EFFECT OF CADMIUM ON RAT MAXILLARY MOLAR JUNCTIONAL EPITHELIUM DURING LACTATION

EFFECTO DEL CADMIO SOBRE EL EPITELIO DE LA ZONA DE UNIÓN
MAXILO-MOLAR DE RATAS DURANTE LA LACTANCIA

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RIBAS, J. P.; LOPES, R. A.; SALA, M. A.; RIBAS, L. M. R.; MATTOS, M. G. C.; SEMPRINI, M.; WATANABE, I. & REGALO, S. C. H. Effect of cadmium on rat maxillary molar junctional epithelium during lactation. *Int. J. Morphol.*, 22(4):257-262, 2004.

SUMMARY: Cadmium in the air, drinking water and food has the potential to affect the health of people, mainly those who live in highly industrialized regions. Cadmium affects placental functions, can cross the placental barrier and directly disturbs fetal development. It is also excreted into milk. The organism is particularly susceptible to cadmium exposure at perinatal period. The effect on rat maxillary molar junctional epithelium a continuous exposure to drinking water containing low level of cadmium during lactation was studied. Female rats received drinking water ad libitum containing 300 mg/l CdCl₂ throughout the whole lactation. Control animals received a similar volume of water without cadmium. Lactent rats (21 day-old) were killed by lethal dose of anesthetic. The heads were separated, fixed in an "alfac" solution for 24 hours; palate region was serially sectioned in frontal plane, at level of first molars. The sections, 6 µm-thick, were stained with hematoxylin and eosin. Nuclear epithelium parameters were estimated, as well as cytoplasm and cell volume, nucleus/cytoplasm ratio, numeric and surface density and epithelial thickness. Mean body weight was 34.86 g for the control group and 18.56 g for the treated group. Histologically, the epithelium was thinner, with more numerous and smaller cells. In this experiment, cadmium induced epithelial hypotrophy, indicating a direct action in oral mucosae cells, besides retarded development in pups.

KEY WORDS: 1. Oral mucosae; 2. Junctional epithelium; 3. Cadmium; 4. Lactation; 5. Morphometry; 6. Stereology.

INTRODUCTION

Cadmium is an environmental pollutant recognized as nephrotoxic compound after chronic exposure. Cadmium is deposited in all internal organs, but mainly in the liver, kidney, and bone, and also in pancreas and salivary glands in animals (Berlin & Ullberg, 1963).

During pregnancy, cadmium is retained in the placenta, which thus acts as an important, but not complete, barrier to protect the fetus from cadmium exposure. This has been shown in rodents (Webster, 1978), as well as in humans (Loiacono *et al.*, 1992). Cadmium transfer via milk is reported to be low in rats and mice, which might be due to binding of cadmium to metallothionein in the mammary tissue (Grawe & Oskarsson, 2000). Metallothioneins are a group of low molecular weight, highly inducible proteins that maintain intracellular zinc homeostasis and protect

against cadmium-induced hepatotoxicity (Templeton & Cherian, 1991).

Since the toxic effects of pollutants are often correlated with their concentration in individual tissues and organs, the purpose of the present investigation was to study the effects of cadmium on the maxillary molar junctional epithelium during lactation.

MATERIAL AND METHOD

Female Wistar rats were mated and kept in separate cages. Standard pellet diet (Purina) and tap water were given *ad libitum*. On the day of parturition, defined as day 1, the

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Supported by CNPq and University of Franca (UNIFRAN).

Part of the PhD Thesis from the first author to the Faculty of Odontology of Ribeirão Preto, University of São Paulo, Brazil.

litters were reduced to ten pups. Cadmium was administered in drinking water (300mg/l CdCl_2) during 21 days (lactation). All pups were sacrificed with 3% Hypnol® at the end of day 21. The heads were separated from the bodies, fixed in a fixative solution consisting of 85ml 80% ethanol, 10ml formalin and 5ml glacial acetic acid, for 24h, embedded in paraffin, cut frontally into semi-serial 6µm thick sections and stained with hematoxylin and eosin.

