Original Article

Stavudine, an anti-retroviral drug induces reactive astrocytes in motor cortex of albino mice

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Abstract

Objectives: Anti-retroviral (ARV) drugs are medications used for the treatment and management of retrovirus infections. Stavudine, one of the commercially available ARVs, is a synthetic ARV agent active against the human immunodeficiency virus type 1. In this study, the effect of stavudine on reactive astrocytes on the motor cortex of albino Wistar mice was investigated. **Materials and Methods:** Twenty-four albino mice weighing 30 g on the average were equally assigned into three groups, A, B and C (i.e., eight mice in each group). Group A served as the control, while Groups B and C were the experimental groups. Group B received 0.6 mg/kg of stavudine, while Group C received 1.2 mg/kg of stavudine by orogastric tubes twice a day for 21 days. No treatment was given to the control group, and all the animals received feed and water *ad libitum* throughout the experimental period. **Results:** Light microscopic study of the reactive astrocytes in the motor cortex of mice revealed few astrocytes stained black in the control group, Groups B and C revealed a significantly ($P < 0.05$) higher reactive astrocytes population, with Group C ($P < 0.05$) having higher reactive astrocytes population compared to Group B. **Conclusions:** These results revealed that stavudine caused hyperplasia of astrocytes in the motor cortex of albino mice, and this may affect astrocyte activity and consequently impair motor functions. The effect was dose dependent.

Key words: Albino mice, astrocytes, motor cortex, stavudine

INTRODUCTION

Anti-retroviral (ARV) drugs are medications used for the treatment and management of retrovirus infections, primarily those that cause disease conditions in human beings, which progressively reduce the effectiveness of the human immune system leaving the individual susceptible to opportunistic infections (Dybul *et al*., 2002). In addition to the lymphoid system, the nervous system is the major target of retroviral infections. Direct infection of neuronal tissue or endothelial cells may be involved as reports point to the presence of human immunodeficiency virus within neurons, oligodendrocytes, astrocytes and CD4 cells on the surface of these cells (Fausi, 1998).

The long-term clinical efficacy of single ARV treatment regimens remains uncertain though the biological rationale for maintaining a clinical response has been established (Weller and Williams, 2009). One regimen currently in use and in combination with other ARV

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drugs is stavudine. Stavudine is a synthetic thymidine analogue active against the human immunodeficiency virus, which inhibits the virus replication in human cells *in vitro*. It is phosphorylated by cellular kinases to the active metabolite – stavudine triphosphate which inhibits the activity of HIV reverse transcriptase both by competing with the natural substrate, deoxythymidine triphosphate, and its incorporation into viral deoxyribonucleic acid (DNA) causing the termination of DNA chain, because stavudine lacks the essential 3-OH group necessary for DNA elongation (Dybul *et al*., 2002; Pharmacare Limited, 2005).

The major adverse effect of stavudine is peripheral neuropathy (de Clerq, 1988). Toxicity of stavudine can also lead to clinical syndrome of weakness, sensory loss, diminishing tendon reflexes or a combination of these symptoms caused by tension of peripheral nerves (Spach and Hodton, 1996). Stavudine crosses the blood-brain barrier (Hamamoto *et al*., 1987), as HIV has been reported to be found in neurons and neuroglia (Fausi, 1998) and has been reported to cause reduction in Nissl bodies in the hippocampus of mice (Ekeoma *et al*., 2010). However, the morphological effect of stavudine on the cerebral cortex has not been reported. Stavudine may pose some deleterious effects on the neurons of the motor cortex, since it has been reported to affect the hippocampus (Ekeoma *et al*., 2010), this may necessitate the proliferation of astrocytes in an attempt to support the neurons. Hence, this motivated the investigation of the effect of stavudine on the motor cortical astrocytes of albino mice.

