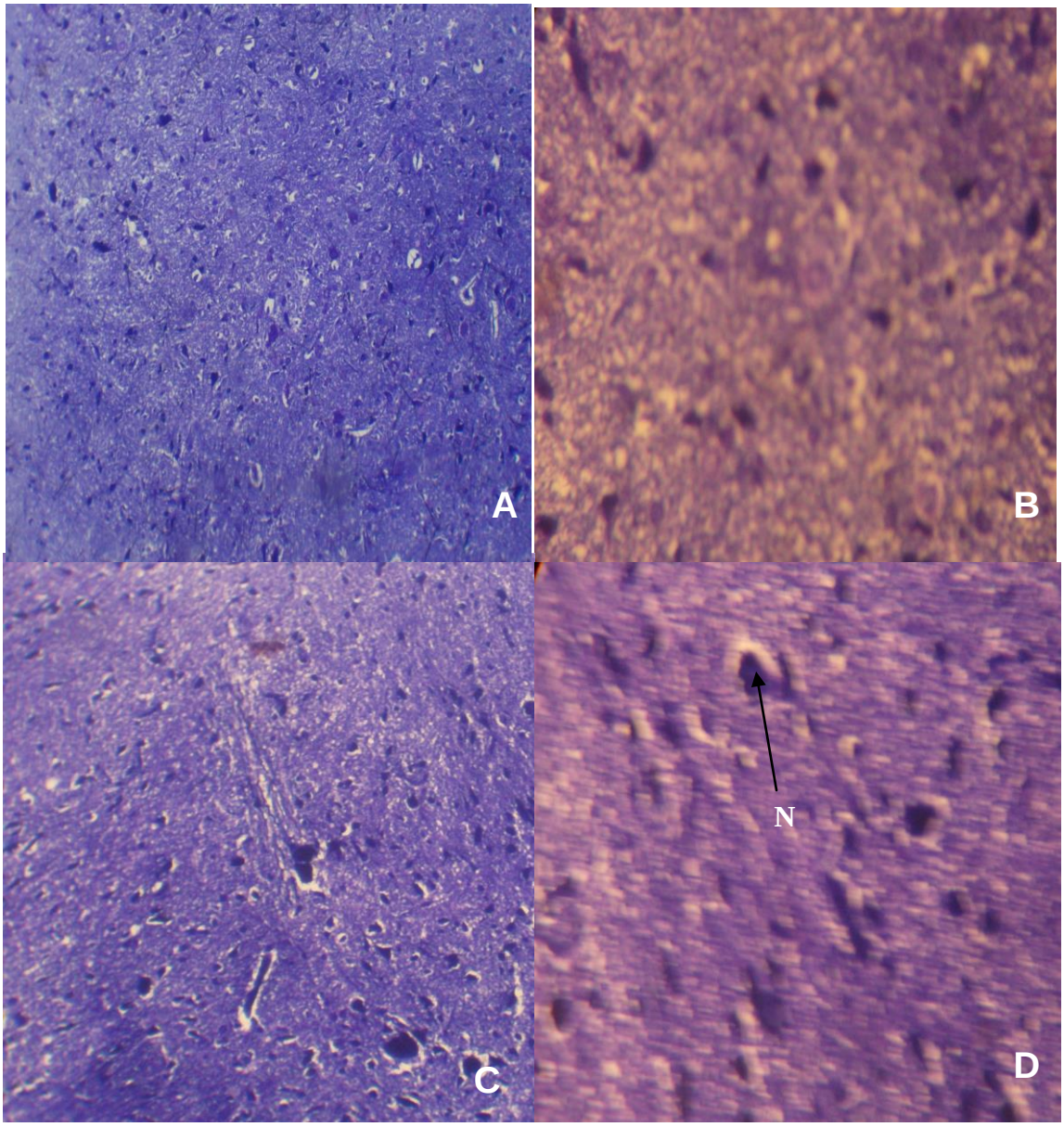

Figure 1: Nissl substance in the superior colliculus (SC) with A and B as control, while C and D as the tested group indicating hypertrophied nissl substance 'N' (Thionin stain)