The following karyometric parameters of the different layers of the gingival and junctional epithelium were estimated according described by Sala *et al.* (1994): the longest axis (D), the shortest axis (d), geometric mean axis, ratio of longest to shortest axis (D/d ratio), perimeter, area, volume, ratio of volume to area (V/A ratio), shape factor, contour index, and eccentricity.

The following stereologic parameters of the different layer of the junctional epithelium were determined: cytoplasm volume, cell volume, nucleus/cytoplasm ratio and cell number density, epithelial surface density and thickness of the epithelium and of the keratin layer (Sala *et al.*, 1992).

Data were analyzed statistically by the Mann-Whitney's non-parametric test.

RESULTS

The pups from dams that had received cadmium during lactation showed significantly lower ($p < 0.01$) body weight (18.56 g) than the control pups (34.86 g).

The maxillary molar of the cadmium-treated rat, on day 21 of postnatal life, showed partial eruption in oral mucosa. The gingiva, in formative phase, was seen and reduced enamel epithelium is present with elongated cells. The junctional epithelium is reduced and the attached gingival epithelium was thinner with small cells (Figs. 1 and 2).

Fig. 1. Histological picture of the maxillary molar junctional epithelium and reduced enamel epithelium of control rat. HE (900 X).

Table 1. Mean values of karyometric parameters of junctional epithelium and reduced enamel epithelium cells of the first maxillary molar of control (C) and cadmium-treated (T) rats. Mann-Whitney test.

Parameters	Junctional epithelium				Reduced enamel epithelium	
	Basal layer		Spinous layer		C	T
	C	T	C	T		
Longest axis (µm)	9.38	6.45*	8.51	6.86*	8.10	7.54
Shortest axis (µm)	6.92	4.91*	6.67	5.18*	6.17	4.69*
Mean axis (µm)	8.02	5.39*	7.77	5.94*	7.04	5.92*
D/d ratio	1.38	1.34	1.39	1.34	1.34	1.63*
Volume (µm ³)	276.32	99.56*	252.64	114.21*	188.60	112.62*
Area (µm ²)	50.93	25.29*	47.85	28.09*	39.35	27.86*
Perimeter (µm)	25.79	17.97*	24.99	19.03*	22.55	19.51*
V/A ratio	5.35	3.73	5.18	3.96*	4.70	3.94*
Eccentricity	0.62	0.55	0.63	0.59	0.56	0.75*
Shape factor	0.95	0.96	0.95	0.96	0.96	0.91*
Contour index	3.63	3.62	3.63	3.62	3.62	3.72*

(*) Significantly different from by Mann-Whitney test, $p < 0.01$

Fig. 2. Histological picture of the maxillary molar junctional epithelium and reduced enamel epithelium of treated rat. Note structural alterations, such thinner junctional and elongated reduced enamel epithelia. HE (900 X).

Table II. Stereological mean values of junctional epithelium and reduced enamel epithelium cells of the first maxillary molar of control (C) and cadmium-treated (T) rats. Mann-Whitney test.

Parameters	Control	Treated
Junctional epithelium		
Basal layer		
Cytoplasm volume (μm^3)	448.40	150.96**
Cell volume (μm^3)	719.63	238.30**
N/C ratio	0.29	0.17
Thickness (μm)	4.54	2.60**
Cell density ($\text{n}^\circ/\text{mm}^3 \times 10^6$)	1.77	4.91**
Spinous layer		
Cytoplasm volume (μm^3)	510.49	261.13**
Cell volume (μm^3)	756.93	371.58**
N/C ratio	0.18	0.15
Thickness (μm)	9.76	7.22**
Cell density ($\text{n}^\circ/\text{mm}^3 \times 10^6$)	1.36	3.02**
Total epithelium		
Surface density (mm^2/mm^3)	49.83	56.38*
Total thickness (μm)	25.24	21.50*
Outer surface/basal layer ratio	0.8314	0.9802
Cell density ($\text{n}^\circ/\text{mm}^3 \times 10^6$)	1.74	3.11**
Reduced enamel epithelium		
Cytoplasm volume (μm^3)	305.09	207.72**
Cell volume (μm^3)	489.40	316.50**
N/C ratio	0.35	0.15**
Thickness (μm)	5.28	10.46**
Cell density ($\text{n}^\circ/\text{mm}^3 \times 10^6$)	2.40	3.08**

