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Full Length Research Paper

Cortical and cerebellar neurodegeneration in cyanide induced toxicity

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One of the most prominent disease conditions in cassava endemic regions of the world is movement disorders. In this study, we investigate the movement disorder from the cortical and cerebellar point of view. Most toxicity studies involving movement disorder has been greatly linked to the motor cortex, thus, we examined neurodegeneration both in the motor cortex and the cerebellar cortex. This study also evaluated the possible role of such degeneration in the etiology of neurodegenerative diseases associated with cassava endemicity. 15 F1 adult Wistar rats were divided into three groups of five animals each. The first group was the control, the second group received 10 mg/kg BW of potassium cyanide (KCN) and the third group received 20 mg/kg BW of KCN for 15 days. The cortical (motor area) and cerebellar tissue were obtained and fixed in formol calcium for cyto-architectural study. In conclusion, toxicity of cyanide in the cortex and cerebellum can involve osmotic imbalance and excitotoxicity at the 10 mg/kg causing increased in cell size and a slower form of degeneration and reactive oxygen species (ROS) generation and lipid peroxidation causing release hydrolytic enzymes from lysosomes that destroys the components of the cytoplasm as observed in the 20 mg/Kg treatment.

Key words: Neuron, cell death, cerebellum, cortex, movement, degeneration, oxidative stress.

INTRODUCTION

In 1981, Osuntokun and colleagues reported a wide range incidence of neurodegenerative diseases in cassava endemic parts of Nigeria, Niger, Zaire and Mozambique. The clinical symptoms expressed showed that patients suffered from upper motor neuron disease leading to movement disorders resembling those observed in Parkinsonism, tropical ataxic neuropathy (TAN), Spastic endemic paraparesis (Konzo) and gradual loss of vision due to degeneration in the visual cortex or as a result of retrobulbar neuritis (Osuntokun, 1981; Soler-Martin et al., 2010). The cerebellum (**"**little brain") is a structure that is located at the back of the brain, underlying the occipital and temporal lobes of the cerebral cortex. Although the cerebellum accounts for approximately 10% of the brain's volume, it contains over 50% of the total number of neurons in the brain (Alexander et al., 2012). Historically, the cerebellum has been considered a motor structure, because cerebellar damage leads to impairments in motor control and posture and because the majority of the cerebellum's outputs are parts of the motor system (Bolduc et al., 2011). Motor commands are not initiated in the cerebellum; rather, the cerebellum modifies the motor commands of the descending pathways to make movements more adaptive and accurate (Filippi et al., 2012). The cerebellum is involved in the following functions: maintenance of balance and posture, coordination of voluntary movement, motor learning and cognitive functions (Alexander et al., 2012; Pekary and Sattin, 2012). The body maintains its equilibrium from sensory inputs from the eyes, the vestibular system in the ears, the proprioceptive system (joints, bones and muscles) regarding the position of the body with respect to the external environment (Bolduc et al., 2011; Pitel et al., 2012). The flocculonodular lobes of the cerebellum

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play an important role in receiving and processing this information. This is why cerebellar injury results in ataxia and an abnormal gait. Literally, every movement of the body, although planned and executed by the motor cortex of the central nervous system, is regulated and smoothly controlled by the cerebellum (Orvis et al., 2012).

The pyramidal motor system (upper motor neurons) controls all of our voluntary movements. Pathological processes which damage the pyramidal motor system are extremely important causes of disability and suffering. The main function of a motor neuron is to convey output signals to muscles for joint control (Ishida et al., 2011). This involves 'upper motor neurons' in the brain relaying signals to 'lower motor neurons' in the spinal cord, which in turn relay signals to skeletal muscles for joint control. Lower motor neurons are also involved in sympathetic function, such as blood pressure regulation (Reiner et al., 2010; Van Elswijk et al*.,* 2010). Thus, the primary basics of movement disorder will not only involve the upper motor neuron but the cerebellum as well. Since the cerebellum controls the instinctive aspect of movement, the broad spectrum of movement disorders and loss of vision associated with cassava endemicity will not only affect the cortical cells but the coordinating centre for visual impulse and information conveyed from the upper motor neurons into the association centres like the red nucleus and substansia nigra all of which are relayed and regulated at the cerebellum (Otellin et al., 2011).

