

# Acute Cadmium Administration Induces Apoptosis in Rat Thymus and Testicle, but not Liver

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## Abstract

The implication of apoptosis in the cytotoxicity of cadmium (Cd) on rat thymus, testicle and liver was investigated. Four days after Cd injection (1.5 and 3.0 mg/kg b.w. i.p) the testicle and thymus relative weight decreased but that of liver increased significantly in a dose-dependent manner. *In vitro*, 6 h incubation of thymocytes in the presence of  $10^{-3}$ M Cd resulted in a typical degradation of DNA into oligonucleotide fragments similar to that observed in the presence of the dexamethasone, suggesting the induction of apoptosis in the cells. The addition of 1 mM ZnSO<sub>4</sub>, a metallic antioxidant, prevented this fragmentation. Nick end-labeling method confirmed the involvement of apoptosis in the thymus and revealed a high percentage of TUNEL-positive cells 48 h following Cd administration mainly in the cortical zone. An apoptotic effect was also observed in the testicle where TUNEL-positive cells were present, primarily in late stages of gametogenesis. In contrast, the liver of Cd-treated rats failed to exhibit significant levels of apoptosis and all hepatocytes showed strictly negative TUNEL-labeled nuclei. In contrast, centrilobular-necrosis was observed reflecting species differences in target cells. These findings suggest that the apoptotic mechanism in thymus and testicle, but not in liver, may contribute to the toxicity of cadmium in rats.

**Keywords:** cadmium, apoptosis, thymus, testicle, liver, rat

## Introduction

Cadmium (Cd) is known to be one of the most toxic environmental and industrial pollutants. Its industrial applications were developed based on its unique chemical and physical properties. Cd is a ubiquitous toxic heavy metal and, unlike organic compounds, it is not biodegradable and has a very long biological half-life [1]. In spite of many studies, the mechanism of its toxicity has not yet been well elucidated and in contrast to other metals, there is no effective therapy for its poisoning.

A variety of noxious agents and chemicals may induce two different processes of cell death through either

necrosis or apoptosis, depending on mode of delivery, concentration and dose administered [2]. Necrosis is an accidental form of cell death, while apoptosis is a form of active cell death characterized by cell shrinkage, membrane blebbing, aggregation of cytoplasmic organelles, chromatin condensation and DNA fragmentation into oligonucleosomal fragments due to the activation of endogenous endonucleases [3]. Apoptosis can be induced by a wide variety of stimuli such as glucocorticoids, radiation and pesticides [4, 5, 6], and it plays an important role in many fundamental biological process such as embryogenesis, metamorphosis, tissue homeostasis, development and regulation of the immune system [7].

This study was performed to investigate the cytotoxic effects of Cd on the thymus, testicle and liver and to de-

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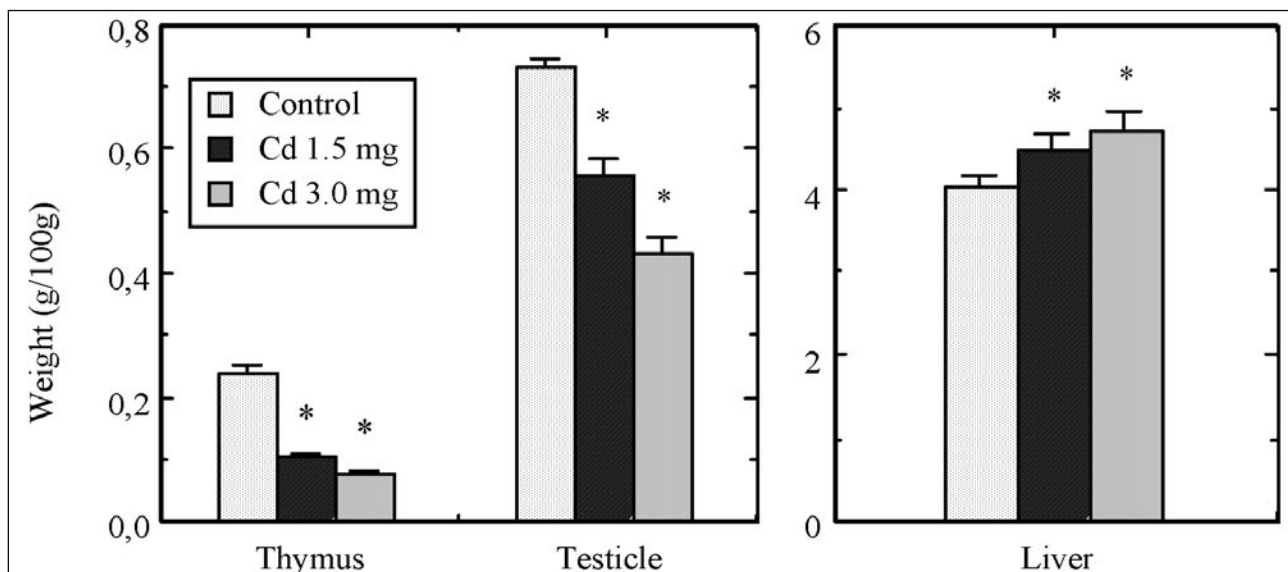


