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Morphological changes and oxidative stress in rat prostate exposed to a non-carcinogenic dose of cadmium

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Abstract

Cadmium chloride is an environmental toxicant implicated in human prostate carcinogenesis. The mechanism of its toxicity is far from fully understood. This study evaluates the effect of exposure to an oral non-carcinogenic dose of cadmium (15 ppm in drinking water for three months) on different parameters of the ventral prostatic lobe of normal and exposed rats. We analyzed the histology by optic light microscopy, activities of antioxidant enzymes (CAT, SOD, GPx and G-6-PDH), expression of iNOS and COX-2 by Western blot, expression of MT-I, MT-II, IGF-I, IGF-BP5 and rtert by RT-PCR.

Histological changes were found: the height of the cells decreased, acinar lumen were enlarged and they lost the typical invaginations. Lipoperoxidation was increased in the Cd group and the antioxidant enzymes changed their activities: SOD increased, CAT and G-6-PDH decreased and GPx did not show variations. iNOS and COX-2 did not change their expressions. MT-I and IGF-BP5 mRNA increased while MT-II, IGF-I and rtert did not show variations.

Cd exposure induces important morphological changes in the prostate, which could be a consequence of lipoperoxidation and oxidative stress, which are not related to iNOS and COX-2. The histology suggests an involution state of the gland, confirmed by the expression of IGF-I, IGF-BP5 and rtert.

Keywords: Rat prostate; Cadmium; Oxidative stress; Involution; Antioxidant enzymes

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1. Introduction

Cadmium (Cd), one of the most important environmental and occupational metallic toxicants, is widely dispersed in the environment. High level exposure to this toxic heavy metal is usually the result of environmental contamination from human activities (Nordberg, 1972). Cadmium has been classified by the International Agency for Research on Cancer as a category 1 (human) carcinogen (IARC, 1993).

Exposure to cadmium has also been considered as one of the causes of human prostatic and testicular cancers (Waalkes and Rehm, 1994). Prostate epithelial dysplasia induced in rats by cadmium presents an increased proliferative activity and high expression of bcl-2 protein, as was described in human prostate intraepithelial cells (Martin et al., 2001). Characteristic DNA fragmentation and histopathologically observed changes characteristic of apoptosis are found in the kidney, prostate, seminal vesicles, testes, and epididymis 48 h after i.p. cadmium administration at a dose of 0.03 mmol/kg (Yan et al., 1997).

On the other hand, it is known that exposure to a changing environment causes cells to face conditions that shift their redox status to a more oxidized state, known as oxidative stress. Excess reactive oxygen species (ROS) are a harmful agent because they react with and modify all classes of cellular macromolecules and critical cellular targets that cause behavioral abnormalities, cytotoxicity and mutagenic damage (Floyd, 1990). It has been proposed that one mechanism of cadmium toxicity would be through the activation of oxidative stress parameters. However, to our knowledge, there are no studies exploring the modification of parameters of oxidative stress in prostate using different doses, exposition time and via of administration of cadmium.

Some studies indicate a possible role for NO in the signaling pathway leading to benign prostatic hyperplasia, and may suggest a different expression and distribution of NOS form in normal and affected prostates. On the other hand, it has been demonstrated that epithelial cells that express iNOS also express ciclooxygenase-2 (COX-2) (Feng et al., 1995). There is some controversy regarding its expression, some studies suggest that prostate cancer tissue shows a marked overexpression of COX-2 (Kirschenbaum et al., 2000). Meanwhile, O'Neill and Ford-Hutchinson (1993) suggest that among the human tissues examined, the highest levels of COX-2 expression were detected in the prostate. However, few studies about COX-2 expression in rat prostate tissues have been made (Harris et al., 2000).

It is well known that metallothioneins (MTs) are small, cysteine-rich proteins that can bind and store Zn and can also bind and detoxify Cd (Tempelton and Cherian, 1991). Four major types of MTs are found in mammals but two main forms of MT, namely MT-I and MT-II have been most extensively studied. These two isoforms are similarly regulated in rodents and are thought to be functionally equivalent (Searle et al., 1984).

There have been few models exploring chronic exposure to orally administered cadmium. For this reason, we used a model in which we try to mimic an environmental oral exposure to cadmium and find out its effect on the prostate, focusing our attention especially in the morphology of the gland and in oxidative stress parameters, as well as in iNOS and COX-2 expression. Simultaneously, we studied the expression of gene markers of involution and transformation processes. We carried out these experiments using a dose of 15 ppm of Cd because this is the level of contamination in the aquatic environment of our interest.

2. Materials and methods

2.1. Experimental model

Twenty-one days old Wistar male rats were separated in two groups: control group (Co) and cadmium exposed group (Cd). Cd group received 15 ppm of cadmium, administered as $CdCl_2 \cdot 2H_2O$ in drinking water (Cd) while Co group received tap water without Cd (Co). The concentration of cadmium in tap water and in the Cd solution was confirmed by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Animals were housed individually in a controlled environment with 12 h light:12 h darkness cycle at 21 °C, during three months and they were fed with rat chow (Cargill, Buenos Aires, Argentina). Food and water were available ad libitum. Care and treatment of rats followed recommended guidelines (US Public Health Service, 1985). During the experiment, body weight gains were registered weekly. Water and food intake were registered daily.

