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Research Article

Effect of lead toxicity on bone calcium content and morphometric parameters

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ABSTRACT

Background: There is large number of pollutants prevailing in the present environment. Among these, lead (Pb) is of particular interest to us because of its wide distribution in the environment. Large existence of lead (Pb) in number of food items has provoked us to investigate the effects of this metal on bone growth in rats. The present study was designed to evaluate the impact of lead poisoning on bone tissue.
Methods: A total of 48 male wistar rats and 30 & 80 days of age were selected for this study. Lead (as lead acetate 250 mg/ml) was provided ad libitum in drinking water for about five weeks to produce subclinical toxicity. Glacial acetic acid was added to the drinking water of lead administered groups at a concentration of 12.5 µl/l to prevent the precipitation of lead acetate. At the termination of treatment period, rats from all four groups were sacrificed by decapitation and their long bones i.e. femur and tibia were excised, cleaned off from soft tissue. Then the bones were preserved in refrigerator (-20oC) and processed for further analysis.
Results: Our study revealed that Lead significantly reduced calcium concentration in both femur ($p<0.001$) and tibia ($p<0.001$) in lead intoxicated rats. Furthermore, morphometric parameters showed significant reduction in the femoral head width upon lead intoxication. Significant decrease in the ash content of both the bones was observed upon lead intoxication for both the age groups, no significant change observed in the length of the femur as well as tibia of all the treated groups.
Conclusions: From this study we can conclude that the lead has induced bone toxicity and has deteriorated the development of bone tissue in the case of growing animals, is the consequence of oxidative stress.

Keywords: Lead poisoning, Bone, Calcium, Morphometric parameters

INTRODUCTION

There is large number of pollutants prevailing in the present environment. Among these, lead (Pb) is of particular interest to us because of its wide distribution in the environment. Drinking water and soil contains particulate amount of lead that has also been shown to be significantly hazardous for children, who are more commonly exposed by ingestion of dust, soil and water.[1] Lead exposure to the human body occurs mainly through the respiratory and gastrointestinal tracts. Once absorbed lead is transported via blood circulation to the soft tissues and then finally deposited in the bones. The primary site of lead accumulation in the human body is bone.[2] It can be stored temporarily on the bone surface and then inside the bone matrix. The lead on the surface can be removed easily; however, lead deposited deep inside the bone structure can be removed in condition of bone resorption.[3] The storage and the mobilization of lead in bones depend on number of factors, like age, lead exposure, pregnancy and race. It has been demonstrated that long-term effects of lead poisoning in living species

induces a reduction in the bone mineralization process.[3] Also, the concentrations of copper, zinc, iron and sodium has been found to reduce in the bones after lead intoxication. The deterioration of bone occurs as a result of increased oxidative stress as shown in our previous study.[9]

Our previous results on bone tissue have motivated us to design this study to evaluate the long-term effects of lead poisoning on the bone composition and strength. Although information is available in literature regarding the adverse effects of lead on number of biological tissues but the information is fairly lacking on the adverse effect of lead on the bone composition and morphometric parameters. Therefore the present study was designed to evaluate the impact of lead poisoning on bone tissue.

METHODS

Experimental modality

For carrying out the present investigation, male Wistar rats were procured from the Central Animal House of Panjab University, Chandigarh. The experimental animals were housed in polypropylene cages and were given free access to clean drinking water (tap water) and standard animal pellet diet (from Ashirwad Industries, Kharar, Punjab, India), throughout the experiment. The animals were acclimatized for a time period of one week to laboratory conditions before the initiation of experiment. The experimental procedures were approved by the Institutional Ethics Committee, Panjab University and conducted according to Indian National Science Academy ethical Guidelines for the use and care of experimental animals.