Fig. 1. Effect of acute administration of cadmium on testes, thymus and liver relative weight in rat. Cadmium (1.5 or 3mg/kg body weight day<sup>-1</sup>) was administered i.p. in 0.9% saline, control animals received the vehicle. Each value is the mean  $\pm$  SE of 12-17 determinations. Animals were decapitated the fourth day. \*  $p < 0.05$  compared to control (Student's t-test).

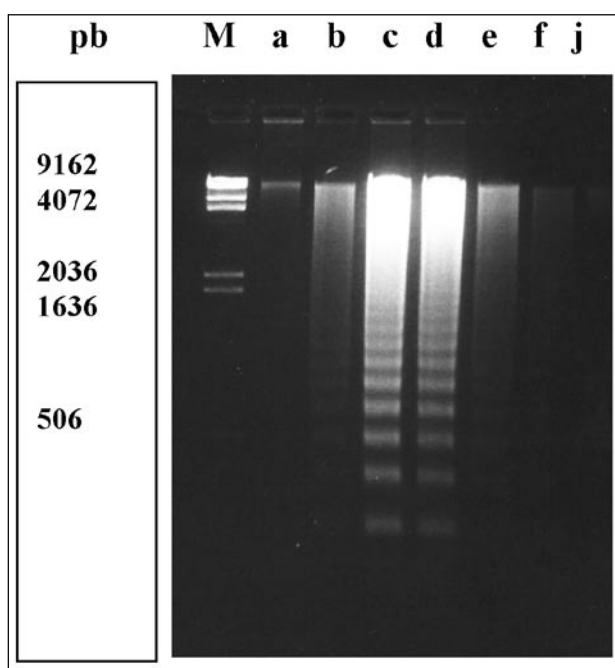


Fig. 2. DNA fragmentation in cadmium-treated rat thymocytes. Agarose gel analysis of DNA samples obtained from thymocytes not incubated (lane a) and incubated 6h in control medium (lane b), medium containing 10<sup>-5</sup>M Dexa (lane c), 10<sup>-3</sup>M Cd (lane d), 10<sup>-5</sup>M Cd (lane e), 10<sup>-7</sup>M Cd (lane f), 10<sup>-3</sup>M Cd + 1mM of ZnSO<sub>4</sub> (lane j). 1kb molecular weight DNA marker (lane M). The DNA samples were fractionated by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide.

termine whether apoptosis may be implicated in this cytotoxicity. Gel electrophoresis ethidium staining method and the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) [8] assays were used to detect fragmentation of DNA resulting from apoptosis.

## Materials and Methods

### Animals and Reagents

Male wistar rats weighing between 150-200 g were used in this study. Rats were housed under controlled conditions of ambient temperature (22  $\pm$  1°C), with 14 h-10 h light/dark cycle. Food and water were provided *ad libitum*. All reagents were of the highest quality and were purchased from the Sigma Chemical Co (St. Louis, MO, USA).

Animals were randomized into 3 experimental groups (n = 5-6) as follows:

1. animals received an intraperitoneally injection (ip) of cadmium chloride in 0.9% saline at dose 1.5 mg/kg body weight (b.wt),
2. animals were administered 3.0 mg of the metal/kg b.wt,
3. control group received equal volumes of vehicle.

Animals were sacrificed by decapitation on the fourth day, the left testis, thymus and liver were dissected and weighed.