2.2. Tissue collection

After the three months treatment, animals were sacrificed by decapitation. Prostates were quickly removed, washed with ice-cold 0.9% saline solution and the ventral lobes were separated and frozen at −80 °C.

2.3. Quantification of Cd concentration on prostate

Prostate tissue was mineralized with 16N nitric acid as described by Clegg et al. (1981). Cadmium concentration was determined on the mineralized material by ICP-AES (Ihnat, 1990) in an atomic emission spectrometer (BAIRD, Model "ICP-2070", Bradford, USA). All the specimens were analyzed in duplicate.

2.4. Histological study

Histological studies were performed by light microscopy. The prostates were carefully excised from prostate–urethra–bladder complex from eight rats in each group. In all instances, representative tissue samples were fixed in Bouin's solution for 5 h. The samples were dehydrated in graded series of ethanol and embedded in paraffin. All sections were obtained from the same region of the prostate for effective comparison. Sections of 5–6 μm thickness were obtained using a Porter Blum Hn40 microtome and stained with hematoxylin–eosin. Photographs were obtained with a Leitz Dialux microscope equipped with a Leica camera.

2.5. Measurement of total metallothionein (MT) concentration

MT levels were determined by a modified Ag-Heme method without radioactive silver, as described by Scheuhammer and Cherian (1991). The content of silver in the samples was estimated by ICP-AES.

2.6. Measurement of parameters of oxidative stress

2.6.1. Tissue homogenates

Samples (300 mg of tissue) were homogenized in 30 mM PBS, 120 mM KCl, pH 7.4 containing a protease inhibitor cocktail, in a Teflon glass homogenizer at 4 °C. The homogenates were centrifuged at 800 × *g* for 10 min at 4 °C to remove nuclei and cell debris. The pellets were discarded and the supernatants were collected and used for the measurement of SOD, CAT, GPx and G-6-PDH activities and of TBARS.

2.6.2. Measurement of enzyme activities and lipid peroxidation

The activity of SOD was determined on the basis of its inhibitory action on the rate of superoxide-dependent reduction of cytochrome *c* by xanthine–xanthine oxidase at 550 nm. The reaction medium contained 50 mM phosphate buffer (pH 7.8), 50 μM xanthine, 20 μM cytochrome *c* and xanthine oxidase to detect 0.025 a.u./min (McCord and Fridovich, 1969). One unit of SOD is defined as the amount of enzyme required to inhibit the rate of cytochrome *c* reduction by 50%.

Catalase (CAT) activity was determined by measuring the decrease in absorption at 240 nm in a reaction medium containing 50 mM phosphate buffer (pH 7.3) and 3 mM H_2O_2. The pseudo-first-order reaction constant (k':k[CAT]) of the decrease in H_2O_2 absorption was determined and the catalase content in units/mg protein was calculated using: $k = 4.6 \times 10^7\ M^{-1}\ s^{-1}$ (Chance et al., 1979; Aebi, 1984). One unit of catalase activity is defined as the amount of enzyme required to decompose 1 μmol of hydrogen peroxide in 1 min.

Glutathione peroxidase (GPx) activity was determined measuring NADPH oxidation at 340 nm in a reaction medium containing 0.2 mM GSH, 0.25 U/ml yeast glutathione reductase, 0.5 mM *tert*-butyl hydroperoxide, and 50 mM phosphate buffer (pH 7.2) (Flohe and Gunzler, 1984). One unit of GPx activity is defined as the amount of GPx required to oxidize 1 μmol of NADPH/min. The enzyme activities were expressed as units of enzyme activity per milligram of protein.

To measure the glucose-6-phosphate dehydrogenase activity, prostates were homogenized with Tris–HCl buffer pH 7.4, containing 1 mM dithiotreitol (DTT). Homogenates were centrifuged at 100,000 × *g* for 1 h and the enzymatic activities were measured in the supernatant. G-6-PDH was determined by the rate of NADPH formation at 340 nm, according to Glock and Mc Lean (1953).

Using the TBA assay (Draper and Hadley, 1990) the levels of lipid peroxidation products, mainly mal-

ondialdehyde (MDA), were determined spectrophotometrically to be thiobarbituric acid reactive substances (TBARS).

2.7. Protein

Protein concentration was determined by the method of Lowry et al. with bovine serum albumin as a standard (Lowry et al., 1951).

