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The effect of Stavudine on the Nissl bodies of the hippocampus of albino mice

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ABSTRACT: Stavudine is a synthetic antiretroviral agent active against the Human immunodeficiency virus (HIV). The increase in production and use of this antiretroviral drug (ARV), and its ability to cross the blood-brain barrier lead us to study the effect of oral administration on the Nissl bodies of the hippocampus of albino mice. Twenty-four albino mice weighing averagely 30g were equally assigned into three groups (A, B, C). Group A served as the control and was not given any treatment, while groups B and C were the experimental groups that received 0.6mg/kg and 1.2mk/kg of stavudine respectively, twelve hourly, for twenty-one days through orogastric tube. Food and water were allowed for the animals *ad libitum* throughout the experimental period. The animals were sacrificed using chloroform anaesthesia and the whole brains were removed, fixed in formal saline, the hippocampus excised. The tissues were routinely processed and stained using cresyl fast violet staining method. Light microscopic study of the Nissl bodies revealed less number of stained Nissl bodies in groups B and C, especially in the pyramidal layer, and this appeared to have been lesser in group C. This result revealed that stavudine may cause reduction in Nissl bodies in the hippocampus which may consequently affect the synthesis of both structural protein and protein for transport in correlation with neuron function thereby influencing their metabolic activity. It may be said that these reduction in the number of Nissl bodies was dose dependent.

Keywords: Stavudine, Hippocampus, Nissl bodies, Albino mice

Introduction

The epidemic of Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS) has continued to grow unabated especially in the African sub-regions (1), and the use of antiretroviral therapies as recommended by the World Health Organization (WHO) for the management of HIV and AIDS has been gathering momentum as its availability has also increased (2). One of the antiretroviral drugs (ARVs) currently used in antiretroviral therapy is stavudine.

Stavudine is a synthetic thymidine analogue active against the human inmmunodeficiency virus. It belongs to the class of antiretroviral drugs known as nucleoside/nucleotide reverse transcriptase inhibitors (3), having a long bioavailability and lower acute toxicity levels (4). It inhibits the replication of HIV in human cells *in vitro*. It enters the cells by diffusion and is phosphorylated by cellular kinases to the active metabolite-stavudine tri-phosphate. Stavudine tri-phosphate inhibits the activity of HIV reverse transcriptase both by competing with the natural

substrate, deoxythymidine tri-phosphate and by its incorporation into viral deoxyribonucleic acid (DNA) causing the termination of DNA chain, as stavudine lacks the essential 3-OH group necessary for DNA elongation. It further inhibits DNA polymerases (beta and gamma), and markedly reduces the synthesis of mitochondrial DNA (5,6).
Stavudine crosses the blood-brain barrier and is distributed in cerebrospinal fluid, and this has been reported to cause neurotoxicity (7). The major adverse effect of stavudine is peripheral neuropathy (8). Toxicity of stavudine can lead to clinical syndrome of weakness, sensory loss, diminished tendon reflexes or a combination of these symptoms caused by tension of peripheral nerves (9) .

Most neurotoxic effects of drugs affect the limbic system with the hippocampus being one of the easiest target. This area sub-serves the function of memory and learning. Almost any type of sensory experiences causes instantaneous activation of different parts of the hippocampus, and the hippocampus in turn distributes many outgoing signals to the hypothalamus and other parts of the limbic system especially through the fornix (10). Neurons that make up the hippocampus contains large aggregations of rough endoplasmic reticulum (ER) that correspond to the Nissl substance in light microscopy (11), which are responsible for synthesizing protein, and thus serve to identify neurons from other brain cells (12, 13).

The ability of the drug, stavudine to cross the blood-brain barrier, and its reported neurotoxic effects led us to investigate the effect of the ARV on the histology of the hippocampal neurons.

Materials and Methods

Twenty-four albino mice with average weight of 30g were used for this study. The animals were procured from the Department of Physiology Animal House, University of Calabar, Calabar. 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 experimental groups. The animals were given normal rat chow and water *ad libitum* throughout the experimental period.

A package containing fifty capsules of stavudine (30g each) was procured from the Pharmacy of the General Hospital, Calabar, Nigeria. 40mg of stavudine is recommended for a 60kg man while 30mg is recommended for children and man with weight less than 60kg. Each capsule of stavudine was dissolved in 100mls of distilled water. The therapeutic dosage was determined per body weight of the animals. This preparation was routinely carried out daily prior to each administration.
Group A were not given any treatment, while animals in groups B and C received 0.6mg/kg and 1.2mg/kg of stavudine suspension each respectively, twelve hourly for twenty-one days, orally with the aid of orogastric tubes.

Twelve hours after the last administrations, the animals were sacrificed by chloroform inhalation method. The brains were extracted by opening of the skull to have access. The whole brains were preserved using formal saline. When properly fixed, the hippocampi were dissected from the medial portions of the temporal lobes, routinely processed and stained using Cresyl fast violet method for Nissl bodies (14).

Results

The tissue section of the control group (group A) showed three hippocampal layers; molecular, pyramidal and polymorphic layers. The Nissl bodies were well stained especially in the pyramidal layer making the neurons easily distinguishable (Plate 1a). Group B treated with 0.6mg/kg of stavudine for 21 days showed Nissl bodies which appeared like the control but having less number (Plate 1b). Group C treated with 1.2mg/kg of stavudine for 21 days showed relatively fewer Nissl bodies in the pyramidal layer (Plate 1c).

