Cadmium Chloride (CdCl2)-Induced Apoptosis in Liver of Mice

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J Fac Med Baghdad
2006; Vol. 48, No.3
Received March 2006
Accepted May 2006

Summary:

Background: Cadmium (Cd) is an industrial and environmental pollutant that affect adversely a number of organs in humans and other mammals.
Objectives: To study the effect of cadmium on liver of mice.
Material and Methods: Male Balb/c mice weighing 30-32 gm, 60 days old, were treated intraperitoneally (ip) with (1-10mg/kg body wt. /CdCl2). The body weight, liver weight, histological examination of liver, SEM, metal analysis along with DNA ladder for apoptosis.
Results: Cadmium induced both a time, and dose dependent increase in apoptotic, severity of necrosis. Liver weight, body weight decreased with increase of dose, while metal content was increased by increase of dose.
Conclusion: It has been concluded that cadmium caused necrotic effect on liver and apoptotic as well as decreased body weight and liver weight.
Key words: Cadmium, mice, metal analysis, apoptosis.

Introduction:

Cadmium (Cd) is an industrial and environmental pollutant that affects adversely a number of organs in humans and other mammals, including the kidneys, liver, lungs and placenta[1-3]. The liver and kidney are the primary organs involved in the elimination of systemic cd [3]. In addition to the direct cytotoxic effects that can lead to apoptotic and/or necrotic events, Cd can have potent carcinogenic effects in target organs, in fact, based on epidemiological and toxicological and also findings indicate that the carcinogenic effects of Cd are related to the activation of protooncogenes[4,5]. In contast, acute exposure leads to accumulation of cd mainly in the liver[6]. Therefore, it is not unexpected that the liver is the target organ in acute cd poisoning. The manifestations of acute cd hepatotoxicity include hepatocyte swelling and fatty change, as well as focal, zonal, and massive necrosis[7]. At the ultrastructural level, the hepototoxic effects of cd include dilatation of the rough endoplasmic reticulum, loss of membrane-associated ribosomes, and nuclear condensation[8].

Apoptosis is a mode of cell death with morphological features quite distinet from those of necrosis[9]. Apoptosis features in the toxicity of many chemical toxicants, DNA is often the target of toxicants, and DNA damage is currently thought to be leading candidates for initiating the process of apoptosis[10]. Cd has been shown to iduce apoptosis in isolated bovine liver nuclei [11].

Therefore, the present study had the apoptosis is an important and currently unrecogenized process occurring in acute cd-induced hepatotoxicity.

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Material and Methods:

Adult male mice, weighing 30-32gm of Balb/c, 60 days old. The animals were treated intraperitoneally (ip), with different doses of cadmium chloride (CdCl2) daly for 15 days, as follows (1mg/kg body wt., 5mg/kg body wt., and 10mg/kg body wt). The animals were divided into four groups each group having 6 mice (one control group on normal diet and water).

Body and tissue weights:

The body weight and liver weight from treated groups was taken along with control after 15 days, when the mice were dissected and percentage change was calculated by the following formula:

$$\frac{\text{Weight of tissue of treated group} - \text{weight of tissue of control group}}{\text{Weight of tissue of control group}} \times 100$$

While body weight after 15 days, weight was taken by the formula:

$$\frac{\text{Weight of body of treated group} - \text{weight of body of control group}}{\text{Weight of body of control group}} \times 100$$

Histological studies:

The liver was cut into small pieces and fixed in Bouins fixative. Histological examination of liver

was carried out by standard histological techniques. Sections of 5 μm thickness were cut and stained with hematoxylin: eosin (H/E).

Metal analysis:

Tissue sample were digested in nitric acid: perchloric acid mixture (2:1), extracted and quantitatively diluted prior to analysis. CdCl2 levels were determined usisng GBC 902 atomic absorption spectrophotometer by acetylene-air flame.

Scanining Electron Microscopy studies (SEM): The liver was cut into small pieces, washed in phosphate buffer and fixed in 2.5% glutaraldehyde in phosphate buffer. The sample was examined under JSM 6100 scanining Electron microscope (SEM).

