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Toxicology

Comparative study on the influence of fluoride on lipid peroxidation and antioxidants levels in the different brain regions of well-fed and protein undernourished rats

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ABSTRACT

Effects of fluoride on the levels of Lipid peroxidation (LP) and antioxidant enzymes in the brain regions of protein undernourished (PU) and well-fed rats (WF) rats exposed to 100 ppm fluoride in drinking water were investigated. The results indicate that the mean body weights and the total brain weights of PU rats as well as those given fluoride (both WF and PU) were significantly ($P < 0.05$) lower than their respective controls. The weights of different brain regions were also significantly reduced ($P < 0.05$) in PU rats compared to WF rats except in the brain stem. Fluoride ingestion diminished the weights of WF and PU rats affecting the cerebrum only (in the case of PU rats) and the cerebellum of both WF and PU rats without an effect on the brain stem of both WF and PU. Additionally, increased LP was observed in the cerebrum and cerebellum of PU rats but after fluoride ingestion, 30% increase in LP was observed only in the cerebrum. In the brain stem however, protein undernutrition was accompanied with a significant reduction in LP but the region seems insensitive to fluoride. There were significant reductions ($P < 0.05$) in CAT, SOD and GSH in all the brain regions (except the GSH level in the brain stem only) of PU rats. Fluoride induced reduction in the activity of CAT in the three brain regions and on SOD activity in cerebrum only for WF rats but no effect of fluoride on all the antioxidants studied in the three brain regions for PU rats. It is concluded that WF and PU rats responded differently to fluoride toxicity. However, it seems that at the dosage used, fluoride toxicity may be a direct effect on the antioxidant enzymes.

Introduction

Fluoride is an essential trace element but the beneficial range is narrow that health may be influenced adversely if excessive fluoride is supplied [1]. Fluorosis has been on the rise since the 1950s and is related to high concentration of fluoride present in drinking water or produced during coal-burning [2,3] and causing damages to the human body characterized by a vast array of symptoms and pathological changes in addition to the well-known effects on skeleton and teeth. Excessive intake of fluoride leading to fluorosis is a slow, progressive degenerative disorder affecting the structure and function of skeletal muscle, brain and spinal cord [4]. Studies have shown accumulation of fluoride in the hippocampus of the brain [2] causing degeneration of neurons and decreased aerobic metabolism and altered free-radical metabolism in the liver, kidney and heart [5,6].

Protein Energy Malnutrition (PEM) is a wasting malnutrition that manifests itself in various clinical forms and affects millions of people worldwide [7]. In children, kwashiorkor and marasmus are the two recognized and devastating forms of PEM. Protein Undernutrition (PU) impacts on mental development and cognition in children and reduces their interaction with environment, which in turn, can lead to problem with attention, inactivity and unresponsiveness. Each of these behaviors compromises children's ability to learn [7,8].

Under normal circumstances, the brain is protected from free radical damage by a careful balance between pro-oxidant and antioxidants mechanisms, which include antioxidant enzymes and free-radical scavenging chemicals [9]. In PU, this balance appears to be disturbed. It has been suggested that many of the clinical

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and pathological manifestations of PU result from the imbalance between free radical defenses and free radical production. Often, the free radical defense system appeared to be disturbed in PU subjects [7]. Some researches concerning fluorosis have been done on rats, rabbits, human beings and pigs [10–12] but rarely in undernourished state in these animals. In this present study, we attempt to assess and compare the effect of the intake of high fluoride levels in drinking water on lipid peroxidation and antioxidants levels of the brain of protein undernourished rats and their well-fed counterparts.

Materials and methods

Animals, chemicals and diets

All reagents including casein were of analytical grade and obtained from BDH Chemical Ltd., Poole, England and Sigma Co., USA. All experimental protocol conducted on rats were performed in accordance with international standard on animal ethics. Male Albino rats obtained from the University of Agriculture, Abeokuta, Nigeria were used for the experiment. Diets were prepared as previously described [7]. The low protein diets contained 5% casein while the normal diet contained 16% casein.