2.8. Activity of iNOS

The specific activity of iNOS (nmol L-citruline/mg protein/30 min) was measured by monitoring L-[^{3}H] citruline formation from L-[^{3}H] arginine as substrate (Galea et al., 1995). Prostates were homogenized with 50 mM Tris–HCl (pH 7.8) containing 0.01% Triton X-100 and protease inhibitor cocktail, and centrifuged at 4 °C. Protein concentrations of the resulting supernatants were determined according to the method of Wang and Smith (1975), using BSA as standard. 20 μl of proteins were incubated at 37 °C in 50 mM Tris–HCl (pH 7.8), 0.5 mM NADPH, 5 μM FAD, 5 μM tetrahydrobiopterin (BH4), 20 μM L-arginine, and 1 μCi L-[^{3}H] arginine, in presence or absence of 1 mM *N*-monomethyl-L-arginine monoacetate (L-NMMA). The assay was made by triplicate. The reaction was stopped by the addition of 20 mM HEPES (pH 5.5) mixed with Dowex AG 50W-X8 RESIN (200–400 mesh, Na^+ form) previously equilibrated with HEPES and briefly centrifuged. L-[^{3}H] arginine was retained in the resin while L-[^{3}H] citruline was recovered in the supernatant and its concentration was determined by liquid scintillation counting.

2.9. Western blot analysis for iNOS

Prostates were homogenized as described previously. Forty micrograms of proteins were mixed with 10 μl of sample buffer (125 mM Tris–HCl, pH 6.8, 4% SDS, 3.5 mM DTT, 0.02% bromophenol blue and 20% glycerol), boiled for 2–3 min and loaded into an 8% SDS-PAGE gel. Protein molecular mass markers were always loaded on each gel. Separated proteins were transferred to PVDF membranes (Polyscreen NEF 1000, which were purchased from NEN Life Science Products) using a blot transfer system (BioRad Laboratories, Hercules, CA). After being blocked with 5% BSA–TBS solution (20 mM Tris, 500 mM NaCl, pH 7.5) overnight, at 4 °C with gentle agitation, membranes were incubated with a primary rabbit anti-iNOS polyclonal antibody solution (Santa Cruz Biotechnology Inc., Santa Cruz, CA.) (1:1000 dilution) for 1 h, at room temperature. After washing three times with TTBS (0.1% Tween 20, 100 mM Tris–HCl, pH 7.5, 150 mM NaCl), membranes were incubated with a secondary goat anti-rabbit IgG antibody linked to biotin for 1 h at room temperature (1:2000 dilution). Membranes were washed again and the color was developed using a Vectastain ABC—detection system (Vector Labs). The intensity of the bands was scanned densitometrically with the image processing and analysis program *Scion Image* and expressed on arbitrary units.

2.10. Western blot analysis for COX-2

COX-2 was determined as described previously for iNOS, but using a primary rabbit anti-COX-2 policlonal antibody solution (Santa Cruz Biotechnology Inc., Santa Cruz, CA) (1:1000 dilution).

2.11. RNA extraction

Total RNA was isolated by using TRIzol (Life Technologies). All RNA isolations were performed as directed by the manufacturers. Gel electrophoresis and ethidium bromide staining confirmed the purity and integrity of the samples. Quantification of RNA was based on spectrophotometric analysis at 260/280 nm.

2.12. Semi-quantitative RT-PCR

2.12.1. Reverse transcription reaction

Ten micrograms of total RNA were reverse-transcribed with 200 units of MMLV reverse transcriptase (Promega Inc.) using random hexamers as primers, following the manufacturers instructions. RT-generated fragments coding for β-actin, MT-I, MT-II, IGF-BP5, IGF-I and rtert were amplified, using PCR.

2.12.2. Polymerase chain reaction

PCR was performed in 35 μl of reaction solution containing 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 1.25 U of Taq polymerase, 50 pmol of each rat specific oligonucleotide primers and RT products (2/10 of RT reaction). The sequences of the different primers are shown in

Table 1
Sequences of the primers used to amplify different genes by RT-PCR and sizes of the fragments generated

cDNA	Forward (5′-3′)	Reverse (5′-3′)	Size (bp)
IGF-I	AAAATCAGCAGTCTTCCAAC	AGATCACAGCTCCGGAAGCA	299
IGF-BP5	TTGCCTCAACGAAAAGAGC	AGAATCCTTTGCGGTCACA	377
Rtert	CGTAAGAGTGTGTGGAGCAAG	TGCAGACTGCGCTTCATCC	145
MT-I	ACTGCCTTCTTGTCGCTTA	TGGAGGTGTACGGCAAGACT	312
MT-II	CCAACTGCCGCCTCCATTCG	GAAAAAAGTGTGGAGAACCG	297
β-Actin	CGTGGGCCGCCCTAGGCACCA	TTGGCCTTAGGGTTCAGAGGGG	243

Table 1. The expected sizes of the PCR products of MT-I and MT-II are 312 and 297 bp, respectively (Ghatak et al., 1996). The expected PCR product of β-actin is 243 bp, IGF-BP 5 is 377 pb, IGF-I is 299 pb and rtert is 145 pb. The samples were heated to 94 °C for 2 min, followed by 35 temperature cycles. Each cycle consisted of three periods: (1) denaturation, 94 °C for 1 min; (2) annealing, 53 °C for IGF-BP5 and IGF-I, 55 °C for β-actin, MT-I and MT-II and 60 °C for rtert for 1 min; (3) extension, 72 °C for 1 min. After 35 reaction cycles, the extension reaction was continued for another 5 min.