DNA ladder for apoptosis: The method[12], used for apoptosis, which appears as a ladder in a garose gel electrophorsis, was modified from[13].

Statistical analysis: Results are reported as mean ± SE . In experiments where the CdCl2 dose was varied, data was analyzed by using student's "t" test.

Fig.1. Dose dependent change in body weight of mice treated with $CdCl_2$ for 15 days. (Mean ± SE, n=6)

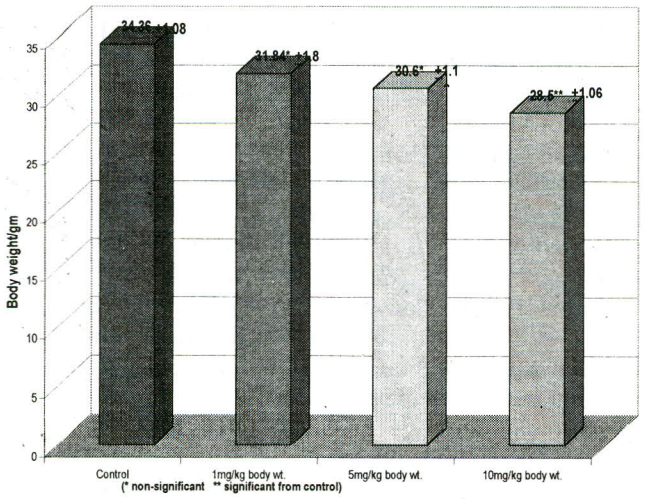
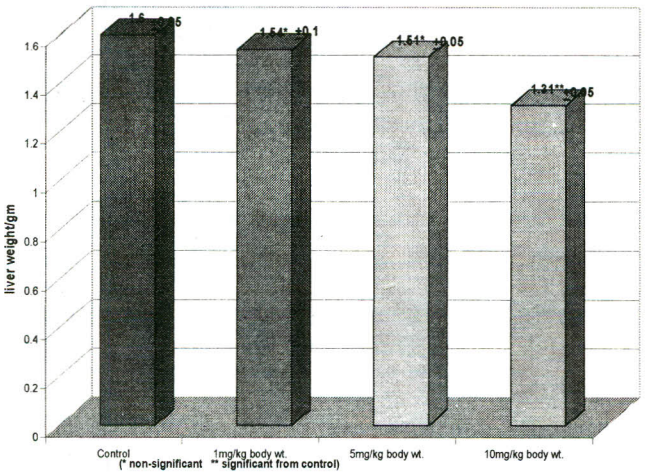


Fig.2. Dose dependent change in liver weight of mice treated with $CdCl_2$ for 15 days. (mean ± SE,n=6)