### Histological Analysis

Testicle, liver and thymus were fixed overnight at room temperature by direct immersion in a 4% para-



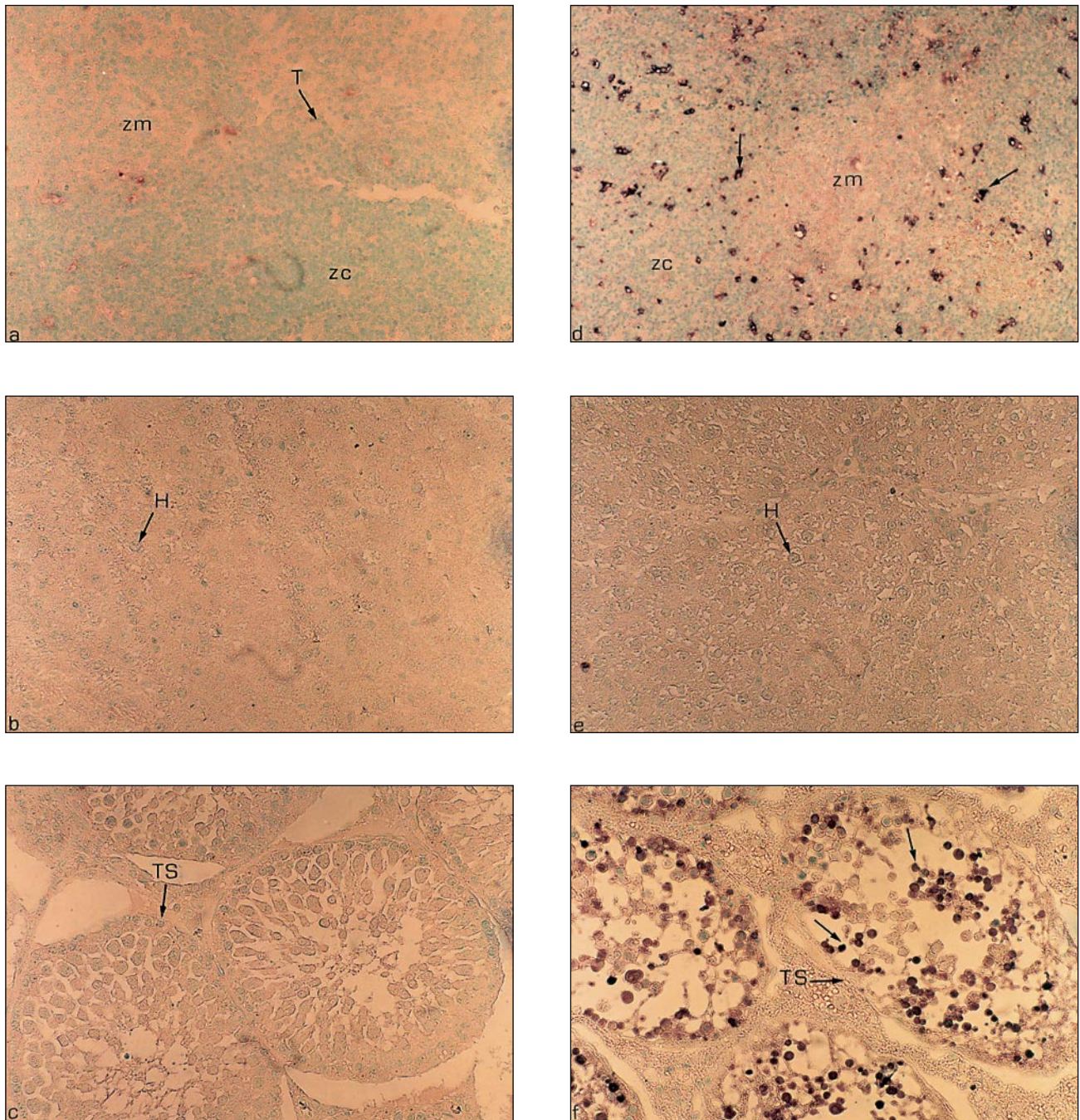


Fig. 3: Photomicrographs of sections of (a) thymus, (b) liver and (c) testes from control rats and (d) thymus, (e) liver and (f) testes from cadmium-treated rats stained by TUNEL technique (magnification x400).

