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Dynamic analysis of exposure to aluminum and an acidic condition on bone formation in young growing rats

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ABSTRACT

The toxic effects of exposure to aluminum (Al) in an acidic condition on bone formation in young growing rats were studied. Wistar rats were divided randomly into Al-treated group (100 mg Al^{3+}/L; pH 5.6) and control group (distilled water). Al-treated rats showed lower body weight, lower serum pH, higher accumulation of Al, in addition to disordered metabolism of calcium and phosphorus compared with control rats. The levels of parathyroid hormone, calcitonin, osteocalcin, procollagen carboxy-terminal propeptide and bone alkaline phosphatase were significantly lower in the Al-treated group than in the control group from days 90, 30, 60, 60 and 90, respectively. The bone mineral density of the distal and proximal femoral metaphysis was significantly lower in the Al-treated group than in the control group on days 120 and 150. These findings suggest that long-term Al exposure in an acidic condition inhibits bone formation and induces bone loss in young growing animals.

1. Introduction

Acid rain is a serious environmental problem. One consequence of acid rain is the release of aluminum (Al) into the environment (de Souza Oliveira et al., 2009). Numerous studies have shown environmentally significant concentrations of Al in soil, lakes and rivers in areas affected by acid rain (Kinraide, 2003; de Vries et al., 2007). Industrial activities and the use of fertilizers have contributed to the increases in Al concentrations, causing significant problems in terrestrial and aquatic environments (Ling et al., 2007). In addition, animals are subject to enhanced Al accumulation as a result of direct exposure to Al and indirectly by the enrichment of the food chain (Liu et al., 2006). The potential harm associated with Al accumulation in humans and animals is severe (Grant et al., 2002; Campbell et al., 2004). Moreover, it has been demonstrated that acid rain enhances the toxicity of Al (Capdevielle and Scanes, 1995; Gensemer and Playle, 1999).

Bone is the main target tissue for the toxic effects of Al and is the primary site of Al accumulation (Hellström et al., 2005). Toxic effects of Al on bone have been confirmed clinically, epidemiologically and experimentally based on its capacity to induce two types of histological lesions, namely osteomalacia and adynamic bone disease (Jeffery et al., 1996; Kumar and Gill, 2009). Clinical and experimental studies have shown that chronic exposure to Al interferes with the mineralization process, and impairs the activity and differentiation of osteoblasts and osteoclasts (Rodriguez et al., 1990). Furthermore, in areas with high Al exposure, acid rain can enhance the absorption, tissue distribution and accumulation of Al in animals and humans (DeVoto and Yokel, 1994).

However, most studies have focused on the metabolism of minerals (Capdevielle et al., 1998); few studies have inves-

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tigated the effects of exposure to Al in an acidic condition on bone formation in young growing animals. Therefore, in the present study, we examined the toxic effects of exposure to Al in an acidic environment on bone formation in young growing animals in terms of: body weight, serum pH, bone total calcium (Ca), phosphorus (P) and Al contents, bone mineral density (BMD), serum levels of parathyroid hormone (PTH), calcitonin (CT), osteocalcin (BGP), procollagen carboxy-terminal propeptide (PICP) and bone alkaline phosphatase (B-ALP). This study should provide valuable information to better understand the environmental toxicity of exposure to Al in an acidic environment.

2. Materials and methods

2.1. Chemicals

Standard solutions of Al (100 μg/mL) and Ca (10 mg/mL) were provided by the National Institute of Metrology (Beijing, China). All other chemicals and solvents (e.g., 99% $AlCl_3{\cdot}6H_2O$, 30% H_2O_2 solution, formic acid, nitric acid and perchloric acid) were of analytical grade, and were obtained from commercial suppliers.

2.2. Animals and treatments

One-hundred, 4-week-old, healthy male Wistar rats weighing 74–96 g were used after 1 week of feeding (standard food). They were then randomly divided into two groups. The Al-treated group was provided with drinking water containing $AlCl_3$ (100 mg/L, Al^{3+}), the pH of the $AlCl_3$ solution was adjusted to 5.6. The control group was given distilled water (0 mg/L, Al^{3+}; pH 7.0). All rats had free access to water (Al-treated rats: $AlCl_3$ solution; control rats: distilled water) and standard food containing 23% protein, 5% lipids, 6% fiber and usual vitamins (A, B_1, B_2, B_6, B_{12}, C, D_3, E, K, nicotinic, folic and pantothenic acids), and a content of P (0.95 g) and Ca (1.30 g) per 100 g of food. Rats were kept in a room with a controlled temperature (23 °C) and relative humidity (50%), and a 12:12-h light/dark cycle. The general health and body weight of the rats were recorded daily.