(*) Significantly different from by Mann-Whitney test, $p < 0.05$

(**) Significantly different from by Mann-Whitney test, $p < 0.01$

The general measurements of the nuclei (longest, smallest and mean axis, volume, area, perimeter and V/A ratio) of cells of the basal and spinous layers of the junctional epithelium showed a statistically significant reduction in the treated group. The same was seen in the reduced enamel epithelium. The eccentricity, contour index and shape factor of the nuclei showed similar values in junctional epithelium and different values in enamel epithelium (Table I).

The cytoplasm and cell volumes, the N/C ratio and the thickness of basal and spinous cells layers were significantly reduced in junctional epithelium of the 1st maxillary molar. The cell number density of both layers was significantly increased in treated pups. The epithelium total thickness was smaller in treated animals.

The surface density and cell number density showed significantly highest values. The reduced enamel epithelium showed significant smaller values for cell and cytoplasm volumes and N/C ratio, and highest values for thickness and cell number density (Table II).

DISCUSSION

In this paper, it was observed a significant lower body weight of pups from animals intoxicated with cadmium. Reduced pups weight was also observed in rats by Crowe & Morgan (1997), in mice by Whelton *et al.* (1988), and in newborn lambs by Floris *et al.* (2000).

Cadmium has a major influence on calcium metabolism (Goyer, 1991). Low dietary calcium induces synthesis of calcium-binding protein, which increases cadmium absorption (Goyer, 1991) and consequently low body weight gain. Increased dietary intake of both iron and cadmium was accompanied by impaired growth rate. In the case of cadmium, growth restriction is considered to be an effect of the toxicity of the metal (Britton *et al.*, 1994).

In this study, the junctional epithelium of pups intoxicated was thinner with smaller and more numerous cells. The reduced enamel epithelium is present with elongated cells. Such picture characterizes a cellular hypotrophy.

The histometric data shows the junctional epithelium thinner in treated pups. The cellular layers were thinner, with

numerous small cells, as observed after stereology, where it was possible to observe small total epithelia thickness, associate with higher surface density, as well as small cytoplasm and cell volumes with cell number density significantly larger. The nuclei were smaller in the basal and spinous layers, as observed by the smaller values for longest, smallest and mean diameters, perimeter, area, volume and V/A ratio. The reduced enamel epithelium showed similar alteration, but with higher cell thickness.

Cadmium is toxic to cellular processes by disrupting mitochondrial function (Miccadei & Floridi, 1993), and can interfere with the transport and metabolism of many essential metals, such as iron, copper and zinc (Chang, 1992). Adequate availability of both zinc and copper is essential for normal growth and development. Insufficient zinc availability in fetal or early postnatal life retards growth (Sandstead *et al.*, 1972). Cadmium directly interferes with iron absorption through the intestine, possibly by competing with iron in the absorptive process (Schäfer & Elsenhans, 1985), and inducing iron-deficiency anemia. Crowe & Morgan observed that this anemia begins during the nursing period retarding growth of the pups.

Cadmium is found bound to metallothionein (MT) in liver. Concentrations of MT are notable in liver of mice in the postnatal period (Wong & Klaassen, 1982). Lucis *et al.* (1972) found significant levels of cadmium in liver and intestines of the neonate. The neonatal intestine accumulated increasing amounts of cadmium with time, containing 17 times more cadmium than the liver after 11 days of lactation.