Thymus, testicle and liver were fixed by direct immersion in a 4% paraformaldehyde in 0.1M phosphate buffer. Serial sections (5 $\mu$ m) were mounted on gelatin-coated glass slides cut and stained using the TUNEL technique (see Materials and Methods). Cd (1.5mg/kg body weight day<sup>-1</sup>) is administered i.p. in saline 0.9%, control animals received the vehicle. Animals were decapitated the fifth day. T: Thymocyte, zc: Cortex, zm: Medulla, H: Hepatocyte, VS: Blood Vessel, TS: Seminiferous Tubule. Apoptotic cells (arrows).

formaldehyde in 0.1M phosphate buffer, pH 7.4. The samples were dehydrated with ethanol and toluene series and embedded in paraffin. Serial sections (5 $\mu$ m) were mounted on gelatin-coated glass slides cut and stained with hematoxylin and eosin or used in the TUNEL procedure [8].

#### TUNEL Assay

After deparaffinization and rehydration, tissue sections were incubated with 0.1% (v/v) Triton X-100 for 2min on ice, followed by washing of the slides twice in PBS (CaCl<sub>2</sub> 2H<sub>2</sub>O 0.8mM, KCl 2.6mM, KH<sub>2</sub>PO<sub>4</sub> 1.4mM, MgCl<sub>2</sub> 6H<sub>2</sub>O



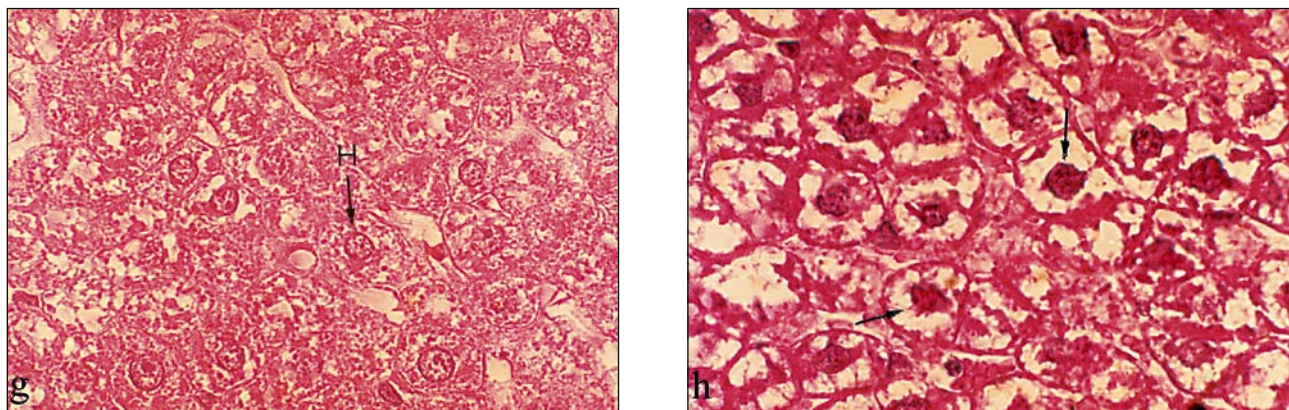


Fig. 4: Histopathological hepatic changes of (g) control- and (h) cadmium-treated fragment of rat liver lobule (magnification x400). Liver was fixed by direct immersion in a 4% paraformaldehyde in 0.1M phosphate buffer. Serial sections (5 $\mu$ m) were mounted on gelatin-coated glass slides cut and stained with haematoxylin and eosin. Cd (1.5mg/kg body weight day<sup>-1</sup>) was administered i.p. in saline 0.9%, control animals received the vehicle. Animals were decapitated the fifth day. Marks of hepatocyte necrosis, cytoplasmic vacuolization and nuclear enlargement (arrows).

0.4mM, NaCl 136mM, Na<sub>2</sub> HPO<sub>4</sub> 8mM, pH 7.2). The specimens were then incubated one hour at 37°C in a solution consisting of 1mM cobalt chloride, 140mM sodium cacodylate and terminal deoxyribonucleotidyl transferase (TdT) at a final concentration of 0.1U/ $\mu$ l to insert biotin-16-dUTP at the 3'-ends of DNA fragments. A straptavidin-peroxydase complex and 3-amino-9-ethylcarbazole served as the detection system for biotin. Sections were lightly counterstained with hematoxylin and mounted in glycerin jelly. Negative control included omission of TdT from the labeling mixture.

