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EFFECTS OF SUBACUTE TREATMENT WITH LEAD ACETATE ON THE MINERAL CONSTITUENTS FROM FEMUR AND TIBIA

Stela-Gabriela JELEA & Marian JELEA
North University of Baia Mare, Science Faculty, 76 Victoriei Street, 430122 Baia Mare, Romania, sjelea@yahoo.com

Abstract: Lead acetate was administered in Wistar rats, by intra-gastric tube, for 7 days, in doses of 10 mg/100g body weight, daily. The effects were monitored after 7 days of treatment and after 7 days post therapeutic pause. A group of animals, which were administered water by the same route, served as stress controls. The animals were sacrificed and were taken femur and tibia. There were intensive positive modifications in the weight and sizes of the investigated bones, tibia and femur, after the 7 days of treatment, which persisted after the end of the therapy. Persistent changes were observed in the mineral composition of the bones, even after the treatment ended.

Bone lead deposits (femur, tibia) influence the weight and the length of these, supporting the metabolic disorders encountered at this level. Presence of lead in the bones alters the mineralization processes, confirmed by our data, indicating high levels of all minerals in this tissue compared to the controls.

Key words: Wistar rats, lead acetate, minerals constituents, bone, zinc protoporphyrines.

1. INTRODUCTION

After the oral administration of the toxic substance, a part of the metal is eliminated from the body, another is distributed in the soft tissues (especially in the cortical zone of the kidney) and another one is deposited in the osseous tissue. The tissue distribution of the lead in the body depends on: the route of administration (Ghergariu, 1980), the form in which the metal gets into the body (Demichele, 1984), the age (Forbes & Reina, 1972; Kostial et al., 1978, 1979; Demichele, 1984), the diet (Krejpcio & Gawęcki, 2002) as well as on other factors. The studies of lead distribution in the soft tissues to rodents (Andráš et al., 2006) show that metal is accumulating of the same species differ according to season (summer-autumn).

As far as the depositing of lead in the osseous tissue is concerned, it is influenced by the: duration of the treatment (Hu et al., 1991; Pounds et al., 1991), concentration and form in which the metal is introduced in the body (Tsaih et al., 1999), the age (Han et al., 1997) and the variety of the osseous tissue (Baloh, 1974; Christofferson et al., 1986; Hu et al., 1991).

Due to the structure of the osseous tissue, the transport of the metal through the osseous matrix is carried out slowly by means of a diffusion process, (Marcus, 1985; Adachi et al., 1998). The mechanism for the transfer of the metal in the osseous tissue is due to the exchanges that take place between the capillary vessels from the Havers channel and the crystals from osteon. After these exchanges the lead arrives in the osseous structure (Escribano et al., 1997). The metal deposited in the proximity of the canaliculi returns immediately in the blood, unlike the lead fixed in the osseous matrix. The lead that has entered the osseous tissue, irrespective of the age of the subject, makes up an endogenous reserve, which contributes later to the maintenance of the plumbemia (Marcus, 1985; Schütz et al., 1987).

The aim of this research is to study the effects of administering the lead acetate on the increase and development of the femur and tibia as well as the evolution of some mineral constituents from the osseous tissue.

2. MATERIALS AND METHODS

The experiments have been carried out on two experimental Wistar rats groups, male, in the pre-puberty period (30 days-old), having the average weight of 65.50 ± 1.35 g. The experimental groups have been treated with lead acetate (Bucharest Reagent), dissolved in bi-distilled water administered by intragastric tube, for seven days. The doses administered daily were of 10 mg/100g body weight. The animals from the first group were sacrificed in the eighth day and those from the second group in the fifteenth day (after a 7 – day post-treatment pause).

There was a control group for each experimental group. Each group was made up of ten animals. The animals from the control groups received a similar volume of liquid to cancel the effects of stress, determined by the manipulation of animals.

The sacrificing of the animals was carried out by beheading, after a previous period of inanition of 16 hours. After the sacrificing, the inferior limbs were cut off and processed by the procedure of manual cleaning (Stoica & Mihăilescu, 1981).

The biometric indexes (weight, length) have been studied in order to estimate the increase and the development of the skeleton. In order to identify the mineral constituents from the sample a wet disintegration has been carried out and after the element entered the solution the photometer measurement has been done. Standard solutions have been prepared for the calibration of the device. On the grounds of the standard solutions calibration curves have been obtained which cover the concentration domain for the analyzed samples.