The PCR products were electrophoresed on 2% agarose gel with 0.01% ethidium bromide. The image was visualized and photographed under UV transillumination. The intensity of each band was measured by densitometric scanning and reported as the values of band intensity units. The relative abundance of each target band was then normalized according to housekeeping gene β-actin, calculated as the ratio of the intensity values of each target product to that of β-actin.

2.13. Statistical analysis

Values are expressed as mean ± standard error of mean (S.E.M.). Significant differences were considered at $P < 0.05$, as determined by Student's *t*-test.

3. Results

3.1. Quantification of Cd concentration on prostate

Prostate tissues were mineralized and cadmium was quantified by atomic emission spectrometry. The concentration of cadmium retained in the prostates of the rats exposed to Cd was significantly higher than in the control (Fig. 1).

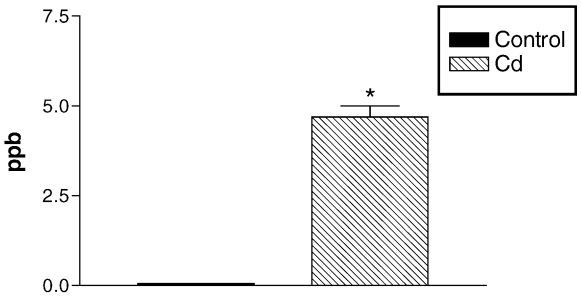

Fig. 1. Quantification of cadmium concentration on prostate. A significant increase of Cd was found in the exposed group (Cd). Asterisk indicates $P < 0.0001$ when compared to controls.

3.2. Body weight gains

After the first month of treatment cadmium exposure resulted in a significant reduction of the body weight gain of the rats (Fig. 2), however, there was no change in the intake of water or food (data not shown). Despite this, prostate weight did not show significant differences after the treatment (control: 0.805 ± 0.06 g; cadmium: 0.725 ± 0.09 g). At the end of the experience,

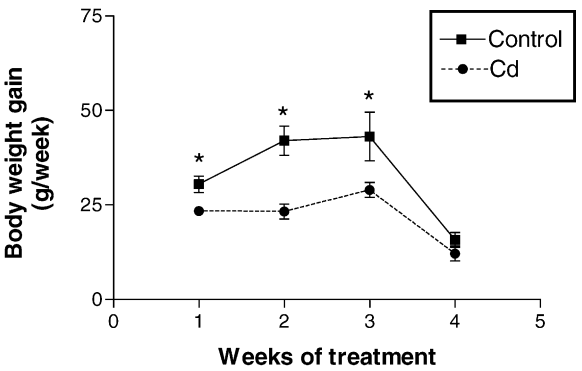

Fig. 2. Values are mean ± S.E.M. for eight animals per group. Asterisk indicates $P < 0.05$ of significance when compared to controls.

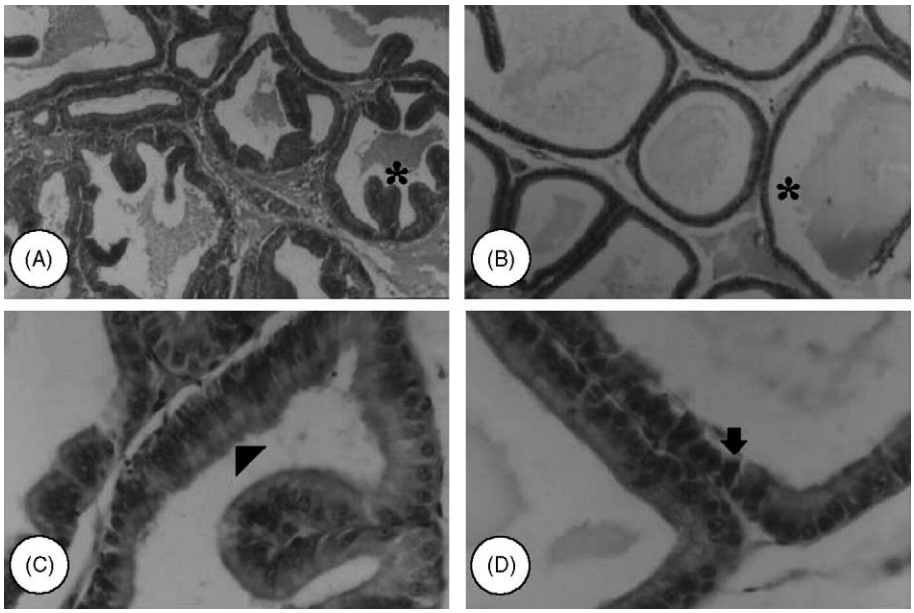

Fig. 3. (A) Control rat (not exposed to Cd). Glandular epithelium consists of tall columnar cells with basal located nuclei. H–E 100×. Abundant invaginations (asterisk) (B) After three months of cadmium exposure, the prostate is highly damaged. Significant decrease of epithelium height. Hypertrophy of the lumen diameter, where a non-homogeneous material without the colloidal normal characteristics is found. Loss of the normal invaginations. Note the presence of atrophic gland (asterisk). H–E 100×. (C) Detail of (A). The height of the epithelium indicates its secretory activity (arrowhead). H–E 400×. (D) Detail of (B). High magnification of the region showed in (B) exhibits cellular atrophy. Presence of picnotic nuclei (arrow). H–E 400×.

the difference of body weight gain was not significant between both groups (data not shown).

