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Biochemical, haematological and histopathological study in relation to time-related cadmium-induced hepatotoxicity in mice

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Abstract

In the present investigation sub-chronic hepatic necrosis was induced by cadmium chloride and was examined biochemically, haematologically and histopathologically in order to study the time-dependent effect and correlation among the parameters. Male balb/c mice were injected with cadmium chloride (2.5 mg/kg bw s.c.) for each other day and, sacrificed on the 7th day, 14th day and 21th day post exposure. Body weight and relative liver weight did not show alteration at any of the time point following the treatment but the tissue cadmium level showed progressive significant increment values with the advancement of time exposure. Most of the biochemical parameters (total protein, DNA, RNA, cytochrome P450 cotents, alkaline phosphatase and UDP glucuronyl transferase activities), haematological parameters (total red blood cells, total white blood cell, differential white blood cell counts, haemoglobin, erythrocyte sedimentation rate, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, plasma protein) indicated either no or less on the alterations/7th day following cadmium exposure. Both the light and transmission electron microscopy, on the other hand, indicated the fact that a minimum of 21 day-exposure was needed to alter the cellular architecture. So, a certain amount of cadmium load might be required to adversely affect the cellular architecture preceeded by biochemical and haematological alterations. In this connection, in the present study a possible mechanism of cadmium-induced hepatoxicity was discussed.

Introduction

Cadmium (Cd) intoxication in humans has been investigated following occupational as well as environmental exposure (Hammond & Foulkes 1986). Its a toxicological important metal accumulates in liver following short-and long-term exposure (Stohs & Bagchi 1995). Bagchi *et al.* (1996) have demonstrated increased hepatic mitochondrial and microsomal lipid peroxidation, glutathione depletion etc. following Cd induction. Recently, Bagchi *et al.* (1997) have also demonstrated that a low dose Cd results in the formation of reactive oxygen species. This group IIB metal has been well known for decades and includes various adverse effects such as disturbances of enzyme function (Vallee & Ulmer 1972), the enhancement of lipid peroxidation (Manca *et al.* 1991), the disarrangement of mitochondrial functions (Southard *et al.* 1974) along with DNA chain break (Tsuzuki *et al.* 1994). The precise mechanism by which metals cause hepatic injury are unknown (Sauer *et al.* 1997). Contrast to the low dose Cd when administered parenterally as a large (bolus) dose, it can induce hepatocellular necrosis (Fowler 1991; Goering *et al.* 1994).

In the present study, the liver was studied because the liver plays an important role in detoxifying

xenobiotics and is protected by number of chemical (Bishayee *et al.* 1997). There are scanty and sporadic reports available regarding the effect of Cd simultaneously on the haematological as well as on the biochemical parameters in addition to the light and electron microscopy of the liver. Considering the lack of pertinent data, the purpose of the present investigation was to determine the time-dependent effect following acute dose of Cd administration in male balb/C mice. Attempts were also made to correlate the biochemical and haematological parameters with the electron and light microscopic observations.

Materials and methods

Animals and treatment

Male balb/C mice used in the present investigation were purchased from the local animal dealer. All animals were kept in polythene cages (5 mice/cage) and allowed to acclimatize for 7 days prior to experimental use. The animals were given food and water *ad libitum* and, were maintained at 12 h/12 h light / dark regimen. The animals of the treated groups received cadmium chloride (Sigma Chem. Co., St. Louis, MO) at the dose of 2.5 mg/kg body weight (*s.c.*) for each alternate day for 7, 14 and 21 days. The control mice received vehicle (normal saline) only for the above-mentioned specified time points. The animals were sacrified by decapitation at these time points.

Hepatic biochemical estimations

DNA and RNA were extracted according to the method of Cherry (1962) and estimated spectrophotometrically by diphenylamine and orcinol reagents, respectively (Plummer 1988). Protein was estimated according to the method of Lowry *et al*. (1951). For the estimation of hepatic alkaline phosphatase (ALP) activity, the tissue was homogenized with ice cold normal saline, centrifuged at 5000 rpm for 20 min in Himac Hitachi Centrifuge (Japan), supernatant was taken and the activity was determined using p-nitrophenyl phosphate as substrate (Sigma Technical Bulletin, 1978). Amount of DNA, RNA and protein were expressed in terms of μg/mg fresh tissue and the activity of alkaline phosphatase was expressed as μ mole p-nitrophenol liberated/min/mg protein at 37 °C.

