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Is cadmium released from metallothionein in rejected human kidneys?

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Summary. Concentrations of metallothionein and metals, i.e. cadmium, copper and zinc, were determined in six rejected transplanted human kidneys and one kidney prepared for transplantation. Tissue samples separated by gel chromatography showed that almost all of cadmium in tissue was in the form of firmly bound cadmium-metallothionein.

Key words: Kidney – Metals – Metallothionein – Rejection – Transplantation

Introduction

Cadmium is a rarely occurring metal which possesses nephrotoxic properties. Environmental contamination of rice and subsequent human exposure, as well as occupational exposure, has resulted in endemic incidendes of renal damage and, in some areas in the worlds, secondary effects on the skeletal system (see reviews by Friberg et al. 1985, 1986).

The first sign of cadmium-induced renal dysfunction is tubular proteinuria which is characterized by increased urinary excretion of low-molecular-mass proteins such as β_2-microglobulin. This is typical for tubular damage (Piscator et al. 1981). The cadmium-induced tubular damage may progress and result in glomerular effects with a drop in the glomerular filtration rate. Eventually, in severe cases of cadmium poisoning, uremia may develop (Friberg and Elinder 1988).

The critical concentration of cadmium in kidney cortex above which tubular damage may occur varies between different animals. Certain species of long-lived marine birds, as well as horses, appear to be more susceptible when compared to monkeys, for example (Kjellström 1986). For humans, the critical concentration of cadmium above which tubular damage may develop is considered to be around 1.8 mmol/kg wet weight, i.e. 200 mg Cd/kg (see review Friberg et al. 1986).

The critical concentration, however, also depends on the form in which cadmium is present. If cadmium is given parenterally bound to metallothionein, severe kidney damage is seen even at a concentration 5% of that necessary to cause damage when ionic cadmium is given (Nordberg et al. 1975). When cadmium bound to metallothionein is administered via intravenous injection it is rapidly transported to the kidney and, as is the case for other small proteins, filtered through the glomerulus and subsequently reabsorbed by the proximal tubular cells.

Tubular damage develops when the cadmium-metallothionein complex is degraded in the tubular cells and non-metallothionein-bound cadmium ions are present (Nordberg 1984). The tubular cells have a specific capacity to produce their own protective metallothionein and damage occurs when this capacity is exceeded.

The concentration of cadmium in kidneys obtained from autopsied adult humans in most countries is in the order of 0.05–0.5 mmol Cd/kg wet weight (Elinder 1985). Smokers, as a rule, have higher concentrations when compared to nonsmokers and it is not unusual to find persons in the general population who have cadmium concentrations in their kidneys exceeding 0.5 mmol/kg (Elinder 1985). If there is not a continual synthesis of metallothionein in the kidneys, these concentrations are sufficient to cause severe damage. The cadmium-burdened kidney may be looked upon as a delayed-action bomb.

As the adult human kidney normally contains potentially toxic concentrations of cadmium, the question has been raised whether this cadmium can be released from metallothionein during storage and after transplantation and subsequently cause kidney damage (Elinder et al. 1984). In a previous study we showed that there was no release of cadmium from metallothionein during 24-h storage in a preservative solution (Elinder et al. 1984).

The objective of the present study was to determine

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what occurs in the human kidney in situ when it has been transplanted into a recipient. In this preliminary report we present data from seven human donor kidneys.

Material and methods

Kidneys. Kidneys from seven donor (aged 36–74) were examined. Six of them had been transplanted but were removed from the recipient within 4 weeks because of malfunction, possibly due to acute rejection or thrombosis. One of the kidneys (no. 7) was, however, not transplanted because no suitable recipient was identified. The pair kidney to no. 7, i.e. taken from the same donor, was transplanted.