Cyanide is capable of generating reactive oxygen species (ROS) and nitric oxide (NO) both of which are capable of peroxidation of lipids and induction of the caspases system that has been described as the basics of cyanide induced neurodegeneration. Cyanide has also been found to be excitotoxic a phenomenon also applicable to cerebellar cells as they possess glutamate receptor (NMDA R1), which can be potentiated by cyanide (Isom et al., 1999). Cyanide also inhibits metalloenzymes like Cytochrome *C* oxidase important for the oxygen carrying capacity of the neuron and alkaline phosphatase responsible for membrane transport functions in the cerebellar cells. Thus, an assault of cyanide will be detrimental on the cerebellum and can sub-serve the primary basics for induction of movement disorder, impaired visual and cognitive functions, ataxia due to neuropathy (Chen et al., 2011).

MATERIALS AND METHODS

15 F1 generation adult Wistar rats (Wild type) were divided into three groups of five animals each. The animals were exposed to standard laboratory conditions and fed on normal rat chow. The animals were also exposed to 12 h alternating light and darkness. The groups were labeled A, B and C where group A received 20 mg/kg BW of cyanide, B received 10 mg/kg BW and C was the control of the experiment. The duration of the treatment was 15 days and the route of treatment was oral. The doses were selected based on a level below the LD_{50} and a concentration obtainable in cassava based diet in humans (4 to 22 mg/kg/Day). The animals were sacrificed by cervical dislocation and the brain was fixed in formol calcium for 24 h. The brain tissue was then removed from the fixative and dissected to remove the cerebellum and the motor area. The tissues were then processed to obtain paraffin wax embedded tissue blocks for histology.

Histology

The tissues were processed and 6 µm thick sections were stained in hematoxylin and eosin using the methods of Park et al. (2012).

RESULTS

General morphology of the cerebellum

Histology of the motor area (cerebral cortex)

Treatment with cyanide induced neuronal cell death at both treatment doses as shown in arrow heads (Cerebellum: Figures 2A and B; Cortex: Figures 4B1 and B2) for the 10 mg/Kg and (Figures 3A and B: cerebellum; Figures 4C1 and C2: cortex) for the 20 mg/kg treatment. The mode of cell death seen in 10 mg/Kg treatment (cerebellum and cortex) was features of necrosis with enlarged cell bodies and distorted membrane. In the 20 mg/kg treatment group, the neuronal damage was pronounced and neuronal connections ware lost (Figures 3A and B: cerebellum; Figures 4C1 and C2: cortex). The nucleus in the 10 mg/kg treatment was prominent while degeneration of cytoplasm and nuclear materials was observed in the 20 mg/kg treatment group (Figures 3A, B; Figure 4C1 and C2); this implies that the form of cell death was caused by oxidative stress which involves production of superoxide anions that can induce peroxidation of lipids and cause damage to membranes. The rate of progression of such damage was more advanced in the 20 mg/kg for the cortex and cerebellum (Figures 3 and 4C) and the extent of degeneration can be said to be proportional to the treatment dose. If this were so, it therefore will imply that the large quantity of radicals thus formed activated nitric oxide (NO) and reactive nitrogen species (RNS). For this study, no biochemical study was conducted. Structural evidence from previous studies suggests that over production of NO can induce rapid degradation of genetic materials via apoptosis as NO is a naturally occurring endogenous modulator of cellular activities (Isom et al., 1999). Comparing the treatment groups in Figures 2 and 3 against the control (Figure 1) (cerebellum) and Figures 4B and C against control (Figure 4A) (cortex), neuronal degeneration can be said to have occurred. Thus, the cerebellar and cortical cell death will increase with the treatment dose.

