Effect of Cadmium on Liver Regeneration after Partial Hepatectomy in Rats

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Cadmium is a nonabundant element that is widely distributed throughout the biosphere and its toxic effects are becoming potentially more serious due to industrialization. It has been reported that cadmium might interact with nucleic acid biosynthesis. In this study we examined the effect of cadmium administration, either 24 hr before or simultaneously to partial hepatectomy, on the liver regenerative process in rats, at different time intervals. The rate of DNA synthesis was suppressed markedly in the cadmium pretreated group and the first peak of liver regeneration was delayed, compared to the simply partially hepatectomized one. The administration of cadmium simultaneously to partial hepatectomy, caused a marked decrease of the rate of DNA biosynthesis, compared to the pretreatment. The rate-determining enzyme thymidine kinase was suppressed in the liver of both cadmium-treated groups. Biochemical parameters and histological findings were also coestimated. The above data suggest that either pre- or simultaneous administration of cadmium, suppressed the liver regenerative process, probably due to the inhibition of thymidine kinase. — Environ Health Perspect 102(Suppl 3):273-276 (1994).

Key words: cadmium, liver regeneration, rats, partial hepatectomy, 3HTdR incorporation, thymidine kinase

Introduction

Cadmium (Cd) is a highly toxic element that is present in food and water and is accumulated in liver and kidney. It is known that Cd is one of the most harmful heavy metals able to induce renal, hepatic and testicular injury (1). Parenteral administration of a soluble Cd salt in rats, causes a rapid accumulation of Cd in the liver. Hepatic necrosis has been reported in rats and mice after acute exposure to Cd (2,3).

The enzyme thymidine kinase (TK, E.C. 2.7.1.21), responsible for the phosphorylation of deoxythymidine and its subsequent incorporation into DNA, has been involved in the inhibition of DNA synthesis in Cd-treated cell cultures (4).

We have shown that the enzyme TK is inhibited in the liver of Cd-treated rats (5). The liver regenerative process following partial hepatectomy (PH), with resection of two thirds of the hepatic mass, is a well established model of rapidly dividing cells. Experimentally, regeneration can be induced by any acute treatment that will remove or kill a large percentage of hepatic mass. Loss of parenchyma rapidly induces a wave of cell proliferation so that the total mass of liver is restored to normal (6).

In this experimental study we examined the effects of Cd administration on liver regeneration, after PH, at different time intervals. Cd was administered prior or simultaneously to PH. The regenerative process was estimated by the rate of DNA biosynthesis and the enzymatic activity of TK, in the liver. Biochemical and histopathological changes were also coestimated.

Materials and Methods

Two hundred and eight male Quinster rats weighing 180 to 220 g each, obtained from the Hellenic Pasteur Institute, Athens, Greece, were used in this study. Animals had free access to food and water ad libitum, were kept in an air-conditioned room 21°C, with a photo-period of 12 hr light/12 hr dark and handled with human care. The rats were randomly selected and assigned to four main experimental groups. Experiments were started between 7:00 and 9:00 A.M. and the animals were fasted for 12 hr before any manipulation. They were subsequently subjected to 70% partial hepatectomy, according to Higgins and Anderson technique (7), or sham operation.

The four main groups of rats were considered as follows: Group sham: operation consisting of gentle manipulation of the liver, under light ether anesthesia. Group I: PH as mentioned above. Group II: PH and intraperitoneal (ip) administration of 2.5 mg CdCl2/kg of body weight (bw), (cadmium chloride pure, CdCl2 + 2H2O, E. Merck, Darmstadt) 24 hr prior to PH. Group III: PH and simultaneous ip. administration of 1.0 mg CdCl2/kg bw, (Cadmium chloride pure, CdCl2+2H2O, E. Merck, Darmstadt).

The animals were sacrificed at 0, 12, 24, 36, 48, 60, 72, and 96 hr postoperatively, under ether anesthesia. All groups of rats were injected ip with tritium deoxythymidine (3HTdR: Amersham UK: 25 μCi/100 g, bw), 1 hr before sacrifice.

Blood samples were collected via cardiac puncture. The samples were allowed to clot and the serum was removed by centrifugation at 1000g for 10 min. All sera were sterile, hemolysis free and were kept at 4°C prior to assay on a Technicon RA-1000 random access chemistry analyzer (Technicon Instruments Corporation, Tarrytown, NY).

