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Apoptosis and p53 gene expression in male reproductive tissues of cadmium exposed rats

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Reverse transcription (RT) PCR technique was used to investigate the mechanism of apoptosis induced by Cd and the change of its related genes in testes and prostate of rats. Adult male rats were given a single (s.c.) injection of $CdCl_2$ 0, 2.5, 5.0, 10 μmol/kg. 48 h and 72 h after administration of Cd, animals were sacrificed. The results indicated that Cd can induce apoptosis in testes via p53-independent pathway. No apoptosis occurred in prostate in any of the Cd-exposed groups. There was a clearly negative relationship in testes between p53 gene expression and Cd exposure and this dose-response relationship was observed both at 48 h and 72 h. There was a very small increase of this gene expression in the dorsolateral lobe of the prostate in Cd exposed groups. The other apoptosis related gene, bcl-x, was not detectable in either control or Cd-exposed group in testes and dorsal prostate. Although the MT-I gene was expressed in testes or dorsal prostate both in control and exposed groups, no overexpression of MT-I gene was found after administration of Cd . The expression of MT-I in the ventral prostate was not detected in the control group, but a weak expression was found after Cd exposure. Since p53 is a tumor suppressor gene which can inhibit tumorigenesis, the consequence of a Cd-induced decrease of p53 in testes may have a relation to the known risk of Cd tumorigenesis in this tissue.

Keywords: cadmium; apoptosis; RT-PCR; p53 gene expression; testes; rat

Introduction

Cadmium-induced toxicity and carcinogenesis in male reproductive tissues are well known phenomena (Parizek 1957; Gunn *et al.* 1963; Waalkes *et al.* 1988) whose molecular mechanisms are largely unknown. A role of metallothionein (MT) has been discussed since the first observations by one of the present authors (Nordberg 1971), but the recent literature has reported variable findings which have been partly conflicting (Nolan & Shaikh 1986; Abel *et al.* 1991; De *et al.* 1991; Shiraishi *et al.* 1993 a,b; Shiraishi and Waalkes 1994). Cadmium can induce tumors in the interstitial tissue of the testicle (Gunn *et al.* 1963) and in the prostate (Waalkes *et al.* 1988). The testicle and the ventral lobe of the prostate are more sensitive to cadmium-induced carcinogenesis than many other tissues (Waalkes *et al.* 1992; Waalkes and Rehm, 1994). An explanation that has been advanced is that there is no cadmium-induced MT gene expression in these tissues. (Shiraishi *et al.* 1993 a, b; Shiraishi and Waalkes 1994). However, this hypothesis and related findings appear not to agree with the findings of other studies that MT is expressed in the testes after Cd exposure (Nordberg 1971; Abel *et al.* 1991; De *et al.* 1991; Nolan & Shaikh 1986).

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Recently, one study has indicated that apoptosis (or programmed cell death) plays an important role in the biochemistry and molecular biology of cadmium damage to testes (Xu *et al.* 1996). Although apoptotic cell death occurs under a variety of physiological situations, it has become apparent that apoptosis also occurs under conditions where cells suffer sub-lethal injury. Apoptosis is regulated by its related genes, such as P53, bcl–2. DNA damage can induce apoptosis via either p53-dependent (Symonds *et al.*, 1994; Lowe *et al.*, 1993; Clarke *et al.*, 1993) or p53-independent pathways (Strasser *et al.*, 1994, Clarke *et al.* 1993). Cadmium can induce apoptosis in testes (Xu *et al.* 1996) and in human tissues or cell lines (Azzouzi *et al.* 1994), however, the mechanism by which Cd can induce apoptosis in testes has been unknown so far and it is not known whether cadmium can induce apoptosis in prostate. Furthermore, p53 gene is also a tumor suppressor gene, and a limited suppression of this gene is considered important for tumorigenesis. Whether or not cadmium can affect p53 gene expression is also unknown. It is of great importance to investigate the apoptosis-related genes in the course of cadmium-induced apoptosis in testes and prostate and their possible contribution to cadmium carcinogenesis.

Thus, reverse transcription polymerase chain reaction (RT-PCR) and Southern blot hybridisation techniques were used in the present study to explore this question. MT-I gene expression was also studied in testes and prostate to investigate the effect of MT-I on early tissue changes from cadmium ultimately leading to carcinogenesis in testes and prostate.