Haematological studies

For the haematological study blood was collected directly by puncturing the heart and kept in EDTA added tube. The total red blood cells (RBCs) and white blood cells (WBCs) counts were performed following routine techniques. For differential count of WBCs, blood was taken directly on slide for making thin film from the tail vein, stained with Wright's stain, different cells were identified and counted. For plasma protein content, ice cold 20% trichloroacetic acid was added to the plasma (1:1) and the mixture was kept in the freeze overnight. Precipitate was isolated by centrifugation at 5000 rpm at 4 °C and was dissolved in 0.1 N NaOH. The stock was suitably diluted to estimate the protein concentration by the method of Lowry *et al*. (1951). For measuring serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT) activities, blood was collected directly puncturing the heart and was allowed to form serum. The serum was separated by centrifuging at 2,000 *g* for 10 min at 4 °C and, the activities were measured by the method of Reitman and Frankel (1979).

Cd level

For Cd determination, the hepatic tissue (1.0–2.0 g) was placed in a test tube and then digested in 1 ml conc. HNO_3 for at least 12 h. Subsequently, the tissue was boiled until it became semidried. Deionized water was then added to make a final volume of 5 ml. Cd standard solution was prepared by dissolving known quantities of Cd salt and the levels were determined in Atomic Absorption Spectrophotometer (Perkin-Elmer Model 560).

Estimation cytochrome P-450 and UDPGT activity

Following 24 h fasting, the liver tissue was excised and homogenized with 1.5% KCl solution containing 0.1 M phosphate buffer (pH 7.4) using teflon/glass homogenizer. The homogenate was then centrifuged at 9,000 *g* for 20 min and the resultant supernatant was recentrifuged at 105 000 *g* for 60 min. The pellet (microsomal fraction) formed was resuspended in homogenizing medium and kept at −70 °C for further use for the assay of cytochrome P-450 (Cyt.P450) and UDP glucuronyl transferase (UDPGT) activity. The Cyt.P450 content in microsome was determined from the reduced carbon monoxide difference as described by Omura and Sato (1964). The UDPGT activity towards p-nitrophenol, on the other hand, was measured

from the microsome by the method of Watanabe et al. (1986).

Light and transmission electron microscopy(TEM)

For light microscopy, in brief, tissue was fixed in Bouin's fixative, embedded in paraffin wax (mp 60 °C–62 °C), cut 7 μ thick sections by hand microtome and stained with haematoxylin and eosin while passing the slides through graded ethyl alcohol and water. For TEM hepatic tissue was fixed in 3% glutaraldehyde (in 0.1 M phosphate buffer, pH 7.4) at 4 °C for 1 h, washed and fixed in 1% osmium tetroxide (in phosphate buffer) at room temperature for 1 h. Tissue was then washed in water, dehydrated in graded ethyl alcohol and emedded in ERL-4602 medium. Ultrathin of about 500–600 Å were cut with glass knife by LKB ultramicrotome (Ultratome 48044). Sections were stained with uranyl acetate and lead acetate (Epstein & Holt 1963; Reynolds 1963).

Statistical analysis

Significance between pair of means for control and treated groups was determined by Student's *t*-test. The data are expressed as mean ± standard error of five mice and the level of significance considered were $P < 0.05$, $P < 0.01$ and $P < 0.001$.

Results

In the present study subchronic and subcutaneously administered Cd was found to exert adverse effect on the biochemistry and morphology of hepatic tissue along with haematological alterations:

1. Body weight and relative liver weight

Table 1 shows body weight and relative liver weight (rlw) of untreated control and Cd treated experimental mice during 21 days study. Any of the time exposure showed no significant difference in bw and rlw between control and Cd treated mice but the value showed a tendency towards increment following Cd exposure along with the advancement of time points.

2. Cd level

The level of Cd in the tissue has also been depicted in Table 1. The accumulation of Cd increased linearly with the time exposure. Each of the time point showed significant increment in Cd concentration compared with the untreated group. While 7 days exposure showed the 14-fold increase ($P < 0.001$), the 14 and 21 days post-exposure indicated a 65-fold ($P < 0.001$) and a 84-fold ($P < 0.001$) increment, respectively. So the highest Cd concentration was observed in the 21 days exposed group.

