THE EFFECT OF AGE ON CADMIUM RETENTION IN ORGANS OF HAMSTERS.

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Abstract.....Age difference of cadmium retention was investigated in hamsters after a short term cadmium exposure. Three types of organs were identified in relation to cadmium retention, i. e., the liver and testes retained an increasing amount of cadmium with increasing age, the kidney and heart showed a gradual decrease in the retention after a transient increase in young ages and the bone showed no retention. These changes seemed to be related to the de novo synthesis of metallothionein in the organ.

Key words : Cadmium, retention, age, difference, metallothionein.

INTRODUCTION

Animals exposed either acutely or chronically to cadmium accumulate this metal in nearly all body tissues, with the liver, kidney and testes exhibiting the highest levels (Shaikh and Lucis 1972, Nordberg and Nishiyama 1972). Analyses of cadmium in organs from general populations and workers occupationally exposed to cadmium have also showed that the accumulation of cadmium takes place in the liver and kidney of human beings (Smith et al 1960, Hamilton et al 1972, Hirst et al 1973, McKenzie 1974). Data assembled from several reports (Schroeder and Balassa 1961, Curry and Knott 1970, Indraprasit et al 1974) provide an interesting tendency of cadmium contents in these organs in relation to age. In both organs, which contain a trace amount of cadmium in newborns, the contents of cadmium increases rapidly up to 25 years of age and then slowly up to 50. Over 50 years of age, however, the content in the kidney slowly decreases in contrast to the slow but ever increasing tendency in the liver.

Given the many factors by which these tendencies might be conducted, it seems important to examine the change with age of cadmium retention ability of organs as one of the contributing factors.

On the other hand, although the mechanism for accumulation of cadmium in these organs is not completely elucidated, metallothionein, a low molecular weight metalloprotein, has been suggested to play a significant role (Nordberg et al 1971, Leber et al 1976, Probst et al 1977). This protein is shown to have affinity to cadmium and to be induced in the liver and probably in other organs in response to cadmium exposure. The de novo synthesis was initiated within a few hrs and maximal metallothionein synthesis occurred between 6 and 12 hrs after a single administration of cadmium (Shaikh and Smith 1975, Probst et al 1977).

In the present investigation we studied cadmium retentions in organs of hamsters in relation to the age of the animals and also to the synthesis of metallothionein in the organs.

MATERIALS and METHOD

Male golden hamsters of a strain originally obtained from the Institute of Preventive Medicine in Japan and bred in our laboratory were used. Each group of the same months old hamsters aged from 3 to 27 months was divided into four subgroups, two control groups and two cadmium-exposed groups. One of the later groups was injected subcutaneously with 1 mg cadmium/kg body weight/day in the form of CdCl_2 dissolved in physiological saline on successive 3 days and another group was treated in the same manner on successive 6 days. On the third day following the last injection several animals from each group were killed and the concentrations of cadmium in the liver, kidney, testes, heart and bone were determined.

Cadmium contents in specimens were analysed by atomic absorption spectrometry according to the method of Yeager et al (1973). To obtain metallothionein fractions (Suzuki 1979) two grams of the organ were homogenized in six times the volume of Tris buffer solution (0.1 M, pH 7.4, 0.25 M glucose) with a polytron homogenizer. The homogenate was heated at 65°C for 60 sec under nitrogen gas to remove heat unstable proteins. The solution was cooled and then centrifuged at 2,300 g for 10 min. The heat-treated supernatant (10 ml) was applied to a Sephadex G-75 column (2.6×90 cm), eluted with Tris buffer solution (0.1 mM, pH 8.6), and collected (5 ml/tube). Metallothionein fractions were identified by measurement of absorbance at 250 nm and cadmium concentration for each fraction.

RESULTS

Table 1 shows the distribution of subcutaneously injected cadmium to organs of hamsters in relation to the dose of cadmium and the age of the animals.

In all the treated groups, the liver most remarkably retained the administered metal and the kidney followed it.

In order to make clear the relationship between the age of animals and the degree of retention in each organs, mean values for groups treated with a total dose of 6 mg cadmium/kg were plotted against ages on a graph (Fig 1). As can be seen, amounts of

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Table 1. The effect of age and dose of cadmium on the distribution of cadmium to organs of hamsters.