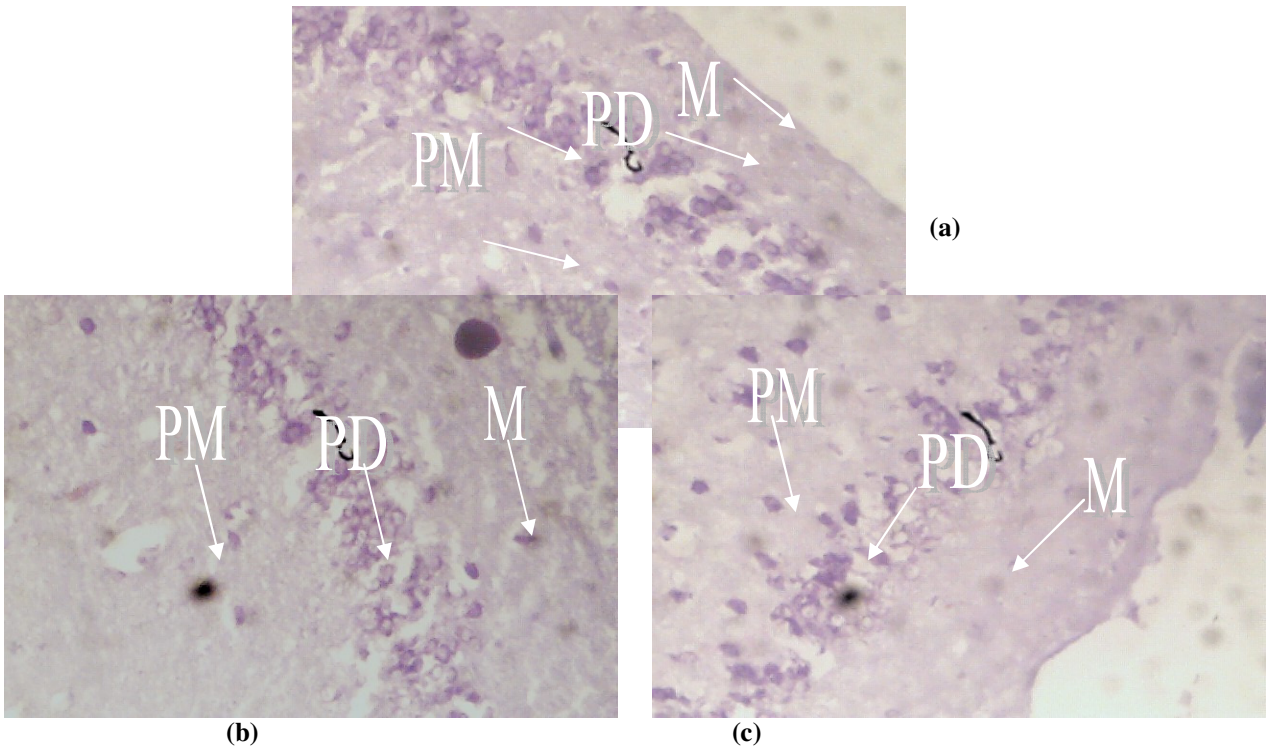

Plate 1: Photomicrographs of hippocampus of mice given stavudine and their control

a- Hippocampus of control mice given feed and water showing many neurons with Nissl's substance in the pyramidal layer and few in the molecular and polymorphic layers.
b- Hippocampus of mice given 0.6mg/kg of stavudine for 21 days showing fewer neurons with Nissl's substance.
c- Hippocampus of mice given 1.2mg/kg of stavudine for 21 days showing relatively fewer Nissl's substance. (Cresyl fast violet x 400 for all plates).

M - Molecular layer
PD - Pyramid layer
PM - Polymorphic layer

Discussion

The neuron is one of the most complex cell in the body, and since it is incapable of dividing after their first few days of life, loss of neurons is irreversible. A conspicuous feature in the perikaryon of neurons are Nissl bodies which are rich in DNA and composed of stacks of rough endoplasmic reticulum and intervening groups of free ribosomes (11). This makes them an important index in tracing neuronal population (12,13). Nerve cells require large amount of proteins to maintain their integrity and to perform their functions, hence in injured neurons, disappearance of the Nissl bodies have been observed in a process described as chromatolysis (13,14).

In this study, there was reduction in the population of Nissl bodies in the sections of the experimental groups compared to the control. The reduction in the population of Nissl bodies was more pronounced in group C treated with 1.2mg/kg of stavudine for 21 days suggesting that the effect of stavudine was dose-dependent.

Chemicals including drugs, toxins and lack of oxygen, cause alterations in the distribution pattern of Nissl bodies, which thereby influenced their metabolic activities (15, 16). The degenerative changes in the Nissl bodies observed in this study may affect the synthesis of protein with correlation with neuronal functions. It is possible that

stavudine may have had an irreversible effect on the Nissl bodies in the hippocampus (though we did not check this) bringing about such microstructural changes in the neurons which manifest as degeneration and loss of Nissl bodies with reduced population of the Nissl bodies. Thus, the loss of function of the Nissl bodies may result in the loss of protein synthesizing ability of the neuron and since protein is the working molecules of the cells, this may ultimately result in cell death. Primarily, the hippocampus is responsible for consolidation of memory and learning and in animals of this nature, play an important function in the determination of smell (17).

This result showed that stavudine caused Nissl bodies reduction especially in the high dose group. In conclusion, if this finding is extrapolated to man, stavudine use especially in high doses may impair memory.

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