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Dose Response of Rat Liver to Low Level Cadmium

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Acute exposure of mammals to a large cadmium (Cd)[2] dose is known to result in overt liver injury. This is preceded by the enhanced formation of metallothionein (MT) and possibly by changes in cellular glutathione (GSH) (Kawata and Suzuki 1983). Moreover, disturbances in the metabolism of zinc, copper and calcium have been reported in various organs of mammals following large single or multiple subcutaneous or oral Cd doses (Stonard and Webb 1976; Ashby et al. 1980; Maitani and Suzuki 1986). These responses were accompanied by various degrees of cell damage.

Recently, the liver has been considered also to represent a major target organ in long term Cd toxicity (Dudley et al. 1985). However, it is unclear whether the continuous intake of a small amount of Cd exerts (sub)cellular effects in liver in the absence of gross toxicity. If such effects do occur they may eventually compromise functional integrity prior to cell damage. In recent studies repeated oral Cd gavage to rats (25 ug Cd/kg) did not enhance hepatic MT (Muller et al. 1986, 1987). This may be to the detriment of the organism in that cadmium ion may not be sufficiently sequestered away from sensitive cellular constituents.

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2 Abbreviations: Cd, cadmium; Zn, zinc; Cu, copper; Ca, calcium; MT, metallothionein; GSH, glutathione

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The previously employed oral low level Cd dosage regimen (25 ug Cd per kg per day) resulted in hepatic Cd levels in rats which approximated those found in environmentally Cd exposed man (Muller et al. 1986). The objective of the present study was to investigate additionally the effect of a lower (2.5 ug Cd/kg) and a higher oral Cd dosage (250 ug Cd/kg) on liver thiols, essential metals and urea as a measure of the metabolic integrity of liver cells. Since in vitro and in vivo studies indicate that mitochondria may be affected by Cd (Sporn et al. 1970; Muller 1986) these organelles were also investigated.

MATERIALS AND METHODS

All chemicals were of the highest purity commercially available. Male Sprague Dawley rats (approximately 350g) from the University of Sydney Animal House had free access to a commercial chow (Allied Foods, Rhodes, NSW) and bidistilled water. One gram of food contained 12 ng Cd, 51 ug Zn, 6.5 ug Cu and 22 mg Ca as determined by atomic absorption spectrometry (see below). The animals were housed in plastic cages and acclimatized at least one week prior to use. Four groups of rats were investigated. Group 1, serving as control, received sodium acetate (0.09 mM in bidist. water; 2.6 ml/kg) five times a week for 6 weeks by a gastric tube (Muller et al. 1986). Contaminants in this solution included Cd (0.09), Zn (45) and Cu (16.6) in ng/ml. Animals similarly treated with 2.5, 25 and 250 ug Cd/kg rat (as Cd acetate) provided groups 2,3 and 4, respectively. The body weight of rats was monitored each day of gavage. Three days after the last dosage rats were anaesthetized with ether and blood was withdrawn from the vena cava for the determination of serum parameters. Subsequently, the liver was perfused to remove blood using ice cold 0.9% NaCl solution. The liver was removed, washed, weighed and immediately homogenized (1 + 3 vol) in ice cold 0.25 M sucrose.

The parameters of tissue injury, aspartate and alanine aminotransferases and creatinine, were determined in serum by adapted standard procedures using a centrifichem and test kits (Roche, Sydney). Tissue and serum urea were determined likewise as urea nitrogen as an estimation of hepatocellular functional integrity. Mitochondria were isolated as previously described (Muller 1986), washed and resuspended in ice cold distilled water. Acid

soluble thiols were measured according to Beutler et al. (1963). Metallothionein was determined in the 10000xg postmitochondrial supernatant by the Cd saturation assay, using radiolabelled Cd (Cd-109) and as the amount of thiol groups in the precipitate resulting from ethanol/chloroform/ethanol treatment, as previously described (Dieter et al. 1986).

Homogenates and mitochondrial suspensions were ashed with H_2O_2 and HNO_3 (65%) (ratio 2.5:1:3 and 7:1:2, respectively) at 120.53C overnight. Cadmium, zinc, copper and calcium were determined at wavelength 228.8, 313.9, 324.8 and 422.7 nm, respectively, using the graphite furnace or the air/acetylene flame of a Varian Techtron AA6 atomic absorption spectrometer (Melbourne, Australia). A deuterium lamp served for background correction. Each metal sample was subjected to 4-6 AAS measurements per metal analysis. Calculations were based on similarly treated blanks and standards. Protein was determined by the Lowry method.

