The Effect of Aluminium on Bone Mineralization - changes in Some Trace Elements and Macroelements

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The aim of this study was to determine how mineral composition of bone is modified after administration of $AlCl_3$ solution, alone or in association with citrate. The experiment was made on biological systems – domestic rabbits. We had three working groups. One control group (C – we administrated NaCl 0.9% solution) and two experimental groups (E_1 – we administrated 50 mg $AlCl_3$/kg b.w. and E_2 – we administrated 50 mg $AlCl_3$/kg b.w. associated with 2% m/v citrate from sodium citrate). The quantitative analyses for Na, K, Mg, Ca, and Al were made on Atomic Absorption Spectroscopy after a wet digestion using a Microwave System. The results showed that the most important changes were in bone mineralization after aluminum chloride administration (for E_1), excepting of aluminum concentration in bone after aluminum administration in association with citrate (for E_2). So, aluminum enhances the concentration of some biometals in bone (Na, K, Ca and Mg) with 15-32%, but after administration of aluminum with citrate the aluminum concentration in bone increased with 76%. The results demonstrate that bone is a target tissue for aluminum accumulation.

Keywords: aluminum chloride, citrate, biological systems, bone

At the present time, aluminum is used for everything from medications to door frames and car bodies. So that, the total uptake of aluminum by humans is so low but this probably reflects its relative non-availability in biological circumstances [1]. It is obvious that certain factors, for example citrate, can contribute towards much greater uptake of aluminum from different sources (ex. diet). Daily intake by humans is estimated to be 1-10 mg. The actual tissue concentration is generally low at about 2 mg/kg weight, with the highest concentrations found in bone, liver, and lung.

There is ample proof that citrate facilitates incorporation of Al^{3+} into mammals. Elevated Al^{3+} levels were found in both the brain and bones of rats fed with a diet containing aluminum citrate or even just citrate.

On the basis of the radii (radius is a long bone of animals' foreleg), Al^{3+} is closest in size to Fe^{3+} and Mg^{2+}. For this reason, in biological systems, Al^{3+} will be competitive with Mg^{2+}. Both Al^{3+} and Mg^{2+} ions helps oxygen donor ligands, especially phosphate groups. Wherever there is a process involving Mg^{2+}, there is an opportunity for interference by Al^{3+} [2, 3].

Ligand exchange rates have special importance for Al^{3+} because they are slow, and systems may be not at equilibrium. Chelated ligands exchange more slowly [4]. The slow ligand exchange rate for Al^{3+} renders it useless as a metal ion engaged in enzyme active site for reactions. Aluminum inhibits enzymes with Mg^{2+} cofactors.

Experimental part

This experiment was made on three groups of rabbits from species Oryctolagus Cunicullus (domestic rabbits).

The experiment was developed during 10 days. Animals were maintained in good physiological conditions, according to the specific laws concerning animal protection in scientific researches [5]. We choose subcutaneous injections in cervical zone due to the easy administration way, and the possibility to inject relatively large volume of solutions (2 ml). This zone between the skin and muscle is well vascularized and the aluminum salt solution absorption in blood is faster.

The control group (C) and two experimental groups (E_1 and E_2) were formed by 5 animals each. Injections were administered on the 6th day and the 8th day, and animals were killed on the 10th day of the experiment, after anesthesia. Animals from control group were injected subcutaneous with 2 mL of physiological salt solution (NaCl 0.9% solution).

Aluminum as aluminum chloride was administered by subcutaneous injection to all experimental groups as follows: to E_1 group, 50 mg $AlCl_3$/kg b.w. and to E_2 group, 50 mg $AlCl_3$/kg b.w. associated with 2% m/v citrate from sodium citrate. All reagents for assays are delivered in the kits for every determination by Roche.

After anesthesia with chloroform, animals were killed by decapitation. After dissection, we took samples of bone (radius). Sampling was made with sterile surgical instruments, and the bone samples were stored in clean glass containers in refrigerator, at -18^{0}C. The glass containers were washed with detergent solution, and rinsed well with water, acidic solution of 5% HNO_3, distilled water, and then dried to 105^{0}C.