Immediately after exsanguination the livers were removed, cleaned, and weighed. Portions of livers were kept frozen at −80°C in order to be analyzed for their DNA content and their TK activity, while another portion was separated and immersed in 10% formalin solution for further histological examination.

Liver portions were homogenized in ice-cold deionized water, and the DNA was extracted from the tissue (8). The determination of DNA in the tissue residue was based on the reaction of deoxyribose with diphenylamine (9). The specific activity of DNA was calculated from the radioactivity measured by a liquid scintillation counter (Wallac LKB 1211 Rackbeta, Sweden) and the amount of DNA that was determined colorimetrically. Results were expressed as

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counts per minute incorporated per ug of DNA (cpm/µgDNA).

The enzymatic activity of TK was assayed in the supernatant fractions of liver portions after homogenization and centrifugation at 105000g for 1 hr at 4°C with a Beckman model L5-75 Ultracentrifuge, according to the method described by Kahn et al. (10). Duplicate aliquots of each sample were spotted onto DEAE cellulose discs and placed in scintillation vials. The discs were counted for their radioactivity content in a liquid scintillation counter (Wallac LKB 1211 Rackbeta, Sweden). The protein content of each sample was determined by the method of Lowry et al. (11). The activity of the enzyme was expressed as counts per minute incorporated, per 1 min, per milligram of protein (cpm/min/mg protein).

Biochemical evaluation of liver function was estimated by measuring serum enzyme activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) determined spectrophotometrically (12,13) with commercially available kits (Technicon Chemicals Company, Tournai, Belgium).

Preparation of livers was performed by fixation in 10% formalin solution, dehydration in graded alcohols, clearing in xylene, and embedding in paraffin wax. Sections were cut at 4 µm and stained with haematoxylin-eosin (H-E). All specimens were randomized and given a code number so that the examiner was unaware of the group to which the specimen belonged.

The statistical analysis of our results was based on the Wilcoxon test for unpaired measurements.

Results

The rate of 3HTdR incorporation in partially hepapectomized rats (group I) was maximum 24 hr after PH (p<0.001) and remained high at the time intervals of 36, 60, and 72 hr (p<0.001) (Figure 1). During the maximum liver regenerative process in group I the enzymatic activity of TK was abruptly increased, between 24 and 48 hr (p<0.001) (Figure 2). The maximum value of TK activity was observed at 36 hr (Figure 2) simultaneously to the first wave of hepatic regeneration, as it is shown in Figure 1. At 60 hr, TK activity was decreased and at 72 hr a slight increase was observed (Figure 2) when at the same time the last peak of DNA synthesis was noticed (Figure 1).

In group II a statistically significant decrease was observed in 3HTdR incorporation values, in comparison to group I, (Figure 1). The peak of DNA biosynthesis was observed at 48 hr in this group, 24 hr later than that of group I. TK activity of group II, was increased mainly at 24, 36, and 48 hr (p<0.001) (Figure 2). The maximum value was observed at 48 hr and was lower (p<0.001) than that of group I.

The administration of CdCl2 simultaneously to PH (group III) caused a decrease of the rate of 3HTdR incorporation at all time intervals examined, (p<0.001) up to 72 hr (Figure 1), compared to groups I and II. TK activity in this group increased 36 hr after PH (p<0.001), with maximum values at 48 and 96 hr (Figure 2).

Nonstatistically significant differences were observed, either for the rate of DNA biosynthesis or for TK activity, in group sham, at all time intervals examined (data not shown).

The enzymatic activities of AST and ALT presented similar profiles in all groups examined (Figures 3,4). The maximum

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The administration of CdCl2 simultaneously to PH (group III) caused a decrease of the rate of 3HTdR incorporation at all time intervals examined, (p<0.001) up to 72 hr (Figure 1), compared to groups I and II. TK activity in this group increased 36 hr after PH (p<0.001), with maximum values at 48 and 96 hr (Figure 2).

Nonstatistically significant differences were observed, either for the rate of DNA biosynthesis or for TK activity, in group sham, at all time intervals examined (data not shown).

