

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

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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 12.5mg/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.

ALTERACIÓN DEL DESARROLLO DE LA CANASTILLA ÓSEA ASOCIADA A LA EXPOSICIÓN DE CROMO HEXAVALENTE DURANTE LA ERUPCIÓN DENTARIA

RESUMEN

Desechos industriales que contienen cromo hexavalente inadecuadamente tratados contaminan el agua de consumo pudiendo afectar a los niños por vía de la leche materna o de la preparación de mamaderas. Por lo tanto, el objetivo del presente trabajo fue determinar el efecto de este tóxico en la actividad del hueso en el alveolo en desarrollo durante la erupción dentaria de ratas Wistar lactantes expuestas a dicromato de potasio. Los animales experimentales recibieron una dosis diaria de 12,5 mg / kg de peso corporal de dicromato de potasio por alimentación forzada durante 10 días; mientras que los controles, un volumen equivalente de solución salina. Se llevaron a cabo estudios histológicos e histomorfométricos de la mandíbula. Los datos fueron analizados estadísticamente utilizando la prueba t de Student; estableciéndose un valor de $p < 0,05$ como esta-

dísticamente significativo. Los animales expuestos a cromo hexavalente mostraron retraso en la erupción dentaria, menor espacio periodontal y volumen óseo; encontrándose disminuidas las superficies en formación y en reabsorción óseas y aumentadas las superficies en reposo ($p < 0,05$) en comparación con los controles. El retraso en la erupción dentaria observado luego de la exposición a cromo hexavalente es el resultado de una menor remodelación ósea en el alveolo en desarrollo. Los resultados obtenidos muestran la importancia del control de sustancias tóxicas en el agua potable, ya que sus efectos pueden alterar el crecimiento y el desarrollo de los individuos que fueron expuestos durante la infancia temprana.

Palabras clave: erupción dentaria; cromo hexavalente; remodelación ósea; agua de consumo.

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 water¹. 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 carcinogenicity⁴.

In the year 2010 the US Environmental Protection Agency (EPA) ⁵ 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 ⁶⁻¹¹. There are studies in the literature associating exposure to hexavalent chromium and risk of bone damage ^{12,13}. Sankaramanivel et al ¹⁴ 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 ¹⁵, 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 ¹⁶ 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.¹⁷ 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 ¹⁸, lead replaces the calcium in the hydroxyapatite crystals and also impairs body growth ¹⁹ and uranium affects bone remodeling, decreasing mandibular growth and delaying tooth eruption ^{20,21}.

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 ^{22,23}. In their 2012 study in Jaipur, India, Tiwari et al ²⁴ 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 ¹⁷ 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 *ad 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 ²⁵ and using current nomenclature as stated by Parfitt ²⁶ and revised by Dempster et al ²⁷.

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: on the wall of the developing alveolus from the highest point of the bone crest to the diaphragm of the Hertwig's root sheath; on the wall of the fundus of the developing alveolus, from the diaphragm of Hertwig's epithelial root sheath on the buccal aspect (Point C) to the diaphragm of Hertwig's epithelial root sheath on the lingual aspect (Point C''); and on the endosteal walls, inside the area demarcated by the black lines, by determining the following parameters:

- *ObS/BS* (%): Bone formation surfaces (surfaces covered with active osteoblasts).
- *ES/BS* (%): Bone resorption surfaces (surfaces covered with active osteoclasts).
- *LCS/BS* (%): Resting bone surfaces, covered with bone lining cells.

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).