Material and methods

Materials

Cadmium chloride was purchased from Mallinchrodt Inc. (Paris, Kentucky). Proteinase K, RNase A were purchased from Boehringer Mannheim (Germany). AquaPhenol was purchases from Appligen-Oncor (Gaithersburg, USA). Ready-to-go T -Primed First-Strand Kit was purchased from Pharmacia Biotech (Uppsala, Sweden). The RNA isolation system was purchased from BIOTECX LABORATORIES, INC. (USA). Other chemicals were purchased from Sigma Chemical Co. (St. Louis. MO, USA).

Animals and treatment

16 Male Wistar rats weighing approximately 150g were obtained from Mollegaard Breeding & Research Center A/S, Denmark. Rats were housed in polycarbonate cages and allowed free access to food and tap water at all times with 12-h light/dark cycle. After 14 days, the average weight of rats was 200±15 g . Permission for these experiments was obtained from the ethics committee for animal experiments in Umeå (permission No. A29/97).

$CdCl_2$ solutions were prepared in physiological saline and injected in a single subcutaneous dose into the dorsal thoracic midline of male Wistar rats. $CdCl_2$ solutions were administrated at 4 ml/kg s.c at levels 0, 2.5, 5.0, and 10 μmol/kg. There were two rats in each group representing a specific time and dose in the present study. 48 h and 72 h after injection of $CdCl_2$, rats were sacrificed after anaesthesia with pentobarbital sodium (60 mg/kg, i.p) and testes and prostates were removed immediately for DNA and for reverse-transcription PCR (RT-PCR) analysis. In a preceding preliminary study on 12 Wistar rats the same analyses were performed (except p53 and bcl-x) on an additional two animals per dose group (doses 0, 2.5 μmol/kg and 10 μmol/kg).

DNA isolation

The DNA was extracted by the methods of Sambrook *et al.* (1989) with some modifications as follows. Tissues were put into liquid nitrogen in a prechilled mortar and then ground to a powder using a pestle. The liquid nitrogen was allowed to evaporate, the powered tissues were added, in portions, to 10 volumes of extraction buffer (10 mM Tris-HCl (pH 8.0), 0.1 M EDTA and 0.5% SDS) in a 50-ml centrifuge tube, proteinase K was added to a final concentration of 50 μg/ml and the mixture was then incubated for 2 h at 50°C. The solution was cooled to room temperature, and an equal volume of phenol equilibrated with 0.5 M Tris-HCl (pH 8.0) was added and gently mixed by slowly turning the tube end over end for 10 min.. The mixture was centrifuged at 5000 X g for 15 min at 4°C. The aqueous phase containing nucleic acid was carefully collected, and the lower phase and interface containing denatured proteins and phenol was discarded. Phenol was placed into the supernatant, gently mixed and then centrifuged as above. This procedure was repeated three times. After that, DNA was precipitated by addition of 20 μl of 3 M sodium acetate/ml extraction solution and 0.7 vol. of 2-propanol, gently mixed and then placed in a freezer at –20°C for 4 h. The DNA was precipitated and centrifuged at 5000 X g for 10 min at 4°C. The supernatant was discarded, the pellet was rinsed twice with 70% ethanol, and briefly dried in vacuum for 5–10 min. DNA was dissolved in 2 ml DEST.H_2O, and then RNase A (1 unit to 50 μg/ml RNA) was added with incubation for 1 h at 37°C. The OD_{260}/OD_{280} absorbency ratio was employed to determine the purity of DNA.

RNA Extraction

RNA isolation procedure was adapted from the method provided by the BIOTECX LABORATORIES, INC. Briefly, 10–100 mg of fresh tissue was homogenised with 1 ml Ultraspec™ RNA reagent in hand-held glass-teflon homogenizer. Following homogenization, the homogenate was stored for 5 min at 4°C to permit the complete dissoci-

