The carcinogenic potential of cadmium in the palatal and gingival epithelium of rats. A morphologic and morphometric analysis

Avaliação do potencial carcinogênico do cadmio no epitélio de revestimento do palato e gengiva de ratos. Estudo morfológico e morfométrico

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Abstract

Cadmium (Cd) is a heavy metal that exerts a variety of toxic effects, chronic and acute, in exposed organisms. The aim of this study was to investigate the carcinogenic potential of Cd in the palatal and gingival epithelium of Wistar rats. Two groups of animals were studied: group 1 consisted of 5 rats exposed to cadmium chloride (CdCl2) in drinking water (300 mg/L) for 6 months; group 2 also consisted of 5 rats placed in the same conditions as those of group 1, but kept free of Cd for 6 additional months. Two other groups (C1 and C2) with the same number of animals, but not exposed to CdCl2, were used as a control for groups 1 and 2, respectively. All animals were weighed before and after the experimental period. After animals being killed, tissues of interest were fixed in solution of 10% formalin, processed by standard histologic techniques, stained with HE, and analyzed under light microscopy using karyometric and stereologic parameters. Loss of body weight, atrophy of the gingiva and soft-palate epithelium were the principal findings of this study, and verified only in the group 1 ($p < 0.05$). In conclusion, at the concentration tested, Cd presented no carcinogenic effect on the oral tissues within the experimental time period.


Introduction

Cadmium (Cd) is an industrial and environmental pollutant of considerable importance that poses a significant health risk to humans and animals. It is widely dispersed into the biosphere by mining and manufacturing processes, and has an extremely long biological half-life that essentially makes it a cumulative toxin.1,2,5 Cd deposited in the environment can give rise to serious intoxication in human beings and other organisms through different routes, which include soil, air, water and plants. This metal may be uptaken by plants and agricultural products, which can result in an increase of oral exposure.4,5,6

It is well documented that chronic exposure to Cd leads to the development of cancer in many organs, especially the lungs, kidneys and liver.4,7 Alterations on the oral epithelium and in the salivary glands, characterized by reduction of the nuclear cell under cadmium exposure, have been reported.3,9,10 There have been only a few studies concerning any possible carcinogenic
effect of Cd in the oral tissues\textsuperscript{11,12}, and none of these revealed any association.

The aim of this study was to investigate the carcinogenic effects of Cd, administered through drinking water, on the palatal and gingival epithelium of rats, using morphologic and morphometric techniques.

\section*{Materials and Methods}

\subsection*{Animals}

Twenty adult male rats (\textit{Rattus norvegicus albinus}, variety Wistar) weighing 160 to 180 g were housed in groups of five in plastic cages under controlled conditions of temperature (21\textdegree{}C to 25\textdegree{}C), relative humidity (45\% to 50\%) and light/dark cycles (12 hours of light/12 hours of darkness). Rats were given \textit{ad libitum} access to a standard rodent maintenance diet (Nuvilab, Curitiba, PR, Brazil) and tap water.

\subsection*{Chemical}

The cadmium chloride (CdCl\textsubscript{2}) used in this study was obtained from Sigma Chemical Company (St. Louis, MO, USA) with the following specifications: hydrate minimum 98\%, water content approximately 2.5 mole/mole. The CdCl\textsubscript{2} was dissolved in drinking (tap) water at the concentration 300 mg/L. The control group drank tap water only.

\subsection*{Experimental design}

CdCl\textsubscript{2} in drinking water was consumed by two groups of five rats. These groups were characterized as follow: 5 animals (group 1) treated with water containing CdCl\textsubscript{2} for 6 months and 5 other animals (group 2) treated with water containing CdCl\textsubscript{2} for 6 months and for another period of 6 months with water free of CdCl\textsubscript{2}. Two other groups of 5 rats, one consuming water free of cadmium for 6 months (group C1) and the other for 12 months (group C2) were used as control for group 1 and 2, respectively. After the experimental period of each group, the animals were individually weighted and subsequently killed by ether anaesthesia. After being killed each animal was submitted to a procedure for removing the structures of interest, i.e, hard and soft palate, and gingiva.

\subsection*{Histopathologic examination}

Tissues were fixed in solution of 10\% formalin for 48 hours. Sections of hard and soft palate and gingiva were processed by standard histologic techniques, and stained with hematoxylin and eosin for light microscopy.

\subsection*{Morphometry}

The histologic sections were examined under light binocular microscopy (Zeiss, Germany) with objective of immersion (magnified one hundred times–100x), aided with a device built for karyometric and stereologic analysis\textsuperscript{13,14}.

\subsection*{Mitotic index}

A minimum of 1,000 cells in the basal layer were counted, and the number of cells showing arrested metaphase was determined.