MATERIALS AND METHODS

Twenty-four albino mice with an average weight of 30 g were used for this study. The animals were procured from the Department of Physiology's Animal House, University of Calabar, Calabar, Nigeria. The animals were randomized into three groups of eight mice each. Group A served as the control group, while Groups B and C were the test groups.

Fifty capsules of 30 mg stavudine were procured from General Hospital Pharmacy, Calabar, Nigeria. Each capsule (containing 30 mg of stavudine, the therapeutic dose) was dissolved in 100 ml of water. The appropriate conversion factors were calculated, and the therapeutic dosage determined as 15 mg/kg. 0.2 ml (0.06 mg) and 0.4 ml (0.12 mg) of the drug suspension were administered to the animals. The stavudine suspension was prepared daily and was administered orally with the aid of orogastric tubes. Group A animals were given food and water *ad libitum* throughout the experimental period, while animals in Groups B and C received 0.06 mg and 0.12 mg of stavudine each respectively, twice daily for 21 days.

On day 22, the animals were sacrificed after being anesthetized with chloroform. The brains were extracted and preserved using neutral formal saline. The motor cortex (anterior half of the cerebral cortex) was dissected and routinely processed, and stained using Hortegas' Lithium method for astrocytes. The astrocytes populations were determined with ImageJ (version 1.77c, National Institutes of Health, USA) software.

Briefly, live images (at the predetermined area) of the sections were captured by the ImageJ software. They were converted to 8-bit images and threshold to 110 at the scale of 1 μ, while ensuring that the scale was in the global mode. Microscopic scale was then set for camera binning of 1 × 1 at ×40 objectives. Nuclei of the cells were then quantified.

Statistical Analysis

Analysis of variance was used to determine the significant difference in astrocyte population between the test groups and the control group. The results were regarded as significant at $P < 0.05$.

RESULTS

The section of the motor cortex of the control group (Group A) showed few scattered astrocytes (stained black). The section of the motor cortex of Group B animals administered with 0.6 mg/kg body weight of stavudine for 21 days showed dense population of astrocytes compared to the control, while the section of the motor cortex of Group C administered with 1.2 mg/kg body weight of stavudine for 21 days showed highly dense population of astrocytes [Plate 1].

The test groups had significant ($P < 0.05$) higher reactive astrocytes population compared with the control. However, Group C treated with 1.2 mg/kg body weight of stavudine had significant ($P < 0.05$) higher reactive astrocytes compared with Group B [Figure 1].

DISCUSSION

Astrocytes are star-shaped cells commonly found between neurons and blood vessels (Shier *et al*., 2004). Astrocytes twist around nerve cells and form the supporting network in the brain; this forms the basis of blood-brain barrier and regulates the entry of substances into the brain tissues (Sembulingam and Sembulingam, 2000). Through modulation of their volumes, compositions and concentration of neurotransmitter, astrocytes exert strong influences on neurons (Peter *et al*., 1998). Damage to the central nervous system (CNS) is invariably accompanied

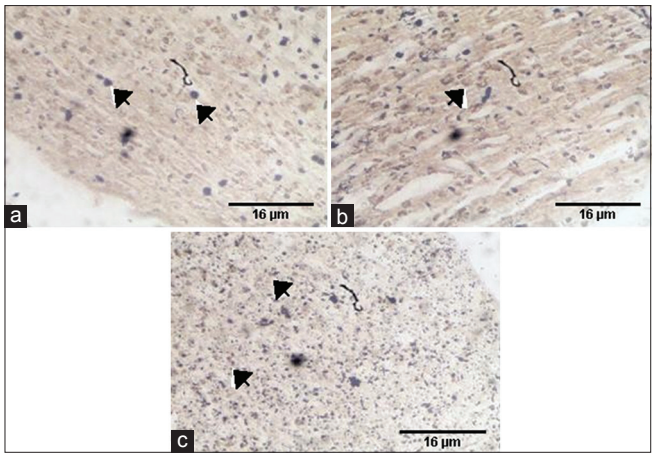

Plate 1: Photomicrographs of motor cortex of mice given stavudine and their control (Hortega's Lithium method, ×400 for all plates). (a) Motor cortex of control mice given feed and water showing few astrocytes stained black (arrowhead). (b) Motor cortex of mice given 0.6 mg/kg of stavudine for 21 days showing numerous astrocytes (arrowhead) compared to control. (c) Motor cortex of mice given 1.2 kg/kg of stavudine for 21 days showing increased population of astrocytes (arrowhead)