3.3. Histological studies

The prostates of both control and Cd rats were fixed, sectioned, stained and examined for evidence of injury. The prostate parenchyma of a control rat is shown in Fig. 3A and C. Dorsolateral and ventral prostates of Wistar rats appeared histologically normal. Tall columnar epithelial cells lined the prostatic acini, which were filled with an homogeneous secretion product. Significant morphological changes in prostate parenchyma were observed in Cd rats when compared to control (Fig. 3B and D). A significant reduction in the height of epithelial cells was noted in the atrophic epithelium. The lumen of the acini was found to contain a different secretion than that observed in control prostates. Likewise, there are some intensely stained nuclei, picnotics, may be in degeneration and the lumens changed its shape and size. This suggests a lack of functionality of the gland, thus a decreased secretory capacity.

3.4. Level of lipoperoxidation (TBARS)

TBARS were determined as indicators of lipid peroxidation (Draper and Hadley, 1990). After three months of exposure to Cd, lipid peroxidation products were significantly higher ($P < 0.001$) in prostates of cadmium rats (Fig. 4).

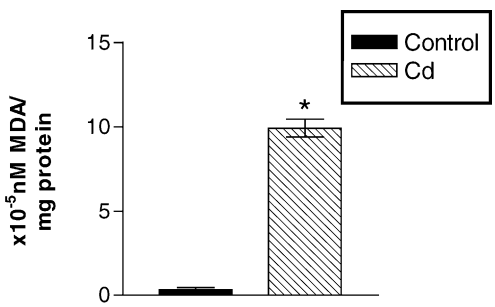

Fig. 4. A significant increase of TBARS was found in the exposed group (Cd). Asterisk indicates $P < 0.0005$ of significance when compared to controls.

Table 2
Antioxidant enzyme activities in the prostate of exposed to Cd and control Wistar rats

Antioxidant enzymes	Control	Cd
Catalase** ($\times 10^{-14}$ mol H_2O_2/(min/mg protein))	3.66 ± 0.42	0.87 ± 0.11
GPx (mmol NADPH/min/mg protein)	5.6 ± 0.74	5.52 ± 0.85
SOD* (UI/mg protein)	148 ± 13	249 ± 19.2
G-6-PDH* (mmol NADPH/min/mg protein)	116.4 ± 1.3	73.6 ± 10.1

GPx, glutathione peroxidase; SOD, superoxide dismutase; G-6-PDH, glucose-6-phosphate dehidrogenase; Cd, cadmium; UI, international units.
* $P < 0.005$.
** $P < 0.0005$.

3.5. Antioxidant defense system

We found a significant decrease of CAT activity while GPx activity did not show variations. The activity of SOD increased significantly in the Cd group. G-6-PDH decreased its activity in the Cd group (Table 2).

3.6. Metallothionein

MT levels were determined as described previously and we found that total MT concentration was increased in the Cd group when compared to controls (Fig. 5A). It is known that there are four types of metallothioneins, but two main forms have been most extensively studied: MT-I, which is rich in Cd, and MT-II, which is rich in Zn (Vallee, 1995). To confirm the previous result, we studied MT-I mRNA expression using RT-PCR and we found that the predicted 312 pb fragment was more expressed in the Cd group. The analysis of MT-II mRNA expression did not show variations (Fig. 5B).

3.7. Effect of cadmium on iNOS

iNOS activity was determined and it did not show differences among both groups either (Fig. 6). We confirmed it by western blot, which did not show variations on iNOS expression among both groups (Fig. 7).

