Mineral Micronutrients in Rabbits Radius After Aluminium Administration

BOGDAN DELEANU[1], MIHAELA SCURTU[2], MIRELA AHMADI[2]*, CAMELIA TULCAN[2], RADU PREJBEANU[1], DOREL DRONCA[3]

[1] "Victor Babes" University of Medicine and Pharmacy Timisoara, Department Orthopedics and Trauma, 2 Eftimie Murgu, Sq., 300041, Timişoara, Romania

[2] Banat's University of Agricultural Science and Veterinary Medicine Timisoara, Faculty of Veterinary Medicine, Department Chemistry and Biochemistry, 119 Calea Aradului, 300645, Timisoara, Romania

[3] Banat's University of Agricultural Science and Veterinary Medicine Timisoara,Faculty of Animal Sciences and Biotechnologies, Department Animal Genetic Improvement, 119 Calea Aradului, 300645, Timisoara, Romania

The aim of this research was to evaluate the aluminum excess effect on bone microminerals metabolism. The study was conducted on experimental animals, rabbits – Oryctolagus Cunicullus, and aluminum administration was as $AlCl_3$, $AlCl_3$ associated with citrate, and $AlCl_3$ associated with fluoride, for a short period of time. From radius bone was analyzed the concentration of Zn, Fe, Mn, Cu and Al with atomic absorption spectrometry method. The results demonstrate that the chemical form of administrated Al is important for minerals metabolism in bone. Thus, Al concentration was higher in bone after excess of Al. $AlCl_3$ administrated simultaneous with citrate increased the concentration of studied microelements in rabbits' radius in most of studied cases. Also, aluminum is accumulating in radius after administration of $AlCl_3$, similar cu increasing the content of Zn and Fe with 27%, Al with 39%, Mn with 124%, while the Cu concentration decreased with 41% compared to control group.

Keywords: aluminum, radius bone, rabbits

Even the availability of aluminum is very high being spread everywhere, this element is considered toxic for biological systems. In most of the waters, the concentration of aluminum is much higher then relevant biocations, such as magnesium (Mg^{2+}), iron (Fe^{3+}) or zinc (Zn^{2+}) [1]. Due to soil composition, aluminum from soil can be solubilized and can interfere with the absorption of calcium and magnesium in plants [2]. Also, because aluminum reacts very simple cu silicon and other compounds, the bioavailability of aluminum is reduces substantially.

As well, aluminum is used as flocculating agent for municipal water system. In medicine it was demonstrated that the dialysis solutions contained high concentration of aluminum [3]. After aluminum arrives in the organism, it is absorbed in the small intestine and is transported to different cells, and very important – in bone. Thus, aluminum starts to change the bone cells activity and starts the demineralization of bone cell growth, resulting pain, renal osteodystrophy, and bone disease such osteomalacia [4].

Magnesium has a very important role for bone protection, because it stabilizes the chemical structures where are involved other cations. Aluminum (Al^{3+}) can form strong ligands in biological systems. Magnesium (Mg^{2+}) binds weakly ligands structures because it acting as catalyst, and can move from one side to other side of reaction mechanism [5-7].

Generally, aluminum toxicity results from its competition with other metal ions from the structure of enzymes and proteins. Thus, aluminum acts on the metals binding site, and substitutes the metal. Changing the metal from protein or enzyme structure induces change function, so the metabolism of the cell where the protein binds the aluminum is altered, with severe consequence on the biochemistry of the organism. The patient will have bone pain – more localized to hips, feet or ankles, and back; and also the patients will have muscle weakness – more common: proximal muscle weakness [8].

Because of the aluminum implications in musculo-skeletal system, the experimental metallobiochemistry can evaluate and elucidate the effect of aluminum on the concentration on some bioelements such as: zinc, manganese, and copper, and also aluminum concentration in bone.