ation of nucleoprotein complexes. Next, 0.2 ml of chloroform was added, samples were covered tightly, vigorously shaken for 15 seconds and placed on ice at 4°C for 5 min. The mixture was centrifuged at 12 000g (4°C) for 15 min. After centrifugation, the volume of aqueous phase should be about 40–50% of the total volume of the homogenate plus chloroform. The aqueous phase (4/5th volume) was carefully transferred to a fresh tube and then equal volume of isopropanol was added, and stored for 10 min at 4°C. The samples were centrifuged at 12 000g (4°C) for 10 min. RNA precipitate formed a white pellet at the bottom of the tube. After removing the supernatant, RNA pellet was washed twice with 1 ml of 75% ethanol by vortexing and subsequent centrifugation for 5 min at 7 500g (4°C). At the end of the procedure, the pellet was briefly dried under a vacuum for 5–10 min. It is important not to let the RNA pellet dry completely, as it will greatly decrease its solubility. At last, the RNA pellet was dissolved in 50–100 μl DEPC-treated water by vortexing for 1 min. Purity of the RNA was determined by the ratio of the absorbance at 280 and 260 nm. The RNA yield was calculated based on absorption at 260 nm (A_{260} of 1.0=40 μg of RNA).

First strand cDNA synthesis

cDNA was synthesized using T-primed First-Strand kit. The buffer and dNTP conditions in the completed first-strand cDNA reaction were 45 mM Tris (pH 8.3), 68 mM KCl, 15 mM DTT, 9 mM $MgCl_2$, 0.08 mM/ml BSA, 1.8 mM each dNTP and *Not* I-d$(T)_{18}$ primer (5′-d[AACTGGAAGAATTCGCGGCCGCAGGAAT$_{18}$]–3′). cDNA synthesis was performed at 37°C for 60 min. 2 μg of total RNA of each sample was used for the synthesis of cDNA.

PCR amplification

Following first strand cDNA synthesis, PCR amplification was carried out in a reaction mixture (100 μl total volume) containing 15 μl first strand cNDA, 2.5 units Taq polymerase (Promega, Madison, WI), 120 pmol forward primer, 120 pmol reversed primer, 0.08 mg/ml BSA, 2.0 mM each dNTP, and 2.0 mM $MgCl_2$. For MT-I, the expected size of the PCR product is 312 bp in length, representing over the entire coding sequence of rat MT-I (Ghatak *et al.* 1996). The expected PCR product of rat ß-actin is 240 bp (Shankland Scholey 1994), and p53 is 270 bp (Will *et al.* 1995), and bcl-x_L is about 800 bp and bcl-x_S is about 600 bp (Boise *et al.* 1993). The samples were heated to 94 °C for 3 min, followed by 35 temperature cycles. Each cycle consisted of three periods: (1) Denature, 94°C for 1 min; (2) annealing, 60°C of ß-actin, and 58°C of bcl-x, and 56°C of P53, and 55°C of MT-I for 1 min; (3) extend, 72°C for 3 min. After 35 cycles of reaction, the extension reaction was continued for another 10 min.

Analysis of DNA and PCR products

All samples including DNA and PCR products were analysed by electrophoresis on 1.5% agarose gel after mixing with loading buffer (0.25% bromphenol blue + 0.25% xylene cyanole FF and 30% glycerol). The gel was visualized and photographed under UV light after reacting with 0.01% ethidium bromide.

Southern blot hybridization

After electrophoresis on 1% agarose gels, P53 PCR products were transferred by capillary blotting onto nylon filters (Boehringer, Germany) according to a standard method (Sambrook *et al.* 1989, the book *Molecular Cloning*). A synthetic oligonucleotide with the sequence GCTACCCGAAGACCAAGAAGG derived from the exon 9 of P53 gene was labelled using the DIG oligonucleotide labelling kit (Boehringer). The hybridization was performed in 5′ SSC (1′ SSC = 0.15 M NaCl, 15 mM trisodiumcitrate) 0.1% N-lauroyl sarcosine, 0.02% sodium dodecyl sulfate (SDS), and 1% blocking reagent (Boehringer). The filters were prehybridized for 2 hours and hybridized for 6 hours. The filter was then washed two times in 2 * SSC, 0.1% SDS for 5 minutes and two times in 0.1* SSC, 0.1% SDS for 5 minutes at 40°C. Chemiluminescent detection was carried out according to the manufacturer's protocol (Boehringer). The filter was exposed to X-OMAT S film (Kodak) at room temperature for 15 minutes. The band densities were quantified by densitometry (Molecular Dynamics, Sunnvale, CA).

