Environmental Health Perspectives
Vol. 54, pp. 275–280, 1984

Metallothionein in Rabbit Kidneys Preserved for Transplantation

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Thirteen rabbits were given repeated cadmium injections to achieve cadmium concentrations in kidney cortex ranging from 0.05 to 1 mmole Cd/kg wet weight. Another four animals served as controls. One kidney from each animal was frozen directly to −70°C whereas the other kidney was kept for 24 hr at +4°C in a preservative (Sachs' solution) to simulate conditions for preservation of human donor kidneys before transplantation. Protein binding of cadmium, zinc and copper in kidney homogenates and the concentration of metallothionein (MT) were measured in the kidney that was frozen directly and in the kidney that had been preserved.

No gross differences in either the protein binding of cadmium, zinc and copper or in the MT content were seen between the directly frozen and preserved kidneys from the same animal. This indicates that MT is not rapidly broken down in rabbit kidneys which have been preserved similarly to human donor kidneys for 24 hr in a standard preservative solution prior to a transplantation.

Introduction

Cadmium is a highly nephrotoxic metal which after long-term exposure gives rise to a typical type of renal damage in animals as well as in humans. The first sign of a cadmium-induced renal effect is an increased excretion especially of low molecular weight proteins. This is an early sign of tubular dysfunction, and the type of proteinuria involved is often designated "tubular proteinuria" (*1*). The critical concentration of cadmium in kidney cortex in order to elicit a tubular type of proteinuria is considered to be around 1.8 mmole Cd/kg wet weight (= 200 mg/kg) (*1,2*). The threshold for toxic effects from cadmium in kidney is, however, considerably lower under certain experimental conditions. When cadmium is given parenterally in the form of metallothionein, severe tubular damage develops at cadmium concentrations in the kidney of about 0.1 to 0.2 mmole Cd/kg (*3,4*). Metallothionein is a low molecular weight protein (MW 6,500) which normally binds more than 80% of the cadmium bound in various tissues, e.g., liver and kidney. This protein is regarded to have a protective role under physiological conditions (*5,6*). When injected, metallothionein will be filtered from the plasma through the glomeruli into the tubular fluid, as is the case for other low molecular weight compounds. Metallothionein will subsequently be resorbed by the tubular cells, probably by pinocytosis (*7–10*). Subsequent to the cadmium-containing metallothionein complex being taken up by the tubular cells, it undergoes degradation into amino acids, lesser peptides and metal ions. New metallothionein will be formed in the cells to sequester released cadmium ions (*11*). When large amounts of cadmium are released from metallothionein during the degradation process, as is the case after a parenteral dose, the tubular cells are unable to produce enough metallothionein, and consequently toxic effects occur (*1,12,13*). A turnover of the metallothionein in the tubular cells also takes place, and therefore metallothionein is constantly resynthesized in the kidney cells in order to prevent toxicity of cadmium (*14,15*).

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The biological half-life of metallothionein varies with organ and concentration of metal. Hepatic rat metallothionein containing zinc or cadmium has a half-life *in vivo* of 10–30 hr or 2.8–3.5 days, respectively (*14*). Renal rat metallothionein with cadmium as major metal component has a biological half-life of about 5 days, which is about twice the hepatic value (*16*).

Adults in Sweden and in various countries (*17,18*) normally have cadmium concentrations in their kidneys of about 0.1 to 0.2 mmole/kg, i.e., concentrations which are toxic to the kidney when this cadmium concentration is reached after parenteral administration of cadmium bound to metallothionein. It could thus be speculated that toxic effects from cadmium could occur in normal human kidneys if normal metallothionein metabolism is changed in any way. Of special interest is storage *in vitro,* which could constitute an inhibiting circumstance, since donor kidneys used for transplantation of kidneys into uremic patients are often stored in a preservative solution up to 24 hr before transplantation.

Tubular dysfunction with varying severity may occur shortly after transplantation (*19,20*). An increased excretion of β_2-microglobulin is not uncommon (*21*).

The objective of the present work was to examine whether cadmium or zinc is released from metallothionein when kidneys from cadmium-exposed rabbits are perfused and stored in a preservative solution under conditions similar to those used for human donor kidneys. The experiment was performed on two occasions but under identical conditions.