#### DNA Extraction and Electrophoresis

DNA extraction and electrophoresis have been performed according to the method described by Genaro et al. (1995) (9). Briefly, at the end of the incubation period, thymocytes were centrifuged at 200g for 10min at 4°C, and the pellet was washed twice with ice-cold PBS. The pellet was resuspended in lysis buffer (5mM tris-HCl, 20mM EDTA, 0.5% triton X-100, pH8) for 15min at 4°C. Nuclei were removed by centrifugation at 500g for 10min and the resulting supernatant was centrifuged at 30,000g for 15min. The fragmented DNA present in the soluble fraction was precipitated with 70% ethanol plus 0.1vol. of 3M ammonium acetate and aliquots were treated for 1h at 55°C with 0.3mg/ml of proteinase K then with RNase for 30 min at 25°C. After two extractions with phenol/chloroform, the DNA was resuspended. 3 $\mu$ g of DNA per sample were loaded into 1.5% agarose gel in 89mM Tris, 89mM boric acid and 2.5mM EDTA (pH 8.0). DNA was visualized using UV illumination (260nm) after ethidium bromide staining.

#### Statistics

Data were analyzed using Stat View 512<sup>+</sup> software (Abacus Concept, Inc). Means were given with standard error and were subjected to the unpaired Student's *t*-test.

#### Results

As shown in Figure 1, four days after acute administration of Cd, the testicle and thymus relative weight decreased significantly in a dose-dependent manner. Compared to control values, the decrease was of 23.7% and 41.2% for testicle and 56.9% and 68.0% for thymus with 1.5 and 3.0mg Cd /kg b.wt. respectively. In contrast, liver relative weight increased in Cd-exposed rats by 11.7% and 17.9%, respectively, for the same doses of the metal.

Nick end-labeling for detection of apoptotic thymocytes revealed a high percentage of TUNEL-positive cells in the thymus 48h following Cd injection. The density of the apoptotic cells was more significant in cortical than in the medullary zone (Figure 2a, d). Similar to the findings in the thymus, an apoptotic effect was also observed in the testicle where TUNEL-positive cells were present primarily in late stages of gametogenesis (Figure 2c, f). In addition, exposure to Cd also induced a pronounced alteration of spermatogenic process with dramatically reductions of spermatozoa produced in the lumen of the seminiferous tubules sections and a decrease of the intratubular tissue volume (Figure 2c, f). However, the liver from Cd-treated rat failed to exhibit significant levels of apoptosis and all hepatocytes showed TUNEL-labeled nuclei to be strictly negative (Figure 2b, e). The predominant hepatic lesions after acute administration of Cd were cytoplasmic vacuolization in the hepatocytes, focal necrosis and nuclear enlargement compared to the liver of control rats (Figure 4a, b).

The *in vitro* Cd-induced apoptotic DNA fragmentation in thymocytes was clearly indicated on the agarose gel as detected by ethidium bromide fluorescence (Figure 3). In the absence of Cd no ladder was observed before incubation and the fragmentation of the DNA remained negligible by 6 hours of incubation of thymocytes (Figure 3, lanes a, b), whereas the pres-

ence of Cd indicated dose dependent typical apoptotic fragmentation of the DNA (Figure 3, lanes d, e, f). The degradation of DNA into oligonucleotide fragments was maximal at  $10^{-3}$ M and identical to that observed in  $10^{-5}$ M Dexamethasone-treated cells, confirming the induction of apoptosis by the metal (Figure 3, lane c). Addition of ZnSO<sub>4</sub> (1mM), a metallic antioxidant, protected the cells and prevented the degradation of the DNA induced by Cd (Figure 3, lane j).

## Discussion

Our results show that acute Cd-administration produced a marked decrease of testicle relative weight in accord with previous data indicating weight loss in testes, seminal vesicles and epididymis accompanied by a loss of reproductive capacity in rats exposed to Cd [10, 11]. The testicular toxicity of Cd appeared to be mediated by a rapid apoptotic process as revealed by the increase of the number of TUNEL-positive cells in the seminiferous tubules of treated rats. This observation agrees with other studies indicating the implication of apoptosis in the mechanism of cytotoxicity of Cd in testes [12, 13] and also in several organs of urogenital systems, including kidney, prostate, seminal vesicles and epididymis [14, 15].