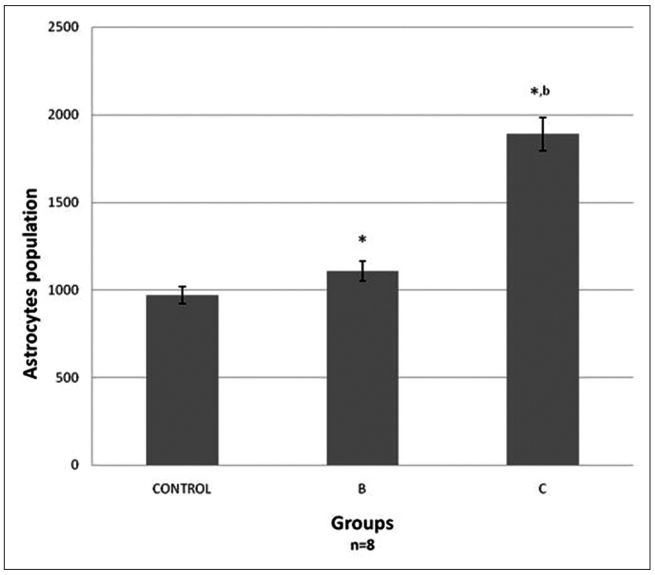

Figure 1: Astrocyte population quantification, *significantly different from control group at $P < 0.05$, [b]significantly different from Group B at $P < 0.05$

by hypertrophy and hyperplasia of astrocytes, a process referred to as "gliosis or astrocytosis (Macsween and Whaley 1992)." In CNS injury, astrocytes acts as neuroprotective sheath, they increase in number, fill injury zone, help in healing and recovery of neurons, such astrocytes are called reactive astrocytes (Peter *et al*., 1998).

In this study, the motor cortex of mice treated with 0.6 mg/kg and 1.2 mg/kg of stavudine showed dense population of astrocytes compared to the control group, an indication of astrocytosis. The presence of reactive astrocytes is an indication of early stage of neuronal cell loss (Abbas and Nelson, 2004). Transformation of astrocytes is induced by cyclic adenosine monophosphate and neurotransmitters released from damaged neuronal cells (Singh and Mathew, 1989). These changes in normal astrocyte morphology and population could impact new functional state which might impair astrocyte activity like uptake and metabolism of monoamine oxide and amino acid neurotransmitters (Cheri *et al*., 2004). Astrocyte hypertrophy is the ultimate cellular manifestation in degenerative conditions due to genetic causes or in acquired metabolic origin (Ghetti and Triarhou, 1992). They play an important role in homeostasis of the CNS both in normal condition and during seconds after brain ischemia. The ischemia stimulates sequential morphological and biochemical changes in glial and induces its proliferation (Singh, 2002). It also reported that altered cortical astrocytic morphology is seen in mood disorders (Torres-Platas *et al*., 2011), which might be its underlying cause.

The motor cortex is region of the cerebral cortex involved in the planning, control and execution of voluntary motor functions (Osim, 2005), thus, lesions to the motor cortex will impair voluntary motor functions. It is reported that the proliferation of astrocytes is an attempt to protect the neurons when there is injury (Guénard *et al*., 1996). This injury could be one of the side effects of stavudine administration, which may then results in sensory loss.

CONCLUSION

The result of this study suggests that stavudine use especially in high dose may impair motor functions.

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