Preparation and storage. Immediately after the transplanted kidney had been removed from the recipient it was placed on ice and, as soon as possible, the whole kidney was sliced and the cortex separated from the medulla. Pieces of about 2 g cortex were placed in acid-washed test tubes and stored at −70° C until analyzed. Later, the kidney cortex was thawed and approximately 1 g taken in duplicate samples for metal analysis by flame atomic absorption spectrophotometry (AAS). The detection limit (in mmol/kg) for the tissue samples was 0.0003 (samples 1–4), 0.0001 (samples 5–7) for Cd, 0.0004 (samples 1–4), 0.0002 (samples 5–7) for Zn and 0.0002 (samples 1–4), 0.0008 (samples 5–7) for Cu. Gel chromatography on a column (366 × 26 mm) of Sephadex G-75 was performed on 1-g pieces from the cortex. The column eluted with 0.01 M Tris/HCl pH 8.0, 0.05 M NaCl, at a flow rate of 14 ml/h; 5-ml fractions were collected. The metals cadmium, copper and zinc were analyzed with AAS. In the fractions detection limits (in mmol/kg) were 0.0001, 0.0004, and 0.0003 for samples 1–4 for Cd, Zn and Cu, respectively, corresponding values for samples 5–7 were 0.0004, 0.0006 and 0.0004, respectively.

Metallothionin. This was quantified using the Onosaka method (Onosaka and Cherian 1981) and calculated from a relationship of 6 mol cadmium atom/mol metallothionein. For the metallothionein analyses the detection limit was 0.0001 mmol/kg.

Results and discussion

Table 1 presents data from each examined kidney. There was a considerable range in cadmium concentrations: <0.0001–0.71 mmol Cd/kg. The highest cadmium concentration was found in kidney no. 7, i.e. the

