Effects of interaction Cd–Zn on serum-PSA level and prostate histology in rats

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ABSTRACT

Objective: To assess cadmium sulfate (CdSO₄) and zinc chloride (ZnCl₂) antagonist effects on the prostate specific antigen and prostatic cell organization in rats.

Methods: The study included 40 adult male rats, divided into four groups: Group 1 (CdSO₄), Group 2 (ZnCl₂), Group 3 (CdSO₄ e ZnCl₂) and Group 4 (control). Animals were treated with CdSO₄ and ZnCl₂ at the same dose (15 mg/L) during 30 days.

Results: It was showed a higher body weight and a lowering serum-prostate specific antigen concentration [(1.8 ± 0.6) ng/mL] in animals treated with CdSO₄. CdSO₄ induced a cyto-nuclear atypia, proliferative lesions, hyperplasia and precancerous foci in prostate tissue. Toxic effects of ZnCl₂ were not recorded in this study.

Conclusions: Protective role of zinc was exhibited against toxic effects of cadmium in prostate gland.

1. Introduction

The Cadmium (Cd) was in trace in waters. Human activities become a major source of emission of Cd in the atmosphere. Cd is a risk toxic metal for public health [1], Cd is classified as a type I carcinogen by the International Agency for Research on Cancer and the National Toxicology Program USA [2]. Studies showed Cd induced different cancers in the kidney, lung, testis and prostate tissues. Cd and zinc (Zn) have similar physicochemical properties [3]. In cells, Cd binds to metallothioneins (MTs). These MTs allow Cd’s detoxification. Therefore, Cd competes with its antagonist Zn [4]. The biological half-life of Cd is 10–30 years and only 10% of the Cd absorbed are excreted in the urine and feces. Cd toxicity induces massive cell injuries and tissue necrosis as lungs, liver and kidney [5]. Cd, in cells, induces synthesis of reactive oxygen species (ROS). These free radicals cause an oxidative damage in the cell membrane [6]. The mechanisms of Cd carcinogenesis are displayed in a simplified diagram (Figure 1). The cellular effect of Cd, in ionic form as Cd²⁺, could suppress some processes while enhancing others (Figure 1). Zn, as an antioxidant, inhibits free radicals synthesis [7]. Studies reported that Zn is able to interact with metals and decrease their tissue concentrations. Consequently, Zn inhibits an oxidative stress process [8]. Zn allows the stability of cell membrane through the synthesis of MTs and inhibition of the tissue Cd absorption and accumulation [9,10]. On the other hand, studies suggested an association of Cd-exposure with risk of prostate cancer [11]. Serum-prostate specific antigen (PSA) is used as a biological tumor marker in early detection of prostate cancer [12]. In patients with prostate cancer, an increased serum-PSA was observed. Studies have explored an association of PSA with Cd administered in animals [13,14]. This study aimed to assess the cadmium-sulfate (CdSO₄) toxic effects, simultaneously with zinc-chloride (ZnCl₂), on the PSA and an cell organization of prostate tissue in rats.

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All experimental procedures involving animals were conducted in accordance to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and approved by the Animal Ethics Committee of the UPPE (Process No. 012974).

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2. Materials and methods

2.1. Animals

Experiments were carried out on 40 adult male Wistar rats at weights ranging from 100 to 300 g. Animals were housed under standard environmental conditions at 22 °C with 12:12 h light–dark cycle and maintained with free access to water ad libitum [15]. Their body weight was measured every week. The experimental period was 30 days. The animals were divided into four groups with 10 rats in each group. In Group 1, rats received orally a dose of CdSO₄ at 15 mg/kg body weight per day. In Group 2, rats received orally a dose of ZnCl₂ at 15 mg/kg body weight per day. In Group 3, rats received a mixture (ZnCl₂ and CdSO₄) in the same experimental conditions. In Group 4, normal rats received a distilled water and standard diet, served as control group.

The experimental protocol was approved by the Animal Ethics Committee of the UFPF (Process No. 012974) in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals [15].

2.2. Preparation of CdSO₄ and ZnCl₂ doses

The toxic doses of CdSO₄ and ZnCl₂ were prepared by dissolving 15 mg Cd and Zn in 1000 mL of distilled water, respectively [16].