Bone tissue was treated as follows: before samples digestion, tissues were weighted on a Mettler Toledo AG204 analytical balance. Digestion was made in a Millestone Microwave System with a special program for samples with fast exothermic reactions (containing a large amount of organic matter). After wet digestion with 5 mL of 65% nitric

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acid (Merck) and 1 mL of 30% H_2O_2 solution, sample solution was transferred into a 50 mL volumetric flask and diluted to volume with double deionized water (< 5μS/cm).

The instrument used for metal determination in solutions was an atomic absorption spectrometer (A Analyst 800), produced by Perkin-Elmer, with Zeeman effect for background correction and transversal heating of graphite tube. Elements Mg, Ca, were determined in air-acetylene flame (Atomic Absorption), Na and K in air-acetylene flame (Atomic Emission) and Al by electro-thermal atomization. We used appropriate ionization control substances for flame and matrix modifiers in graphite tube. For calibration of the instrument, we used standard single element solutions of 1000 mg/L, produced by Merck, making dilutions for calibration standards, to obtain a calibration curve in linear range. The calibration curve control was made with a multielement standard solution (Merck). The results were obtained in mg/L or μg/L in solution, and reported after calculations in μg/g w.t., considering the initial weight of bone tissue and the volume of volumetric flask used (50 mL).

Statistical data were obtained using descriptive statistics (EXCELL). The results were reported as mean (X) ± standard deviation (S.D.). In calculations, information about uncertainty of calibration curve, volume of flasks and pipettes and mass measurement uncertainty were not considered.

Results and discussions

The results with metals concentration (Na, P, Mg) are graphically presented in figure 1.

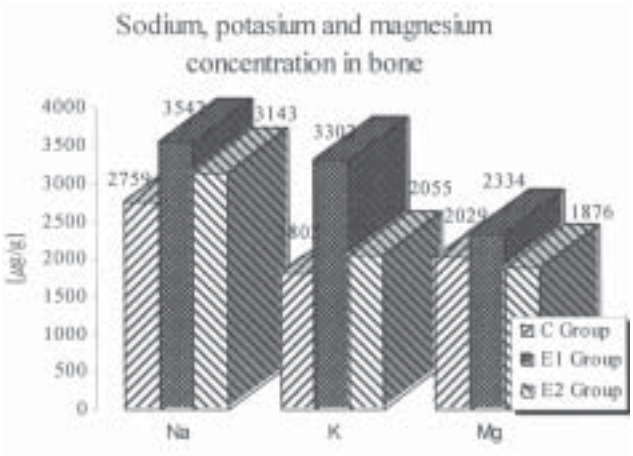

Fig. 1. Some metallic elements concentration in radius after aluminum administration

Sodium and potassium concentrations are higher in experimental groups compared to control group (fig. 1). Potassium increased significantly after aluminum chloride administration, and had moderate increase after administration of aluminum chloride associated with citrate. Magnesium had a small increase in bone which is unexpected for E_1 group, but has a little decrease when citrate is administered in E_2 group. The excess of citrate determines calcium and magnesium elimination from bone (figs.1, 2). The study of Carlisle researches in 1984 demonstrated that aluminum binds strongly to ATP instead of the essential magnesium but is removed by complexation with citrate [2].

The researches showed that Ca^{2+} radii is much larger than Al^{3+}, and in biological systems Al^{3+} will be competitive with Mg^{2+} rather than Ca^{2+} [6, 7]. It was established that injection of aluminum caused an acute elevation of calcium and phosphorus from serum with metastasis