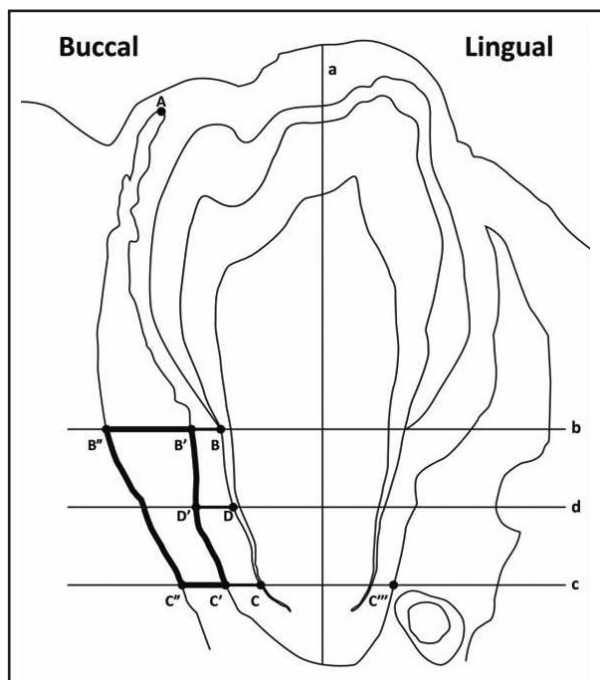


Fig. 1: Method used for the histomorphometric study of the developing tooth alveolus.

Buccal-lingual section.

Line a drawn through the longitudinal axis of the developing tooth.

Line b drawn perpendicular to line "a" through the cementum-enamel junction.

Line c drawn perpendicular to line "a" through the diaphragm of Hertwig's epithelial root sheath.

Line d drawn parallel to and equidistant from lines "b" and "c".

Point A is the highest point of the bone crest.

Point B at the cementum-enamel junction.

Point C at the diaphragm of Hertwig's epithelial root sheath.

Point D on line "d", equidistant from points B and C on the surface of the developing root.

Point B' drawn where line "b" goes through the periodontal plate of the developing alveolus.

Point C' drawn where line "c" goes through the periodontal cortical plate of the developing alveolus.

Point D', where line "d" goes through the periodontal cortical plate of the developing alveolus.

Point B'', on line "b", 100 microns from point B'.

Point C'', on line "c", 100 microns from point C'.

- The degree of tooth eruption was measured from point A to point B'.

- Periodontal width was determined, measuring segments B-B' C-C' 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

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).

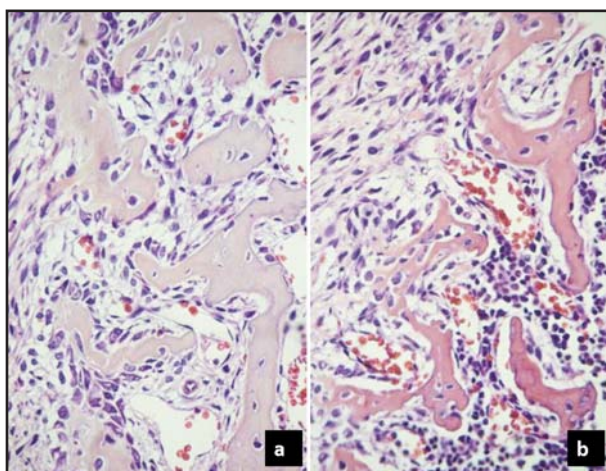


Fig. 2: Bone activity in the endosteal walls of the developing alveolus. Orig. Mag. X400. a: Control; b: Experimental. Note the large areas of bone formation with a large number of osteoblasts in the control section. Quiescent bone surfaces with inactive osteoblasts predominate in the experimental section; the latter also shows fewer and thinner trabeculae than the control section.

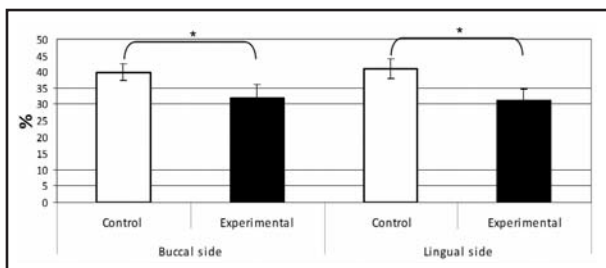


Fig. 3: Histomorphometric values of alveolar bone volume. Bone volume on both the buccal and lingual aspects was significantly lower in experimental animals than in controls (* $p < 0.05$).