\subsection*{Statistical analysis}

Data were analyzed using Mann-Whitney test, and significance was set at \( p < 0.05 \).

\section*{Results}

\subsection*{Body weight}

The difference between the mean of the body weight of rats from group 1 (367.60 ± 35.50g) and that of the control group C1 (541.80 ± 32.32g) was statistically significant (\( p = 0.004 \)). On the other hand, the difference between the mean of the body weight of
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Rats from group 2 (526.60 ± 38.19) and that of the control group C2 (541.40 ± 34.67) did not reach statistical significance (p = 0.210).

**Histopathologic and morphometric findings**

Analysis of tissue sections by standard techniques, using hematoxylin-eosin staining, showed a decrease of the epithelial thickness, along with variation in the size and shape of nuclei in the epithelium of the soft palate and gingiva of only the animals from group 1 (Figures 1 and 2). A detailed, comparative analysis of morphologic variation of nuclei between animals exposed to CdCl₂ and their respective controls was carried out based on measurement obtained using a karyometric device, and no statistical difference was found in this respect (p > 0.05). The stereological values (mean) for epithelial thickness are shown in Tables 1 and 2. Significant differences were found between group 1 and C1 with respect to the soft palate and gingival epithelium. For rats of group 2 and C2 there were no statistical differences.

**Table 1**

<table>
<thead>
<tr>
<th>Sites</th>
<th>Epithelial thickness (µm)</th>
<th>Group 1</th>
<th>Group C1</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Keratin layer</td>
<td>Keratin layer</td>
<td></td>
</tr>
<tr>
<td>Hard palate</td>
<td></td>
<td>15.06 ± 3.32 **</td>
<td>12.32 ± 1.45</td>
<td>p = 0.061</td>
</tr>
<tr>
<td></td>
<td></td>
<td>31.17 ± 4.99 **</td>
<td>33.29 ± 5.83</td>
<td>p = 0.274</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15.56 ± 1.69 **</td>
<td>16.46 ± 2.86</td>
<td>p = 0.274</td>
</tr>
<tr>
<td></td>
<td></td>
<td>61.80 ± 9.58 **</td>
<td>62.09 ± 8.07</td>
<td>p = 0.345</td>
</tr>
<tr>
<td>Soft palate</td>
<td></td>
<td>8.43 ± 0.96 **</td>
<td>10.81 ± 2.72</td>
<td>p = 0.111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20.13 ± 2.80 **</td>
<td>21.01 ± 3.40</td>
<td>p = 0.421</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.82 ± 2.51 **</td>
<td>12.52 ± 0.83</td>
<td>p = 0.274</td>
</tr>
<tr>
<td></td>
<td></td>
<td>39.39 ± 3.45 **</td>
<td>44.29 ± 5.62</td>
<td>p = 0.210</td>
</tr>
<tr>
<td>Gingiva</td>
<td></td>
<td>10.24 ± 0.68 **</td>
<td>10.63 ± 1.10</td>
<td>p = 0.274</td>
</tr>
<tr>
<td></td>
<td></td>
<td>23.91 ± 3.90 **</td>
<td>26.36 ± 3.94</td>
<td>p = 0.274</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19.55 ± 2.27 **</td>
<td>17.36 ± 2.01</td>
<td>p = 0.111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52.56 ± 3.75 **</td>
<td>52.35 ± 6.37</td>
<td>p = 0.300</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD
** nonsignificant; p > 0.05 (Mann-Whitney test)
* significant; p < 0.05 (Mann-Whitney test)

**Table 2**

<table>
<thead>
<tr>
<th>Sites</th>
<th>Mitotic index (n)</th>
<th>Group 1</th>
<th>Group C1</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard palate</td>
<td></td>
<td>29.8 ± 2.83 **</td>
<td>25.4 ± 2.57</td>
<td>p = 0.210</td>
</tr>
<tr>
<td>Soft palate</td>
<td></td>
<td>20.8 ± 2.54 **</td>
<td>20.4 ± 2.13</td>
<td>p = 0.160</td>
</tr>
<tr>
<td>Gingiva</td>
<td></td>
<td>26.2 ± 2.47 **</td>
<td>18.4 ± 1.26</td>
<td>p = 0.072</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD
** nonsignificant; p > 0.05 (Mann-Whitney test)

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Figure 2
Photomicrograph of a section of the gingiva of control (a) and cadmium-treated rats for 6 months (b), showing a decrease of the epithelial thickness in the epithelium of animals treated with Cd compared with controls. Hematoxylin and eosin stain (x200)

Discussion

In the present study the carcinogenic potential of Cd in the oral epithelium of rats was evaluated by morphologic and morphometric techniques. This investigation was carried out by experimental exposure of rats to CdCl₂ in drinking water (300mg/L). This concentration approaches the critical level for Cd toxicity, thus allowing for an investigation of its harmful action in the animal tissues.¹⁵,¹⁶