Experimental design

Forty eight male Wistar rats were taken in two age groups i.e. 30 day's old (controls /CI/ and experimental /Lead I/) and 80 day's old (controls /CII/ and experimental /Lead II/) animals. Lead (as lead acetate 250 mg/ml) was provided ad libitum in drinking water for about five weeks to produce subclinical toxicity. Glacial acetic acid was added to the drinking water of lead administered groups at a concentration of 12.5 µl/l to prevent the precipitation of lead acetate. At the termination of treatment period, rats from all four groups were sacrificed by decapitation and their long bones i.e. femur and tibia were excised, cleaned off from soft tissue. Then the bones were preserved in refrigerator (-20^{o}C) and processed for further analysis.

Calcium content in bone tissue

Calcium (Ca) contents in the bone tissue (both femur as well as tibia) were estimated using atomic absorption spectrophotometer (AAS).

Bone sample preparation for atomic absorption spectrophotometer (digestion method)

Method of Szpunar et al was partially modified for bone digestion.[5] One gram of sample was placed in a 250 ml digestion tube and 10 ml of concentrated Nitric acid (HNO_3) was added. The sample was heated for 45 min at 90^{o}C, and then the temperature was increased to 150^{o}C at which the sample was boiled for at least 8 h until a clear solution was obtained and the digestion mixture volume was reduced to about 1ml. After cooling the digestion mixture, 5 ml of 1% HNO_3 was added and then filtered with Whatman filter paper. Trace elemental analysis for bone samples were done using the Atomic Absorption Spectrometer (Perkin Elmer, Model 3100).

Atomic absorption spectrophotometer parameters for analysis of Calcium

Ca hollow cathode lamp was operated at 0.7 nm slit width which was selected to isolate 422. 7 nm lines using air-acetylene gas (temperature about 2300^{o}C). Lamp current for Ca was kept at 20 mA respectively. For calcium, the standard (1000 ppm) was prepared in the laboratory using calcium carbonate. Working standard solutions of Ca were prepared with ultrapure water with a specific resistivity of 18 MΩ cm obtained by filtering double-distilled water through a Millipore-Q purification (Lab pure series Bio-age) system. Before calibrating the system, the blank was measured. Auto zero operation stores the blank curve, which was then automatically subtracted from all the subsequent readings. Then the system was calibrated using different concentrations of standards by a method of non-linear calibration. After the calibration, the analytical dissolution was directly aspirated into the flame of atomic absorption spectrophotometer to determine Ca concentrations. These concentration of calcium was obtained in parts per million (ppm) and then converted into mg/g of bone tissue.

Bone morphometric parameters

The length and the femoral head width of all the bone samples were recorded with the aid of digital Vernier Calliper (Mitutoyo corp. Japan). After recording these measurements, the dried bone specimens from each group were kept in silica crucibles and were subjected to a temperature of 500^{o}C for 8 hours (in an electric furnace) to burn off the organic matrix. The residual ash was then weighed to determine the mineral content.

Statistical analysis

Statistics analysis of the data was performed by analysis of variances (one way ANOVA). Following one way ANOVA post Hoc test using least significance difference (LSD) and by student 't' test at p=0.05 using SPSS statistical software data for individual parameters represents average value calculated from three parallels.

RESULTS

Calcium content in bone tissue

Mean values of calcium concentration in both femur ($p<0.001$) and tibia ($p<0.001$) were found to be significantly reduced in the lead intoxicated groups when compared to the normal control. Also the difference in the calcium content was found among both the age groups.

Table 1: Effect of lead toxicity on the calcium concentration of the bone tissue.

Groups	Femur calcium (mg/g of tissue)	Tibia calcium (mg/g of tissue)
C I	241±14	191±6
Lead I	157±5[c]	153±8[c]
Control II	256±15	201±7
Lead II	164±10[c]	164±7[c]

Each value is the mean ± SD (n=6). Values with a superscript are significantly different from the control group ([a], $p<0.05$; [b], $p<0.01$; [c], $p<0.001$)

Bone tissue morphometric parameters

Table 2: Effect of lead intoxication on femur morphometric parameters.