For reading the metals the following wavelengths have been selected: 422.7 nm for calcium; 589.0 nm for sodium; 766.5 nm for potassium; 285.2 nm for magnesium; 213.9 nm for zinc and 283.3 nm for lead. The readings have been done as compared to a blank sample of distilled water. The extinctions are read on a standard curve carried out previously with standard solutions that cover the concentration domains for the analyzed samples. The reagents used were produced by the Merck Company.

The zinc protoporphyrines have been determined from blood, by fluorometry (Alessio et al., 1978) and from the blood serum the calcium and lead have been studied. A wet disintegration was done for dosing the calcium and the lead, and after the elements had entered the solution the measurement with the spectrometer was done (Ghergariu et al., 2000) at the spectrometer of atomic absorption Perkin-Elmer model 3110.

The reading of the calcium from the sample was done over a wavelength of 422.7 nm as compared to a 0.1 lanthanum chloride blind sample. The dosing of the lead from the blood serum was done by diluting the samples with deionized water in proportion of 1:5. The reading was done at the wavelength of 283.3 nm comparatively with deionized water.

The data obtained have been processed statistically by the Student's "t" test, and the aberrant values have been eliminated according to the Chauvenet criterion (Snedecor & Cochram, 1978; Weber, 1980). The statistic consideration has been considered from de la $p<0.05$.

3. RESULTS AND DISCUSSIONS

The results obtained in case of the animals from the 1st experimental group have shown the significant increase of the biometrical indexes, (Tab. 1). Thus the weight of the femur exceeded the control with 20.40% ($p<0.01$) and in case of tibia with 26.51% ($p<0.001$). At the same time the length of the femur was 7.84% ($p<0.001$) as compared with the control and that of tibia with 6.07% ($p<0.01$).

Table 1. Biometric indexes (GrF - femur weight, GrT - tibia weight, LF - femur lengths, LT - tibia length) in case of the rats treated with lead acetate.

Treatment	7 days		7 days break	
Experimental group	Control	Treated	Control	Treated
Gr F X ± ES	93.88 ± 0.43	113.04 ± 3.49	123.77 ± 1.65	129.15 ± 0.04
D%		+20.40		+4.34
p		<0.01		NS
GrT X ± ES	75.70 ± 0.28	95.77 ± 1.17	97.51 ± 0.38	108.53 ± 1.99
D%		+26.51		+11.30
p		<0.001		<0.01
LF X ± ES	20.66 ± 0.33	22.28 ± 0.07	23.28 ± 0.36	24.81 ± 0.22
D%		+7.84		+6.57
p		<0.001		<0.001
LT X ± ES	25.20 ± 0.61	26.73 ± 0.15	28.22 ± 0.28	27.57 ± 0.23
D%		+6.07		-2.30
p		<0.01		NS

The table includes the average ± the standard error (X ± ES), percentage differences as compared to the control (D%) and statistic significance considered from $p<0.05$. The statistically non-significant values are marked with NS.

The interruption of the treatment for seven days maintained the increase weight of the tibia (+11.30%, $p<0.01$) as compared with the control. As for the length of the femur, it exceeded the control value with 6.57% ($p<0.001$), (Tab. 1).

As far as the mineral constituents from the femur are concerned (Pb^{+2}, Ca^{+2}, Mg^{+2}, Zn^{+2}, Na^{+}, K^{+}) the results have shown that the continuous administration of the lead acetate for seven days increased the Pb^{+2} content (+287.03%, $p<0.001$) and decreased the Mg^{+2} (-15.98%, $p<0.01$) from the osseous tissue. The intoxication with lead determines the decrease of the calcium content, because the lead replaces the calcium by the process of cationic exchange from the hydroxylapatite crystal (Carmouche et al., 2005). The studies on the mice exposed to lead pointed out the same changes (Jelea et al., 2001).

If the treatment was interrupted an increased deposit of Pb^{+2} (+270.43%, $p<0.01$), continues to be maintained while the Zn^{+2} and K^{+} concentrations decreased significantly (-18.07%, $p<0.02$ respectively -58.33%, $p<0.05$), and Mg^{+2} recorded an increase of 10.57% ($p<0.01$) as compared to the control, (Fig. 1).

