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Fluoride and aluminium disturb neuronal morphology, transport functions, cholinesterase, lysosomal and cell cycle activities

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Abstract

Fluoride and aluminium have been reported to cause severe alterations in the brain. However, their exact mechanisms of neurotoxic activities remain unknown. **Aim:** This study was designed to investigate the role of fluoride and aluminium in neuronal transport, lysosomal, cell cycle protein and acetylcholinesterase activities. **Method:** Adult Wistar rats were given low and high doses of fluoride, aluminium and a combination of both with the control group receiving distilled water for 30 days. Blood sera and brain homogenates were quantified for alkaline phosphatase (biomarker for neuronal transport) activities. Brain sections were stained with cresyl fast violet to detect neuronal cell damage. Histochemical demonstration of acetylcholinesterase (AChE) activity and the immunohistochemical detection of cell cycle protein (anti-cyclin D) and lysosomal protein (anti-cathepsin D) were done using the antigen retrieval method. **Result:** Results showed severe histomorphologic alterations, dysregulation of membrane transport activities, inhibition of AChE activities and increased expression of lysosomal and cell cycle proteins. **Conclusion:** These findings confirm that excessive fluoride and aluminium intake induces the progression of cell death which inhibit AChE activities and trigger the release of lysosomal and cell cycle proteins.

Keywords: Nissl body; Alkaline phosphatase; Cyclin D; Cathepsin D; Fluoride; Aluminium; Acetylcholinesterase

1. Introduction

Fluoride is an environmental pollutant existing only in combination with other elements as fluoride compounds. It is present in dental products, food, pesticides and water. However, the main source of fluoride for humans is the intake of groundwater contaminated by geological sources (maximum concentrations reaching 30–50 mg/l) [1,2].

Many developing countries have been reported to have endemic fluorosis [3]. Amongst other effects, fluorosis has been said to affect cells from soft tissues, such as renal, endothelial, gonadal, and neurological cells [1,4]. The central nervous system is highly susceptible to chronic fluorosis [5,6], and the prefrontal cortex has been implicated in playing significant roles in attention processes, decision-making, goal-directed behaviour, working memory, processing of emotional stimuli, temporal organization of behaviour, rule learning and behavioural flexibility [7].

Neuronal membrane transport and integrity are important in neuronal metabolism [8,9]. In the adult brain, phosphatases have been reported to be essential for synaptic functions (neurotransmission) and in the regulation brain functions such as learning and memory [10]. The dysregulation of the activities of alkaline phosphatase has been linked to some neurological disorders [11].

Acetylcholinesterase (EC 3.1.1.7) is a serine hydrolase that belongs to the esterase family within higher eukaryotes. Apart from its catalytic function in hydrolyzing acetylcholine, acetylcholinesterase (AChE) is said to play a role in cell proliferation, differentiation and responses to various insults [12,13].

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Cyclin D has been reported to play a key role in cell cycle regulation which is associated with cell proliferation, DNA damage and cell death [14]. Recent studies suggest that the regulatory targets for cyclins D are the molecules responsible for the G1/S phase transition of the cell cycle. In mammalian somatic cells, cyclin D is synthesized in late G1 and appears to be required for progression into S phase [15].

Cathepsin D is a soluble lysosomal aspartyl protease that plays a crucial role in normal cellular functions [16]. It has been shown to be indispensable for postnatal tissue homeostasis. Numerous physiological functions of cathepsin D have been suggested based on its ability to cleave structural and functional proteins and peptides, these include metabolic degradation of intracellular proteins, activation and degradation of polypeptide hormones and growth factors, activation of enzymatic precursors, processing of enzyme activators and inhibitors, brain antigen processing and regulation of programmed cell death [17].

Reports have indicated that fluoride interacts with aluminium to form a fluoro-aluminium complex [18]. This interaction has been implicated in mimicking metabolic activities of phosphate analogues proffering them with the ability to activate G-protein 2nd messenger receptor system on cell membranes [18–20]. It has been suggested that the synergistic action of fluoride and aluminium in the environment, water and food chains can lead to the formation of various pathologies such as alterations of homeostasis, metabolism, growth and differentiation of the living organisms [21].