Results

Cadmium induced apoptosis in testes and prostate of rats

The occurrence of Cd-induced apoptosis in rat testes is indicated by DNA fragmentation demonstrated on agarose gel (Fig 1–1). Both medium dose of $CdCl_2$ (5.0 μmol/kg) and high dose of $CdCl_2$ (10 μmol/kg) can induce such changes in testes at both 48 h (lane 4, 5) and 72 h (lane 7, 8) after administration of cadmium. There is no apoptotic DNA fragmentation at the dose of 2.5 μmol/kg $CdCl_2$ at 48 h or 72 h after administration of cadmium. Comparison of the 10 μmol/kg $CdCl_2$ group between 48 h and 72 h shows more DNA fragmentation after 72 h than after 48h. This indicates that Cd-induced apoptosis at high dose may be progressively aggravated with increasing time after the cadmium treatment. This phenomenon is not apparent at the dose of 5.0 μmol/kg $CdCl_2$. There is no evidence that cadmium can induce apoptosis in ventral or in dorsolateral lobes of prostate in rats (Fig. 1–2, and Fig. 1–3). The doses of cadmium used in this study failed to give rise to apoptosis both at 48 h and 72 h after administration of cadmium when detected by DNA gel electrophoresis method.

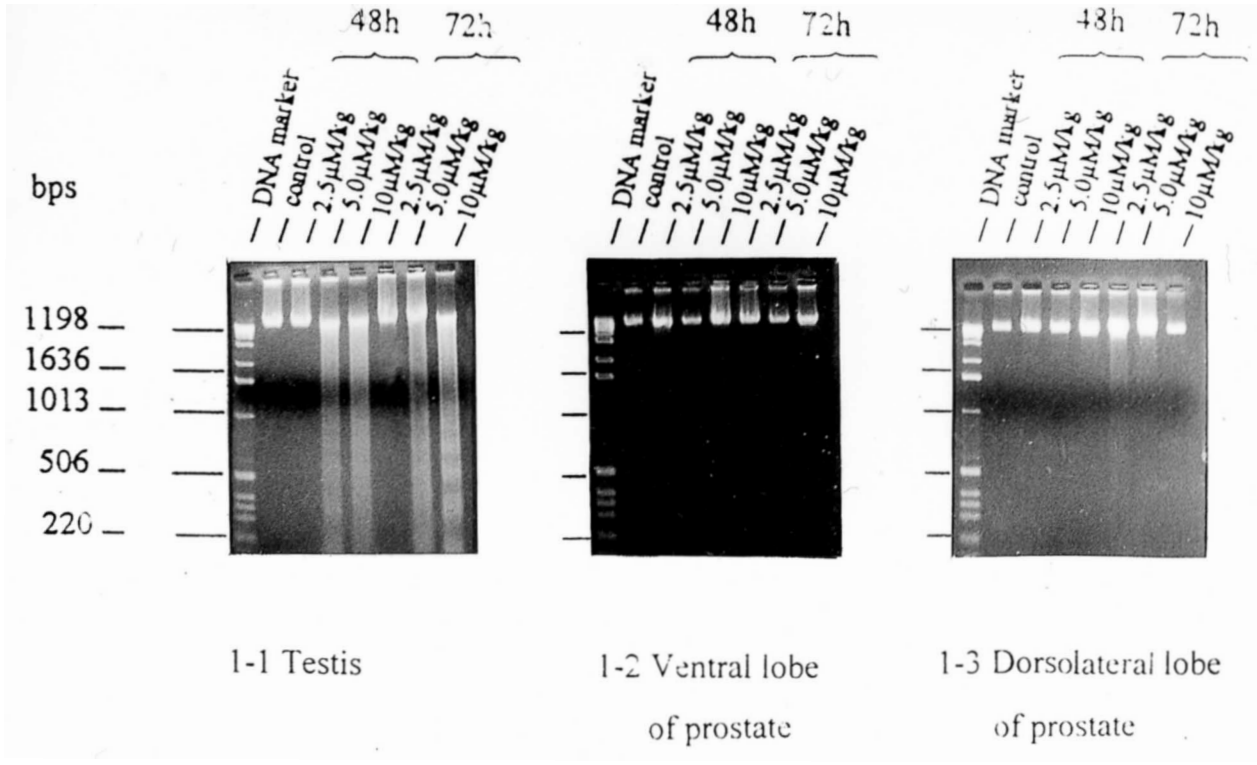

Figure 1. Apoptotic DNA fragmentation in testes and prostates from Wistar rats at 48 or 72 hours after administration of various dose levels (0, 2.5, 5.0 and 10.0 μmol/kg) of Cd as a single s.c. dose.