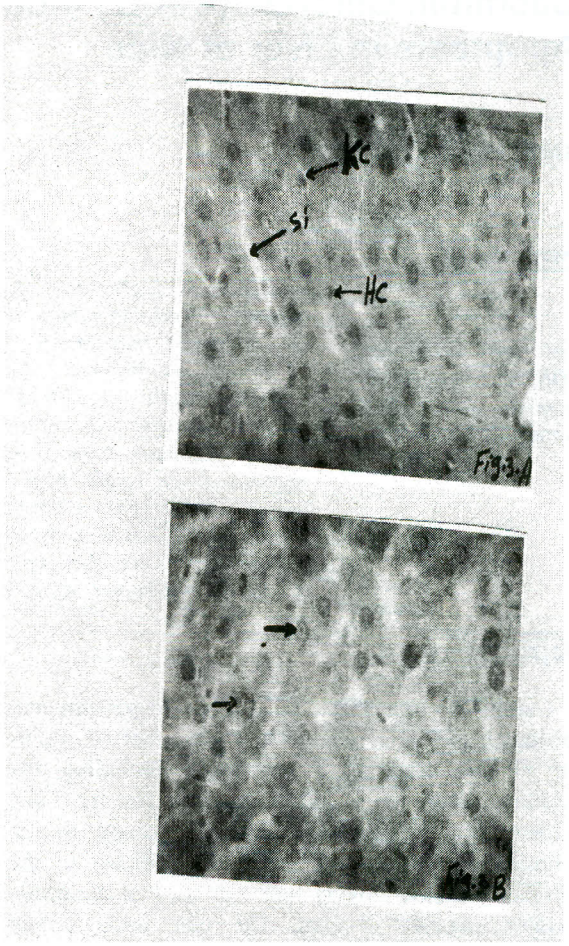


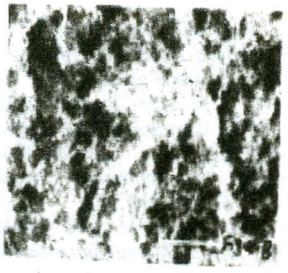
Fig.3. A. liver of control mice showing normal kupffer cells (kc), Sinusoids (si) and hepatocytes (HC). (X40)
B. Hepatocytes showing necrosis, pycontic nuclei (arrow) with mild degenerative change after exposure to 10mg/kg body wt.,CdCl2.

Results:

The intake of feed and water by treated mice reduced as compared to control. Moreover, the decrease was dose dependent. The liver weight and body weight significant decrease with increased of doses. (Figs. 1,2). Histological observation of liver after various doses of CdCl2 treatment showed marked alteration (Fig.3,A,B). The metal treatment cased marked changes in liver such as swelling and massive fatty degeneration in hepatocytes and large vacuoles in cytoplasm. Cytoplasm of hepatocytes showed vacuoles and nuclei were pycontic and staining affinity of nucleus was comparatively poor. The degree of apoptosis was quantified histologically with SEM study, the sinusoids were wide and bound by hepatic cells, these have kupffer cells (Fig.4,A). The sinusoids were covered with debris due to necrosis of cells as seem in (Fig.4,B). due to damage to hepatic cells after treatment with CdCl2. The damage of hepatic cells increased with

increase of dose were observed. Apoptosis was observed at 10 mg/kg body wt., CdCl2 administration (Fig.5).

The metal content in liver of different groups studied, there was progressive increase in the hepatic content in liver of animals after the treatment. (Fig.6)



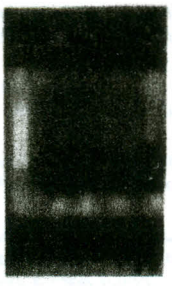
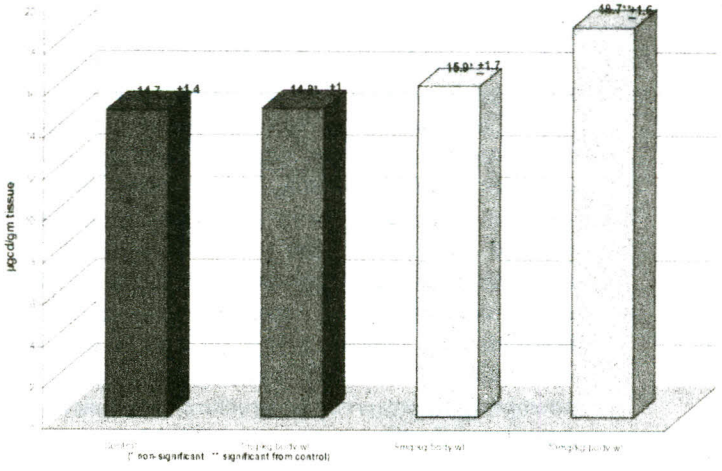


Fig.4. Scanining Electron Microscopy (SEM) of liver of mice showing.
A. Hepatic cells (HC), with sinusoids (Si) having kupffer cells (kc). Control. X2500.
B. Observe the depris of badly damaged hepatic cells.(10mg/kg body.wt., CdCl2).x2500.

Fig.5. Autoradiograph of agarose gel analysis of liver DNA fragmentation after Cd injection (labels indicate doses of cd injected ip, in mg/kg body wt.) DNA ladders were evident at 10mg /kg lane 5, lane 2, from control mice, lane 3,1mg/kg, lane 4, 5mg/kg, showed no ladder. Lane 1, indicate DNA molecular weight marker.