The results obtained with testicles are similar to those observed in thymus with a marked decrease of the organ relative weight in a dose-dependent manner. The thymolytic effect of Cd has been shown by Morselet et al. [16], who noted a decrease in thymus relative weight with the presence of Cd in the target organ, confirming that Cd acts directly on the thymocytes. Agarose gel electrophoresis of cellular DNA is the usual method for demonstrating apoptosis, with the appearance of a characteristic ladder pattern [17]. *In vitro*, thymocytes exposure to Cd resulted in a DNA ladder similar to that observed in Dexamethasone-treated cells confirming the induction of apoptosis by the heavy metal. The same result has been observed for mouse thymocytes [18], human T cell line [19], human histiocytic lymphoma cell line [20], human promonocytic cells [21] and normal human mononuclear cells [22].

The addition of ZnSO<sub>4</sub> (1mM) protected the cells and prevented the degradation of the DNA induced by the Cd. The mechanism of the anti-apoptotic action of zinc is poorly understood. Zinc may prevent apoptosis by inhibiting the Ca<sup>2+</sup>/Mg<sup>2+</sup>-dependant endonuclease [23] and the apoptotic protease, the caspase 3 [24] or by promoting DNA synthesis and the anti-apoptotic proteins Bcl-2 activation [25]. On the other hand, zinc is also an antioxidant [26]. Thus, it may be concluded that an oxidative pathway is involved in Cd-induced DNA fragmentation. In support of this argument, previous studies have demonstrated that Cd administration to rats resulted in the formation of reactive oxygen species and increased hepatic and brain lipid peroxidation [27].

Nick end-labeling revealed a high percentage of TUNEL-positive cells in the thymus, 48h after Cd injection compared to control. The density of the apoptotic cells is

more significant in cortical than in the medullary zone. This result is comparable with that obtained by using dexamethasone [28], a synthetic glucocorticoid well known for its potent apoptotic effect. As in testicles, these results indicate that the toxicity of Cd in thymus implied an apoptotic mechanism.

It's well documented that liver is the major target organ of acute and chronic Cd toxicity in laboratory rodents [29]. Contrary to the testicle and the thymus, acute administration of Cd increased significantly the relative weight of liver. This observation closely resembles that described by Habeebu et al. [30], who also reported granulomatous inflammation and proliferating nodules in mouse liver parenchyma. Apoptosis is rare in adult healthy rodent livers [31] but it can be enhanced after exposure to carbon tetrachloride [32], ethanol [33], thioacetamide [34] and dioxin [35]. In our experiments, liver from Cd-treated rat failed to exhibit significant levels of apoptosis and all hepatocytes TUNEL-labeled nuclei were strictly negative. This result is somewhat surprising because the Cd used with a comparative dose induced apoptosis in mouse liver and the apoptotic index peaked at 9-14 hours after Cd administration and then decreased from 14 hours onwards. In this period, liver necrosis was most severe [36]. In rat liver, the lack of Cd-induced apoptosis may not be attributed to the long time exposure since we observed no difference between the apoptotic index values of control and 2, 6, 12, 24 and 48 hours after Cd injection. This result confirmed that acute Cd hepatotoxicity in rat occurred without signs of apoptosis but likely by necrosis features reflecting species differences in target cells.

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## References

1. GOYER R. Toxic effect of metal: Casarett and Doull's toxicology. The basic Science of poisons. Klaassen CD Eds. 5th Ed, Mc Graw-Hill, New York, pp 691-736. **1995**.
2. PITOT H. Hepatocyte death in hepatocarcinogenesis. *Hepatology*. **28**, 1, **1998**.
3. WYLLIE A.H., KERR J.F., CURRIE A.R. Cell death: The significance of apoptosis. *Int. Rev. Cytol.* **68**, 251, **1980**.
4. COHEN J.J., DUKE R.C. Glucocorticoid activation of a calcium-dependent endonuclease in thymocyte nuclei leads to cell death. *J. Immunol.* **132**, 38, **1984**.
5. UMANSKY S.K. Apoptosis: Molecular and cellular mechanisms *Mol. Biol.* **30**, 285, **1996**.
6. TEBOURBI O, BEN RHOUMA K, SAKLY M. DDT induces apoptosis in rat thymocytes. *Bull. Contam. Toxicol.* **61**, 216, **1998**.
7. ARENDS M.J., WYLLIE A.H. Apoptosis: mechanism and role in pathology. *Int. Rev. Exp. Pathol.* **32**, 223, **1991**.
8. GAVRIELLI Y., SHERMAN Y., BEN SASSON S.A. Identification of programmed cell death *in situ* via specific label-