Significant differences were evaluated by analysis of variance and Duncan's multiple range test. Trend analysis was performed using Kendall's rank correlation coefficient, Tau. The level of significance was set at $p<0.05$. The significance of linear correlation was determined using the correlation coefficient R^2 and biometrica tables.

RESULTS AND DISCUSSION

Rats treated for 6 weeks with repeated oral doses of 2.5, 25 and 250 ug cadmium per kg, respectively, did not display significant alterations with respect to controls (con) in animal weight gain (con = 69.3 $\pm$ 4.3g; means $\pm$ SE, n = 4) or liver weight (con = 16.7 $\pm$ 3.1g). Moreover, serum levels of aspartate aminotransferase (96.9 $\pm$ 13.8 mU/l), alanine aminotransferase (38.5 $\pm$ 7.3 mU/l), creatine (82.2 $\pm$ 5.5 ug/mg protein) and protein (81.9 $\pm$ 3.7 mg/ml) remained unchanged after each of the Cd doses. This adds to recent reports in which no effect of extended oral administration of 25 ug Cd/kg on animal health was observed (Muller et al. 1986, 1987).

Cadmium increased in liver tissue in a dose related manner ($R^2 = 0.9966$, $p<0.001$) whereas a significant increase in mitochondrial Cd was found only at the highest Cd-dose (figure 1). Tissue Cd in rats approximated the values in livers of European people and, even at the highest Cd dose, was almost ten

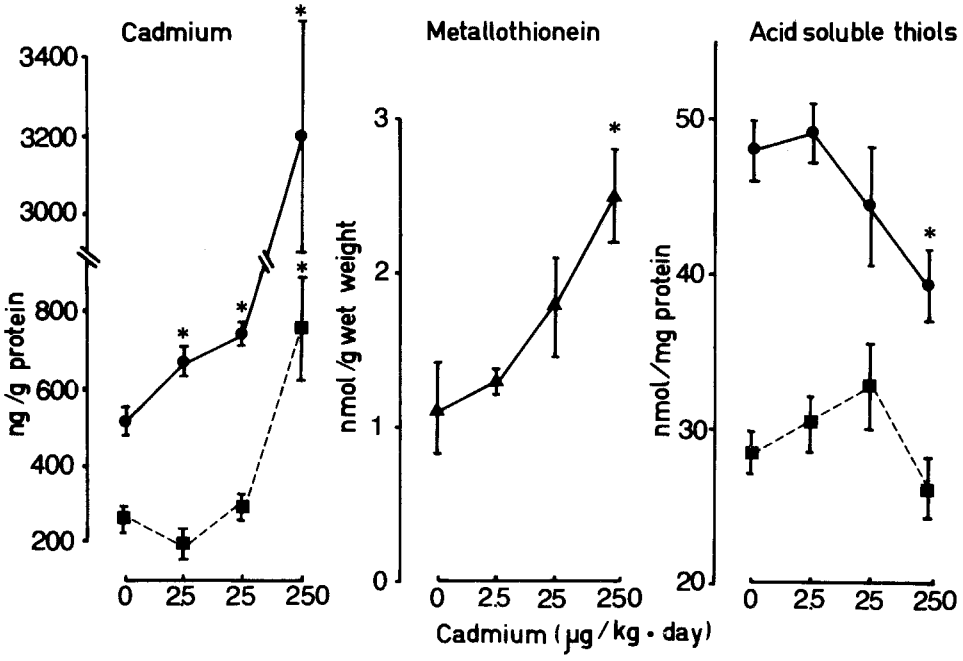

Figure 1.

Response of cadmium, metallothionein and acid soluble thiols in livers of rats orally exposed to various cadmium doses. Means ± SE of 3-4 rats in liver tissue (solid line) and mitochondria (dashed line) are represented. Asterisks indicate significant differences to controls.

times less than that found in some Japanese (e.g. 4-10 mg/kg; Elinder 1985).

Simultaneously, a dose dependent increase in metallothionein (MT) (R^2 = 0.9894, $p<0.05$) was found which showed statistical significance only at the 250 ug Cd dose level (figure 1). No significant alterations in tissue and mitochondrial zinc (con = 158.1 $\pm$ 2.2 and 63.5 $\pm$ 3.9 ng Zn/mg protein, respectively) and copper (con = 21.4 $\pm$ 1.8 and 15.5 $\pm$ 0.3 ng Cu/mg protein, respectively) were observed at any Cd dose level. In contrast, challenge of rats with large amounts of Cd has been shown to increase hepatic Zn (Stonard and Webb 1976) and Cu (Ashby et al. 1980) which have been related to enhanced MT-synthesis and inhibition of biliary metal excretion, respectively.