MT-I gene expression in testes and prostate of rats treated with cadmium

MT-I gene expression in testes was detected both in control and Cd-exposed groups by RT-PCR methods (Fig. 2–1, row 1 and Table 1). However, there are no clearly detected changes of MT-I gene expression in the $CdCl_2$ groups 48 h or 72 h after administration of cadmium. This indicates that cadmium fails to induce MT-I gene expression in the testes of rats under the conditions of the present experiment.

Basal MT-I gene expression in ventral lobe of prostate (Fig. 2–2, row 1, lanes 1 and 5 and Table 1) was not detected in this study, however, there was strong expression of MT-I gene in dorsolateral lobe in control group (Fig. 2–3, row 1, lanes 1 and 5 and Table 1). 48 h and 72 h after treatment with different doses of cadmium, there were no obvious changes of MT-I gene expressions in dorsolateral lobe (Fig. 2–3, row1, lanes 2,3,4,6,7 and 8 and Table 1). In ventral lobe, weak Cd-induced MT-I gene expression could be seen only in Cd exposed groups (Fig. 2–2, row 1, lanes 3, 7 and 8).

Apoptosis related gene expression in testes and prostate of rats treated with cadmium

There is apparently negative dose-response relationship between cadmium and p53 gene expression in the testes (Fig. 2–1, line 2 and Table 1). This suggests that cadmium can decrease the expression of p53 in the testes of rats. The findings after 48 h of administration of cadmium are identical with those at 72h. The p53 gene expression is gradually decreased with increasing doses of cadmium. It is hardly detectable at 10 μmol/kg $CdCl_2$ group at 48 h and 72 h after administration of cadmium.

As shown in Fig. 2–2 and Fig. 2–3 and Table 1, there was more of basal p53 gene expression (control groups) in ventral lobe of prostate than in dorsolateral lobe of prostate. However, the Cd-induced p53 gene expression in ventral lobes did not change in 2.5 μmol/kg Cd groups(Fig. 2–2, row 2, lane 2, 6 and Table 1) and there was an uncertain subtle decrease in 5.0 μmol/kg and 10 μmol/kg Cd groups (Fig. 2–2, row 2, lanes 3,4, 7,8 and Table 1); a little enhanced expression of p53 gene could be observed in dorsolateral lobes in all Cd-exposed