Materials and methods

Present research used animal experimental model, rabbit species Oryctolagus Cunicullus. We worked with four rabbits groups, each group having 8 animals, and the weight was varying between 700-800g. The hybrid rabbits that we used came from a group of Double Cross Hybrids, obtained after crossing Large Chinchilla and New Zeeland White, both breeds being used for rabbit meat production.

During the 10th days of experiment the animals had good physiological conditions, according with specific laws concerning animal protection in scientific researches [9].

Administration of aluminum was done subcutaneous injections, in cervical zone, because this is anatomical part allow to inject relatively large volume of solutions (2 ml), and because is an easy administration route. Also, the cervical region, between the skin and muscle, is well vascularized, and the absorption of aluminum salt solution in blood is faster.

We worked with four experimental animal groups: one control group (C) and three different experimental groups (E_1, E_2, E_3). First days were used for preparing the animals for experiment. Injections were administered on the 6th day and the 8th day, and animals were sacrificed on the 10th day of the experiment, after anesthesia.

We administrated for all animals from control and experimental groups a total volume of 2 mL solution. For animals from control group (C) we injected subcutaneous

* *email: mirelaahmadi@yahoo.com*

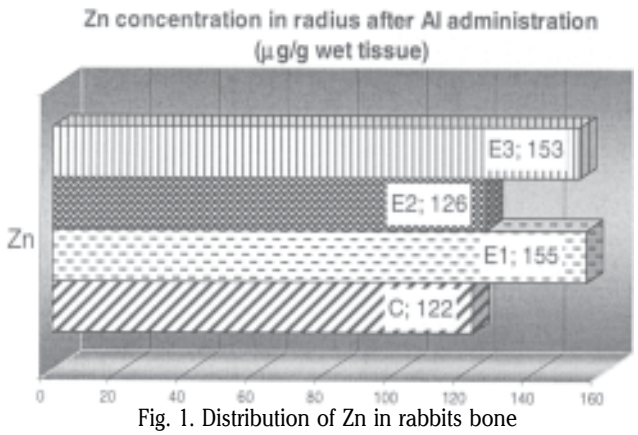

Fig. 1. Distribution of Zn in rabbits bone after Al administration (μg/g wet tissue)

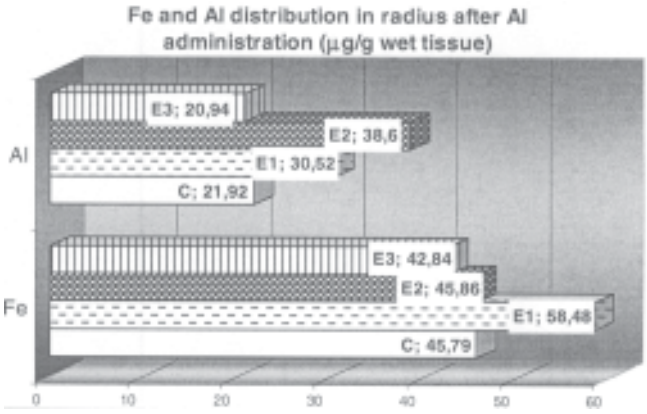

Fig. 2. Concentration of Al and Fe in bone after Al administration (μg/g wet tissue)

2 mL of physiological salt solution (NaCl 0.9% solution). For animals from experimental groups we administrated by subcutaneous injection aluminum chloride ($AlCl_3$) as follow: to E_1 group, 50 mg $AlCl_3$/kg body weight (b.w.); for E_2 group, 50 mg $AlCl_3$/kg b.w. associated with 2% m/v citrate from sodium citrate; and for E_3 group, 50 mg $AlCl_3$/kg b.w. associated with 2mg NaF/kg b.w. All reagents for assays were delivered as kits by Roche.

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

Bone tissues were weighted on an analytical balance, and then the 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 acid (Merck) and 1 mL of 30% H_2O_2 solution, sample solution was transferred into a 50 mL volumetric flask and dilute to volume with double deionized water (< 5μS/cm).