Table 1. Effect of cadmium (2.5 mg/kg body weight) on body weight, relative liver weight and heptic cadmium level of male balb/c mice following 7 days, 14 days and 21 days of exposure.

Days	Body weight (g)	Relative liver weight	Cd level (μg/mg tissue)
0	21.98 ± 1.52	3.90 ± 0.14	0.25 ± 0.16
7	22.16 ± 1.45	4.01 ± 0.25	$10.12 \pm 1.00^{***}$
14	23.01 ± 0.92	4.65 ± 0.50	$16.25 \pm 1.00^{***}$
21	24.00 ± 0.95	4.98 ± 0.72	$21.19 \pm 1.59^{***}$

$^{***}P < 0.001$ (compared with the untreated control group).

3. Haematology

Total RBCs count indicated an adverse effect of Cd as evident in Table 2. Seven days following Cd exposure indicated an insignificant decrease of 12% from the control value, but thereafter the value decreased significantly to 27% ($P < 0.05$) in the 14 days post-treated group. The highest depleted value was observed in the highest duration exposure group (44%; $P < 0.001$). Other haematological parameters like Hb, ESR and total WBCs showed similar response as found in the case of RBCs following Cd treatment. In each case, concerning the 7 days following exposure, Cd failed to show any significant alteration of any value compared with the controlled animals. In the later time point, while Hb and WBCs showed significant depleted values, ESR, on the other hand, showed significant increased values and in each case the highest altered level was observed in the highest time exposed group.

In case of differential WBCs count, the percentage of lymphocytes responded differentially from that of neutrophils following the metal exposure (Table 3). Highly significantly ($P < 0.001$) depleted value of lymphocytes from the control group was evident in the 21 days exposed mice and a 14 days following exposure showed low significant value as to the animals ($P < 0.05$). The 7 days exposure, on the other hand, did not show any alteration. Neutrophil value increased progressively significantly at each of the three time points and similar response was also recorded in case eosinophil count. The monocyte count showed more/less similar response as found in case of lymphocyte count after treatment.

Table 2. Effect of cadmium (2.5 mg/kg body weight) on haemoglobin (Hb), total reb blood cell (RBC) count, total white blood cell (WBC) count, erythrocyte sedimentation rate (ESR) and blood plasma protein level of male balb/c mice following 7 days, 14 days and 21 days of exposure.

Days	Hb (g %)	RBC (10^6/mm^3)	ESR (%)	WBC (10^3/mm^3)	Plasma protein (g/100 ml)
0	11.90 ± 0.90	6.89 ± 0.52	2.20 ± 0.20	13.09 ± 1.75	8.8 ± 0.92
7	10.20 ± 0.20	6.07 ± 0.48	2.71 ± 0.20	12.52 ± 2.00	6.7 ± 0.70
14	$8.99 \pm 0.90^{*}$	$5.00 \pm 0.35^{*}$	$3.22 \pm 0.30^{*}$	$9.11 \pm 0.62^{*}$	$5.2 \pm 0.42^{**}$
21	$7.25 \pm 0.80^{**}$	$3.87 \pm 0.30^{***}$	$4.02 \pm 0.20^{***}$	$8.27 \pm 0.80^{*}$	$3.6 \pm 0.30^{***}$

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ (compared with untreated control mice).

Table 3. Effect of cadmium (2.5 mg/kg body weight) on differential count of peripheral blood leucocytes of male balb/c mice following 7 days, 14 days and 21 days of exposure.

Days	Lymphocyte(%)	Neutrophil(%)	Monocyte(%)	Eosinophil(%)
0	75.16 ± 5.06	20.57 ± 1.52	5.00 ± 0.26	0.92 ± 0.09
7	63.00 ± 6.52	$30.11 \pm 1.85^{*}$	$3.03 \pm 0.23^{*}$	$2.46 \pm 0.09^{**}$
14	$55.21 \pm 3.92^{*}$	$39.92 \pm 2.03^{***}$	$2.44 \pm 0.18^{*}$	$1.83 \pm 0.21^{**}$
21	$43.08 \pm 3.98^{***}$	$44.11 \pm 2.98^{***}$	$9.59 \pm 0.15^{***}$	$2.76 \pm 0.19^{***}$

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$ (compared with untreated control mice).