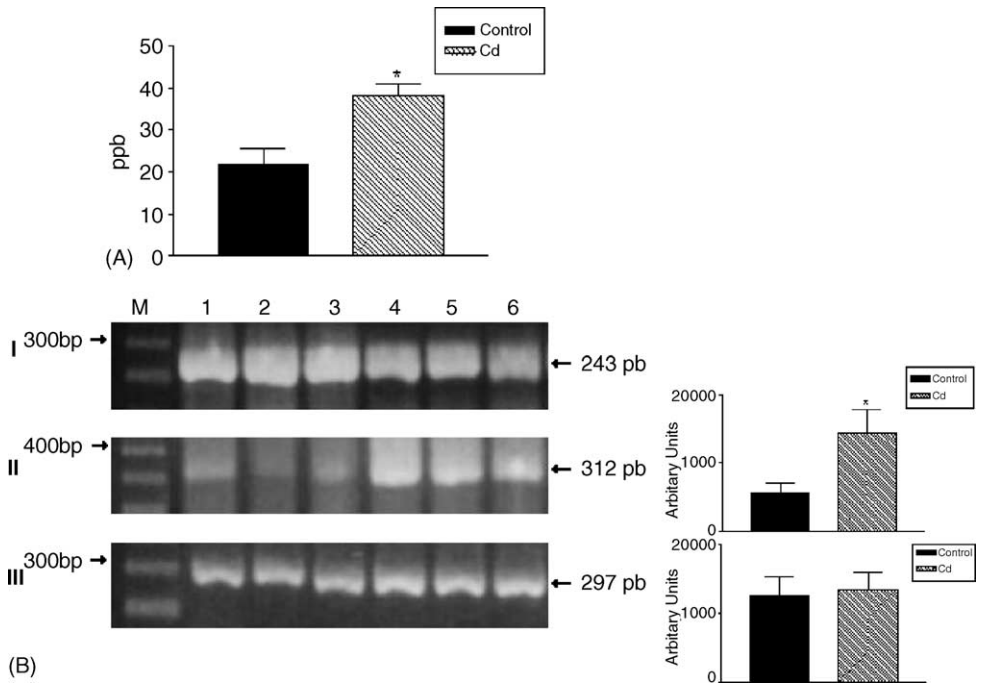

Fig. 5. (A) Total MT concentration on rat prostate. RT-PCR. (I) Ethidium bromide-stained agarose gel of β-actin PCR products, used as an internal control. (II) Ethidium bromide-stained agarose gel of MT-I PCR products. (III) Ethidium bromide-stained agarose gel of MT-II PCR products. M: molecular weight marker. Lanes 1–3: control prostates. Lanes 4–6: Cd prostates. On the sides, quantification of the intensity of the fragment bands in relation to the intensity of the internal control bands. Asterisk indicates $P < 0.005$ of significance when compared to controls.

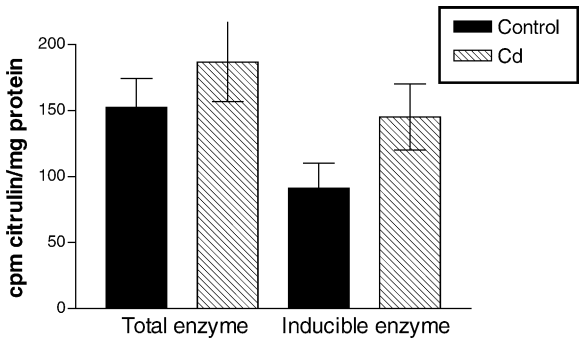

Fig. 6. Cadmium does not modify the activity of iNOS in rat prostate. Values are mean ± S.E.M. for eight animals per group. Bars represent mean ± S.E.M.

3.8. Effect of cadmium on COX-2

The next criterion tested was to determine if there was a contribution of COX-2 in prostate injury as a consequence of cadmium intoxication. We analyzed COX-2 by Western blot, which did not show variations on its expression in Cd prostate when compared to controls (Fig. 8), as expected.

3.9. RT-PCR

Given the results of the histological studies, we decided to study some molecular markers in order to know if there was an involution state or a transformed one.

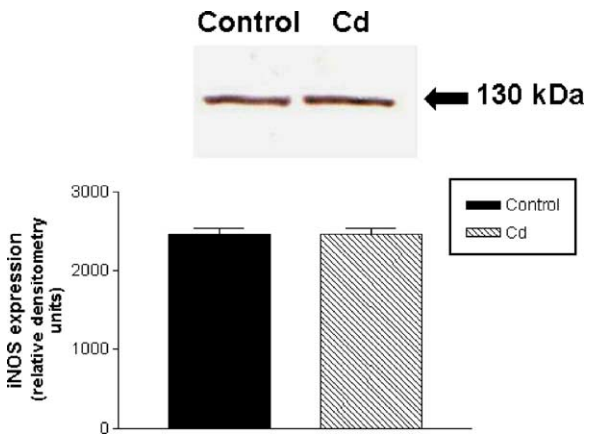

Fig. 7. Cadmium does not modify the expression of iNOS in rat prostate. iNOS was detected by immunoblotting. One representative Western blot of four experiments is shown. Densitometric analysis of the blots is represented as relative densitometry units ($n = 8$). Bars represent mean ± S.E.M.

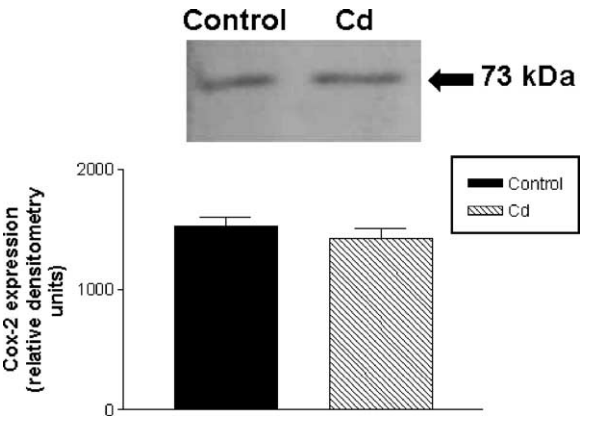

Fig. 8. Cadmium does not modify the expression of COX-2 in rat prostate. COX-2 was detected by immunoblotting. One representative western blot of four experiments is shown. Densitometric analysis of the blots is represented as relative densitometry units ($n = 8$). Bars represent mean ± S.E.M.