- ing of nuclear DNA fragmentation. *J. Cell. Biol.* **119**, 493, **1992**.
9. GENARO A., HORTELANO S., ALVAREZ A., MARTINEZ C., BOSCAL. Splenic B lymphocyte programmed cell death is prevented by nitric oxide release through mechanisms involving sustained Bcl-2 levels. *J. Clin. Invest.* **95**, 1884, **1995**.
  10. LASKEY J.W., REHNBERG G.L., LAWS S.C., HEIN J.F. Reproductive effects of low acute doses of cadmium chloride in adult male rats. *Toxicol. Appl. Pharmacol.* **73**, 250, **1984**.
  11. SAYGI S., DENIZ G., KUTSAL O., VURAL N. Chronic effects of cadmium on kidney, liver, testis and fertility of male rats. *Biol. Tr. Elem. Res.* **31**, 209, **1991**.
  12. XU C., JOHNSON J.E., SINGH P.K., JONES M.M., YAN H., CARTER C.E. *In vivo* studies of cadmium-induced apoptosis in testicular tissue of the rat and its modulation by a chelating agent. *Toxicology.* **107**, 1, **1996**.
  13. JONES M.M., XU C., LADD P.A. Selenite suppression of cadmium-induced testicular apoptosis. *Toxicology.* **116**, 169, **1997**.
  14. XU C., PRAMOD K.S., JONES M.M., JOHNSON J.E., DIETRICH M.S. Cadmium-induced apoptosis in the urogenital organs of the male rat and its suppression by chelation. *J. Toxicol. Environ. Health.* **52**, 149, **1997**.
  15. YAN H., CARTER C.E., XU C., SINGH P.K., JONES M.M., JOHNSON J.E., DIETRICH M.S. Cadmium-induced apoptosis in the urogenital organs of the male rat and its suppression by chelation. *J. Toxicol. Environ. Health.* **52**, 149, **1997**.
  16. MORSELET A., LEENE W., DE GROOT C., KIPP J., EVERS M., ROELOFSEN A., BOSH K. Differences in immunological susceptibility to cadmium toxicity between two rat strains as demonstrated with cell biological methods. *Toxicology.* **48**, 127, **1988**.
  17. COMPTON M.M., CIDLOWSKI J.A. Rapid *in vivo* effects of glucocorticoid on the integrity of rat lymphocyte genomic deoxyribonucleic acid. *Endocrinology.* **118**, 38, **1986**.
  18. FUJIMAKI H., ISHIDO M., NOHARA K. Induction of apoptosis in mouse thymocytes by cadmium. *Toxicol. Lett.* **115**, 99, **2000**.
  19. EL AZZOUZI B., TSANGARIS G., PELLEGRINI O., MANUEL Y., BENVENISTE J., THOMAS Y. Cadmium Induces apoptosis in human T cell line. *Toxicology.* **88**, 127, **1994**.
  20. LI M., KONDO T., ZHAO Q.L., LI F.J., TANABE K., ARAI Y., ZHOU Z.C., KASURA M. Apoptosis induced by cadmium in human lymphoma U937 cells through Ca<sup>2+</sup>-calpain and caspase-mitochondria-dependent pathways. *J. Biol. Chem.* **275**, 39702, **2000**.
  21. GALÁN A., GARCÍA-BERMEJO L., TROYANO A., VILABOIA N.E., FERNÁNDEZ C., DE BLAS E., ALLER P. The role of intracellular oxidation in death induction (apoptosis and necrosis) in human promonocytic cells treated with stress inducers (cadmium, heat, X-rays). *Eur. J. Cell. Biol.* **80**, 312, **2001**.
  22. de la FUENTE H., PORTALES-PEREZ D., BARANDA L., DIAZ-BARRIGA F., SAAVEDRA-ALANIS V., LAYSECA E., GONZALEZ-AMARO R. Effect of arsenic, cadmium and lead on the induction of apoptosis of normal human mononuclear cells. *Clin. Exp. Immunol.* **129**, 69, **2002**.