Our initial findings showed a decrease of body weight only in those animals exposed to Cd throughout the entire experimental period. In the animals of group 2, those that besides being exposed to Cd spent another equivalent period of time taking water free of Cd did not show a significant loss of body weight in comparison to their control group C2. This suggests that although Cd has a toxic effect on the corporal mass of the animals, this effect is likely to vary directly with the amount of Cd ingested and the time period of exposure. Other similar studies also described loss of weight in animals exposed to Cd.⁸,⁹,¹⁰

The changes in the tissues examined in this study were basically limited to a reduction of epithelial thickness in any of the tissue sections studied.

Mitotic index

No statistical difference was found with respect to the mitotic index of the oral epithelium of the animals exposed to CdCl₂ in comparison to their respective controls. These data are demonstrated in table 3.

Table 3
Influence of Cd on the mitotic index (n) in the oral epithelium of rats exposed to CdCl₂ (groups 1 and 2) and their controls (groups C1 and C2)

<table>
<thead>
<tr>
<th>Sites</th>
<th>Epithelial thickness (µm)</th>
<th>Group 1</th>
<th>Group C1</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hard palate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratin layer</td>
<td>17.14 ± 6.26^ns</td>
<td>17.57 ± 5.11</td>
<td>p = 0.421</td>
<td></td>
</tr>
<tr>
<td>Spinous layer</td>
<td>29.11 ± 7.39^ns</td>
<td>32.49 ± 4.32</td>
<td>p = 0.345</td>
<td></td>
</tr>
<tr>
<td>Basal layer</td>
<td>15.51 ± 2.23^ns</td>
<td>16.09 ± 0.90</td>
<td>p = 0.421</td>
<td></td>
</tr>
<tr>
<td>Total epithelium</td>
<td>56.00 ± 3.79^ns</td>
<td>64.37 ± 4.35</td>
<td>p = 0.080</td>
<td></td>
</tr>
</tbody>
</table>

| Soft palate  |                           |         |          |          |
| Keratin layer | 7.54 ± 1.43*              | 10.37 ± 0.44 | p = 0.004 |
| Spinous layer | 19.71 ± 4.06*             | 25.73 ± 4.89 | p = 0.028 |
| Basal layer | 12.83 ± 1.82^ns            | 14.25 ± 1.60 | p = 0.111 |
| Total epithelium | 40.09 ± 4.75*           | 50.35 ± 4.65 | p = 0.016 |

| Gingiva      |                           |         |          |          |
| Keratin layer | 9.30 ± 1.08^ns            | 9.20 ± 0.81 | p = 0.500 |
| Spinous layer | 22.28 ± 3.73*             | 27.29 ± 3.34 | p = 0.028 |
| Basal layer | 15.80 ± 2.50^ns            | 17.81 ± 1.49 | p = 0.111 |
| Total epithelium | 45.89 ± 6.68*          | 54.00 ± 3.59 | p = 0.048 |

Data are expressed as means ± SD
* mitotic index: number (n) of cells (of 1,000) showing arrested mitosis in the basal layer
^ nonsignificant; p > 0.05 (Mann-Whitney test)
Resumo

Cádmio (Cd) é um metal pesado que exerce uma variedade de efeitos tóxicos, crônicos e agudos, em organismos expostos. O objetivo deste estudo foi investigar o potencial carcinogênico do Cd no epitélio do palato e da gengiva de ratos Wistar. Dois grupos de animais foram estudados: grupo 1 que consistiu de 5 ratos expostos a cloreto de cádmio (CdCl₂) em água de bebedouro (300 mg/L) por um período de 6 meses; grupo 2 que também consistiu de 5 ratos submetidos às mesmas condições dos animais do grupo 1, mas permaneceu livre de Cd por um período adicional de 6 meses. Dois outros grupos (C1 and C2) com o mesmo número de animais, entretanto não expostos ao CdCl₂, foram usados como controle para o grupo 1 e 2, respectivamente. Todos os animais foram pesados antes e após o período experimental. Após terem sido sacrificados, os tecidos de interesse para o estudo foram fixados em formalina a 10%, processados por meio de técnica histopatológica padrão, corados em HE, e analisados sob microscopia de luz, utilizando parâmetros cariométricos e estereológicos. Perda de peso, atrofia do epitélio da gengiva e do palato mole foram os principais achados deste estudo, e verificados apenas no grupo 1 (\(p < 0.05\)). Em conclusão, Cd não produziu efeito carcinogênico nos tecidos orais, nas condições experimentais empregadas neste estudo.

Palavras chave:
- Cloreto de cádmio.
- Cancer oral.
- Carcinogênese.
- Rato

References