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Original Research Article

Spinal ossification in mouse embryo is severely compromised by the lead acetate treatment

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ABSTRACT

Background: Bone growth is the vital process that continues throughout the life of living beings. Several pollutants may cause poorer ossification and bone formation. Vertebral ossification is directly correlated to growth, strength and spinal stability therefore the determination of ossification pattern in vertebral column is important to evaluate the bone formation. Bio-accumulation of lead in the body has been known to cause the adverse effects on the bone development through the disruption of mineralization.
Methods: The present study was designed to evaluate the impact of lead exposure on the embryonic ossification in mice during development. For this study 28 pregnant female mice were selected and treated with the lead (as lead acetate 0.5 ppm) for 14 and 21 days periods. At the end of the treatment periods pregnant mice were sacrificed and embryos were excised and processed for further analysis.
Results: Present study revealed damaged and ruptured spinal ossification center with premature chondrocytes present in lyzed condition in lead treated group compared to control.
Conclusions: It is concluded that lead exposure induced bone toxicity that has deteriorated the spinal ossification in the growing mice.

Keywords: Bone ossification, Lead acetate, Mouse embryo, Bone toxicity

INTRODUCTION

During embryonic development and in growing mammals calcium is essential for formation and growth of bone. The ossification decides the formation of calcium constituents in the bone. The amount of calcium passing through umbilical cord from maternal blood circulation and enters in the foetal area will decide the framework of foetal skeleton. Subsequently the ossification becomes rapid and furnishes the formation of whole foetal skeleton. There are large numbers of pollutants prevailing in the present environment cause damages to vital body organs. Among this lead (Pb) is of particular interest to us because of its wide distribution in the environment. Lead serves no useful purpose in the body and its presence in the body leads to toxic effects. 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. 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 human body is the bone. Lead posses a substantial threat to pregnant mother and their developing embryos. Blood lead readily crosses the placenta, putting the developing embryos at risk.[1] Lead adversely influenced bone development through disruption of mineralization during growth. Lead causes bone malformations in the rat and mouse foetus and

inhibit bone formation in dogs.[2] It has been demonstrated that long term effects of lead poisoning in living species induces a reduction in the bone ossification process. Information is available in literature regarding the adverse effect of lead on number of biological tissue however the studies are fairly lacking on correlation between vertebral morphology and ossification process during embryonic life. Therefore; present study designed to evaluate the impact of lead poisoning on spinal ossification process in developing embryos of mice.

METHODS

Animals

Female and male Swiss albino mice (Musculus albinus) were procured from veterinary college Mhow (M.P.). The study was conducted in the lab of school of studies in zoology and biotechnology, Vikram university Ujjain, Madhya Pradesh, India during the period of October 2007 to October 2011. The experimental animals were housed in polypropylene cages and were given free access to clean drinking water and standard animal pallet diet throughout the experiment. The animals were acclimatized for a time period of one week to laboratory conditions before the initiation of experiment. Healthy sexually mature female and male mice were selected and grouped together in the ratio of 2:1 in each cage. After copulation the vaginal plug was observed (considered zero day of pregnancy) and then male mice were separated from pregnant female mice.

Treatments

Lead was used for present study in the form of lead acetate [Pb $(CH_3COO)_2$]. The dose of lead acetate was selected after calculating the LC_{50} value. LC_{50} was found to be 0.5 ppm/l. The 0.5 ppm of lead acetate was given to pregnant mice orally till the last day of experiment.

Experimental design

The 28 pregnant mice were divided into 4 groups for 14 and 21 days of treatments. The control group consisting of 14 mice were fed on standard food and plain tap water (without administration of lead acetate) for the same period, At the end of the 14 and 21 days of the treatment the pregnant mice of control and treated groups were anaesthetized and dissected out. Embryo/foetus were separated and immediately fixed into 4% paraformaldehyde. The research article is based on histopathological and descriptive study of embryonic spinal region which are depicted in microphotographs hence there is no use of any statistical tool or software to analyse data.