We studied the genes IGF-I and IGF-BP5, whose expression is modified in prostate involutions states after castration (Nickerson et al., 1998) and rtert, the catalytic portion of the telomerase, which is increased on transformed cells (de Kok et al., 2000). β-Actin was used as an internal control.

The RT-PCR performed on our models showed a significant increase of IGF-BP5 without a significant variation of IGF-I expression, although it showed a trend to decrease. Under these experimental conditions the expression of rtert showed no alterations (Fig. 9).

4. Discussion

The goal of these studies was to examine the effect of chronic exposure to cadmium on the histology of the ventral prostate and to analyze if its variations are related to changes in different parameters of oxidative stress as well as to modifications in markers associated to involution or cellular transformation. For this purpose, a rat model that mimics the environmental and occupational exposure to cadmium was used.

Many studies have been made in order to shade light on cadmium intoxication effects, but most of them have been made in vitro or by injection administration (Martin et al., 2002). Our model, in which the animals were subjected to oral cadmium exposure for

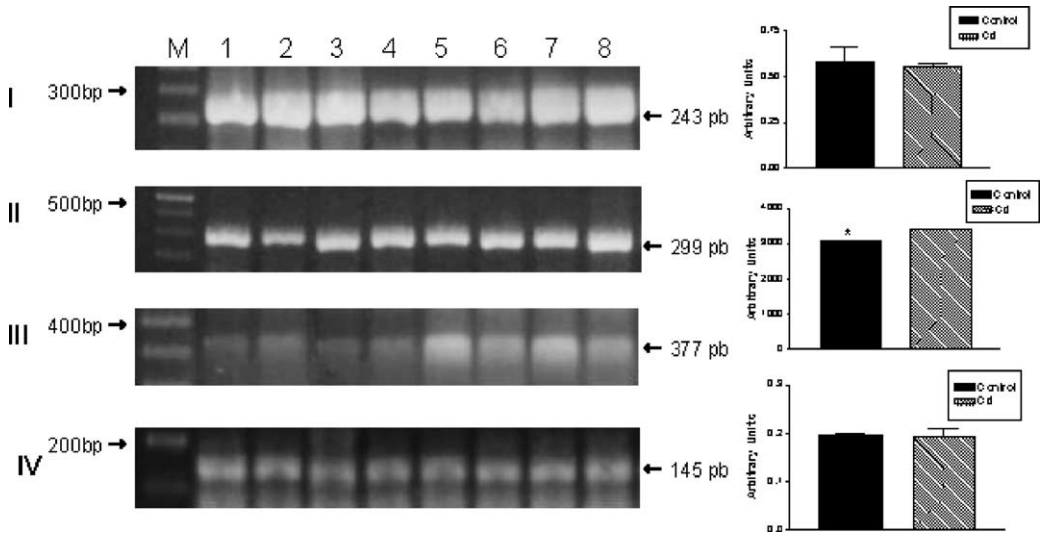

Fig. 9. (I) Ethidium bromide-stained agarose gel of β-actin PCR products, used as an internal control. (II) Ethidium bromide-stained agarose gel of IGF-I PCR products. (III) Ethidium bromide-stained agarose gel of IGF-BP5 PCR products. (IV) Ethidium bromide-stained agarose gel of rtert PCR products. M: molecular weight marker. Lanes 1–4: control prostates. Lanes 5–8: Cd prostates. On the sides, quantification of the intensity of the fragment bands in relation to the intensity of the internal control bands. Asterisk indicates $P < 0.005$ of significance when compared to controls.

three months, has the advantage that it is physiologically more relevant.

In the present study, oral cadmium exposure influenced the weight gain of the rats, which is consistent with what Zeng et al. (2003) showed; however there was no change in the intake of water or food (data not shown).

All the changes observed suggest an involution or a degenerative state, which could be a consequence of cellular oxidative damage. It is known that cadmium induces oxidative stress in many organs or cells (Ramirez, 2003). For instance, in this same model, different parameters of oxidative stress were assayed in liver, such as TBARS and activity of some enzymes of the antioxidant defense system (SOD, CAT and GPx). These experiments showed an increased level of lipoperoxidation and modified activity of the enzymes, what suggest a severe oxidative environment (unpublished data). On the other hand, there are no studies that show modifications of parameters of oxidative stress in prostate. In order to determine if the morphological changes observed in the ventral prostate are associated to a redox balance alteration, we analyzed several parameters of oxidative stress. Concerning the effect of Cd on the antioxidant defense system, and in agreement with the observation done by other authors in other organs, (Jentzsch et al., 1969; Ramírez and Gimenez, 2002) we found that cadmium intoxication increases peroxidation products, as indicated by prostate TBARS. It is known that TBARS produced by lipid peroxidation may cause cross-linking and polymerization of membrane components (Spatz, 1992). Besides, this may alter intrinsic membrane properties, such as fluidity, ion transport, enzyme activity and the aggregate state of cell surface determinant (Rong et al., 1996). Oxidative stress induced by Cd exposure could alter cellular membranes, modifying the lipid composition and functionality of the cells (Koizumi et al., 1996).