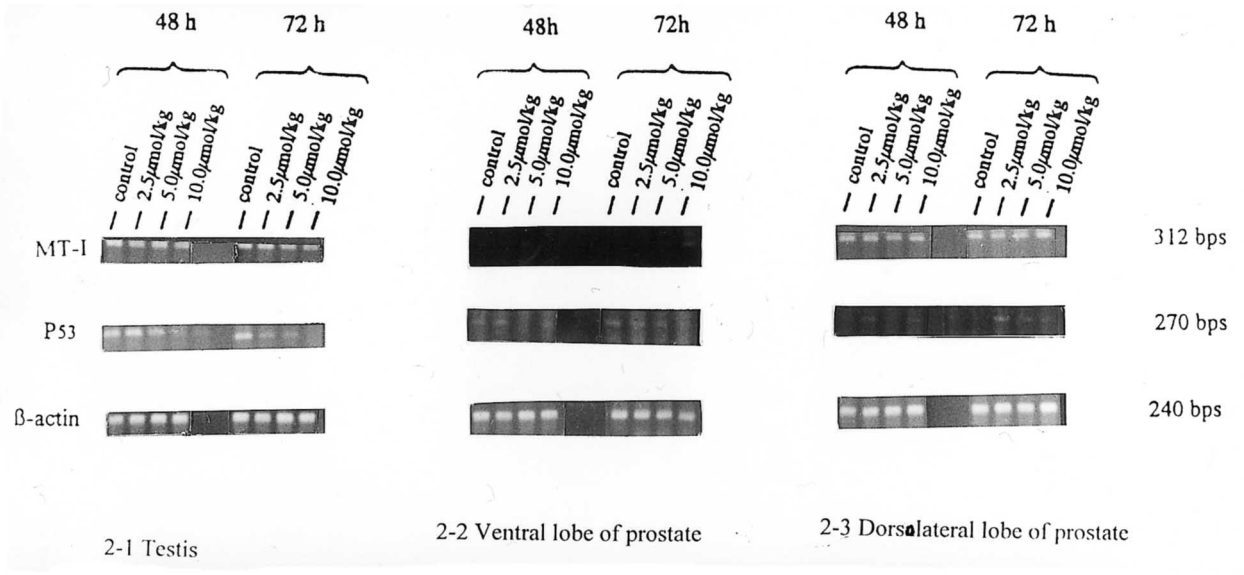

Figure 2. The expressions of MT-I, p53 and ß-actin in testes and prostates from Wistar rats at 48 or 72 hours after administration of 0, 2.5, 5.0 and 10.0 μmol/kg of Cd as a single s.c. dose.

Table 1. Semiquantitative estimation of p53, MT-I and β-actin gene expression in Wistar rats at 48 or 72 hours after the administration of various dose levels (0, 2.5, 5.0, 10.0 μmol/kg) of Cd as a single s.c. dose

Time	48 h				72 h			
Dose (μmol/kg)	0.0	2.5	5.0	10.0	0.0	2.5	5.0	10.0
Testes								
p53	+++	+++	+	–	+++	+	±	–
MT-I	++	++	++	++	++	++	++	++
β-actin	+++	+++	+++	+++	+++	+++	+++	+++
Ventral lobe of prostate								
p53	+	+	±	+	+	+	+	±
MT-I	–	+	±	+	–	+	+	±
β-actine	+++	+++	+++	+++	+++	+++	+++	+++
Dorsolateral lobe of prostate								
p53	–	+	±	+	–	+	+	±
MT-I	++	++	++	++	++	++	++	++
b-actin	+++	+++	+++	+++	+++	+++	+++	+++

β-actin gene expression as reference (+++) compared with p53 and MT-I gene expression

groups (Fig. 2–3, row 2, lanes 2,3,4,6,7,8 and Table 1), but the dose-response relationship between Cd and p53 gene expression in dorsal prostate was not clear.

The specificity of p53 PCR products was further confirmed by the southern blot analysis (Fig. 3–1.). By measurements of the density of the bands, the relative amount of synthetic DNA from different groups was determined (Fig. 3–2). When the control group was used as a standard (100%), the relative densities in 2.5 μmol/kg Cd groups were 89.0% in 48 h and 88.0% in 72 h, those in 5.0 μmol/kg Cd groups were 75.5% in 48 h and 70.3% in 72 h and those in 10.0 μmol/kg Cd groups were 82.6% in 48 h and 57.5% in 72 h. There was a dose dependent decrease of the relative densities in 72 h after Cd administration, but the density in 10.0 μmol/kg group was somewhat higher than that in 5.0 μmol/kg group at 48 h after Cd administration.

The bcl-x gene expression in testes and prostate was also measured by RT-PCR technique. However, neither basal (control group) nor cadmium-induced (cadmium-exposed group) bcl-x gene expression were detectable (data not shown) in testes and prostates at 48 h and 72 h after treatment with cadmium.

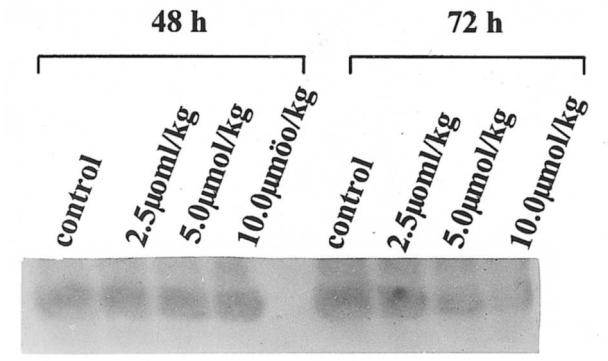

Figure 3–1. Southern blot hybridization results of p53 PCR products of testes from Wistar rats at 48 or 72 hours after administration of 0, 2.5, 5.0 and 10.0 μmol/kg of Cd as a single s.c. dose.