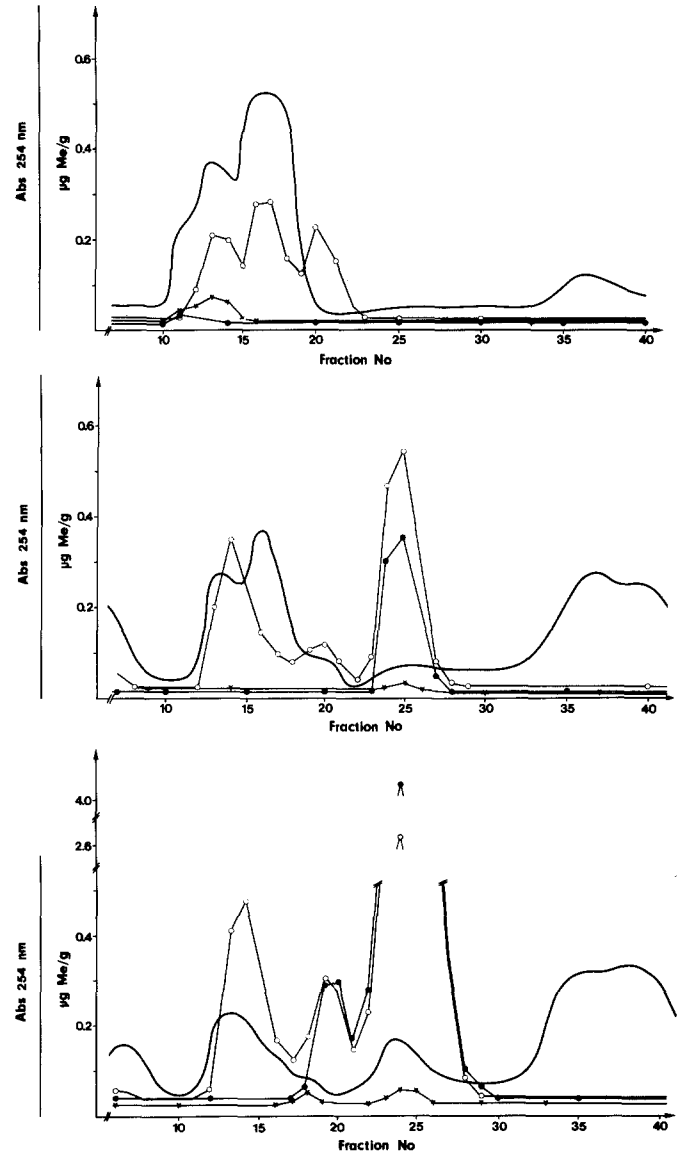

Fig. 1. Gel chromatography on Sephadex G-75 of kidney cortex homogenate from kidneys 1, 3 and 7. Column dimensions were 366 × 26 mm. Elution with 0.01 M Tris/HCl pH 8.0, 0.05 M NaCl, at a flow rate of 14 ml/h. Volume of fractions was 5 ml. Absorbance at 254 nm was continuously monitored. (●) Cadmium concentration; (○) zinc concentration; (×) copper concentration

Table 1. Age, cadmium, copper, zinc concentrations in kidney cortex, dry/wet mass ratio, cadmium and zinc concentration in metallothionein (MT) fractions and MT concentration in kidney cortex (mean of duplicates)

No.	Age of		Metal concentration (mmol/kg wet mass)			Dry/wet mass ratio	Cd in MT fractions (mmol/kg)	Zn in MT fractions (mmol/kg)	Cu in MT fractions (mmol/kg)	MT (mmol/kg)
	R	D	Cd	Cu	Zn					
1.	33 (f)	36 (m)	≤d.l.	0.017	0.26	0.21	≤d.l.	≤d.l.	≤d.l.	0.001
2.	52 (m)	46 (f)	0.036	0.017	0.34	0.17	0.03	0.09	0.009	0.009
3.	56 (f)	74 (m)	0.038	0.023	0.28	0.17	0.04	0.11	0.006	0.013
4.	25 (f)	59 (f)	0.028	0.031	0.22	0.20	0.02	0.05	0.002	0.006
5.	71	36 (f)	0.071	0.031	0.26	0.16	0.04	0.06	0.013	0.008
6.	51 (m)	45 (m)	0.049	0.028	0.25	0.16	0.03	0.07	≤d.l.	0.010
7.	—	43 (f)	0.71	0.044	1.12	0.20	0.42	0.48	0.012	0.118

The ages and sex (f = female, m = male) of recipient (R) and donor (D) are given. ≤d.l. = below or equal to detection limit. Kidney no. 7 was not transplanted, although its pair was (not included in this study)

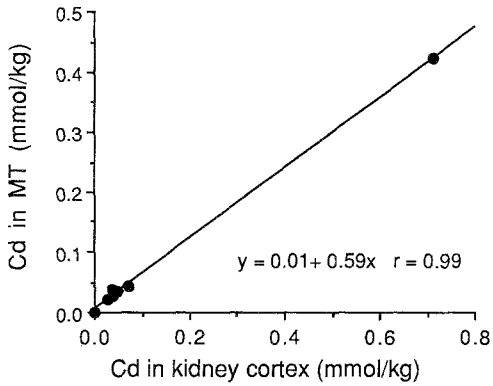

Fig. 2. Cadmium concentration in metallothionein (mmol/kg) in relation to cadmium in kidney cortex ($n=7$)

one not transplanted. The pair kidney was transplanted and later rejected. Unfortunately, we were unable to obtain samples from this rejected kidney. It is obvious from this very small sample ($n=7$) that even kidneys containing relatively high concentrations of cadmium may be successfully transplanted.

As is the case with normal (not transplanted) kidneys, all detectable cadmium was recovered in the Sephadex G-75 fractions containing metallothionein (Fig. 1). There was a linear relationship between the cadmium concentration in the renal tissue and the amount recovered in metallothionein fractions (Fig. 2). This observations seems to hold true even when the one kidney containing a very high cadmium concentration (no. 7) is excluded from the data (Fig. 3). Thus, there is no indication that cadmium is released from metallothionein in poorly functioning transplanted kidneys.