DISCUSSION

The motor area, entire cerebellar cortex, the deep

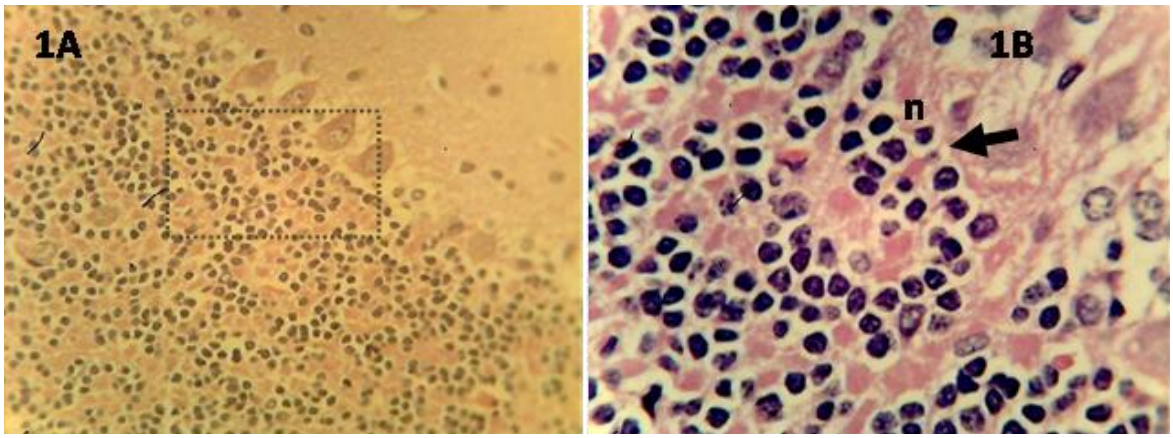

Figure 1. General morphology of the normal cerebellum (Purkinje cell layer: arrow head). n Shows the normal cells (Magnifications 1A: X400 and 1B: X1,000). Area in shaded square represents the magnified region of the slide. (Magnifications 1: X400 and 1B: X1, 000).

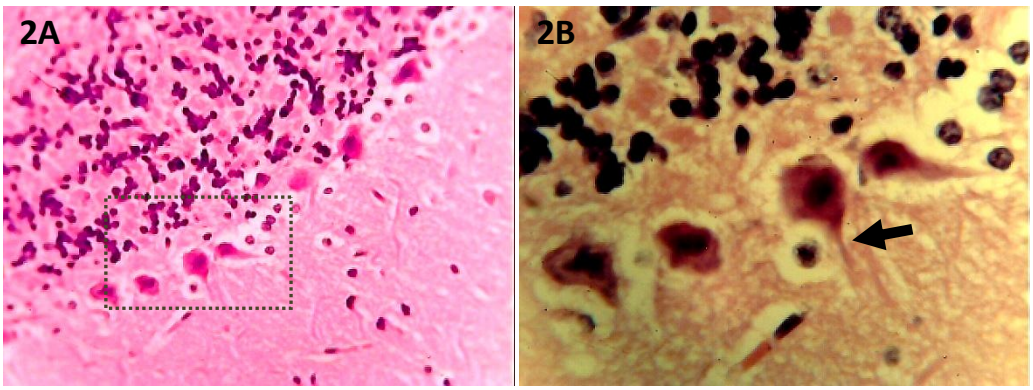

Figure 2. General morphology of cells for treatment 10 mg/Kg. The cell shows enlarged cell bodies (arrow head), pale stained cytoplasm and prominent projections from the cell body indicating degeneration by osmotic imbalance as the nuclear materials appears intact and membranes distorted. Such structural changes are signs of degeneration by necrosis resulting from distortion of membrane which leads to osmotic imbalance (Magnifications 2A: X400 and 2B: X1, 000).