Histopathological analysis

For histological analysis embryos were fixed overnight at 4ºC in 4% paraformaldehyde in PBS, rinsed in PBS, dehydrated through ethanol series, cleared in xylene embedded in paraffin wax and cut into 5 µm sections. The sections of all groups were stained with haematoxylene-eosin (H and E).

RESULTS

Skeletal growth is an integral component and primary stimulation of somatic growth. During embryonic development bone formation occurs by 2 different means i.e., intramembranous and endochondral ossification. Development of foetal vertebral skeleton is highly regulated process. It is known that lead accumulates in the skeleton and has direct effect on osteoblast function.[3]

In the present study spinal section of embryo exhibited premature chondrocytes in spinal ossification center and round shaped notochordal segments in control group while lead acetate treatments group revealed damaged and lyzed premature chondrocytes with burred, appearance, disrupted, broken fetal spinal sclerotome and compact cellular arrangement in vertebral area. Histological studies show that the spinal section of 14 days embryo of control group exhibited only premature chondrocytes, located in the spinal ossification center. The size of chondrocytes were small and notochordal segment exhibited round in shape (Figure 1).

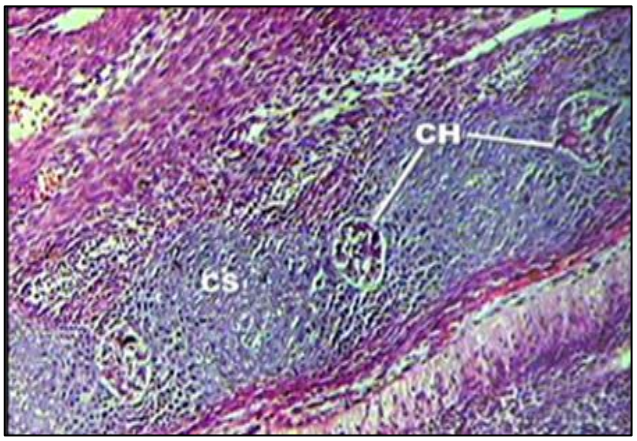

Figure 1: Control group chondrocyte with rounded notochord (14 days).

The 14 days lead treated embryo revealed damaged spinal section. The premature chondrocytes were seen in lyzed condition. Damaged chondrocytes and blurred appearance of notochordal segment were prominently seen (Figure 2).

In control group of 21 days treatment the fetal sclerotome vertebral area were seen with compact and fine arrangement of the tissue enclosing triangular fetal lung. The pleural cavity was arranged compactly. The fetal spinal and vertebral area in this group showed compact and patch like arrangement of vertebrae, embedded by surrounding intervertebral tissue with prominent differentiation (Figure 3).

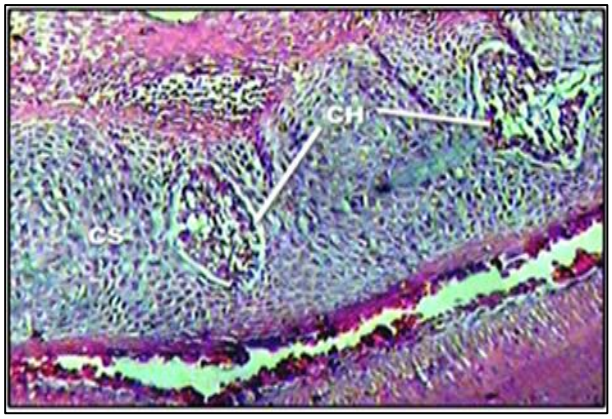

Figure 2: Treated group chondrocyte with ruptured notochord (14 days).