2.3. Blood-PSA assay

At the end of the experiment, animals were anesthetized by the intraperitoneal injection of sodium pentobarbital 40 mg/kg body weight. Blood samples were obtained from the hearts of animals and allowed to clot for 20 min in laboratory temperature and then centrifuged at 3000 r/min for 10 min for serum separation. Serum-PSA levels were measured by mini VIDAS automate analyzer (BioMérieux, Marcy-l’Étoile, France). The method used was an enzyme-linked fluorescent assay which is an enzyme immunoassay ELISA “sandwich” in heterogeneous phase where the molecules of PSA are caught between two monoclonal antibodies of murine nature. The levels of the PSA were obtained in two stages to a final detection by fluorimetry. A quality control was performed for each used kit VIDAS-PSA to validate the results.

2.4. Blood Cd²⁺ assay

Blood Cd²⁺ assay was carried out by the spectrophotometric analysis. A portion of blood was also collected in ethylene diamine tetracetic acid tubes to be analyzed by automate instrument named Abacus 4 Hematology Analyzer (Diatron, Budapest, Hungary).

2.5. Histological study

Animals were dissected to isolate prostate tissues. After draining the blood, prostate samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine serum albumin for 6 h. Paraffin sections were taken at 5 mm thickness, processed in alcohol-xylene series. For light microscopy, semi thin sections of prostate tissue were stained with alun haematoxylin-eosin (H & E) and examined with an Olympus BH-2 light microscope and photographed with Sony DSC-W610 digital camera (Sony Corporation Konan, Minato-ku, Tokyo, Japan).

2.6. Statistical analysis

Data are expressed as mean ± SD, with a value of P < 0.05 considered statistically significant. Statistical evaluation was performed by One-way ANOVA followed by the Tukey’s t-test for multiple comparisons. All analyses were made with the statistical software SigmaPlot (version 11.0).

3. Results

As shown in Table 1, animals were monitored and controlled during the experimental period. CdSO₄ induced significant decrease in body weight in animals of Group 1 compared to normal control rats (Group 4). Lowering of body weight was respectively illustrated by the following values (113.00 ± 8.10) g vs. (203.10 ± 30.10) g whereas animals treated with ZnCl₂ (Group 2) and with the CdSO₄–ZnCl₂ (Group 3) showed a statistically significant increase in body weight compared to experimental animals administrated with CdSO₄ (Group 1), and their body weights were respectively (125.40 ± 26.30) g and (172.20 ± 15.30) g vs. (113.00 ± 8.10) g.

A rising of prostate gland weight (Group 1) explained the higher Cd levels in the prostatic cells whereas in animals treated with Zn (Groups 2 and 3), prostate gland weight increased significantly (Table 1). These data elucidated an antagonist function of Zn that eliminated Cd and detoxified the prostate tissue from this toxic heavy metal. Although a slight increase of prostate gland weight was observed in animals of Group 1 [(3.10 ± 1.80) g], it remained statistically insignificant (P > 0.05) to other groups of animals, including Group 2 [(1.20 ± 0.10) g] and Group 3 [(3.00 ± 0.01) g] (Table 1).

Blood-PSA concentrations were nearly identic, in animals treated with CdSO₄ after 10 and 15 days, while after 20 days it showed a significant increase of blood-PSA in the same animals (Group 1). The blood-PSA concentrations were
(17.10 × 10^{-2} ± 2.60) ng/mL (Group 1) vs. (1.80 × 10^{-2} ± 0.60) ng/mL (Group 2) and (6.80 × 10^{-2} ± 1.00) ng/mL (Group 3) (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Prostate weight (g)</th>
<th>PSA (×10^{-2} ng/mL)</th>
<th>Cd^{2+} (×10^{-2} μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>113.00 ± 8.10^{2}</td>
<td>3.10 ± 1.80</td>
<td>17.10 ± 2.60</td>
<td>879.80 ± 152.10^{2}</td>
</tr>
<tr>
<td>Group 2</td>
<td>125.40 ± 26.30</td>
<td>1.20 ± 0.10</td>
<td>1.80 ± 0.60^{b}</td>
<td>74.00 ± 4.50</td>
</tr>
<tr>
<td>Group 3</td>
<td>172.20 ± 15.30</td>
<td>0.90 ± 0.01</td>
<td>6.80 ± 1.00</td>
<td>142.60 ± 50.30</td>
</tr>
<tr>
<td>Group 4</td>
<td>203.10 ± 30.10</td>
<td>0.40 ± 0.00</td>
<td>0.40 ± 0.30^{b}</td>
<td>3.20 ± 2.40</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.001</td>
<td>&gt; 0.05</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. a: Significant difference at P < 0.05; b: Highly significant difference at P < 0.001.