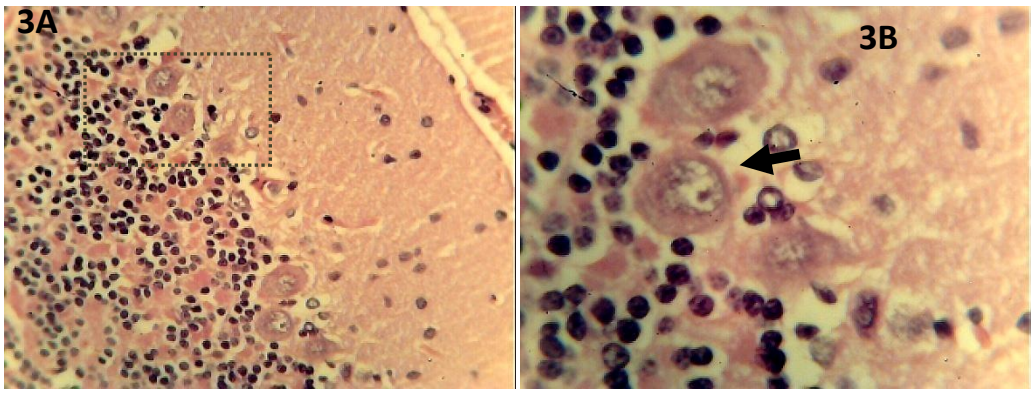

Figure 3. General morphology of cells undergoing both apoptosis and necrosis induced by oxidative stress treated with 20 mg/Kg of KCN. The cell bodies show enlargement and fragmented cytoplasm and nucleus caused by ROS and NO, respectively. The NO is generated in excess amount considering the increased ROS formation as against the 10 mg/Kg seen in Figure 2. The connections of the axons are free ended and vacuolar spaces are observed around the cells probably due to peroxidation of lipids and fast progressing degeneration of cell body and axons. (Magnifications 3A: X400 and 3B: X1, 000).