Fig.6. Dose dependent change in hepatic control of cdcl2 in mice treated with CdCl2 for 15 days (mean ± SE, n=6)



Discussion:

Necrosis as a feature of acute Cd hepatotoxicity is well established. This report characterizes apoptosis in the liver, and its relationship to liver necrosis, following acute cdmium poisoning. Several approaches were applied to investigate apoptosis in the liver following ip injection of Cd into mice. The result demonstrate that apoptosis is an important mode of eliminating damaged cells in Cd hepatotoxicity.

Liver necrosis resulting from Cd toxicity is well documented[7,14]. Focal necrosis was observed early (3h after 30 μmole cd/kg ip). Zonal necrosis (periportal) was present by 9hr, and wassive necrosis was seen by 24h. This progression of necrosis is consistent with earliar reports[7]. In the dose-response studies, focal, zongal and massive necrosis were also observed and were dose-dependent. The dose dependence was reflected in the histology and decreased in weight of liver. The progression of necrosis in consistent with earliar observation[14,15].

Peliosis hepatis, "blood lakes" in the liver showing no endothelial has lining, peliosis hepatis is usually a complication of androgenic/ anabolic steroid therapy[15]. The pathogenesis of peliosis is unknown, but one ultrastructural study, suggested that damage to sinusoidal endothelium[16].

In the dose-response studies, liver cell regeneration was dose- dependent. Apoptosis and liver cell regeneration are also observed in chronic Cd- induced hepatotoxicity[17].

The mechanism by which cd induces apoptosis in the liver is unknown. Cd is rendered inert in liver by being complexed to metallothionein, alow-molecular- weight scavenger protein, rich in cysteine residues. Excess free cd in the cytoplasm binds to cellular organelles, including the nucleus, and disrupts their functions. Cd is genotoxic in vitro, causisng single- strand breaks in DNA[18], frame- shift mutation[19], and chromosomal

aberrations[20]. Acute cd exposure induces the immediate early response genes c-jun, c-fos, and c-myc, and the tumor suppressor gene p53[21,22]. These genes play various roles in apoptosis[23], thus partly explaining the presence of apoptosis in cd toxicity.

In summary, we have presented evidence that apoptosis is an important and predictable event in acute Cd hepatoxicity. Apoptosis shows consistent time- course and dose- reponse patterns, and it precedes necrosis. While Cd – induced liver necrosis is sustained for at least 10mg/kg and also induced apoptosis. The early occurrence of apoptosis in acute cd poisoning in the mouse. The early occurrence of apoptosis in acute cd hepatotoxicity suggests a role for apoptosis in the elimination of critically injured liver cells while attempting to preserve the structural and functional integrity of the liver.

References:

1- *ATSDR. Toxicological profile for cadmium. Agencey for Toxic substances and Disease Registry, Atlanta, GA. 1999.*

2- *Bhattacharyya, M.H., Wilson, A.K., Tajan, S.S. and jonah, M. Biochemical pathways in cadmium toxicity. In: Zalups, P.K., Koropatnick, J.(Eds.), Molecular Biology and Toxicology of Metals, Taylor and Francis, London, 2000; PP: 34-74.*

3- *Rudolfs, K. and Sarfaraz, A. Molecular and handling of cadmium in transporting epithelelia. Toxicol. And appli. Phramc. 2003; 186; 163-188.*

4- *Joseph, P., Muchnok, T.K., Klishis, M.I., Roberts, j.R., Antoniai, J.M., whong, w.z. and ong, T. Cadmium-induced cell transformation and tumosigenesis are associated with transcriptional activation of C- Fos, C. Jun, and C-mue proto- Oncogenes: role of cellular cadmium and reative oxygen species. Toxicol.sci.2001;61, 295-303.*

5- *Joseph, P., Lei, Y.X., whong, W.Z. and ong, T.M. Oncogenic potential of mouse translation factor.1 delafor anovel cadmium-responsive proto- oncogene. J.Bio 1.Chem.2002; 277,6131-6136.*