For metal analysis we used an atomic absorption spectrometer (A Analyst 800), produced by Perkin-Elmer, with Zeeman effect for background correction and transversal heating of graphite tube. Elements Fe and Zn were analyzed in air-acetylene flame (Atomic Absorption), Cu, Al, and Mn were analyzed 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 wet tissue, considering the initial weight of bone tissue and the volume of volumetric flask used (50mL) [10].

Statistical data were obtained using descriptive statistics (EXCELL). The results were reported as mean ($\overline{X}$) ± 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

Most important in accidental intake of aluminum for long time, is that aluminum is sequestered in bone and its effect is cumulative. But because the blood concentration of aluminum is not a clear marker of absorption or organ aluminum load, only mineralization reflects most important bone histopathological changes. Therefore, the biopsy of bone is the only method for a clear diagnosis of aluminum-related bone disease [11].

Bone is formed by an organic matrix formed by calcium salts. Crystallization salts from organic matrix of bone are mainly represented by calcium and phosphate, and the principal compound is hydroxyapatite, but along calcium and $(PO_4)^{3-}$ are magnesium, sodium, potassium and carbonate. At the rats feed with low content or calcium diet, the aluminum and iron are stored in bone, inhibiting the normal osteogenesis [12].

Results of our experiment are presented in figure 1 – for zinc distribution in radius bone; figure 2 – for aluminum and iron presented in bone and figure 3 – for manganese and copper distributed in radius bone after aluminum administration.

From our presented data in figure 1 and 2 we can observe that zinc and iron is not significant modified after aluminum administration. Zinc concentration in bone is increased in administration of aluminum as $AlCl_3$ (with 27%) and $AlCl_3$ associated with fluoride (with 25%), suggesting that fluoride does not have any influence in the retention of zinc in bone. Also, iron concentration from bone is moderate increased after $AlCl_3$ administration – with 27%, but the iron content in others experimental groups were not modified too much.

Zinc, iron, manganese and copper content in bone after administration of aluminum chloride associated with citrate (E_2) did not show important variations compared to control group, and this can be explained that the citrate moderate the accumulation of these elements in radius bone.

Manganese concentration in radius was significantly increased for E_1 – administration of $AlCl_3$ (with 124%) and E_3 – $AlCl_3$ associated with fluoride (with 394%) compared to control.

Contrary to zinc and manganese, the copper content in bone is lower for E_1 (with 40%) and E_3 (with 60%) after aluminum chloride administration.

Also, aluminum concentration from bone, after $AlCl_3$ administration, was significantly increased for E_1 (with 39%) and E_2 (with 76%), and had a very low decrease in E_3 (with 5%), which may suggest that fluoride protects the bone for aluminum accumulation.

Because aluminum (Al^{3+}) has similar radius with Fe^{3+} and Mg^{2+}, the aluminum will be competitive with magnesium. Both ions have affinity for oxygen donors, specially the phosphatidic groups.

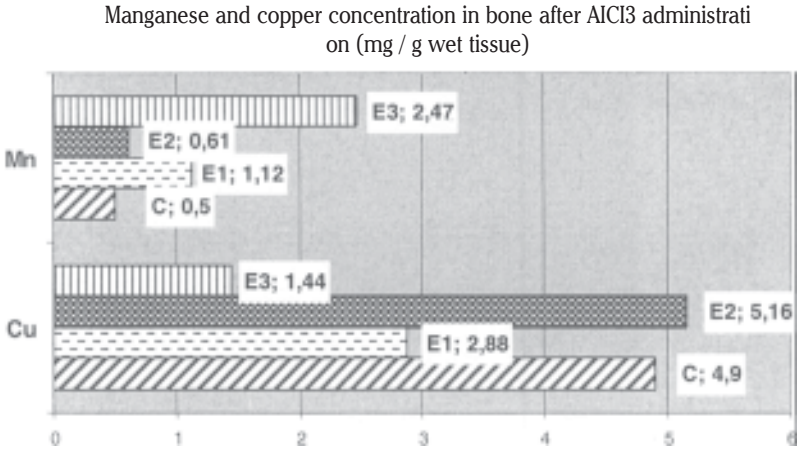

Fig. 3. Manganese and copper distribution in bone after $AlCl_3$ administration (μg/g wet tissue)

