IMPAIRMENT OF BONY CRYPT DEVELOPMENT ASSOCIATED WITH HEXAVALENT CHROMIUM EXPOSURE DURING TOOTH ERUPTION

Luciana M. Sánchez, Marianela Lewicki, Romina C. De Lucca, Ángela M. Ubios
Department of Histology and Embryology, School of Dentistry, University of Buenos Aires. Buenos Aires, Argentina

ABSTRACT
Improperly treated hexavalent chromium-containing industrial wastes contaminate drinking water, potentially affecting children taking breast milk or baby bottles prepared with infant formula. Thus, the aim of the present work was to determine the effect of this toxic on bone activity in the developing alveolus during tooth eruption of suckling Wistar rats intoxicated with potassium dichromate. Experimental animals received a daily dose of 12.5 mg/kg body weight of potassium dichromate by gavage for 10 days; controls received an equivalent volume of saline solution. Histologic and histomorphometric studies of the mandible were performed. The data were statistically analyzed using Student’s t test; statistical significance was set at a value of p < 0.05. Experimental animals exhibited delayed tooth eruption, decreased periodontal width and bone volume, a lower percentage of bone formation surfaces, and higher percentage of quiescent surfaces (p < 0.05) compared to controls. The delay in tooth eruption observed after exposure to hexavalent chromium is the result of a lower rate of bone remodeling in the developing alveolus. The obtained results show the importance of controlling toxic substances in drinking water, since their effects may alter the growth and development of subjects who were exposed during early infancy.

Key words: tooth eruption; hexavalent chromium; bone remodeling; drinking water.

INTRODUCTION
Hexavalent chromium compounds produced by the chemical industry are used for the manufacture of dyes and pigments, leather tanning, and wood preserving. Wastes from electroplating, petrochemical industry, leather tanning, and textile industry can be released into the air or the soil, or be discharged into waterways, contaminating drinking water 1. The general population can be exposed to hexavalent chromium directly through skin contact, by inhaling air, or by drinking or eating foods contaminated with chromium 2,3. After entering the cell, Cr VI is reduced to Cr III, resulting in the formation of reactive intermediates which contribute to the cytotoxicity, genotoxicity, and carcinogenicity 4.
In the year 2010 the US Environmental Protection Agency (EPA) established that the maximum allowable concentration of total chromium which includes all forms of chromium including chromium-6 in drinking water should not exceed 0.1mg/l or 100 parts per billion (ppb). Nevertheless, these recommendations are not met in some countries in America, Europe and Asia. There are studies in the literature associating exposure to hexavalent chromium and risk of bone damage. Sankaranivel et al reported that Cr VI has been found to enter the inorganic bone matrix of vertebrae, femur and calvaria of adults male rats, altering the tissue and interfering with bone formation and resorption, thus, leading to altered bone turnover.

According to the Agency for Toxic Substances and Disease Registry, babies could be exposed to high environmental levels of chromium through inhalation and consumption of contaminated foods -including breast milk- and water -used to prepare baby formula. Very few studies have investigated the effects of chromium exposure on children. However, it is likely that children would have the same health effects as adults. Soudani et al found that exposure of rat dams to potassium dichromate before and after delivery affected growth and decreased bone mineral content of their progeny and De Lucca et al demonstrated a decrease in body growth of suckling rats receiving potassium dichromate solutions. The association between other toxic substances and bone alterations is well documented: iron decreases bone formation and inhibits endochondral ossification, lead replaces the calcium in the hydroxyapatite crystals and also impairs body growth and uranium affects bone remodeling, decreasing mandibular growth and delaying tooth eruption.

Tooth eruption is a highly dynamic biological process, in which bone tissue plays a crucial role. Little is known about bone remodeling in the walls of the alveolus as the tooth drifts during tooth eruption. Studies in rat molars, which, like human teeth, are teeth of limited eruption, have shown that bone resorption and formation are essential during the intraosseous and mucosal penetration stages of tooth eruption, when the walls of the dental alveolus develop. In their 2012 study in Jaipur, India, Tiwari et al demonstrated the presence of hexavalent chromium in the blood of children working in gem polishing industries. It is of note that the studied children were at the age when the permanent second molars erupt (10-12 years). Although De Lucca et al demonstrated that the exposure of suckling rats to hexavalent chromium resulted in decreased body and mandibular growth and delayed tooth eruption, it remains to be clarified whether these observations are the result of an alteration in bone remodeling. Thus, the aim of the present work was to determine the effect of hexavalent chromium exposure on the developing alveolus during tooth eruption in suckling Wistar rats intoxicated with potassium dichromate.