Animals, with higher PSA (17.10 × 10^{-2} ng/mL), showed a higher blood-Cd^{2+} concentration (879.80 × 10^{-2} μg/L) (Table 1), but the statistically significant difference of the distribution of the blood-Cd^{2+} concentrations was reported among Groups 2 and 3 (Table 1).

Histological study in control group showed a normal prostate tissue, with acini in spherical forms and a single layer of epithelial cells in prismatic size (Figure 2A). The prostate glands, in animals of Group 1, had an atypical hyperplastic structure. It was also observed large glands with fine and pronounced papillary folds. A large prismatic epithelium, projecting into the gland lumen, corresponded to an atypical hyperplasia (Figure 2B). Prostate atypical hyperplasia was the result of an excessive proliferation and an irregular differentiation associated to a loss of epithelium integrity, stroma and changes in nuclei forms. It was also reported large prostate glands with pseudo-papillary multi-layered epithelia. Prostate glands, in animals treated with only ZnCl₂ and with the mixture (CdSO₄ + ZnCl₂), had normal cells with organized structure and an accentuated glandular hyperplasia (Figure 2C, D).

### 4. Discussion

Studies suggested that Cd is involved in the carcinogenesis process of different tissues including prostate, liver and pancreas [17]. In this study, a lowering body weight of CdSO₄ treated animals is similar to the results reported by Bosland [18]. Although this study showed the Cd toxic effects in prostate tissues of animals, a rigorous caution has been considered in the analysis of the results about their extrapolation to human organism because the anatomy and histology of the prostate in both human and rat (vertebrate mammals) are considerably different. In other studies, the microscopy of murine prostate tissue could not detect impending neoplastic changes after CdSO₄ subcutaneous administration during an experimental period of two years [19]. Another study, conducted on rats and mice orally administered with CdSO₄ during two years, revealed similar results [20]. Furthermore, Olisekodiaka et al. reported a prostate adenocarcinoma in CdCl₂ intra prostatic injection rats at the dose used (80–400 mg/kg) [21]. Studies have supported the hypothesis that Cd, by orally way, has been associated with prostate cancer in humans [22]. In rat, Cd, orally administered, induced proliferative lesions and cell injuries at the prostatic ventral and lateral lobes. These lesions showed multiple foci in prostate lateral lobe, similar to those found in this study. Results of this study attest the carcinogenic potential of Cd on prostate lateral lobe. It was suggested that the prostatic lateral lobe of rat has structural and functional properties nearly similar to a human prostate. In this study, it has been revealed that the blood-PSA concentration was higher in animals administrated with CdSO₄ whereas this tumoral marker was lower in non-treated rats. This result incriminates Cd as a risk factor in the genesis and progression of prostate tumors.

Zn is another well-known antioxidant. Although it's an essential trace-element, it also plays important roles in testosterone production and spermatogenesis [23]. Furthermore, Zn reduces toxic effects of Cd and its antioxidant mechanism is not yet elucidated [24]. Nevertheless, molecular mechanisms were proposed for Zn protective activity. Zn stabilizes cell membranes and protects lipid from the free radical peroxidation [25]. Consequently, Zn induces hepatocellular MT production,
which is involved in protecting tissues against Cd toxicity [26]. Blood Cd and Zn concentrations increase proportionally with the age [27–29].

Zn and Cd had exhibited significant antagonist effects in prostate tissue. However, further studies are required to elucidate the mechanism of this antagonism.

Conflict of interest statement

We declare that we have no conflict of interest.

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References