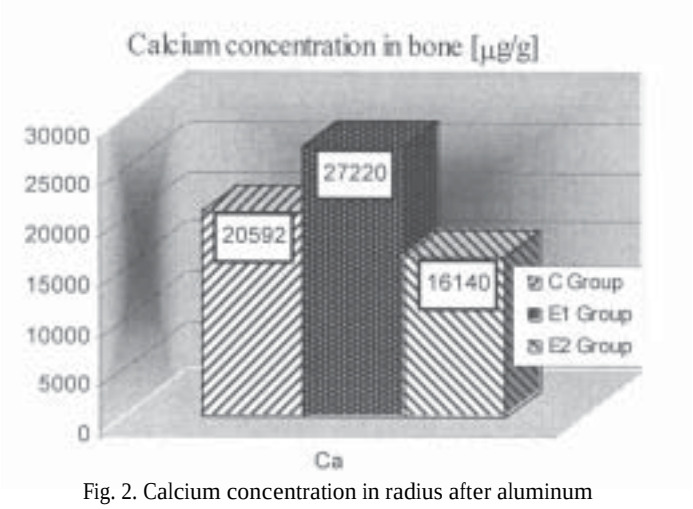

Fig. 2. Calcium concentration in radius after aluminum administration

calcification in rats [2]. Also, alkaline phosphatase from blood serum is correlated with bone mineralization and bone's health status. Usually alkaline phosphatase has an enhanced activity in liver disease, in disease of the skeletal system, hyperparathyroidism, osteomalacia, fractures and malignant tumors [8-10].

Aluminum toxicity and long term accumulation of this element in humans has been blamed as a cause of osteoporosis. In this study is obviously that aluminum excess intake do not decrease the level of calcium in bone as expected (fig. 3).

Comparing with control group, aluminum in bone is enhanced by administration of aluminum as chloride (with 39%) and much more when aluminum chloride is administered in association with citrate (with 76%).

The combination which appears to have the greatest influence on aluminum absorption is administration of a soluble aluminum compound in association with opening the tight junction between small intestinal cells. This is exemplified by administration of aluminum citrate [11]. Aluminum citrate is not only relatively soluble as compared to other aluminum compounds, but citrate, probably as a result of chelating calcium, opens the paracellular pathways. This results in very rapid enhanced aluminum absorption (fig. 3), because aluminum in bone increased when citrate was administered.

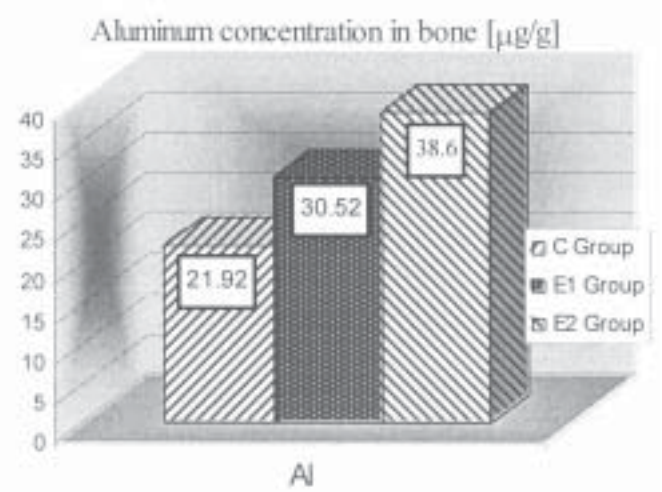

Fig. 3. Aluminum concentration in radius after aluminum administration

Plasma aluminum levels cannot be correlated well with other tissue stores of aluminum [12, 13].

After aluminum chloride administration, in experimental E_1 group it was noticed a significant increase in aluminum.

The trace metals as copper, zinc, and manganese are involved in enzymes activity, and are catalysts for oxidative processes. This disturbance of bone mineralization can lead to oxidative stress appearance in bone [14, 15]. Concentration of aluminum is directly dependent on the administration dose [16]. The increase of aluminum concentration in radii demonstrates that bone is also a target tissue for aluminum accumulation.

Conclusions

After aluminum chloride administration, the concentration of Na, K increased in bone, but concentration of Mg and Ca decreased after aluminum chloride associated with citrate.

Aluminum accumulates in bone after aluminum chloride administration and even more, after association with citrate, confirming that bone is a target tissue for aluminum intoxications, and also the concentration of aluminum was dose-dependent. All studied metals and trace metals are suffering modifications, generally the augmentation of their concentration in bone after aluminum chloride.

Generally, the association of aluminum with citrate maintained the level of studied metals and trace metal around the control group concentration, excepting aluminum, which increased. In the presence of citrate, studied macroelements are not suffering significant changes, except calcium.

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