6- *Kotsonis, F. N. and Klaassen, C. D. Toxicity and distribution of cadmium administered to rats sublethal doses. Toxicol. Appl. Pharmacol.1997; 41.667-680.*

7- *Dudley, R. E., Svoboda, D.J. and Klaassen, C.D. Acute exposure to cadmium causes severe liver injury in rats. Toxicol. Appl. Pharmacol. 1982; 65. 302-313.*

8- *Dudley, R. E., Svoboda, D.J. and Klaassen, C.D. Time- course of cadmium- induced ultrastructural changes in rat liver. Toxicol. Appl. Pharmacol.1984; 76, 150-160.*

9- *Wyllie, A. H., Kerr, J. F. and Currie, A. R. Cell death: The significance of apoptosis, Int. Rev. Cytol.1980; 68,251-306.*

10- *Corcoran, G.B., Fix, L., Jones, D. P., Moslen, M.T., Nicotera, P., Oberhammer, F. A. and Buttyan. R. Apoptosis: Molecular control point in toxicity. Toxicol. Appl. Oharamacol.1994; 128,169-181.*

11- *Lohmann, R. D. and Beyersnsmann, D. Effects of zinc and cadmium on apoptotic DNA fragmentation in isolated bovine liver nuclei. Environ. Health perspect.102(Suppl 3), 1994; 269-271.*

12- *Kerr, J.F., winterford, C.M. and Harmon, B.V. Apoptosis its significance in cancer and cncer therapy. Cancer, 1994; 73: 2013-2026.*

13- *Subramanian, T., Tarodi, B. and clinadurai, G. P53- independent apoptotic and necrotic cell deaths induced by adenovirus infection suppression by EIB 19k and bcl-2 proteins. Cell growth Differ.,1995; 6: 131-137.*

14- *Gering, P. L. and Klaassen , C.D. Tolerance to cadmium-induced hepatotoxicity following cadmium pretreatment. Toxicol. Appl. Pharmacol. 1984; 74, 308-313.*

15- *Taxy, J. B. Peliosis: A morphologic curiosity bbecoumes an iatrogenic problem, Hum. Pathol.1978; 9,331-340.*

16- *Zafani, E. S., Cazier, A., Baudelot, A. M. and Feldmann, G. Ultrastructural leasions of the liver in human peliosis. Areport of 12 cases. Am. J. pathol.1984; 114, 349-359.*

17- *Habeebu, S . S. M., Lin. Y. and Klaassen, C. D. Metallothionein-1/11 knock-out mice are vulnerable to chronic CdCl2–induced hepatotoxicity. The Proceedings of the Fourth International Metallothionein Meeting. 1997; Abstract No. 147 (Proc MT-IV. Abst # 147).*

18- *Coogan, T. P., Bar,R. M. and Waalkes, M. P. Cadmium-induced DNA strand damage in cultured liver cells: Reduction in cadmium genotoxicity following zinc pretreatment. Toxicol. Appl. Pharnmacol. 1992; 113, 27-233.*

19- *Biggart, N. W. and Murphy, E., Jr. Analysis of metal-induced mutations altering the expression or structure of aretroviral gene in a mammalian cell line. Muta, Res. 1988; 198, 115-129.*

20- *Hartwig, A. Role of DNA repart inhibition in lead- and cadmium induced genotoxicity: A review. Enviren. Health Perspect.1994; 3, 45-50.*

21- *Matsuoka, M., and cell, K. M. Cadmium- induced expression of immediate early genes in LLC-PKI cells. Kidney Int. 1995; 48, 383-389.*

22- *Zheng, H., Liu, J., Choo, K., Michalska, A. E. and Klaassen, C. D. Metallothionein-1 and-11 knock-out mice are sensitive to cadmium- induced liver mRNA expression of c-jun and p53. Toxicol. Appl. Pharmacol.1996; 136, 229-235.*

23- *Anderson, A. J., Pike, C. J. and Cotman. C. W. Differential induction of immediate early gene proteins in cultured neurons by β - amyloid (Aβ): Association of c-jun with Aβ- induced apoptosis. J. Neurochem.1995; 65, 1487-1498.*