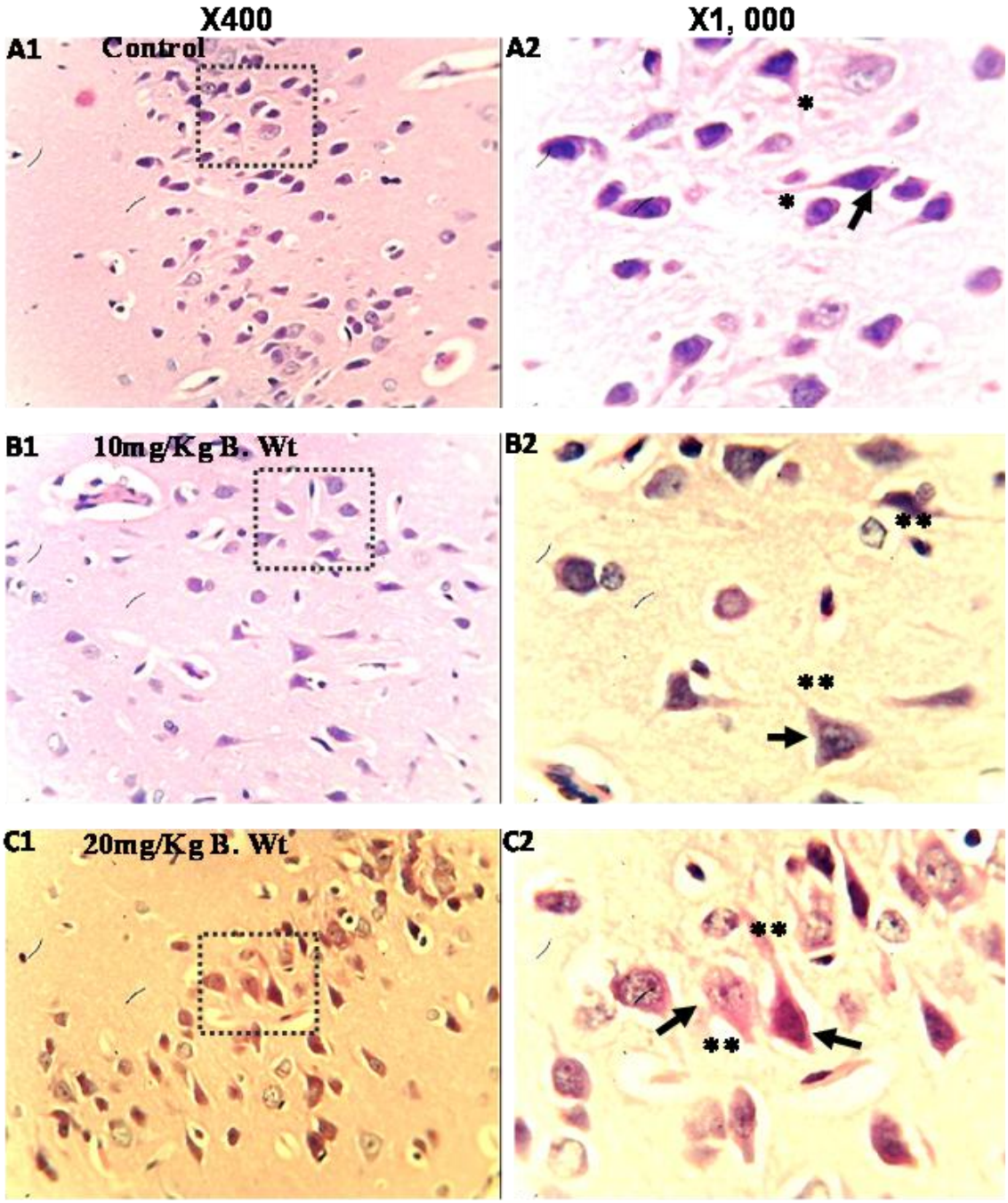

Figure 4. General morphology of the pyramidal cell layer of the cortex. Fig 4A1 and 4A2: shows the histology for the control group at *x400* and *X1, 000* respectively. Arrow head indicates normal pyramidal cells. *Fig 4 B1* and *4B2* shows degenerating pyramidal cells, arrow head shows the enlarged darkly stained cytoplasm indication signs of excitotoxicity. (*) indicates prominent axonal projections. Similar features were also observed in *Fig4C1* and *C2* where the 20mg/Kg treatment showed signs of neurodegeneration with prominent distortion of membrane and cytoplasmic content; this is probably due to formation of reactive oxygen species combines with excitotoxicity. The degenerative process progression shows rapidly degenerating pyramidal cells in this group. Dotted square represents the magnified section in the X400 shown in the X1, 000.

cerebellar nuclei, pontine relay nuclei and cerebellar peduncles constitute the cerebellar system that works in association with the basal ganglia to form two distinct modulatory systems for the purpose of motor function (Thürling et al., 2012). Thus lesions of the cerebellar cortex will lead in a first instance to in coordination of

ongoing movement (Mizusawa, 2012). This defect is called cerebellar ataxia which represents the inappropriate operation of muscles that rely on sensory feedback to produce coordinated movement (Mizusawa, 2012; Hoche et al., 2012). In this experiment, the toxicity of cyanide has been seen to produce structural lesions in the cerebellar cortex and the motor area; some of the animals showed signs of tremor (especially in the 20 mg/kg BW treatment group) with constant tilting of the head to one side. The tremor observed could be as a result of a wide range of effects in the brain, but if such tremor is owned to lesions of the cerebellum only it is called intention tremor as opposed to the tremor at rest observed in patients with Parkinsonism. In 1981, Osuntokun and colleagues reported series of movement disorders in cassava endemic populations in some parts of Africa. Several movement disorders were observed some of which includes tropical ataxic neuropathy, spastic endemic paraparesis (Konzo) and other symptoms resembling those observed in Parkinsonism, thus supporting the idea that movement disorders linked with toxicity of cyanide in diet is a function of both the motor area and the cerebellar cortex.