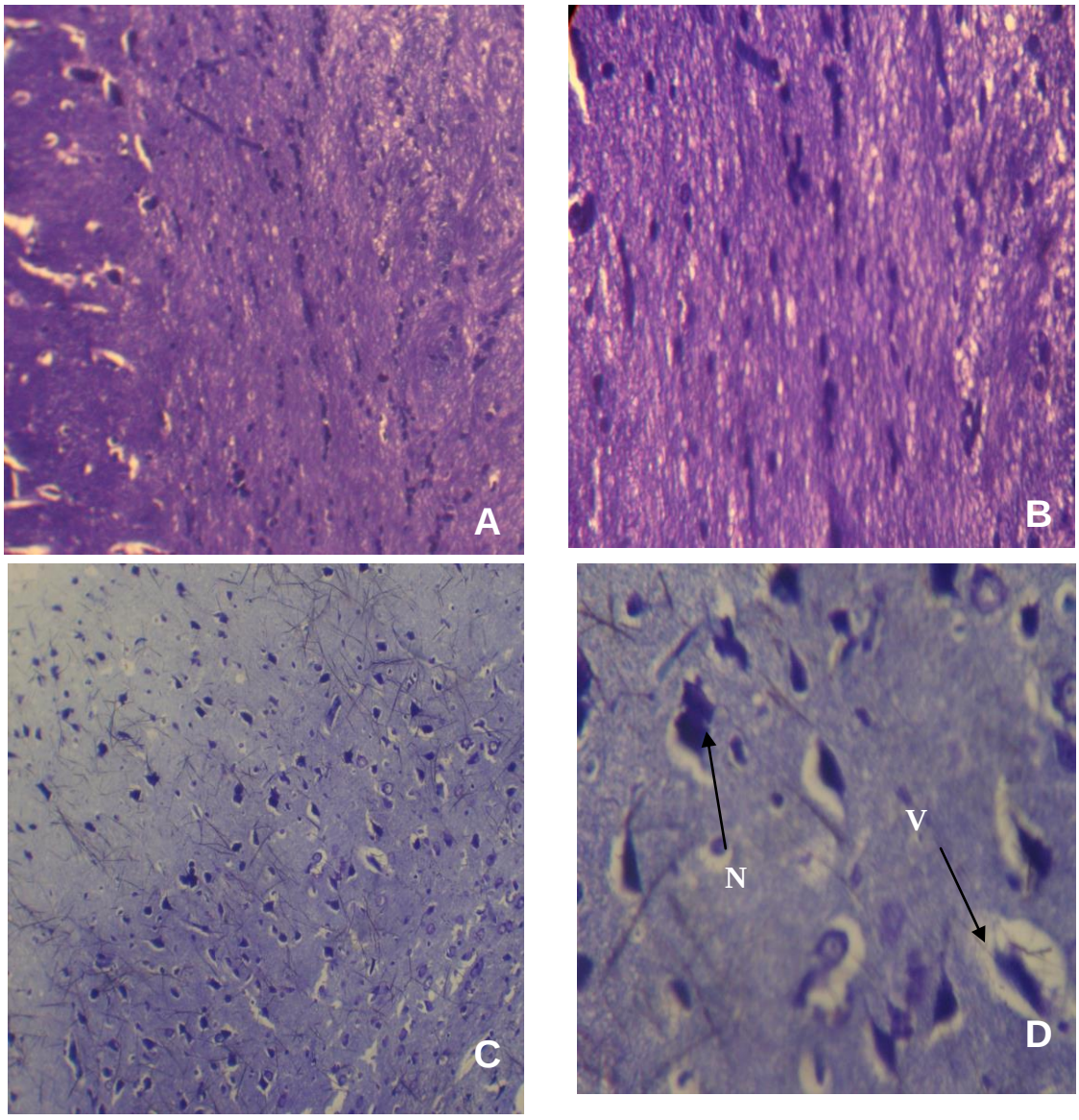

Figure 2: Nissl substance in the lateral geniculate body (LGB). With A and B as control, while C and D as the tested group indicating hypertrophied nissl substance 'N' and vacuolation 'V'. (Thionin stain)