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

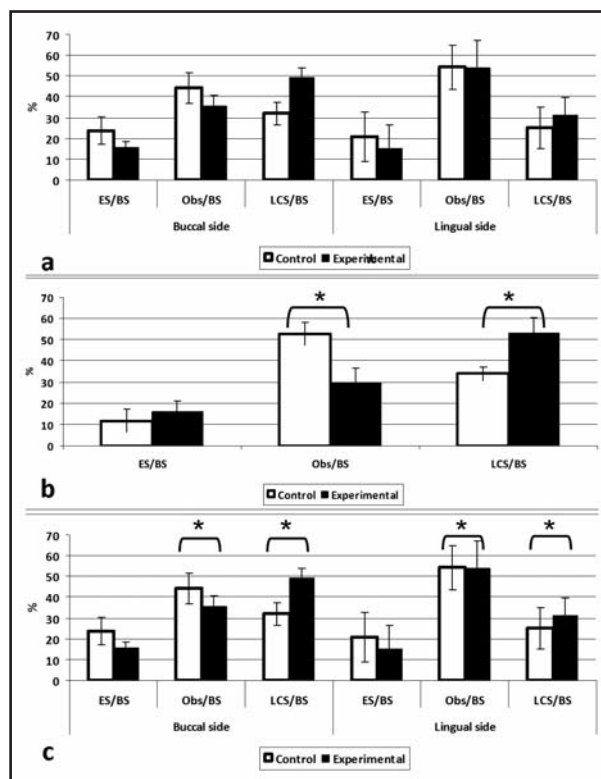


Fig. 4: Histomorphometric study of bone activity. Bone activity in the wall of the developing alveolus. Bone activity in the fundus of the alveolus. Bone activity inside the developing alveolus. A lower percentage of bone formation and resorption surfaces and a higher percentage of resting surfaces were observed in the buccal and lingual plates of the developing alveolar bone (c) and in the fundus of the alveolus (b) of experimental animals, as compared to controls. The differences between groups were statistically significant (* $p < 0.05$). No significant differences in bone activity in the developing alveolus (a) were observed between groups.

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 Lucca et al.¹⁷ this effect can be observed morphometrically as a delay in tooth eruption. 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 function²⁸. Thompson and Puleo²⁹ reported that chromium interferes in the differentiation and function of osteoblasts derived from mesenchymal cells. Ning and Grant³⁰ 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 Puleo³³ 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^{21,22}; the present results confirm those findings.

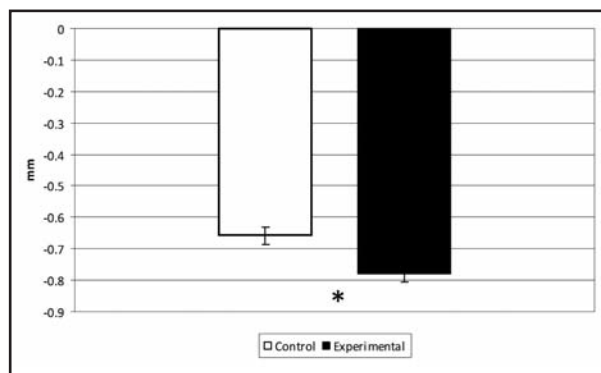


Fig. 5: Morphometric values of tooth eruption. The degree of tooth eruption was significantly lower in experimental animals compared to controls (* $p < 0.05$).

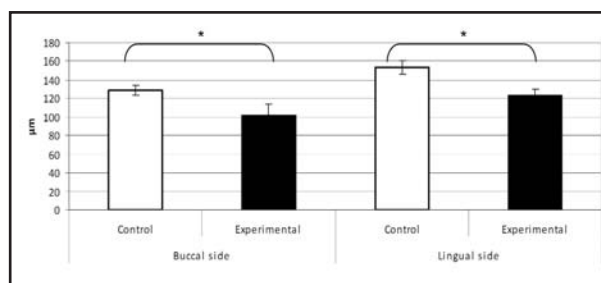


Fig. 6: Histomorphometric values of periodontal width. Periodontal width on both the buccal and lingual aspects was significantly lower in experimental animals as compared to controls (* $p < 0.05$).

The results of our 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 is similar to what occurs in adynamic bone disease. The latter disease has been observed for example, in cases of aluminum toxicity in humans³⁵ and in an experimental model of iron overload¹⁸.

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

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 cytoskeleton³⁶ and DNA alterations³⁷ 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.

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