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Interplay of glia activation and oxidative stress formation in fluoride and aluminium exposure

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Abstract

Background: Oxidative stress formation is pivotal in the action of environmental agents which trigger the activation of glial cells and neuroinflammation to stimulate compensatory mechanisms aimed at restoring homeostasis. **Aim:** This study sets to demonstrate the interplay of fluoride (F) and aluminium (Al) in brain metabolism. Specifically, it reveals how oxidative stress impacts the activation of astrocytes (GFAP), mediates proinflammatory responses (microglia and B-cells: CD68 and CD 20 respectively) and shows the pattern of lipid peroxidation in the brain following fluoride and (or) aluminium treatment in vivo. **Method:** Male adult Wistar rats were treated with low and high doses of fluoride, aluminium or combination of fluoride–aluminium for 30 days. The control group received distilled water for the duration of the treatment. Blood and brain tissue homogenates were prepared for colorimetric assay of stress biomarkers [malonialdehyde (MDA) and superoxide dismutase (SOD)]. Subsequent analysis involved immunodetection of astrocytes (anti-GFAP), microglial (anti-CD68) and B-cells (anti-CD20) in coronal sections of the prefrontal cortex using antigen retrieval immunohistochemistry. **Result and conclusion:** Aluminium, fluoride and a combination of aluminium–fluoride treatments caused an increase in brain lipid peroxidation products and reactive oxygen species (ROS) formation. Similarly, an increase in glial activation and inflammatory response were seen in these groups versus the control. Oxidative stress induced glial activation (GFAP) and increased the expression of B cells (CD20). This also corresponded to the extent of tissue damage and lipid peroxidation observed. Taken together, the results suggest a close link between oxidative stress neuroinflamation and degeneration in aluminium-fluoride toxicity.

Keywords: Oxidative stress; Prefrontal cortex; Astrocytes; Microglia; B cells

1. Introduction

Fluoride is one of the very few chemicals that has been shown to cause significant effects in people through drinking-water [1,2]. Fluoride is abundant in the environment and exists only in combination with other elements as fluoride compounds; these include constituents of minerals in rocks and soil [3]. Fluoride has beneficial effects on the teeth at low concentrations in drinking-water. However, excessive exposure to fluoride in drinking-water, or in combination with fluoride from other sources may result in a serious public health problem called Fluorosis [1]. Fluorosis is characterized by dental mottling and skeletal manifestations such as crippling deformities, osteoporosis, osteosclerosis, and may also affect soft body tissues as the brain [1].

Aluminium is highly ubiquitous in the environment and it is often found as a component of materials used in manufacturing (clays, glasses, and alum), cooking, pharmacological

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agents and antiperspirants [4]. Through its various applications in daily life, bioaccumulation of aluminium can occur in the human system following prolonged use or exposure [4].

Aluminium is a well-established neurotoxin and it has been suspected to be linked with various neurodegenerative diseases including Alzheimer's disease (AD) [5–7], amyotrophic lateral sclerosis (ALS), motor deficits and motor neuron degeneration [8], parkinsonism, and the gulf war syndrome [9].

Recently, much attention has been given to the possible synergistic role of aluminium and fluoride in some observed toxicity and neurodegenerative disorders [10]. Studies have shown that fluoride enhances the absorption of aluminium from the gastrointestinal mucosa and aids its passage across the blood–brain barrier [11]. Furthermore, aluminium and fluoride are known to readily form chemical complexes resembling phosphate analogues that are capable of activating G-Proteins, a membrane linked to second messenger activation [11,12].

Separate treatments or exposure to either aluminium or fluoride are capable of eliciting oxidative stress and pro-inflammatory response in the central nervous system (CNS) [13]. An important aspect of oxidative stress-mediated inflammation is the infiltration of the CNS by cells of the peripheral immune system [13]. Neuronal injury caused by oxidative stress also involves activation of microglia cells and other blood line phagocytes which are believed to precede formation of reactive oxygen species (ROS), cell death and the infiltration of B cells [13].

Previous studies have reported a narrow margin between the recommended intake and the neurotoxic doses of fluoride and aluminium [14,15]. Compelling evidence indicates that fluoride and aluminium produces injury to the central nervous system (CNS) by inducing free radical generation and lipid peroxidation in the brain [11]. Hence, to further shed more light on the mechanism of fluoride and aluminium induced neurotoxicity, this study is designed to elucidate the role of fluoride and aluminium in oxidative stress formation, glial activation and neuroinflammation. This was done by examining the levels of several oxidative stress markers, and the immunohistochemical localization of astrocytes, microglia and B cells.

2. Materials and methods

2.1. Animals and treatment

Male Wistar rats were obtained from the breeding colony of the National Veterinary Research Institute (NVRI) in 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 available 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), average room temperature ($22 \pm 2\,^{\circ}C$; 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 [16].

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 (LN), high dose NaF group (HN), low dose Aluminium Chloride ($AlCl_3$) group (LA), high dose $AlCl_3$ group (HA), low dose mixed/combined treatment of NaF and $AlCl_3$ (NaF+ $AlCl_3$) group (LM) and the high dose NaF+ $AlCl_3$ group (HM). The LN group received 2.1 ppm NaF; this corresponds with the WHO recommended value for fluoride in drinking water [1]. Following previous studies, the HN group received 10 ppm respectively [17]. Bearing in mind 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 [20], The LA and HA groups received 10 ppm and 100 ppm respectively [18,19]. The LM and HM groups received 1.05 ppm NaF+ 5 ppm $AlCl_3$ and 5 ppm NaF+ 50 ppm $AlCl_3$ respectively [21] and the control group (C) was given the distilled water as vehicle (to rid the water of ions). The animals were treated for 30 days following which they were sacrificed by cervical dislocation with the brains being excised and stored in either 10% formal saline or 0.25 M sucrose solution for immunohistochemical and colorimetric assays respectively.

2.2. Colorimetric assays

Blood: Peripheral blood was obtained by cardiac puncture, kept in vials and immediately centrifuged to obtain the serum which was stored at $-20\,^{\circ}C$ for subsequent colorimetric assays.

Brain: The brain tissues were harvested by careful dissection of the cranium using bone forceps, following which the whole brains were excised and immediately placed in 0.25 M sucrose solution. The tissues were then homogenized with *Potter-Elvhjem* and centrifuged at $15{,}000 \times g$ for 5 min at $4\,^{\circ}C$. The supernatants were collected and stored for subsequent colorimetric assays.

Enzyme assays: The resulting supernatants were used to quantify the levels of malondialdehyde (MDA; a lipid peroxidation product) and superoxide dismutase (SOD; an enzyme responsible for the conversion of superoxide anions into oxygen and hydrogen peroxide).

SOD (*superoxide dismutase*): The tissue homogenates were diluted in carbonate buffer (pH 10.2) in preparation for the SOD assay (1:10). The colorimetric reaction utilizes the ability of SOD to inhibit free radical production form epinephrine following the effect of xanthine oxidase. 0.1 ml of microsome was diluted in 0.9 ml of distilled water to make a 1 in 10 dilution of microsome. An aliquot of 0.2 ml of the