However, acid soluble thiols (mainly consisting of reduced glutathione) were decreased after 250 ug Cd/kg (figure 1). A significant decrease was not observed in mitochondria. A decrease in GSH has been reported in intraperitioneally Cd treated mice (Kawata and Suzuki 1983). The strong linear relationship between MT and GSH responses at various Cd doses in the present study (R^2 = 0.9980, $p<0.001$) suggests an association of the two Cd-induced effects. This may support the hypothesis that GSH-moieties may contribute to hepatic MT synthesis in Cd-exposed mammals (Kawata and Suzuki 1983).

Cadmium also increased tissue calcium (Ca) in a Cd-dose related manner (R^2 = 0.9870, $p<0.05$) without alteration of mitochondrial Ca (figure 2). Similarly, a Cd-induced accumulation of Ca has been found in human red blood cells (Plishker 1984) and in testes of mice subcutaneously treated with 3.4 mg Cd/kg (Maitani and Suzuki 1986). Our results are consistent with the increase in hepatocellular phosphorylase a activity as reported after long term Cd exposure of rats by drinking water (Sporn et al. 1970).

In figure 3 the effect of Cd on hepatic urea is illustrated. A trend to a Cd dependent decrease in urea (R^2 = 0.9999, $p<0.001$) was observed which was significant as estimated by using Kendall's rank correlation coefficient, Tau. This effect was reflected by a decrease in serum urea due to 25 and 250 ug Cd (figure 3). These results suggest a Cd-induced dysfunction in a metabolic parameter that involves both mitochondrial and cytosolic compartments.

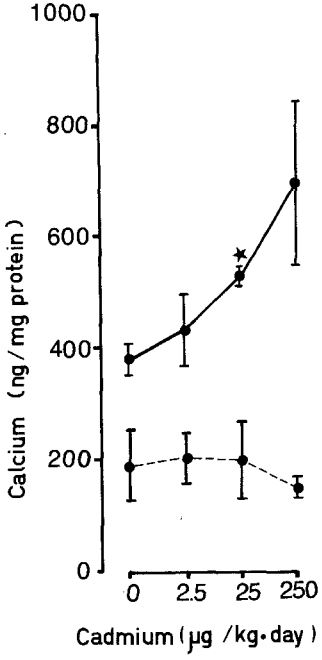

Figure 2.

Response of liver calcium to cadmium

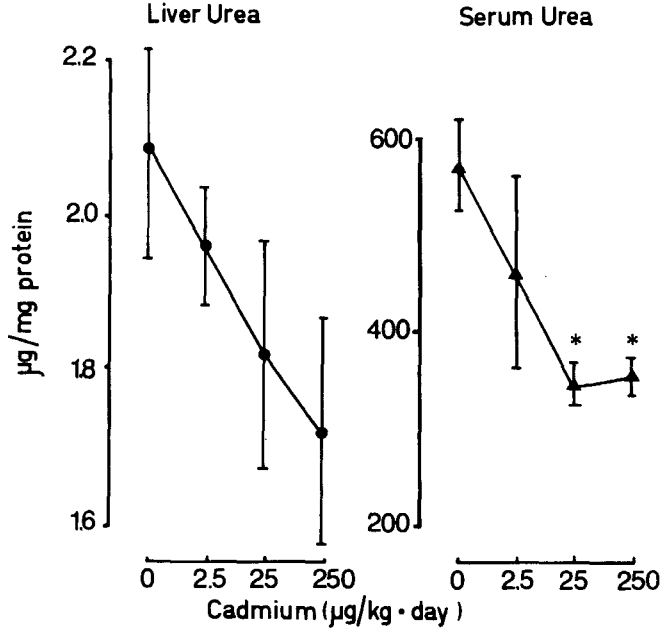

Figure 3.

Response of liver and serum urea in rats to cadmium

Means ± SE of 3-4 rats in mitochondria (dashed line), liver tissue and serum (solid line), respectively, are represented. The asterisks indicate significant differences to controls.

The described Cd effects were predominantly confined to the extra-mitochondrial compartment. However, the involvement of Cd induced mitochondrial dysfunction (Sporn et al. 1970; Muller 1986) in cellular responses to low level Cd gavage to rats cannot be excluded (Muller et al. 1987).

In summary, the present study demonstrates that after extended gavage of low level cadmium to rats disturbances in hepatic GSH, calcium and urea metabolism are observed in the presence of marginally increased metallothionein. These effects may contribute to deterioration in cellular metabolic integrity resulting in subclinical organ dysfunction.

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