4. Hepatic functions

Biochemical evidence of Cd-administered hepatic injury has been depicted in Tables 4 and 5. As early as 7 days after the treatment of the metal significant elevation in SGOT ($P < 0.05$), SGPT ($P < 0.05$) and hepatic ALP ($P < 0.001$) were observed. But other biochemical parameters like hepatic Cyt.-P450 content, UDPGT activity, DNA and RNA levels, total plasma protein did not show any significant alterations in value. At this time point, SGOT, SGPT and hepatic ALP showed 31%, 69% and 136% elevation as compared to the control counterparts, respectively. Further, a marked rise in SGOT, SGPT and ALP activity occurred with the increment of time exposure. In each case the highest value was recorded in the 21 days exposed group. The alterations in values of SGOT, SGPT and ALP activity were found to be more than double in the 21 days exposed groups compared with that of the 7 days exposed counterparts. Similar patterns were observed with respect to Cyt.P450 and UDPGT following Cd exposure. Fourteen days after Cd administration Cyt.P450 concentration was 22% less than that found in control microsome ($P < 0.05$) and by the 21 days exposure, the Cyt.-P450 value decreased to 33% that observed in control mice. Following the 14 days exposure, UDPGT activity inhibited by a approximately 40% ($P < 0.001$) and the highest exposure (21 days) had decreased the value to more than half (58%) of which was observed in untreated control mice. Table 4 illustrates time-dependent alterations of blood plasma protein, DNA and RNA concentrations following Cd induction. In each case, early time exposure (7 days) failed to produce any effect as mentioned earlier but the moderate (14 days) and highest (21 days) time exposure showed significantly depleted values in each of the parameter. While moderate depletion occurred in modertate time point following Cd treatment, the highest time exposure indicated the highest significantly depleted values. The percentage depletion of control values were found to be more or less similar in case of plasma protein (40%), DNA (36%) and RNA (43%) during 14 days and 21 days (plasma protein, 60%; DNA, 56%; RNA, 66%) exposure following Cd administration. The RNA/DNA indicating the transcriptional capacity decreased as the time progressed in 7 days (1.77), 14 days (1.74) and 21 days (1.50) from the control value.

5. Hepatic morphology

Liver from Cd treated groups did not exhibit any macroscopic alterations in structure. Following light microscopy observation, early and moderate time points (7 and 14 days) failed to produce any significant change in structure but after 21 days following Cd treatment some marked alterations were observed and that led us to the TEM study of that particular group (21 days). Microscopic examination of hepatic

Table 4. Effect of cadmium (2.5 mg/kg body weight) on total hepatic protein level, total hepatic DNA content and total hepatic RNA content of male balb/c mice following 7 days, 14 days and 21 days of exposure.

Days	Protein (μg/mg tissue)	DNA (μg/mg tissue)	RNA (μg/mg tissue)
0	225.77 ± 7.12	6.25 ± 0.78	12.25 ± 1.50
7	$186.11 \pm 7.25^{*}$	5.09 ± 0.80	9.00 ± 0.98
14	$150.27 \pm 5.15^{***}$	$3.98 \pm 0.50^{*}$	$6.92 \pm 0.82^{*}$
21	$121.09 \pm 5.00^{***}$	$2.75 \pm 0.62^{**}$	$4.12 \pm 0.76^{**}$

section of the group (21 days) revealed signs of cell injury (Figure 1A) compared with that of control untreated mice (Figure not shown). Parenchymal cell swelling and cytoplasmic eosinophilia were clealy evident from all hepatic sections examined from the treated group. In addition numerous pycnotic nuclei and necrotic hepatocytes were evident along with fatty metaphorphosis. Kupffer cells were clearly visible in treated groups. The figure clearly demonstrates that histological changes were diffuse and not localized in any specific area. As morphological changes were diffuse, it suggests that Cd acts as general hepatotxin. Representative TEM observations (Figure 1B) depict ultrastructural changes. Less and disorganised rough endoplasmic reticulum along with irregular shaped nucleus were observed in Cd exposed mice as compared to the normal RER and perfectly round nucleus in control mice (Figure not shown). Compartmentalization in mitochondria with surrounding and central zone were clearly evident from the figure in the treated group. Lipid droplets were also visible in the cytoplasm.