Material and Methods

Animals

Seventeen rabbits (chinchilla in the first series and New Zealand white in the second), weighing about 2.8 kg each when exposure started, were used. The animals were kept in separate cages and given free access to drinking water and ordinary pelleted food. Thirteen of the animals were given subcutaneous injections of cadmium chloride three times/week for 1 to 3 weeks. The total dose given was 2.7, 6.7, 13, 20, 27, and 40 μmole/kg body weight for different subgroups of animals. Four rabbits (two of each race) served as controls and were given an equal volume (0.2 mL) of 0.9% NaCl subcutaneously.

Between 15 and 20 weeks after cessation of exposure the rabbits were anesthetized by intravenous administration of mebumal. The abdomen was opened while the animals were still alive, and perfusion of the kidneys with ice-cold modified Sachs' solution started, containing 4.76 g KH_2PO_4, 9.70 g $K_2HPO_4 \cdot 3H_2O$, 2.30 g $KHCO_3$, 1.23 g $NaHCO_3$, 0.76 g $MgCl_2$ and 37.50 g mannitol in 1000 mL of distilled water. This solution is routinely used for preservation of human cadaveric kidneys prior to transplantation (*22*).

Preparation of Kidneys

As soon as possible, the kidneys were removed under continuous perfusion with ice-cold Sachs' solution into the renal artery. After a successful perfusion, one of the kidneys was put directly into liquid nitrogen and subsequently kept at −70°C. The other kidney was placed in an ice-cold beaker with Sachs' solution and kept at 4°C. The latter procedure is identical to preservation of human kidneys after transplantation. After 24 hr the preserved kidneys were also frozen in liquid nitrogen and then stored at −70°C until further analysis.

Biochemical and Metal Analysis

Directly frozen and preserved kidneys from the same animals were examined parallel to each other. The kidneys were thawed, and samples of approximately 1 g of kidney cortex were taken for gel filtration chromatography using Sephadex G-75 and metal analysis. Before analysis of total metal the samples were dried at 110°C overnight and the dry/wet weight ratio determined. The gel filtration was performed after homogenization and ultracentrifugation by exactly the same equipment and methods earlier described (*23*). Cadmium, zinc and copper in protein fractions obtained from the G-75 filtration, and tissues, were determined by atomic absorption spectrophotometry (*24*). Quantitative measurements of metallothionein, based on the cadmium saturation method as described by Onosaka and Cherian (*25*), were carried out on kidneys obtained from nine of the animals. The cadmium saturation method is a quick and easy method for quantitative assay of metallothionein. The concentration of MT can be calculated from a 6 g cadmium atom–MT relationship (*25*). More information about animals, exposure and methods used is given in a separate report (*26*) derived from the same group of exposed animals.

Results and Discussion

The 13 exposed rabbits acquired cadmium concentrations in kidney cortex ranging from 0.05 to 1.0 mmole Cd/kg wet weight. The four control rabbits had cadmium concentrations below 0.02

mmole Cd/kg. Gel filtration on G-75 of ultracentrifugated kidney cortex homogenates showed that most of the cadmium in kidneys was recovered in fractions having a V_e/V_0 ratio of about 2.3. This V_e/V_0 ratio corresponds to the molecular size of metallothionein, as shown in previous experiments using an identical methodology and apparatus (*12*). These fractions also contained zinc, and the zinc content in the MT fractions increased with increasing cadmium content, indicating that metallothionein binds both cadmium and zinc. There was no evidence of an increase in the copper content in the metallothionein fractions, with increasing cadmium content. A mathematical relation between cadmium and zinc in metallothionein is described in another report (*26*) based on the same animals. The cadmium saturation method also confirmed that metallothionein concentration increased with increasing concentrations of cadmium in kidney cortex.

The main objective of the present work was to investigate whether cadmium and zinc are released from metallothionein, or whether metallothionein is degraded, during 24 hr storage in a preservative solution. Each animal was its own control as one kidney was fresh frozen and the other placed for 24 hr in the preservative solution. Table 1 presents detailed data on total cadmium and zinc concentrations, the amount of cadmium and zinc in metallothionein fractions obtained from the G-75 filtration and, for nine animals, quantitative data on metallothionein content in fresh frozen and preserved kidneys from the same animal. There is a clear tendency towards somewhat higher values for the preserved kidneys compared to the fresh frozen ones, due to the increase in the dry/wet weight ratio, which on an average increased by 18% in exposed animals ($n = 12$). When total cadmium concentrations were compared on a dry weight basis, there was no difference between fresh and preserved kidneys. The average concentrations for the exposed animals ($n_1 = 12$) were 1.88 and 1.92 mmole/kg, respectively. The regression equation gave the following values: $a_1 = 0.03$, $b_1 = 1.01$ ($y = a_1 + b_1x$). One rabbit (No. 13) was excluded from these calculations, since there is strong suspicion that the samples for determination of total cadmium and dry/wet weight ratio had been exchanged.