Age (month)	Total dose* (mg/kg)	Cadmium contents (Mean±S. D.) (μg/g)				
		Liver	Kidney	Heart	Testes	Bone
3	0	0.103±0.015 (4)**	0.340±0.040 (4)	0.326±0.045 (4)	0.074±0.008 (4)	1.42±0.16 (4)
	3	14.1 ±5.6 (2)	6.84 ±2.70 (2)	1.93 ±1.38 (2)	0.363±0.088 (2)	1.46±0.16 (2)
	6	29.2 ±6.2 (4)	17.6 ±3.8 (4)	3.30 ±0.42 (4)	0.682±0.072 (4)	1.48±0.18 (4)
6	0	0.206±0.191 (2)	0.569±0.180 (2)	0.320±0.092 (2)	0.100±0.051 (2)	1.33±0.20 (2)
	3	14.1 ±1.4 (4)	13.6 ±2.2 (4)	1.98 ±0.22 (4)	0.450±0.041 (4)	1.54±0.20 (4)
	6	33.2 ±9.6 (3)	31.0 ±4.8 (3)	3.84 ±0.29 (3)	0.803±0.058 (3)	1.56±0.34 (3)
9	0	0.234±0.114 (3)	0.366±0.073 (3)	0.505±0.309 (3)	0.074±0.026 (4)	1.25±0.08 (4)
	3	21.9 ±9.8 (5)	9.86 ±2.12 (5)	1.96 ±0.40 (5)	0.453±0.073 (5)	1.46±0.36 (3)
	6	35.7 ±9.4 (4)	21.0 ±6.6 (4)	3.08 ±0.88 (4)	0.762±0.149 (4)	1.60±0.10 (4)
12	0	0.200±0.020 (3)	0.360±0.111 (4)	0.272±0.135 (4)	0.104±0.034 (6)	1.43±0.18 (4)
	3	20.8 ±1.6 (6)	10.7 ±1.0 (6)	2.63 ±0.38 (6)	0.673±0.194 (6)	1.60±0.23 (6)
	6	31.8 ±9.5 (2)	21.2 ±3.6 (3)	3.55 ±tzqj (3)	0.706±0.010 (3)	1.71±0.002 (3)
18	0	0.277±0.112 (4)	0.293±0.060 (4)	0.377±0.212 (4)	0.074±0.059 (4)	1.52±0.17 (4)
	3	20.4 ±2.9 (4)	8.78 ±2.45 (4)	2.63 ±0.33 (4)	0.855±0.513 (3)	1.65±0.32 (4)
	6	40.4 (1)	17.1 (1)	3.63 (1)	0.841 (1)	1.52 (2)
21	0	0.173±0.060 (6)	0.300±0.033 (5)	0.283±0.088 (4)	0.052±0.012 (6)	1.30±0.28 (6)
	3	23.3 ±3.0 (6)	8.9 ±0.93 (6)	2.38 ±1.12 (5)	0.753±0.178 (6)	1.67±0.16 (6)
	6	39.6 ±5.4 (6)	18.2 ±2.6 (6)	3.25 ±0.62 (5)	0.993±0.453 (6)	1.72±0.35 (6)
24	0	0.286±0.109 (6)	0.334±0.118 (2)	0.446±0.185 (3)	0.116±0.038 (3)	1.38±0.17 (7)
	3	21.8 ±3.9 (5)	7.65 ±1.13 (5)	2.45 ±0.69 (5)	0.784±0.192 (5)	1.62±0.06 (4)
	6	41.5 ±7.8 (6)	16.7 ±4.2 (5)	3.02 ±0.62 (4)	0.905±0.226 (5)	1.63±1.18 (6)
27	0	0.441±0.196 (4)	0.370±0.082 (3)	0.406±0.198 (4)	0.222±0.127 (3)	1.21±0.65 (4)
	3	24.4 ±2.0 (4)	7.31 ±0.48 (4)	1.55 ±0.19 (4)	0.818±0.085 (3)	1.79±0.25 (3)
	6	43.5 ±9.0 (4)	12.3 ±2.3 (4)	2.72 ±0.48 (4)	1.02 ±0.17 (4)	1.59±0.08 (4)

*1 mg Cd (as CdCl₂)/kg/day was injected subcutaneously for 3 or 6 successive days.

Animals were killed on the third day after last dose. **indicates numbers of animal.

Fig. 1. Changes with age of cadmium retentions in organs of hamsters following acute administration of cadmium. Only mean values for groups treated with a total dose of 6 mg cadmium/kg are plotted.

cadmium retained in the liver during the same period after the injection of the same dose of cadmium per body weight showed a tendency to increase substantially with increasing age. In contrast to this, those in the kidney showed a gradual decrease with ageing in the age ranges of 6 to 27 months after giving a transient, but obvious increase at the age of 6 months. Testes showed a tendency similar to the liver, but the degree of cadmium retention was far less as compared with that of the liver.

The retention ability of the heart increased at the age of 9 to 12 months and decreased thereafter. The pattern of the retention tendency was similar to that of the kidney, but the degree was less than that of the kidney. The bone showed a retention ability less than two times of control throughout the all ages, giving no constant tendency.