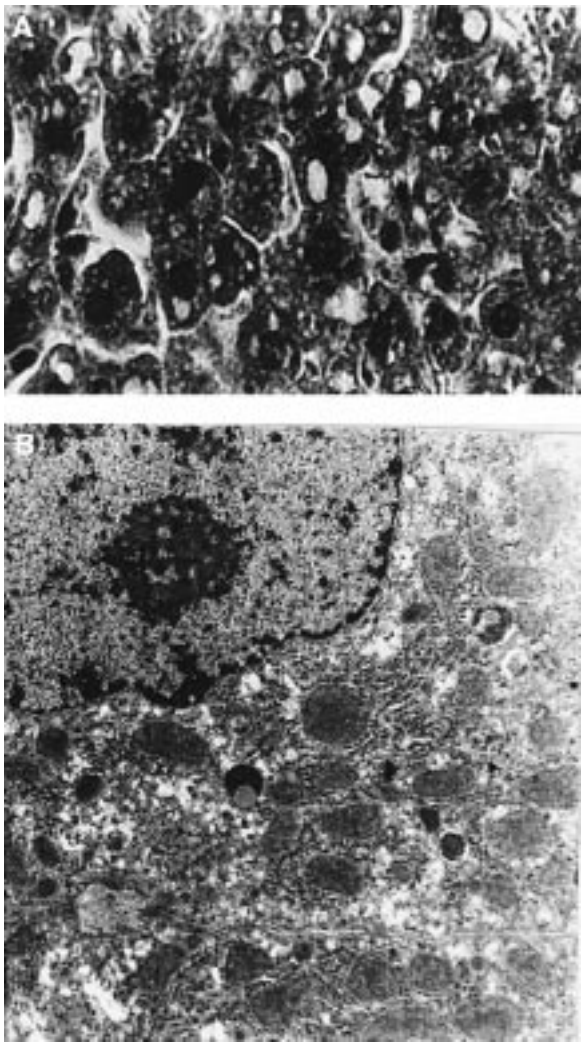

Figure 1. A. Cadmium (2.5 mg/kg bw)-induced hepatic microscopic pathology of male balb/c mice following 21 days of treatment. Liver sections were fixed in Bouin's fixative and stained in haematoxylin and eosin as described in materials and methods. Note the parenchymal cell swelling, eosinophilia, numerous pycnotic nuclei and necrotic hepatocytes. Fatty metamorphosis is also evident. Clealrly visible activated Kupffer cells. Note also diffuse histologic changes and not localised to any specific area (x 1250). B. Transmission electron micrograph of hepatic tissue of balb/c male mice 21 d after cadmium exposure (2.5 mg/kg bw) showing less rough endoplasmic reticulum, irregularly shaped nucleus, lipid droplets. Note also compartmentalisation in mitochondria with surrounding and central zones. Mitochondria are also swollen and irregularity in shape exists (A = x7000, B = x12000).

Discussion

In the present study hepatic tissue damage was estimated by measuring the hepatic protein, DNA, RNA, Cyt.P450 content and, UDPGT and ALP activities in addition to haematological and morphological indices. In most of the previous studies toxicity associated with Cd involves low dose chronic exposure. The results of the present case clearly indicate that subchronic Cd administration for alternate days results in time-dependent decrease in hepatic DNA, RNA, Cyt.P450 contents, inhibition of UDPGT activity and, elevation in ALP activity, SGOT, SGPT. Some of the blood parameters like Hb decreased along with total RBCs

Table 5. Effect of cadmium (2.5 mg/kg body weight) on total hepatic alkaline phosphatase (ALP) activity, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) activities, cytochrome P450 (Cyt.P450) level, UDP-glucuronyl transferase (UDPGT) activity of male balb/c mice following 7 days, 14 days and 21 days of exposure.