DISCUSSION

The superior colliculus and lateral geniculate body of the tested rats showed nissl substances that were less stained as compared to the constant intense stained observe in the neurons of the control rats. Apart from the characteristics vacuolations of the parenchyma, there appeared more spaces between the axonal mesh around the sparsely distributed neurons in the tested rats. The tested section of superior colliculus showed neurons with faintly stained nissl substances of various sizes with evidence of hypertrophy, while the tested section of the lateral geniculate body showed less intense and enlarge stained nissl substances with characterized microcytic changes. Nissl substances had been reported to play key roles in cellular metabolism (Adams, 1965). Its depletion in efavirenz treated rats could

be of health hazard especially at neuronal level. Under the light microscope, nissl substances range in appearance from rhomboid blocks in large neurons to particulate materials in the cell bodies of smaller neurons. Nissl bodies are nodal points in the endoplasmic reticulum, which permeate the cell body and dendrites. They are absent at the axon and axon hillock (Noback and Demarest, 1981). Hence their absence in neurons could negatively affect their functions. It had been reported that nissl body comprises of broadsheet of rough or granular endoplasmic reticulum, free ribosomes and ribosomes in clusters or rosette. These ribosomes had been found in large amounts in the cytoplasm of neurons as cytoplasmic RNA. Under the control of nuclear deoxyribonucleic acid (DNA), cytoplasmic RNA is concerned with protein synthesis. The protein synthesized is transported down the axon by axoplasmic flow or transport. If nerve cells require large amounts of protein to maintain their integrity and perform their functional activities (Noback and Demarest, 1981); decreased protein synthesis due to decreased nissl substance leading to decreased neuronal intensity and function as observed in locomotor activities of the treated rats.

Degeneration of nissl substances is usually characterized by disintegration resulting in powdering remains that are confined to the periphery of the cell. The cytoplasm appear homogenous, chromatolysis is accompanied by a concomitant decrease in RNA and protein synthesis caused by certain diseased conditions. Other alterations in nissl substances are characterized by a change of membrane configuration forming lamella bodies such as anulate lamellae as elaborated with electron microscope (Davis and Robertson, 1991). In support of our observations, nissl substances have been reported to be altered by chemicals, toxins, certain drugs and oxygen-lack causing loss of function or interference in normal metabolism (Davis and Robertson, 1991). Neuronal degeneration had been reported earlier to cause a decrease in nissl bodies as chromatolysis occurs (Martin et al., 1978). During necrotic cell death, ribosomes are dispersed from the rough endoplasmic reticulum and polyribosomes in disassociate resulting in a number of monomeric ribosomes that are found free in the cytoplasm (Martin *et al*., 1978). In this study, neuronal degeneration was observed in sections of the superior colliculus and lateral geniculate body in rats tested with efavirenz. The decrease in the staining intensity of the nissl substance in the tested group may be due to the deleterious effects of efavirenz on the neuronal integrity and its nissl substances with consequent decrease in cellular metabolism. 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 tested superior colliculus and lateral geniculate body as compared to the control group (Adjene and Momah, 2010; Adjene et al., 2010). In this study, efavirenz was observed to cause depletion of nissl substances in the superior colliculus and lateral geniculate body of the tested rats. This observation could underline the possible interference in protein synthesis in the superior colliculus and lateral geniculate body. It is however probable that this consequence of efavirenz on the neuronal metabolism on the superior

colliculus and lateral geniculate body may offer possible explanation on the effects of the drug on the nervous system as observed by various workers especially in human and animal model.

In conclusion this study, neuronal degeneration was observed in the sections of the superior colliculus and lateral geniculate body in the tested rats with efavirenz. There was a decrease in the staining intensity of the nissl substance, which may be due to the deleterious effects of efavirenz on the neuronal integrity and its nissl substances with consequent decrease in cellular metabolism. Efavirenz was observed to cause depletion of nissl substances in the superior colliculus and lateral geniculate body with a characteristic vacuolations in the parenchyma of the superior colliculus and lateral geniculate body. There appeared to be more spaces between the axonal mesh around the sparsely distributed neurons in the tested rats with that of the lateral geniculate body more prominent.

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