Experiments were carried out in accordance with the guiding principles in the use of animals in toxicology, adopted by the Chinese Society of Toxicology. The study protocol was approved by the Ethics Committee on the Use and Care of Animals, Northeast Agricultural University (Harbin, China). Ten rats in each group were light anesthetized (ether) and were euthanized by decapitation every 30 days. Whole blood was collected and centrifuged (1200 × g for 15 min) to obtain serum. Hind limbs were removed. Femurs were dissected, cleaned, and stored at −70 °C.

2.3. Contamination control

To avoid contamination, all reagents (formic acid, nitric acid, perchloric acid, and so on) were detected for the Al contamination. All glassware (volumetric flasks, pipet tips, and vials) was immersed for at least 48 h in a 10% (v/v) HNO_3/ethanol solution and, shortly before use, washed with distilled water. Other laboratory wares were plastic materials. The materials used for sample preparation (scalpel, surgical scissors, and so on) were also washed with distilled water.

2.4. Measurement of serum pH

Serum pH was measured with an Orion 868 pH meter (Thermo Electron Corporation, Waltham, MA, USA).

2.5. Determination of bone Al, Ca and P content

Femurs were dried for 24 h at 65 °C. Femurs were weighed, placed in 25-mL tubes. Ten milliliter of formic acid and 2 mL of nitric acid were added, respectively. One milliliter of 30% H_2O_2 was placed in the tube after 10 min (Bohrer et al., 2008). The solution was digested repeatedly using a MDS-COD microwave digestion oven (Sineo Microwave Chemistry Technology Company, Shanghai, China). The digestive solution was transferred to volumetric flask (500 mL) and diluted to 500 mL with distilled water. Standard Al and Ca solutions were used to prepare the working standard solution and a blank solution was prepared at the same time. The Al and Ca contents were measured using a PEAA800 flame atomic absorption spectrophotometer (PerkinElmer, Fremont, CA, USA). The concentration of P was detected using a diagnostic kit (Sigma–Aldrich, St Louis, MO, USA) using the molybdenum blue method (Tsang et al., 2007).

2.6. Determination of serum B-ALP and PICP levels

Serum PICP was analyzed by a sandwich ELISA kit (R&D Systems, Inc., Minneapolis, USA) using polyclonal antibodies recognizing PICP as the capture antibody and horseradish peroxidase-labeled polyclonal PICP antibodies for detection. Serum B-ALP was determined using a sandwich ELISA kit (R&D Systems, Inc., Minneapolis, USA), as described for PICP. The absorbance at 450 nm was measured in a Tecan Sunrise microplate reader. All samples were assayed in duplicate. The levels of PICP were expressed as nanograms PICP per deciliter (ng/dL) serum and B-ALP as international units per liter (U/L) of serum.

2.7. Determination of PTH, CT and BGP levels in serum

The serum concentration of PTH, CT and BGP were detected using ^{125}I radioimmunoassay (RIA) kits (Jiancheng Bioengineering Institute, Nanjing, China) with competitive RIA method (Firling et al., 1999). The kit is based on competition of PTH, CT or BGP contained in the standard or sample with ^{125}I-labled PTH, ^{125}I-labled CT or ^{125}I-labled BGP for binding to the antibody, respectively. The radiation intensity was measured with a GC-2010 RIA arithmometer (Anhui Ustc Zonkia Scientific Instruments Co., Ltd., Hefei, China). All samples were assayed in duplicate. The levels of PTH were expressed as nanograms per deciliter (ng/dL) of serum, CT as nanomoles per liter (nmol/L) of serum and BGP as nanograms per milliliter (ng/mL) of serum.

2.8. BMD determination

The BMD (mg/cm^2) of the distal and proximal femoral metaphysis was measured by dual-energy X-ray absorptiometry (DXA)