Days	ALP[a]	SGOT[b]	SGPT[b]	Cyt.P450[c]	UDPGT[d]
0	1.11 ± 0.25	44.25 ± 2.53	21.28 ± 2.77	3.29 ± 0.22	16.80 ± 0.95
7	$2.62 \pm 0.33^{***}$	$58.10 \pm 3.92^{*}$	$35.88 \pm 1.92^{*}$	3.00 ± 0.25	15.11 ± 1.10
14	$4.01 \pm 0.52^{***}$	$67.77 \pm 3.90^{***}$	$41.09 \pm 2.75^{**}$	$2.58 \pm 0.20^{*}$	$9.99 \pm 0.82^{***}$
21	$5.86 \pm 0.62^{***}$	$78.23 \pm 5.00^{***}$	$57.78 \pm 3.00^{***}$	$2.20 \pm 0.19^{**}$	$7.12 \pm 0.80^{***}$

a = μ mole p-nitrophenol liberated/ mg protein/ min at 37 °C.
b = activity expressed in terms of UL/L.
c = *n* mol/ mg protein.
d = *n* mol of glucuronide formed/min/mg protein.
*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$ (compared with untreated control mice).

and, WBCs count but the ESR increased. The differential leucocyte count showed low lymphocyte and high neutrophil values in treated groups. In the present investigation most of the alterations in biochemical, haematological and morphological parameters were observed after 14 days of treatment with a maximum value in the 21 days exposed group. Thus, the results clearly indicated the cumulative effect of Cd.

The degree of accumulation of Cd is markedly influenced by the duration of Cd exposure (Bokori *et al.* 1996). The toxicity of Cd may involve a reaction of reactive oxygen species (ROS) as described by Manca *et al*. (1991) and our previous investigation also showed elevated LPO in the liver following Cd exposure (Karmakar *et al.* 1998). The decreased DNA content following the Cd administration (Schilderman *et al.* 1997) may be due to increased ROS generation that could cause increased single strand break (Bagchi *et al.* 1997) along with the time following Cd exposure. The ultrathin section also indicated a nuclear morphological alteration in the treated group. The decreased RNA concentration in the present study may also be correlated with the adverse effect of Cd on DNA that could time-dependently inhibit the transcriptional capacity on the one hand, and destruction of polysomes on the other hand as shown by the decreased RER in TEM study. The trasncriptional capacity has been shown by RNA/DNA which was found to be 1.96 in untreated control mice, decreased sharply to 1.77 and 1.74 in the 7 days and 14 days exposed groups respectively. The lowest value (1.50) was observed in highest time-point exposed group indicating the highest adverse effect of Cd according to the present study. In our previous study we observed decreased glutathione following Cd administration which could be one of the causes of oxidative stress along with increased LPO.

A plausible mechanism may involve Cd concentration in hepatic tissue following exposure may exceed the intracellular metallothionein and glutathione concentration to bind that may enable the Cd to interact with the cellular organelles that could disrupt biochemical processes. According to the present investigation the morphological changes were secondary. Early and moderate time points following Cd administration failed to show any marked alterations in morphology following light and electron microscopy studies excepting the biochemical alterations. The morphological alterations may be the consequences of toxic effects of Cd.

The plasma protein level depletion in the treated groups could be a case of time-dependent hepatotoxicity and produces evidence that Cd had no early effect as recorded in the present experiment. The initial lack of severe pathology following the 7 days and 14 days exposure may be due to the presence of protective mechanism that was operative at that time points but the later time point showed liver necrosis which was an indicative of failure of that operative system. Thus, it could be stated that large hepatic concentration of Cd results in the damage in a cumulative manner.

Increased heterochromatin following Cd as shown in the Figure 1 may be associated with the inhibition of protein synthesis through the inhibition of RNA synthesis. Thus, RNA and protein, syntheses are sensitive to Cd interference. Mitochondrial structure disarrangement as noticed in the present study may be associated with the energy requirement process impairment. Cd accumulates in mitochondria and inhibits oxidative stress (Saris & Jarvisalo 1979) and

the accumulation may cause the change in matrical structure of the mitochondria.