Cadmium exposure in postnatal life induced CdMT synthesis and consequent displacement of zinc and copper of the MT channels (Panemangalore & Cherian, 1983). When the metal exceeds the amount of MT, it begins its toxicant effects, as observed in this paper.

Perfusion of isolated hepatic system as well as in vitro studies showed that cadmium inhibits the synthesis of proteins.

It also interferes with mRNA transcription binding with specific sites of chromatin. Cadmium breaks up polyribosomic structures (Gamulin *et al.*, 1997), increasing the effect on mRNA transcription and, finally, it leads to peroxidation in lipids (Stacey & Klaassen, 1980), which can affect the synthesis of excretory proteins.

Cadmium in excess or free in cytoplasm binds with cell organelles and with nuclei altering their function. Cadmium is also genotoxic, causing DNA to break up leading (Coogan *et al.*, 1992), to mutations (Biggart & Murphy, 1988), and chromosomal aberrations (Hartwig, 1994). It acts on genes playing multiple roles in apoptosis (Yonish-Rouach *et al.*, 1993).

Cadmium is also known to cause adverse effects on numerous and important cell processes from lead metabolism interruption to the eventual death of cells (Morselt, 1991). Cadmium has high affinity for membranes of the sulfhydryl group (Lijnen *et al.*, 1991), which may account for the cell membrane disorganization (Visser *et al.*, 1993). Cadmium inhibits Na⁺/K⁺-ATPase (Lijnen *et al.*) as well as Ca²⁺-ATPase leading to an increase in intracellular calcium concentration (Visser *et al.*; Vorbodt *et al.*). Vorbodt *et al.* showed that cadmium inhibits Ca²⁺-ATPase in endothelial cell cultures and is specifically pronounced in the interendothelial fissures that are junctional formation sites.

Calcium interacts with many heavy metals (Shamoo, 1987) and may be an important factor in pathophysiologic mechanisms. The increase in intracellular calcium concentration caused by cadmium results from an increase in permeability in the protoplasmic membrane demonstrated in the Ca²⁺-ATPase inhibition mediated by calcium efflux (Visser *et al.*). These facts may account for the results observed in this study.

Intoxication caused by cadmium in pups during lactation led to slowed growth and reduced body weight. The epithelium of the junctional epithelium was significantly affected, showing cell hypotrophy.

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RESUMEN: El cadmio presente en el aire, agua potable y alimentos, afecta la salud de las poblaciones, principalmente en regiones altamente industrializadas. El cadmio afecta la función placentaria, puede atravesar la barrera placentaria y alterar directamente el desarrollo fetal. Puede ser también excretado en la leche. El organismo es particularmente susceptible a la exposición al cadmio durante el período perinatal. Fue estudiado el efecto de la exposición continua, durante la lactancia, a agua potable conteniendo bajos niveles de cadmio, sobre el epitelio de la zona de unión maxilo-molar. Ratas hembras recibieron agua potable ad libitum conteniendo 300mg/l de CdCl₂ durante todo el período de lactancia. Los controles recibieron un volumen similar de agua sin cadmio. Ratas lactantes (21 días de edad) fueron sacrificadas con dosis letal de anestésico, sus cabezas separadas, fijadas en alfac por 24 h y la región palatina seccionada frontalmente, al nivel de los primeros molares. Los cortes de 6 µm de espesor fueron teñidos con hematoxilina y eosina. Se estimaron los parámetros nucleares del epitelio, así como los volúmenes citoplasmático y celular, relación núcleo/citoplasma, densidad numérica y superficial y grosor epitelial. El peso medio fue 34,86g para los controles y 18,56g para los tratados. Histológicamente, el epitelio fue más fino, con células más abundantes y menores. El cadmio indujo hipotrofia epitelial, indicando una acción directa sobre la mucosa oral, además de retardo del desarrollo de las crías.

PALABRAS CLAVE: 1. Mucosa oral; 2. Epitelio de unión; 3. Cadmio; 4. Lactancia; 5. Morfometría; 6. Estereología.

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Received : 26-07-2004

Accepted: 02-09-2004