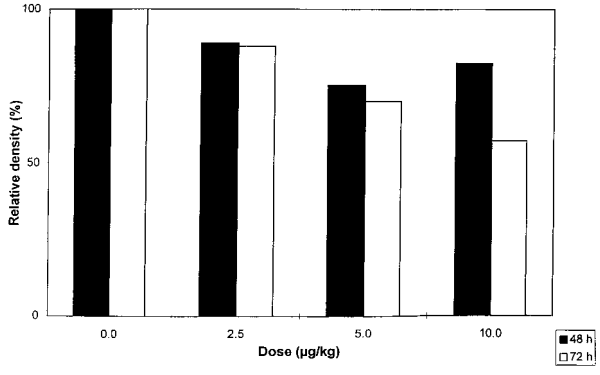

Figure 3–2. Densitometric quantification of hybridization of p53 PCR products of testes from Wistar rats at 48 or 72 h after administration of 0, 2.5, 5.0 and 10.0 μmol/kg of Cd as a single s.c. dose (cf. Fig. 3–1).

Evidence showing complete and intact mRNA

β-actin was used as the index showing the complete and intact mRNA in the present study. β-actin mRNA in all testicular and prostatic samples was expressed very well (Fig. 1 and 2, row 3 and Table 1). This guaranteed that all mRNAs used for p53, bcl-x and MT-I genes expression are complete and intact.

Repeatability of results

For control and dose groups 2.5 and 10 μmol/kg the same results were obtained in a preliminary study on 12 additional animals (cf. Section 2.2)

Discussion

The present results indicate that cadmium can induce apoptosis in the testes of rats and reduce p53 gene expression. This suggests that cadmium can induce apoptosis in testes by a p53-independent mechanism. Wild-type p53 was first noted to cause growth arrest and was manifested as a tumor suppressor gene. The capacity of p53 to induce apoptosis has also been recognized when overexpressed in some cultured cells (Shaw *et al.* 1992; Yonish-Rouach *et al.* 1991) and is required for DNA damage-induced apoptotic cell death of mouse thymocytes (Lowe *et al.* 1993; Clarke *et al.* 1993). It has also been suggested that p53 suppressor gene plays a role in the meiotic process of spermatogenic differentiation and maturation (Schwarts *et al.* 1993). But this needs to be studied further. Thus p53 gene expression should be increased if cadmium would induce apoptosis in testes via p53-dependent pathway. In this study, cadmium reduced p53 gene expression and it can therefore be concluded that apoptosis was induced in testes by a pathway independent of p53. It would be of interest in future studies to investigate if the decrease in p53 induced by cadmium is related to a general loss of cells carrying the p53 gene or by some other mechanism. Studies of apoptotic phenomena in specific testicular cells, interstitial cells and those of the sperniogenic epithelium, can be performed by immunohistochemistry e.g. In Situ End Labelling (Westin *et al.* 1995).

Although Cd-induced apoptosis occurred in testes, apoptosis in prostate of rats exposed to Cd was not observed in this study. This might indicate that the testes of rat are more sensitive to Cd damage than prostate. Whether or not apoptosis can occur in prostate at a later time after exposure to Cd or after exposure to higher doses of Cd than used in the present study deserves further investigation.

Although the basal level of p53 gene expression in ventral prostate was more pronounced than that in dorsal prostate, a tendency towards decreased p53 gene expression in ventral prostate at 5 or 10 μmol/kg and a subtle tendency towards an increase in Cd-induced expression of this gene in dorsal prostate of Cd exposed groups was found in this study. This provides limited suggestive evidence that the p53 gene was differently affected by Cd in these two lobes of the prostate. Since p53 is a tumor suppressor gene, loss or inactivation of p53 increases the probability of malignant transformation. The p53 gene is frequently mutated or lost in human cancers of many different tissue types (Hollstein *et al.* 1991). Cells lacking p53 fail to arrest in G1 phase of cell cycle following DNA damage (Kuerbitz *et al.* 1992), and this may lead to genetic instability (Livingstone *et al.* 1992; Yin *et al.* 1992). Thus, the loss of p53