Cyt.P450-dependent monooxygenase is the principal 'phase-I' enzyme and similarly, UDPGT is the principal 'phase-II' enzyme than can utilize the monooxygenase product to form glucuronides, the hydrophilic substance which is ultimately excreted via urine. According to the present study, Cyt.P450 and UDPGT activity responded in such a way confirming the notion that they are in some way coordinately regulated. Along with the advancement of time, the significant decrement in both the parameters is indicative of total failure of RER as observed in figure (Dematties & Aldridge 1978). The decreased value of Cyt.-P450 as recorded in the present investigation could be attributed to the enhanced LPO as noticed in our previous work (Karmakar *et al.* 1998) and an increased activity of haemooxygenase may be the other possibility.

It is accepted that the functional studies in toxicology should be coupled with the appropiate histological studies, because appropriate morphological studies are useful especially during the anatomical localization of action of toxin (Kramp *et al.* 1974) and electron microscopy study might be possible to identify the toxic effect on plasma membrane and/or subcellular organelles (Brendt *et al.* 1989). In the present study we recorded histopathological alterations during the later period only, but on the other hand the biochemical and haematological parameters showed in some cases alterations as early as 7 days following the treatment. Thus, the biochemical and haematological changes occurred first, then followed by the alterations of the cellular structure. It may be stated that a certain amount of Cd load is required to cause architectural alterations and a similar argument was also stated by Bhattacharyya (1996).

According to the present study, dose-dependent elevation of SGOT, SGPT and other blood parameters bear a close resemblance to that of hepatic biochemical parameters in addition to the histopathology. Marked inhibition of GST activity following Cd administration (Karmakar *et al.* 1999) may have some relation with alteration of Cyt.P450 and UDPGT, because GST is associated with the cellular defense mechanism. In this respect, it could be said that for the alteration of biochemical and haematological parameters less Cd load is sufficient compared with the concentration required for histopathological alterations and initial alterations of biochemical as well as haematological indices.

Although the peripheral cell counting is not sufficient to indicate a change in quality and quantity of precursor cells, it is still the way to determine the toxicity on haematological indices (Schofeld 1986). The destruction of RBCs indicates the abnormality in hepatocellular functions and morphological alterations of the cells and also indicates the changes in the membrane cholesterol and phospholipid ratio and content (Sherlock & Dooley 1993). The adverse effect of Cd in a time-dependent manner probably influences the Hb synthesis mechanism as shown by the depressed level of Hb and RBCs count and Cd-induced alterations in differential count of leucocytes may be due to alterations of the lymphoid:myeloid ratio. Activated neutrophils produce ROS during inflammatory reactions and if neutrophils accumulate, tissue would be exposed to large quantities of potentially injurious neutrophil content (Rollet-Labella *et al.* 1998). In this regard, it could be stated that following Cd administration in the present investigation, neutrophil count increased time-dependently.

The liver is known to play an essential role in the synthesis of plasma protein and different globulins. Kupffer cells of the liver (RE system) are well known for the antigenicity as they phagocytose many antigens. The decreased plasma protein following Cd administration is the indicative of the immunosuppression and at the same time Cd treatment activated the resident macrophages as evident in the figure. The significant reduction in RBCs indicated the fact that Cd adversely affected the erythropoiesis in time-dependent fashion (Kasuya 1994) and an increased ESR supported the anaemia (Schumann *et al.* 1996). Elevated levels of SGOT and SGPT are the conventional indicator of hepatic damage. The marginal increment in rlw following Cd exposure may be due to accumulation of lipid and triglycerides which is in good agreement with the previous observation from this laboratory (Mandal *et al.* 1992). The changes in UDPGT is due to the changes in protein-lipid interactions, because it is an oligomer whose activity is dependent on the proper association of monomeric polypeptides (Castuma *et al.* 1989). Since stimulation of LPO following Cd administration was reported by different workers and also noted in our personal observation (Karmakar & Chatterjee 1998), it is more likely that altered membrane environment results in the disaggregation of UDPGT.

In summary, the current results being in conjuction with previous studies indicate that subchronic administration of Cd to mice results in a hepatic injury

during the later period and this may be due to the disruption in DNA replication, RNA synthesis, mitochondrail metabolism and inhibition of Cyt.-P450 and UDPGT activity during the early time points. The hepatic Cd load may be one of the key factors to bring about the changes. A threshold level of alteration of biochemical parameters is necessary for the morphological changes. In future studies more time points should be considered using an additional dosing pattern to get an insight into the precise mechanism of Cd-induced hepatotoxicity.

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