**MATERIAL AND METHODS**

Sixteen 4-day-old suckling Wistar rats were assigned to one of two groups: an experimental and a control group. Under topical anesthesia [Xylocaine (Xilocaína®, Astra Zeneca Argentina)], experimental animals received 12.5mg/kg body weight of potassium dichromate (Biopack, Argentina) daily by gavage through a flexible PVC tube. Control animals received an equivalent dose of saline solution under the same conditions as experimental pups.

The litters were adjusted to 8 pups per dam and were housed with their mother in individual cages with wood-chip bedding, and kept on a controlled light-dark cycle (lights on at 7 am and off at 7 pm) and under constant humidity (40-70%). The mothers were fed a solid diet and water at libitum. After each procedure, the pups were returned to the cage with their mother.

All the pups were euthanized on day 15 of the experiment and the mandibles were resected. The hemi-mandibles were fixed in 4% buffered formalin for 48 hours, decalcified in 10% EDTA pH 7, and embedded in paraffin. Buccal-lingual sections of the hemi-mandibles at the level of the mesial root of the first lower molar were obtained and stained with hematoxylin-eosin in order to perform histologic and histomorphometric studies under a stereoscopic microscope. Digital microphotographs of the histologic sections of the hemi-mandibles were analyzed using the Image Pro® Plus software, version 5.1 (Media Cybernetics) to measure the histomorphometric parameters listed below, based on stereologic principles and using current nomenclature as stated by Parfitt and revised by Dempster et al.
Parameters measured in the alveolar bone of the developing alveolus (Fig. 1):
* The degree of tooth eruption, expressed in millimeters, was determined as the distance between the highest point of the bone crest on the buccal aspect of the developing alveolus and the cementum-enamel junction. Therefore, the result is 0 when tooth eruption is complete and is a negative number when the tooth is not fully erupted.

The following parameters were measured on both the buccal and lingual aspects. To simplify reading, only measures on the buccal aspect are explained in Fig. 1.
* Periodontal width was measured at three sites: B-B', C-C' and D-D'; values were averaged and results are expressed in microns.
* Bone volume, defined as the fraction of total volume corresponding to trabecular bone, was measured in the area demarcated by the black lines. Total volume is defined as the volume of trabecular bone tissue plus the volume of developing bone marrow; results are expressed as a percentage.

Bone activity was evaluated in three different regions:
- From point A to point C'
- From point C' on the buccal aspect to point C' on the lingual aspect
- On the endosteal walls in the area delimited by points B' C' E and F

- The degree of tooth eruption was measured from point A to point B'.
- Periodontal width was determined, measuring segments B- B', C- C' and D-D'.
- Bone volume was measured in the area delimited by points B' C' B'' and C''.
- Bone activity was assessed in three different regions of the alveolar wall:
  - From point A to point C'
  - From point C' on the buccal aspect to point C' on the lingual aspect
  - On the endosteal walls in the area delimited by points B' C' E and F

Statistical analysis: The data were statistically analyzed using Student’s t-test; statistical significance was set at a value of \( p < 0.05 \).

Ethical principles: All procedures were performed in keeping with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH publication 85-123 Rev.2010) and the study was approved by the Ethics Committee of the School of Dentistry of the University of Buenos Aires (FOUBA-UBACYT 2011-2014-3).
RESULTS
Our study showed that exposure to potassium dichromate in the form of chromium VI caused a significant delay in the eruption of the first lower molar, which was associated with a clear alteration in the development of the alveolus. The bone of the developing alveolus of experimental animals exhibited thinner and more spaced trabeculae than that of controls. Chromium-exposed animals showed fewer osteoclasts and active osteoblasts and more bone lining cells on the endosteal surfaces, the wall of the developing bone crest, and on the fundus of the alveolus (Fig. 2). The histomorphometric study showed significantly decreased bone volume in the alveolus of experimental animals compared to controls, on both the buccal and lingual aspects (Fig. 3).

The surfaces of the endosteal bone trabeculae on the buccal and lingual aspects and fundus of the alveolus of the experimental group exhibited a lower percentage of bone formation and resorption surfaces and a higher percentage of resting surfaces than in the corresponding controls (Fig. 4). The distance between the highest point of the bone crest on the buccal aspect of the developing alveolus and the cementum-enamel junction was greater in experimental animals than in controls, showing a significant delay in the eruption of the first lower molar. Hence, the result of the histomorphometric analysis was a negative number (Fig. 5).