would serve as an initiating event in tumorigenesis by permitting the propagation of cells that have suffered damage (Lane 1992). So, cadmium-induced decrease of p53 gene expression, found in testes and possibly in ventral prostate in the present study, may enable these damaged cells to escape the G1 arrest and such events may give rise to an accumulation of DNA aberrations. This may lead to the occurrence of tumors in testes and ventral prostate. It is known that cadmium can induce tumors in testes and ventral prostate in rats (Gunn *et al.* 1963; Waalkes *et al.* 1988). The mechanism of cadmium tumorigenesis has not been well understood. Thus, a decrease of p53 gene expression could be a probable mechanism of cadmium carcinogenesis in testes and in prostate, more probable than the lack of Cd-induced MT-I gene expression in these tissues, previously advanced by other authors.

Bcl-x is a bcl-2-related gene that can function as a bcl-2-independent regulator of apoptosis (Boise *et al.* 1993). Bcl-x contains two types of cDNA, one cDNA, bcl-x_L, contains an open reading frame with 233 amino acids. The other cDNA, bcl-x_S, encodes a 170 amino acids protein. Bcl-x_L rendered cells resistant to apoptotic cell death upon growth factor deprivation, while bcl-x_S could prevent overexpression of bcl-2 from inducing resistance to apoptotic cell death. In the present study, we detected no expression of either bcl-x_L or bcl-x_S in any of the tissues or treatment groups. Bcl-x gene has been highly conserved in vertebrate evolution (Boise *et al.* 1993), and bcl-x mRNA is expressed in a variety of tissues of chicken and human, with the highest levels of mRNA observed in the lymphoid and central nervous system (Boise *et al.* 1993). A possible explanation of our findings with no expression of bcl-x gene in testes and prostate could be that bcl-x gene expression maybe tissue specific and that there is no expression in testes and prostate of rats. This speculation needs further investigation.

In the present study, we found that there exists a basal expression of MT-I gene in testes and dorsal prostate of rats, but there was no enhancement after exposure to Cd. This suggested that the MT-I gene in the testes and dorsal prostate does not respond to cadmium stimulation, although MT-I gene exists. Our findings are identical with those of other studies which found no Cd -induced expression of the MT-I gene in these tissues when animals were exposed to Cd or some other MT inducing agents (Shiraishi *et al.* 1993 a, b; Shiraishi & Waalkes 1994). One reason for the lack of MT-I induction in the present study may be that only a single dose of Cd was used. In the early studies by one of the present authors, repeated exposure was employed when demonstrating induced synthesis of Cd-binding metallothionein-like protein in testes (Nordberg 1971). Some other studies have shown that MT is consititutively expressed in the whole testes or specific testicular cells at levels higher than in other organs, e.g. liver, and that in vivo or in vitro Cd exposure increases MT gene expression in the testes (Abel *et al.* 1991; De *et al.* 1991; Nolan & Shaikh 1986). In rodent, testicular Cd appears to be localized in the interstitial tissue (Nordberg 1972) and a high incidence of testicular interstitial cell (TIC) tumors occur following Cd exposure (Gunn and Gould, 1970). Most of the studies on MT gene expression, including this present study, used the whole testes as targets, which may not reflect the real MT activity in these specific cells. One recent study, which focused on interstitial cells, has shown that MT-I mRNA was expressed at high basal levels in isolated TICs of control rats, and that there was a significant increase of MT-I mRNA level in TICs (1.92-fold) at 24 h after treatment with 4 μmol/kg Cd. However the Cd-induction of MT-I mRNA transcripts did not translate into increase of MT protein levels in TICs (McKenna *et al.* 1996). Since the present study did not provide the data on MT-I mRNA in TICs after exposure to Cd, it is not possible to evaluate to what extent MT-I could protect testes from the damage of Cd.

In summary, our observations indicated that cadmium-induced apoptosis in testes was via p53 -independent pathway. MT-I appeared not to be induced in testes or dorsal prostate by a single dose of cadmium. The expression of MT-I in ventral prostate was not detected in control group, but a weak expression was found after Cd exposure. The cadmium-induced decrease of p53 gene expression in testes might be a possible mechanism of testicular carcinogenesis by cadmium.

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