The exact mechanisms by which cadmium induces oxidative stress remain to be clarified. The observed alterations in components of the oxidant defense system associated to cadmium exposure, such as glutathione depletion (Wahba et al., 1990; Koizumi and Li, 1992) and the inactivation of antioxidant enzymes such as SOD or GPx (Omaye and Tappel, 1975; Hussain et al., 1987) may lead to increases in the steady-state level of oxidants.

Previous investigators have documented that O_2^- can inactivate catalase and peroxidase (Metodiewa and

Dunford, 1989). Perhaps, this is one of the probable reasons for the decreased activity of CAT. On the other hand, our finding suggests that SOD may be able to minimize the negative effects of O_2^- on cellular catalase. Furthermore, SOD may decrease the superoxide anion radicals that are available to react with H_2O_2, resulting in the formation of hydroxyl radicals (Liochev and Fridovich, 1994). SODs can also inhibit any free radical chain reaction in which superoxide serves as initiator and/or propagator and hence decrease the net production of H_2O_2 (Grisham, 1994). However, G-6-PDH showed a decrease on its activity under Cd treatment. The lower levels of NADPH as a result of the decreased activity could increase the oxidative stress observed by us. Besides, Tavazzi et al. (1999) determined that pyridine coenzymes could be considered as new target molecules of ROS. Thus, prostate damage in terms of lipid peroxidation can be explained by the disturbed defense mechanism activities. The elevated activity of SOD would lead to increased superoxide radicals, which could inactivate CAT and therefore, create a toxic environment for the cell.

On the other hand, it is known that Cd also acts indirectly by inducing the production of metallothionein (Waalkes and Goering, 1990). MT can bind metals with known pro-oxidant activity and alternatively it can scavenge hydroxyl radicals and singlet oxygen (Oteiza et al., 1996). However, there is still some controversy regarding the expression of MT mRNA in the ventral prostate of the rat (Coogan et al., 1995). We found an increased expression of MT-I in the ventral lobes of prostates exposed to cadmium, which could reflect a protective response to Cd; while MT-II expression did not show variations. Consequently, we can affirm that the elevated concentration of total MT is due to an increased expression of MT-I.

Controversial information was previously published respect to the effect of Cd on NO production. For example, Tian and Lawrence (1996) have found an inhibitory effect of Cd on NO production. On the other hand, Hassoun and Stohs (1996) have observed a Cd-dose-dependent increment in NO production after 48 h of incubation. Most of these studies have been made on macrophages. Then, under our experimental conditions, cadmium exposure did not modify the expression of iNOS and the production of NO. This suggests that the oxidative stress induced by the heavy metal is not related to the generation of NO.

There is wide evidence that implicates COX-2 in inflammation (Feng et al., 1995). In our studies, we did not find alterations in the expression pattern of COX-2 in prostate exposed to Cd. This is consistent with the histological findings, which did not show infiltrations or inflammatory processes.

Given the results of the morphological assays, we studied molecular markers of involution. It is known that insulin like growth factor-I (IGF-I) is an intraprostatic growth factor, which promotes cell growth and differentiation in many tissues, including the prostate, through interactions with IGF-receptors (Cohick and Clemmens, 1993). Regulation of IGF activity occurs by the intervention of insulin-like growth factor-binding protein (IGFBP). IGFBPs can inhibit IGF activity by reducing its bioavailability through receptor competition (Perkel et al., 1990). It has been shown that after castration, the prostate undergoes an involution state, characterized by an IGF-I decrease and IGF-BP5 increase (Nickerson et al., 1998). In our study, we found that the expression of IGF-I mRNA showed a trend to decrease while IGF-BP5 increased significantly, as was observed in castrated rats by Nickerson et al. (1998).

As previous studies have described preneoplastic changes caused by cadmium exposure (Waalkes and Rehm, 1992; Martin et al., 2001), we also analyzed the expression of rtert, the catalytic portion of the telomerase, which is increased on differentiated and tumor cells (de Kok et al., 2000). Its expression was not modified under our experimental conditions.

These results suggest that cadmium exposure induces involutive changes in the prostate, rather than preneoplastic ones. Anyway, we have to consider that the previous studies that suggested those neoplastic states were made under higher Cd concentration and during a longer time of exposition (Martin et al., 2001), while the one used here is considered a non-carcinogenic concentration and a situation closer to reality (Koizumi and Li, 1992).

Thus, chronic cadmium intoxication produces important morphological alterations in the ventral prostate, due to modifications in the activity of enzymes of the antioxidant defense system and to a consequent high level of lipoperoxidation. This oxidative stress is apparently produced by ROS, but not by the activity of iNOS or COX-2. Those morphological alterations could be associated with an involution state, as suggested by the increase of IGF-BP5.

Our results are a contribution to the knowledge about the mechanism of action of cadmium on prostate gland when it is ingested in non-carcinogenic doses. It is also important as a risk assessment study by correlating the exposure risk of low dose cadmium with prostatic tissue injury and oxidative stress.

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