The regression line for each age group between the doses and contents of cadmium showed a linearity only for the liver and kidney. The equations for the regression lines

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Table 2. The equations for regression lines between the doses and contents of cadmium.

Age (month)	Equations	
	Liver	Kidney
3	$y = 5.00x - 0.22$	$y = 3.15x - 1.31$
6	$y = 5.50x - 0.65$	$y = 5.08x - 0.15$
9	$y = 5.90x + 1.57$	$y = 3.45x + 0.07$
12	$y = 5.26x + 1.68$	$y = 3.47x + 0.34$
18	$y = 6.68x + 0.25$	$y = 2.90x + 0.22$
21	$y = 6.91x + 0.35$	$y = 2.98x + 0.19$
24	$y = 6.88x - 0.52$	$y = 2.73x + 0.03$
27	$y = 7.18x - 1.2$	$y = 1.99x + 0.70$

X represents total doses of cadmium expressed as mg/kg body weight and y represents contents of cadmium in organs expressed as $\mu\text{g/g}$ wet tissue.

Table 3. Mean cadmium contents (nmol/g tissue) in metallothionein fractions from the liver and kidney of hamsters exposed to total cadmium dose of 6 mg/kg body weight.

Age (month)	3-6	9-12	18-24	27-
Liver	187	205	250	264
Kidney	149	127	109	79

Only mean values for two animals are represented in order to note the tendency.

were summarized in Table 2. The direction coefficient of the lines for the liver was moderately high and showed an increase parallel to ageing. Those for the kidney showed an increase at the age of 6 months and decreased thereafter.

Changes with age of cadmium contents in metallothionein fractions from the liver and kidney of hamsters administered with cadmium were consistent with those of the cadmium contents in the corresponding whole organs (Table 3).

DISCUSSION

A large number of studies in experimental animals have demonstrated that the accumulation of cadmium in animals is contingent upon several factors including levels of exposure, duration of exposure, routes of administration and species and strain dependent responses (Friberg et al 1971). However, little information is available on the effect of age on the accumulation of cadmium in connection with the age-dependent response of organ.

Although the distribution of cadmium to organs of hamsters was consistent with the earlier reports (Decker et al 1957, Shaikh et al 1972) that have shown that a short term exposure to a relatively high amount of cadmium caused high concentrations in the liver and relatively low concentrations in the kidney, the present study clearly showed

that the age of the exposed animals is a significant factor which influences the accumulation of cadmium in organs. In addition, our result showed that there are types of organs in relation to the change with age of the cadmium retention ability when exposed acutely to cadmium. The first type includes the liver and testes and shows a continuous increase in the ability along with ageing. The kidney and heart represent the second type in which the ability increases from birth to a given age and declines thereafter. In the third type, as observed in the bone, there occurs almost no retention even with the exposure to a moderately large amount of cadmium.

It is generally accepted that the cadmium content in metallothionein after cadmium exposure represents the amount of induced metallothionein in the organ and that the distribution of cadmium depends largely on the content of metallothionein in the organ. The parallelism of the cadmium content in the whole organ with that in metallothionein fraction of the organ in the present study not only supports the above acceptance, but also indicates that the change with age of the retention of cadmium in each organ reflects the change of the ability of the organ to synthesize metallothionein in response to cadmium exposure. In this respect, it should be noted that the response of the liver and testes is kept increasing until the old age.

Nordberg (1972) suggested that cadmium is bound to metallothionein in the liver which subsequently is transported to the kidney and becomes concentrated in the renal cortex. The hypothesis is supported by the observation that animals receiving an intravenous injection of cadmium bound to purified metallothionein demonstrated a rapid preferential accumulation of the cadmium in the kidney (Nordberg et al 1975, Tanaka et al 1975). However, our finding that cadmium contents in metallothionein in the liver showed a continuous increase and those in the kidney showed a continuous decline with increasing age is contrary to the hypothesis and seems to indicate that the moderately accumulated cadmium in the kidney at the initial stage of cadmium exposure is related to the de novo synthesis of metallothionein in the kidney. Furthermore, the finding might offer a rationale for the contrasting changes with age of cadmium contents between the liver and kidney of human beings as stated in Introduction.

SUMMARY

The change with age of cadmium retention was compared among organs of hamsters aged 3 to 27 months after a short term cadmium response.

The liver and testes retained an increasing amounts of cadmium with increasing age, while the kidney and heart showed gradual decrease in cadmium retention after a transient increase in young ages. Almost no retention was observed in the bone through all ages.

Cadmium contents in metallothionein fractions of the liver and kidney showed similar changes with age as those of the total cadmium contents in the corresponding whole organs. These results indicate that cadmium accumulating ability as a biological response to acute cadmium exposure is different among ages as well as among organs,

and is related to the ability of the organs for the de novo synthesis of metallothionein.

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