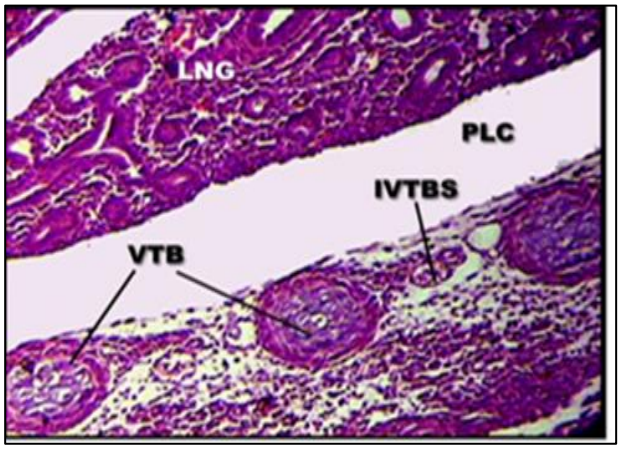

Figure 3: Control group uniformly arranged spinal sclerotome (21 days).

There was a marked damage found at the 21 days lead treated group. Spinal section exhibited disrupted, broken fetal spinal sclerotome vertebral area and deformed tissue of lung. The fetal vertebral area along with fetal lung tissue showed broken and blurred appearance (Figure 4).

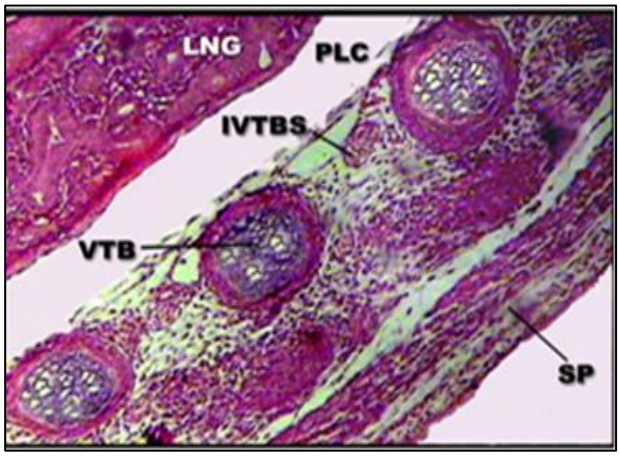

Figure 4: Treated group disrupted broken fetal sclerotome (21 days).

DISCUSSION

As shown in results the disorganization of cells during bone development may cause the embryonic growth retardation and loss of zonal structure and columnar array of the cartilage in mouse embryo.[4]

Other studies also found the inhibition of growth plate development in female wistar rat treated with 17 mg/kg lead acetate.[5] Lead suppresses the expression of phenotypic markers of growth.[6] The chondrocytes were the important target of lead toxicity. Lead delays the fracture healing at higher doses by inhibiting the progression of ossification and association with delay in cartilage mineralization in mouse.[7] Lead accumulated in the skeleton throughout the development and localized in area of bone mineralization and growth. In the present study the administration of lead acetate to pregnant mice resulted in delayed skeletal ossification in embryo/fetus. Ossification was retarded irrespective to embryonic tissue of origin. The reduced activity of catalyze glutathione S-transferase and superoxide dismutase were observed upon lead intoxication suggesting that lead exposure damage the tissue by elevating the oxidative stress and also lead significantly reduced the nucleic acid content and the activity of alkaline phosphate that has been considered as biomarkers of osteoblast's function. Lead accumulates in the hydroxyapatite crystals during calcification and also inhibit the proper functioning of chondrocytes. Thus, lead interferes with calcium homeostasis and calcium regulated secondary messenger system via disruption of cAMP signals.[8]

This study has some limitations due to small sample size although every mouse underwent the entire protocol, some data were omitted during analysis due to technical artifacts.

CONCLUSION

We conclude that lead treatment induces bone toxicity and deterioration in the development of spinal ossification The major mechanism behind the bone toxicity and altered ossification is the consequence of lead induced oxidative stress. The increase in the oxidative stress due to lead treatment and its detrimental effects on bone toxicity and ossification may cause delayed bone growth.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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