Groups	Length (mm)	Femoral head width (mm)	Ash content (g)
C I	29.12±0.17	6.12±0.24	0.461±0.031
Lead I	28.51±1.37	5.62±0.10[c]	0.292±0.004[c]
Control II	28.61±0.63	6.07±0.24	0.343±0.008
Lead II	29.45±1.76	6.26±0.24	0.305±0.007

Tables 2 and 3 tabulate the morphometric parameters for the femur and tibia bone that includes length, head width and ash content.

Length

There is no significant change observed in the length of the femur as well as tibia of all the treated groups.

Head width

The OVX group showed significant decrease ($p<0.001$) in the femoral and tibial head width of lead treated group when compared to the control group.

Ash content

Significant decrease in the ash content of both the bones was observed upon lead intoxication for both the age groups.

Table 3: Effect of lead intoxication on tibia morphometric parameters.

Groups	Length (mm)	Tibia head width (mm)	Ash content (g)
Control I	30.19±0.44	4.33±0.19	0.464±0.020
Lead I	31.15±0.49	3.59±0.51[c]	0.337±0.010[c]
Control II	31.33±1.30	4.95±0.52	0.437±0.023
Lead II	30.46±2.05	4.08±0.55	0.333±0.012[c]

DISCUSSION

Lead is a metal poison that accumulates inside the body and causes abnormal functioning of number of organs. Lead is more toxic in the developmental stages in comparison to the developed stage.[6] Also, in the case of bone tissue, it has a greater impact on the metaphyseal growth plate. It gets associated with the calcium and phosphorous present in the bone matrix.[7] The current study has been carried out to investigate the effects of lead toxicity on the bone calcium content and morphometric parameters.

A significant reduction in the bone calcium content upon lead intoxication has been observed. This decrease in calcium content may be because of the increased bone resorption. Lead is one of the risk factor for the development of osteoporosis by altering the bone mineral metabolism.[8] Osteopenia, osteoporosis, and osteomalacia, with increased bone fragility in humans and experimental animals, were observed as a result of exposure of lead exposure.[9] Long-term exposure to lead leads to damage in different body tissues Also, in the bone tissue, the increase in lipid peroxidation was observed that may be attributed to changes in the antioxidant defense system. The reduced activities of catalase, glutathione S-transferase and superoxide dismutase, was observed upon lead intoxication suggesting that it causes oxidative stress. Also, lead significantly reduced the nucleic acid content and the activity of alkaline phosphatase that has been considered as biomarkers of osteoblast's function.[9] So we can say that the osteoblast function has been impaired which might have caused increased bone resorption. Earlier studies also showed increased serum calcium levels in lead intoxicated rats.[11,12] From the above mentioned findings, we can say that the decreased calcium content in the bone is the result of enhanced bone resorption. This impairment is because of the inhibitory action of lead on the osteoblast cells and vitamin D synthesis for osteocalcin. Lead interferes with the calcium homeostasis and calcium regulated secondary messenger system via disruption of cAMP signals.[12]

The femoral head width was found to decrease in lead treated rats, a very important finding in the current study. The active growth plate is found in this region. We are dealing with the rats which are in the growing phase, and growth plate closure has not occurred since then.

Therefore lower femoral head width observed in case of lead intoxicated rats could be attributed to the interaction of lead with that of the growth plate. This result is consistent with the earlier study reporting lesser growth plate thickness in lead exposed rats.[13] One another report in human population indicates the shorter stature in lead-intoxicated children which is thought to be attributed to the effect of lead on the growth plate function.[14] Lead actually accumulates in the hydroxyapatite crystals during calcification and also inhibits the proper functioning of the chondrocytes.

CONCLUSION

The present study indicates that the lead has induced bone toxicity and has deteriorated the development of bone tissue in the case of growing animals, is the consequence of oxidative stress. Further, it has caused changes in the bone composition and femoral head width which may cause bones to become susceptible to fractures.

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