Toxicity of aluminum due to its accumulation after a long exposure was considered cause of osteoporosis. Aluminum concentration in bone is significantly increased after $AlCl_3$ administration associated with citrate [4].

Conclusions

Aluminum administrated as aluminum chloride modifies the concentration of some microelements in rabbits bone depending on the chemical form.

Thus, when aluminum is administrated in excess, it accumulates in bone. Even for a short time administration of aluminum in moderate concentration, the content of aluminum in bone was 40% higher for E_1 rabbits and increased with 78% for E_2 rabbits, when with administrate $AlCl_3$ associated with citrate. Administration of $AlCl_3$ associated with fluoride protects the bone from Al accumulation.

Fluoride associated with aluminum chloride administration decreases the content of iron, copper and aluminum from bone. Also, copper concentration from analyzed radius was lower with 70% for E_3 group ($AlCl_3$ associated with fluoride), and with 41% for E_1 group ($AlCl_3$), that demonstrate the citrate associated with aluminum chloride limited the copper loss from bone (copper demineralization).

References

1. BERTHON G. (2002) Coord. Chem. Rev., 9, 28, 319
2. HILL M.K. (2004) Understanding Environmental Pollution, Chapter 6. Acidic deposition (pp, 142-153), Cambridge University Press.
3. DAUGIRDAS J.T., BLAKE P.G., ING T.S. (2007) Handbook of dialysis, 4th edition, Wolters Kluwer/Lippincott Williams and Wilkins Handbood, Philadalphia.
4. VERMESAN H., PUP (SCURTU) M., AHMADI M., OLARIU L., VERMESAN D., PREJBEANU R., Rev. Chim. (Bucharest), **59** no. 8, 2008, p. 891
5. NICOLINI M., ZATTA P.F., CORAIN B., Aluminum in chemistry, biology and medicine, Cortina international-Verona, Raven Press, New York, 1, 1991
6. POPESCU R., COJOCARU A., MANEA A.C., Rev. Chim. (Bucharest), **58**, no. 6, 2007, p. 507
7. OPPENHEIM W.L., NAMBA R., GOODMAN W.G., SALUSKY I.B., J Bone Joint Surg Am, 1989, 71(3), 446-452
8. COBURN J.W., NORRIS K.C., Diagnosis of aluminum-related bone disease and treatment of aluminum toxicity with deferoxamine, Semin Nephrol, 1986, Dec; 6(4 Suppl 1), 12-21.
9. Romanian Law nr 205/2004 (Art. 7, 8, 22), publ. in M.O. of Romania, Part I, Nr. 531/14.06.2004.
10. PUP (SCURTU) MIHAELA, Teză de Doctorat: Efecte dishomeostazice induse de microelemente metalice, Conducător ştiinţific: Prof. Dr. Gârban Zeno, Univeristatea "Politehnica" Timişoara, Facultatea de Chimie Industrială şi Ingineria Mediului, Timişoara, 2006
11. MALLUCHE H.H. (2002) Aluminum and bone disease in chronic renal failure, Nephrol. Dial. Transplant, 17(Suppl), 2:21-24.
12. KONISHI Y., YAGYU K., KINEBUCHI H., SAITO N., YAMAGUCHI T., OHTSUKI Y. (1996) Chronic effect of aluminum ingestion on bone in calcium deficient rats, Pharmacol. Toxicol., 78(6), 429-434.

Manuscript received: 8.01.2015