In addition, the periodontal space was narrower in the potassium dichromate-exposed animals.
than in controls, and the histomorphometric study showed it was significantly lower in experimental animals compared to controls, on both the buccal and lingual aspects (Fig. 6).

**DISCUSSION**

The results of the present study showed that hexavalent chromium has a toxic effect on the bone cells involved in the bone remodeling process that takes place in the bone tissue of the developing tooth alveolus. According to De Luca et al.\(^7\) this effect can be observed morphometrically as a delay in tooth eruption in animals exposed to potassium dichromate. It is well documented that bone remodeling involves the coupled action of osteoblasts (bone matrix-forming cells) and osteoclasts (cells that resorb the bone matrix).

**In vitro** studies have reported that exposure to hexavalent chromium can affect human osteoblast and osteoclast survival and function.\(^{28}\) Thompson and Puleo\(^{29}\) reported that chromium interferes in the differentiation and function of osteoblasts derived from mesenchymal cells. Ning and Grant\(^{30}\) showed that hexavalent chromium reduced to trivalent chromium is a potent inducer of cytotoxicity in osteoblasts. Lohman et al.\(^{31}\) found changes in cell morphology and in the differentiation capacity of osteoblasts. In addition, a study by Anisian et al.\(^{32}\) showed that high concentrations of chromium decreased osteoblast activity. Nichols and Puleo\(^{33}\) found that sub-lethal and physiological concentrations of hexavalent chromium affected the formation and function of osteoclasts. Thus, chromium would interfere in the differentiation of osteoclastic cells derived from precursor cells in the bone marrow, and would inhibit the Ca\(^{2+}\) receptors in osteoclasts. The receptor binding site is highly sensitive to di- and tri-valent cations. Hence, given that hexavalent chromium reduces to trivalent chromium, the latter would bind to the receptor, increasing the cytosolic concentration of calcium and decreasing bone resorption. Neale et al.\(^{34}\) demonstrated the inhibitory effect of chromium on osteoclastogenesis in human monocytes cultured with chromium particles. Previous studies conducted at our laboratory showed that bone resorption and formation are indispensable during the intraosseous and mucosal penetration stages of tooth eruption, which is when the walls of the alveolus develop.\(^{20,22}\) The present results confirm those findings.

![Fig. 5: Morphometric values of tooth eruption. The degree of tooth eruption was significantly lower in experimental animals compared to controls (* p<0.05).](image)

The results of the present study show that exposure to hexavalent chromium leads to significantly decreased bone resorption and formation in the endosteal surfaces of the developing tooth alveolus and in the fundus of the alveolus, as shown by the presence of fewer osteoclasts and active osteoblasts. The larger proportion of areas of resting bone on the endosteal surfaces and fundus of the developing alveolus in experimental animals as compared to controls is similar to what occurs in adynamic bone disease. The latter disease has been observed for example, in cases of aluminum toxicity in humans and in an experimental model of iron overload.\(^{18}\)

The present study showed decreased bone formation and resorption, and a predominance of bone quiescence in the developing alveolus of animals exposed to potassium dichromate as compared to controls. These findings show that hexavalent chromium affects bone turnover, as shown by the lower proportion of both areas of bone resorption and formation.
bone formation and the predominance of resting bone, which in turn results in the decrease of bone remodeling. These observations explain the delay in tooth eruption.

Hexavalent chromium has been found to induce damage to the cytoskeleton and DNA alterations in exposed fibroblast cultures. These findings could explain the decreased periodontal width observed in the present study, which would be related to the inhibition of formation of fibroblasts that impairs periodontal ligament remodeling.

The results obtained in the present study allow concluding that because hexavalent chromium inhibits osteoclasts and osteoblast function, the delay in tooth eruption observed in animals exposed to hexavalent chromium would be due to a lower rate of bone remodeling in the developing tooth alveolus. Taking into account that in addition to the well known health consequences of hexavalent chromium exposure the latter can also affect tooth eruption in children who intake water contaminated with this toxic substance, it is crucial to create further awareness worldwide about the importance of avoiding environmental contamination, and ensuring compliance with waste-water treatment regulations with the aims to protect future generations.

ACKNOWLEDGMENTS
The authors thank H.V. Ivana Sánchez Rojas for her technical assistance and Ignacio Emanuel Sánchez for his collaboration in preparing the graphs of the manuscript. This study was supported by GRANT 2011-2104 UBACYT 20020100196 (University of Buenos Aires).

REFERENCES