Experimental protocol

Animals were allowed to acclimatize for a period of two weeks and maintained at 12 h light–dark cycles at room temperature. At the end of the two weeks, animals were randomly assigned to four groups: A, B, C, and D with six rats per group and kept in different wire cages. Animals in groups A and B were placed on normal (16% casein) diet and the two other groups (C and D) were placed on low protein (5% casein) diet. Food and water were supplied *ad libitum* for eleven weeks. At the end of eleventh week, animals in groups B and D were exposed to 100 ppm fluoride (sodium fluoride, NaF) in drinking water for one week [10,13]. At the end of one week treatment, animals were sacrificed by cervical dislocation after mild anesthesia with diethyl ether. The heads were dissected and brains removed, rinsed briefly in ice cold isotonic saline and were immediately separated into cerebrum, cerebellum and brain stem. These different regions of the brains were homogenized and kept frozen in the freezer at −4 °C till the following day for analyses.

Preparation of brain homogenates

Two sets of brain homogenates were prepared. 100 mg of each part of the brain of individual sample was homogenized in 1 mL 0.01 M phosphate buffer, pH 7.4, kept frozen in the freezer and used for catalase, glutathione and lipid peroxidation analyses while another 100 mg of each of the brain sample was homogenized in 1 mL of isolation buffer (250 mM sucrose, 5 mM tris, 1 mM mercaptoethanol and 0.5 mM phenylsulphonyl-flouride (PMSF) pH 7.4) also kept frozen in the freezer and used for protein determination and superoxide dismutase analyses.

Lipid peroxidation assay

Brain lipid peroxidation assay was carried out by measuring the thiobarbituric acid-reactive (TBAR) products using the procedure of Vashney and Kale [14]. The method is based on formation of pink colored product when malondialdehyde treated with 2-thiobarbituric acid which has a maximum absorbance at 531.87 nm.

Glutathione assay

Assay for glutathione (GSH) was done by the method of Beutler et al. [15]. This method is based on the development of a stable yellow color when a 2-nitrobenzoic acid is added to sulfhydryl compounds. The absorbance was measured at 412 nm and the value of GSH is proportional to the absorbance at this wavelength.

Catalase assay

The catalase activity of each brain samples was determined by the method of Sinha [16] but with a slight modification. 0.1 mL of each brain part was mixed with 4.9 mL of distilled water. 1 mL of the mixture was added to H_2O_2–phosphate buffer mixture. The principle is based on the formation of chromic acetate when hydrogen peroxide (H_2O_2) reacts with dichromate–glacial acetic mixture at 100 °C. The decomposition of H_2O_2 when acted upon by catalase and reduction in the green coloration is measured spectrophotometrically at 570 nm.

Superoxide dismutase (SOD) assay

SOD activity was determined by the method of Del-Maestro et al. [17]. This assay is based on the ability of SOD to scavenge superoxide anion radical ($O_2\bullet-$) which, by shortening reaction chains, decreases the overall rate of pyrogallol autoxidation and the change in optical density at 420 nm was recorded.

Statistical analysis

Statistical analysis was carried out by using one-way Analysis of Variance (ANOVA). Differences between means were determined by the use of Duncan multiple range tests (SPSS software).

Results

The results of this investigation as presented in Table 1 indicate that the mean body weight, total brain weight as well as the weights of different brain regions of PU rats were significantly ($P < 0.05$) lower than that of the control. Also, the body and total brain weights of well-fed and PU rats given fluoride were significantly lower ($P < 0.05$) than their respective controls. The weights of different brain regions were also significantly reduced ($P < 0.05$) in PU rats compared to well-fed rats except in brain stem. Fluoride ingestion diminished the brain weights of well-fed and protein-undernourished rats affecting the cerebrum only (in the case of protein-undernourished rats) and the cerebellum of both well-fed and PU rats without effect on the brain stem of both well-fed and protein undernourished rats. In Tables 2 and 3, increased lipid peroxidation (LP) was observed in the cerebrum and cerebellum of protein-undernourished rats when compared with well-fed controls. Fluoride increased LP in cerebrum by 30 per cent but no effect was observed in the cerebellum of PU and well-fed rats, respectively. In the brain stem however, as shown in Table 4, protein undernutrition was accompanied with a significant reduction in LP but the region again seems insensitive to fluoride. The antioxidant levels of these brain regions varied. Tables 2–4 show that there were significant reductions ($P < 0.05$) in catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) in all the brain regions of PU rats compared to the well-fed controls except that the GSH level in the brain stem of PU rats was not significantly different from the values obtained for the well-fed controls. Fluoride induced reduction in the activity of CAT in all the three brain regions of well-fed rats but the reduction in the activity of SOD was observed only in the cerebrum without significant effect in both cerebellum and brain