  23. WALTON M.I., WHYSON D., O'CONNOR P.M., HOCKENBERY D., KORSMEYER S.J., KOHN K.W. Constitutive expression of human Bcl-2 modulates nitrogen mustard and camptothecin induced apoptosis. *Cancer. Res.* **53**, 1853, **1993**.
  24. PERRY D.K., SMYTH M.J., STENNICKE H.R., SALVESSEN G.S., DURIEZ P., POIRIER G.G., HANNUN Y. Zinc is a potent inhibitor of the apoptotic protease, caspase 3: A target for zinc in the inhibition of apoptosis. *J. Biol. Chem.* **272**, 18530, **1997**.
  25. ISHIDO M., SUZIKI T., ADACHI T., KUNIMOTO M. Zinc stimulates DNA synthesis during its antiapoptotic action independently with increments of an antiapoptotic protein, Bcl2, in porcine kidney LLC-PK1 cells. *J. Pharmacol. Exp. Therap.* **290**, 923, **1999**.
  26. BETTGER W.J. Zinc and selenium site-specific versus general antioxidant. *Can. J. Physiol. Pharmacol.* **71**, 721, **1993**.
  27. BAGCHI D., JOSHO S.S., BAGCHI M., BALMOORI J., BENNER E.J., KUSZYNSKI C.A., STOHS S.J. Cadmium- and chromium-induced oxidative stress, DNA damage, and apoptotic cell death in cultured human chronic myelogenous leukemic K562 cells, promyelocytic leukemic HL-60 cells, and normal human peripheral blood mononuclear cells. *J. Biochem. Mol. Toxicol.* **14**, 33, **2000**.
  28. BEN RHOUMA K., SCHIMCHOWITSCH S., STOECKEL M.E., FELIX J.M., SAKLY M. Implication des récepteurs glucocorticoïdes de type II dans l'action apoptotique de l'aldostérone sur les thymocytes de rat. *Arch. Int. Physiol. Biochim. Biophys.* **105**, 216, **1997**.
  29. DUDLEY R.E., SVOBODA D.J., KLAASSEN C.D. Acute exposure to cadmium causes severe liver injury in rats. *Toxicol. Appl. Pharmacol.* **65**, 302, **1982**.
  30. HABEEBU S.S., LIU J., KLAASSEN C.D. Metallothionein-null mice are more sensitive than wild-type mice to liver injury induced by repeated exposure to cadmium. *Toxicol. Sci.* **55**, 223, **2000**.
  31. SHULTE-HERMANN R., BURSH W., GRASL-KRAUPP B. Active cell death (apoptosis) in liver biology and disease. Boyer J.L., Ockner R.K., eds. *Progress in liver disease*. vol 13, Philadelphia: Saunders, 1-35. **1995**.
  32. SHI J., AISAKI K., IKAWA Y., WAKE K. Evidence of hepatocyte apoptosis in rat liver after the administration of carbon tetrachloride. *Am. J. Pathol.* **153**, 515, **1998**.
  33. NANJI A.A. Apoptosis and alcoholic liver disease. *Semin. Liver. Dis.* **18**, 187, **1998**.
  34. LEDDA-COLUMBANO G.M., CONI P., CURTO M., GIACOMINI L., FAA G., OLIVERIO S., PIACENTINII M., COLUMBANO A. Induction of two different modes of cell death, apoptosis and necrosis in rat liver after a single dose of thioacetamide. *Am. J. Pathol.* **139**, 1099, **1991**.
  35. STINCHCOMBE TS, BUCHMANN A, BOCK KW, SCHWARZTOHS M. Inhibition of apoptosis during 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin-mediated tumor promotion in rat liver. *Carcinogenesis.* **16**, 1271, **1995**.
  36. HABEEBU S.S., LIU J., LIU Y., KLAASSEN C.D. Cadmium-induced apoptosis in mouse liver. *Toxicol. Appl. Pharmacol.* **149**, 203, **1998**.