Of interest in this study are the Purkinje cells of the cerebellum; given the degree of abortivation of dendritic fibers around the Purkinje cells, they appear to be cells specialized in accommodating and integrating large inputs from the adjacent cells especially the GABAergic climbing Mossy fibers (Figures 1A and B) (Tsai et al., 2012). However in the treatment groups, the specific interaction between the mossy fibers and the terminal regions of the Purkinje cells were lost owing to the fact the degeneration had occurred in both set of neurons. Some of the features of such degeneration are retraction of the axonal systems and loss of connections to adjacent neurons (Figures 2 and 4B). Another possibility of the essence of the vacuolar spaces may be as a result of peroxidation of lipids. The cells generally showed increase in cell size in the 10 mg/kg group and loss or fragmented cytoplasm in the 20 mg/kg treatment group.

A major question is that: does the toxicity of cyanide depend on the dose of treatment and if this were so, could such dependence be based on the mechanism of toxicity that is predominantly adopted at such dose? There is no straight answer to this question but the hypothesis to explain the difference in the damage to the cells based on dose is that; firstly, cyanide is capable of potentiating NMDA R1 (a form of glutamate receptor). Since the cerebellum contains more than 50% of the neuronal cells in the brain and glutamate neurons are the most abundant, this will make the cerebellum a major site for excitotoxicity of cyanide and a major contributor to movement disorder just as the widely studied motor area (Mead et al., 2012).

Cyanide is capable of potentiating NMDA R1 as against up-regulating the receptor. Potentiating the receptor will imply the ability of cyanide to bind to the receptor keeping it active for a prolonged period of time, thus since the neuronal and astrocytic cells does not possess metabolic machinery to break down cyanide, the activity of cyanide is continuous in the neuron thus causing prolonged excitation in the first instance. In a second instance, this will affect the proposed glutamate-glucose exchange required for energy metabolism, thus the NMDA R1 is kept active causing glutamate transport to be impaired. Since glutamate cannot be easily released, glucose is deficient in the neurons as glutamate is always exchanged for glucose. A possibility is that more glutamate will be produced by the cells to request for glucose thus leading to accumulation of glutamate in glutamate toxicity (de Rivero et al., 2012). In a third instance, potentiation of NMDA R1 is associated with transient firing of the neurons and influx of calcium ions causing osmotic imbalance and a resultant increase in osmotic pressure in the neurons thus causing increase in size (Dong et al., 2012).

A second method of cyanide toxicity is the inhibition of the *Heme a3-Cuβ* bi nuclear centre of cytochrome C oxidase. Cyanide can inhibit this mitochondria enzyme in its three redox states. The enzyme is a member of the cytochrome P 450 super family concerned with the conversion of molecular oxygen to water to drive ATP production. Thus inhibition of this enzyme causes accumulation of molecular oxygen and electrons. When these two components react, reactive oxygen species (ROS) are generated and also nitric oxide and other reactive nitrogen species (RNS)/nitric oxide (NO). ROS can cause peroxidation of lipids and release of lysosomal enzymes, since ROS are capable of peroxidation of lipids, the bilayer of endosomes, peroxisomes and lysosomes are also damaged causing the release of hydrolytic enzymes accounting for loss of cytoplasmic content as seen in the 20 mg/kg treatment. NO is a naturally occurring endogenous modulator of cellular activities but under cyanide treatment, the NO will increase initiating signals capable of driving the cell into apoptosis or necrosis; of important note is the fact that over production of NO will give signals to initial cleavage of DNA which is seen in the 20 mg/kg treatment as fragmentation of the cytoplasm and nuclei (Min et al., 2012).

In conclusion, toxicity of cyanide in the cerebellum and cortex can involve osmotic imbalance and excitotoxicity at the 10 mg/kg causing increase in cell size and a slower form of degeneration and ROS generation and lipid peroxidation causing release of lysosomes and digestion of the components of the cytoplasm was a prominent occurrence in the 20 mg/kg treatment group.

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