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activity in group I was observed at 12 hr, in group II at 24 hr and in group III at 12 hr (Figures 3,4). ALP values presented peaks at 36, 72, and 96 hr in group I and at 48 and 72 hr in group II, as it is shown in Figure 5. In group III, ALP activity showed alternative minimum and maximum values (Figure 5). The number of mitotic figures observed in each group correlated well with DNA biosynthesis in liver at the same time. In both groups II and III, a decrease of mitotic activity was observed, in comparison with group I. The mitotic activity was higher in group II compared to group III.

**Discussion**

Thymidine kinase (TK) catalyzes the conversion of deoxythymidine in the presence of ATP to deoxythymidinomonophosphate (dTMP) that progressively is converted to the triphosphate form, able to be incorporated into DNA (14). It has been shown that this enzyme is closely correlated with DNA biosynthesis. Its activity increases dramatically in rapidly proliferating cells, such as those of regenerating liver and cancer cells (14,15).

We have shown that Cd administration in intact rats decreased the activity of TK in liver tissue (5). It has also been shown that the activity of TK in serum was decreased by the time, in Cd-treated rats (16). In previous studies it has been mentioned that Cd pretreatment affects the liver regenerative process after PH (17). Our findings are in accordance to those of other investigators (18,19). Cihak and Inoue observed a decrease of DNA synthesis and TK activity in Cd-treated rats 24 hr after PH(18). Stoll et al. have also reported that DNA synthesis was decreased 20 hr after PH in Cd-treated rats (19). In these studies Cd was administered simultaneously (18) or 1 hr after PH (19) and DNA synthesis was estimated at one time point only. In our study Cd was administered either prior or simultaneously to PH and the effect of Cd on 3HThdr incorporation and TK activity was examined at different time points up to 96 hr. Both administered doses were below the LD50 for ip injection of this toxic agent in rats (19) and no mortality was observed after its administration in different rat strains (20). Our results showed a marked inhibition of the deoxynucleotide-synthesizing enzyme TK and DNA synthesis in the liver of partially hepatectomized rats in the presence of Cd, at the time intervals of 0 up to 36 hr. The inhibition of TK activity was parallel to the inhibition of 3HThdr incorporation into hepatic DNA in groups II and III.

Cd pretreatment (group II) delayed the appearance of the first peak of liver regenerative process from 24 to 48 hr (Figure 1), possibly due to the inhibition of TK activity at the early steps of liver regeneration. The administration of Cd at the reduced dose of 1.0 mg CdCl2/Kg bw, simultaneously to PH (group III), caused inhibition of TK activity up to 36 hr and subsequently a further delay of liver regeneration. Cd was administered at this low dose, because liver mass and function were impaired due to PH. The appearance of stable values for DNA synthesis and TK activity from 48 up to 96 hr in the group where Cd was administered simultaneously to PH, was also an interesting finding. As there is no information in the literature about the inhibitory effect of Cd on the activity of purified TK in vitro, our work supports some evidence for this inhibition in vivo.

The elevation of transaminase values after PH, in all groups examined, was attributed to the trauma of the liver removal and to the necrosis which followed insertion of hemostatic sutures (10). The observed values of enzymes' activities in the group subjected to PH Cd or nontreated, were extremely high, compared to the values that had been observed in Cd intoxicated rats (21). The ALP activity in groups I and II was increased after the first peak of liver regenerative process. Concerning the ALP activity in group III, one could suggest that the maximum value at 12 hr possibly was due to the Cd intoxication (21). Although the precise biochemical basis of cellular regeneration in liver still remains to be elucidated, numerous studies indicate that alterations in polyamine metabolism play an important role in hepatic growth processes (18). The observation that chronic heavy metal administration reduced liver weight lends support to the view that Cd interferes with normal hepatic growth, as evidenced by the decreased polyamine formation (22). It is known that mitotic index can be used as a reliable marker of liver proliferation (23). The observed histological findings in all groups examined were in accordance to the rate of 3HThdr incorporation and TK activity in the liver tissue.

To the best of our knowledge there are no other studies published concerning the effect of Cd administration prior or simultaneously to PH on the liver regeneration, by the time in rats. Our findings suggest that Cd inhibits TK at the first hours of regenerative process after PH and by this way an inhibition of liver regeneration was occurred.

In order to elucidate the mechanism of Cd toxicity and its inhibitory effect on liver regeneration, further investigations should be undertaken.

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