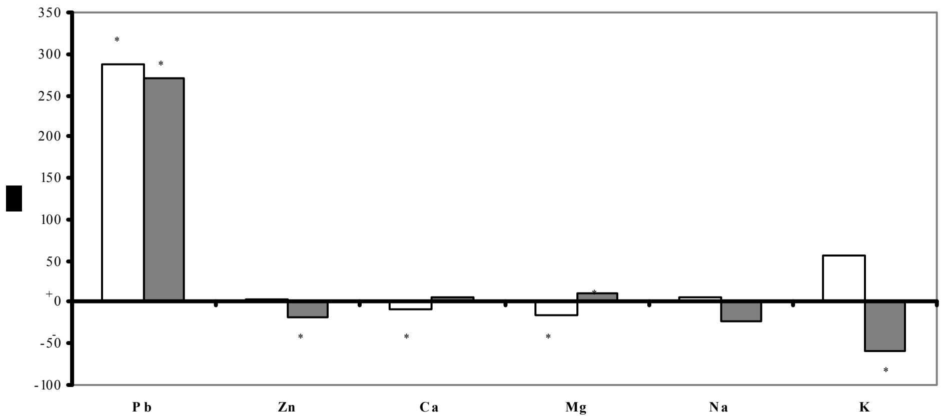

Figure 1. Percentage differences as compared with the control of some mineral constituents (Pb^{+2}, Zn^{+2}, Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}) from the femur of the rats treated with lead acetate. The white columns represent the 1st experimental group. The grey columns represent the 2nd experimental group. The control is marked with 0. The statistic significance is marked with asterisk.

The same mineral constituents studied in the femur were studied in case of tibia as well, (Fig. 2). The treatment with a duration of seven days determined an increase of all the researched elements, to significant differences as compared with the control (+42.64%, $p<0.01$ for Zn^{+2}; +220.36%, $p<0.001$ for Pb^{+2}; +22.57%, $p<0.001$ for Ca^{+2}; +13.35%, $p<0.05$ for Mg^{+2} and +79.36%, $p<0.01$ for K^{+}) with the exception of Na^{+} which decreased under the value of the control with 44.62% ($p<0.01$).

These modifications are maintained in most of the cases after the period of seven days as well, a period in which the treatment with lead acetate was not carried out. Thus, the chemical analysis of the mineral constituents from the osseous tissue pointed out an increase, as compared to the control, of the content of: Zn^{+2} with +40.46% ($p<0.02$), Pb^{+2} with +318.91% ($p<0.001$), Ca^{+2} with +9.53% ($p<0.01$) and Mg^{+2} with +18.03% ($p<0.05$). At the same time the concentrations of Na^{+} and K^{+} decreased with 35.11% respectively 49.60% ($p<0.001$) as compared with the control.

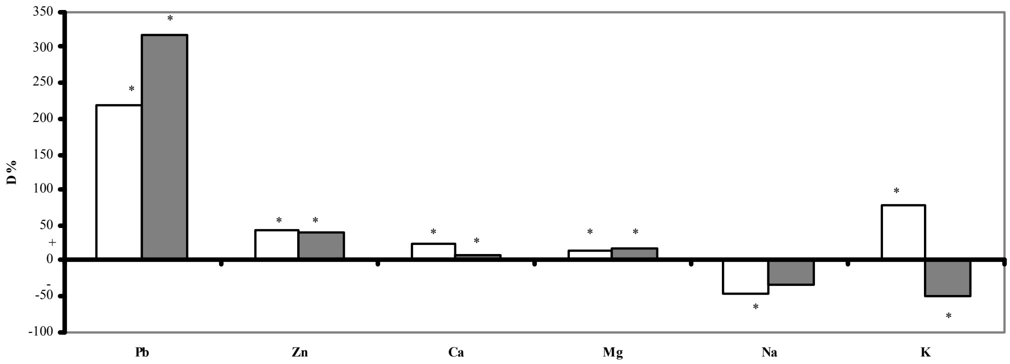

Figure 2. Percentage differences as compared with the control in case of some mineral constituents from tibia in case of Wistar rats treated with lead acetate; (Explication see figure 1).

The calcium and the lead were dosed from the blood serum, (Tab. 2). The results show that after 7 days of administration of the lead acetate the concentration of lead from the blood increased as compared with the control group with 65.82% ($p<0.001$) coming back to normal after the post-treatment interruption of 7 days, when the metal was not administered. As for the calcium, it decreases significantly in the seven days of treatment but increases with 10.28% ($p<0.001$) after the period of interruption.