We do not know whether metallothionein is resynthesized in the transplanted kidney or whether the metallothionein that was present in the kidney when it was removed from the donor remains in the transplanted kidney. If the organ is functioning well, there should be resynthesis since there is a constant turnover of all proteins in the body.

The Sephadex G-75 fractions in which metallothionein and cadmium were eluted also contained zinc (Table 1). This was not surprising as most mammalian renal metallothionein is known to bind cadmium and zinc and, to a lesser extent, copper. In kidneys containing high cadmium concentrations the relative content (on a molar basis) of zinc in metallothionein was less than that for cadmium. The relative content of cadmium and zinc in metallothionein in relation to the cadmium concentration in cortex is presented in Fig. 4. The pattern is very similar to that previously observed in horses environmentally exposed to cadmium (Nordberg et al. 1979) and experimentally exposed rabbits (Elinder et al. 1987).

Acute tubular necrosis frequently develops in transplanted kidneys, about 30%–60% of the recipients being affected (Brophy et al. 1980). A high age of the donor and a long cold ischaemic time period are factors known to increase the risk for necrosis (Brophy et al. 1980; Foster et al. 1988). Acute tubular necrosis is, however, rarely the cause of rejection or long-term transplant failure. If there are no other causes of graft failures, such as acute or chronic rejection from an immunological host reaction towards the transplanted organ, or technical problems such as poor perfusion because of thrombosis, a transplanted kidney with acute tubular necrosis only usually starts to function sooner or later (Foster et al. 1988). The cause for rejection in the kidneys examined in this study was not always clear, but in no case could it be related to tubular necrosis only. It is difficult to determine what happens to metallothionein-bound cadmium in a functioning transplant or in a kidney which is solely affected by acute tubular necrosis.

The present, preliminary, findings cannot rule out the possibility that cadmium released from metallothionein in transplanted kidneys is in some way involved in the mechanisms underlying acute post-transplantation tubular necrosis. However, it is noteworthy that in the rejected kidneys examined in this study, cadmium was always bound to metallothionein and there were no indications of non-metallothionein-bound cadmium in the kidney. This is contrary to the hypothesis

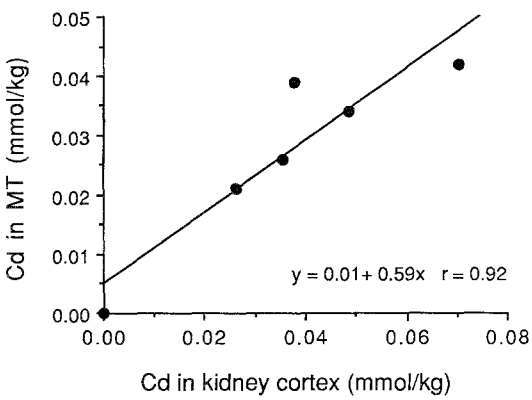

Fig. 3. Cadmium concentration in metallothionein (mmol/kg) in relation to cadmium in kidney cortex excluding one kidney not transplanted ($n=6$)

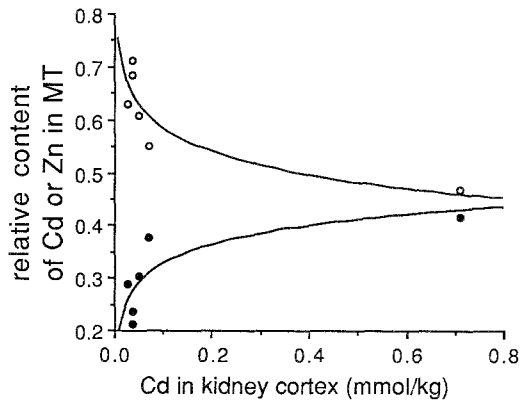

Fig. 4. The relative content of cadmium and zinc in metallothionein in relation to the cadmium concentration in kidney (mmol/kg). ● Cd; ○ Zn

that cadmium plays a role in the rejection of transplanted human kidneys.

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