Although observations report that fluoride and aluminium are capable of inducing cortical dysfunction, their exact mechanisms of actions still remain largely unknown. The present study aims at elucidating the underlying mechanisms of fluoride and aluminium induced neurotoxicity.

2. Materials and methods

2.1. Animals and treatment

Male Wistar rats were obtained from the breeding colony of the National Vertinary Research Institute (NVRI), Jos, Nigeria. The animals were kept in the animal holding facility of the Department of Anatomy, Bingham University, Nasarawa, Nigeria. They were given standard rat pellets and water *ad libitum* and were allowed to acclimatize for two weeks. They were kept in aerated cages under standard laboratory conditions of 12 h light/dark cycle (lights on at 0800 h) and average room temperature (22 ± 2 °C; relative humidity, 50–60%). All experimental protocols were followed upon approval by the local institutional research committee and in accordance with the guidelines for animal research, as detailed in the NIH guidelines for the care and use of laboratory animals [22].

2.1.1. Treatment

$N = 35$ male rats weighing between 180 and 250 g were randomly divided into seven (7) groups of $n = 5$ animals each. The groups comprised of the control group (C), low dose sodium fluoride (NaF) group (L-NaF), high dose NaF group (H-NaF), low dose aluminium chloride ($AlCl_3$) group (L-Al), high dose $AlCl_3$ group (H-Al), low dose combined treatment of NaF and $AlCl_3$ (NaF^+ $AlCl_3$) group (L-NaF/Al) and the high dose NaF^+ $AlCl_3$ group (H-NaF/Al). The L-NaF group received 2.1 ppm NaF, which corresponds with the WHO recommended value for fluoride in drinking water [23]. Following previous studies, the H-NaF group received 10 ppm, respectively [24]. Following reports that the oral median lethal dose (LD_{50}) of aluminium chloride in rats ranges between 200 and 1000 mg of aluminium per kilogram of body weight [25], the L-Al and H-Al groups received 10 ppm and 100 ppm, respectively [26,27]. The L-NaF/Al and H-NaF/Al groups received 1.05 ppm NaF + 5 ppm $AlCl_3$ and 5 ppm NaF + 50 ppm $AlCl_3$, respectively [28] and the control group (C) was given the distilled water (to rid the water of ions) as vehicle.

Thirty (30) days after treatment, animals were sacrificed by cervical dislocation and the brains were excised and stored in either 10% formal saline or 0.25 M sucrose solution for histological, immunohistochemical and colorimetric assays.

2.2. Nissl body staining

Fixed tissue samples were embedded in molten paraffin wax after which serial coronal sections were obtained at 5 μm thickness at various levels through the prefrontal cortex (Bregma 3.72–2.52) [29]. Cortical sections were mounted on glass slides and processed in the following order: 100% ethanol, 2 min; xylene, 2 min; 100% ethanol, 2 min; 70% ethanol, 2 min; distilled water, 5 min; cresyl fast violet, 3 min; distilled water, two dips; 70% ethanol, 5 min; 80% ethanol, 2 min; 90% ethanol, 2 min; 95% ethanol, 2 min; 100% ethanol, 5 min; xylene, 5 min; and then mounted with DPX and air dried for microscopic observation.

2.3. Biochemical determination

Blood was obtained by cardiac puncture and serum was stored in −20 °C freezer. The excised brain tissues were kept in cold 0.25 M sucrose solution following which they were homogenized with *Potter-Elvhjem* homogenizer and centrifuged at 15,000 × *g* for 5 min at 4 °C. The resulting sera and brain tissue supernatants were used for the colorimetric determination of alkaline phosphatase (ALP).

Alkaline Phosphatase kit (Quimica Clinica Applicada SA (QCA), Spain) was used for the colorimetric assay of alkaline phosphatase levels in both sera and tissue homogenates. This assay kit uses the phenolphthalein monophosphate substrate (end point colorimetric). Four test tubes were labelled appropriately as test, standard, control, and blank. The test contained 1 ml water, 100 μl serum/tissue homogenate,