Table 2. Concentration of calcium (Ca^{+2}) and of lead (Pb^{+2}) from the blood serum in case of the Wistar rats in the pre-puberty period treated with lead acetate.

Treatment	7 days		7 days interruption	
Experimental group	Control	Treated	Control	Treated
Ca^{+2} X $\pm$ ES D% p	55.31 $\pm$ 3.86	46.72 $\pm$ 2.07 -15.57 NS	138.36 $\pm$ 8.45	152.58 $\pm$ 1.12 +10.28 <0.001
Pb^{+2} X $\pm$ ES D% p	1.99 $\pm$ 0.006	3.30 $\pm$ 0.14 +65.82 <0.001	1.99 $\pm$ 0.006	1.97 $\pm$ 0.71 +1.01 NS

Explication see table 1.

The subacute treatment with lead acetate determined the significant increase of zinc protoporphyrines as compared with control, with 123.70% at the 1st experimental group while in case of the 2nd experimental group the increase were not significant, of 4,24%. All these effects can be due to the interaction between lead and some enzymatic processes, responsible for the synthesis of hem (Baranowska-Bosiacka et al., 2000; Sakai, 2000; Borošová et al., 2008).

Actually, many researchers have shown that lead operates especially by an enzymatic mechanism, inhibiting the activity of some enzymes (delta-aminolevulinic dehydrase, ferrochelatase).These inhibiting processes are the result of the metal fixation on the sulphidrylic components of the proteins (Cézard & Haguenoer, 1992) as well as of the shifting of some active biological ions from metal-enzymes.

4. CONCLUSIONS

In the experimental model applied to the Wistar male rats, in the pre-puberty period, to which the lead acetate was administered by daily intragastric tube probing for 7 days, and then a 7 - day post-treatment interruption followed, one can notice that in the osseous tissue (the femur and the tibia) this metal is deposited in big quantities. This demonstrates that the bone has a great affinity for the lead, but also the fact that once fixed in the bone, it is quite difficult to mobilize from this level, a proof that after a 7 – day post-treatment it is present in high concentration in the bone. The lead is deposited in the compact bone and in the spongy one in its trabeculae but the fixed quantity depends on the metabolism of this tissue and the turnover of this metal at the level of the osseous structure.

The presence of lead in the osseous tissue alters its mineralization (Adachi et al., 1998). Our data show that the depositing of the mineral constituents (lead, calcium, potassium, sodium, magnesium and zinc) is much more prominent in tibia as it is in the femur, especially in case of the rats sacrificed immediately after the seven days of treatment. In this case all the mineral elements are found in concentrations much higher as compared with the control group, with the exception of the sodium which is low. But if the analyses on the tibia and femur are carried out after the 7 days post-treatment interruption the depositing of the mineral elements is different from that of the rats treated 7 days with lead acetate and sacrificed in the 8^{th} day, but generally similar in the two bones. The lead is in competition with the intra- and extra- cell calcium ions. In osteoblast cultures the lead addition makes it penetrate their membrane more than the calcium ions (Schirrmacher et al., 1998).

The effects of lead on femur and tibia, in case of the 7 days treatment are also evident in the significant changes concerning their weight and lengths, which present increases. These effects are also maintained in case of the second experimental group for which the treatment was interrupted, with the exception of the tibia length, which is within the limits of the values of the control group. These results demonstrate that the remaining lead in the two bones continues to act on the bone.

There is a significant increase of the zinc protoporphyrines concentration. All these effects are due to the interaction between lead and some enzymatic processes responsible for the hem synthesis (Jelea & Jelea, 2007).

In conclusion:

- The lead acetate administered to the Wistar rats in the pre-puberty period, in a daily dose of 10 mg/100g body, for 7 days, did not cause behavior changes, did not influence the appetite nor did it lead to the animals' mortality.
- The lead accumulated in high quantity in the osseous tissue, femur and tibia, the latter ones also containing high quantities of other ions (calcium, potassium, magnesium and zinc).
- These increased metal concentrations from the two bones are associated with increase in length and weight.

- Against the background of administering the lead acetate, the biochemical modifications are generally more reduced, which demonstrates that the actions of the lead on the structural elements precede the functional ones.
- The 7 days of interruption of the treatment reduce within normal limits the effects produced by the administration of the lead acetate, which demonstrates that the actions of the heavy metal are reversible, although its presence is obvious in the osseous tissue.

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