A linear regression analysis between the cadmium content in metallothionein fractions in fresh frozen kidneys (x-axis) and in preserved kidneys (y-axis) yields the following constants: $a_2 = 0.01$, $b_2 = 1.10$ ($y = a_2 + b_2x$), and a regression coefficient (r_2) of 0.97 ($n_2 = 17$). The corresponding regression constants for zinc in metallothionein fractions of fresh frozen and preserved kidneys was $a_3 = -0.01$, $b_3 = 1.17$ and $r_3 = 0.96$ ($n_3 = 17$). The metallothionein concentration as measured by the cadmium saturation method in fresh frozen and preserved kidneys also correlated very well, $a_4 = -0.004$, $b_4 = 1.24$, $r_4 = 0.97$ ($n_4 = 9$). Thus, the preserved kidneys, on the average, had about 15% higher levels of cadmium, zinc and metallothionein compared to the fresh frozen kidneys from the same animal, which is explained completely by the change in dry/wet weight ratio. It has indeed been noticed that human donor kidneys also change the dry/wet

Table 1. Cadmium concentration kidney cortex, cadmium and zinc content in metallothionein fractions and MT concentration in fresh frozen and preserved kidneys from cadmium-exposed rabbits.

		Fresh frozen kidneys					Preserved kidneys				
Animal and no.	Injected dose, μmole Cd/kg	Cd concn, mmole/kg	Dry/wet weight ratio	Cd in MT fractions, μmole/g	Zn in MT fractions, μmole/g	MT, μmole/g	Cd concn, mmole/kg	Dry/Wet weight ratio	Cd in MT fractions, μmole/g	Zn in MT fractions, μmole/g	MT, μmole/g
New Zealand											
1		0.008	0.256	≤0.013	0.061		0.012	0.258	<0.013	0.063	
2		0.013	0.221	≤0.019	0.054		0.01	0.277	<0.019	0.056	
3	2.7	0.046	0.218	0.041	0.063		0.057	0.258	0.046	0.068	
4	2.7	0.073	0.217	0.056	0.070		0.088	0.240	0.073	0.066	
5	6.7	0.166	0.219	0.124	0.118		0.203	0.255	0.144	0.134	
6	6.7	0.172	0.234	0.125	0.151		0.174	0.242	0.114	0.135	
7	13.3	0.209	0.230	0.166	0.169		0.271	0.256	0.151	0.171	
8	13.3	0.267	0.238	0.185	0.177		0.280	0.253	0.201	0.177	
Chinchilla											
9		≤0.023	0.170	0.006	0.114	0.020	0.018	0.199	0.006	0.109	0.028
10		≤0.023	0.225	0.011	0.041	0.018	0.022	0.267	0.012	0.053	0.017
11	13.3	0.509	0.236	0.358	0.265	0.130	0.587	0.276	0.346	0.275	0.155
12	13.3	0.298	0.210	0.183	0.201	0.122	0.361	0.255	0.223	0.275	0.119
13	20.1	0.584	0.218	0.355	0.296	0.185	0.443	0.173	0.534	0.360	0.210
14	26.7	0.942	0.220	0.699	0.299	0.222	1.17	0.264	0.870	0.334	0.299
15	26.7	0.969	0.219	0.851	0.322	0.209	1.20	0.271	0.813	0.309	0.288
16	40.1	0.804	0.216	0.515	0.320	0.236	0.980	0.265	0.670	0.402	0.252
17	40.1	0.322	0.133	0.238	0.214	0.111	0.498	0.185	0.365	0.323	0.143

weight ratio subsequent to storage in a preservative (27). Another way to eliminate the influence from changes in wet/dry weight ratios on the comparability between fresh and preserved kidneys is to use the zinc to cadmium ratio in metallothionein fractions. If, for example, zinc should be released from metallothionein during storage, this would be reflected by a change in the ratio between zinc and cadmium. There was, however, no indication that such a change took place in the present study. A linear regression line between the ratio of zinc and cadmium in metallothionein fractions from fresh and preserved kidneys of exposed rabbits gave the following constants: $a_5 = 0.03$, $b_5 = 0.93$, $r_5 = 0.93$ ($n_5 = 13$).

There was no indication that kidneys with either a low or a high cadmium concentration released cadmium or zinc from metallothionein when stored in the preservative (Table 1).

The elution profiles obtained from G-75 filtra-

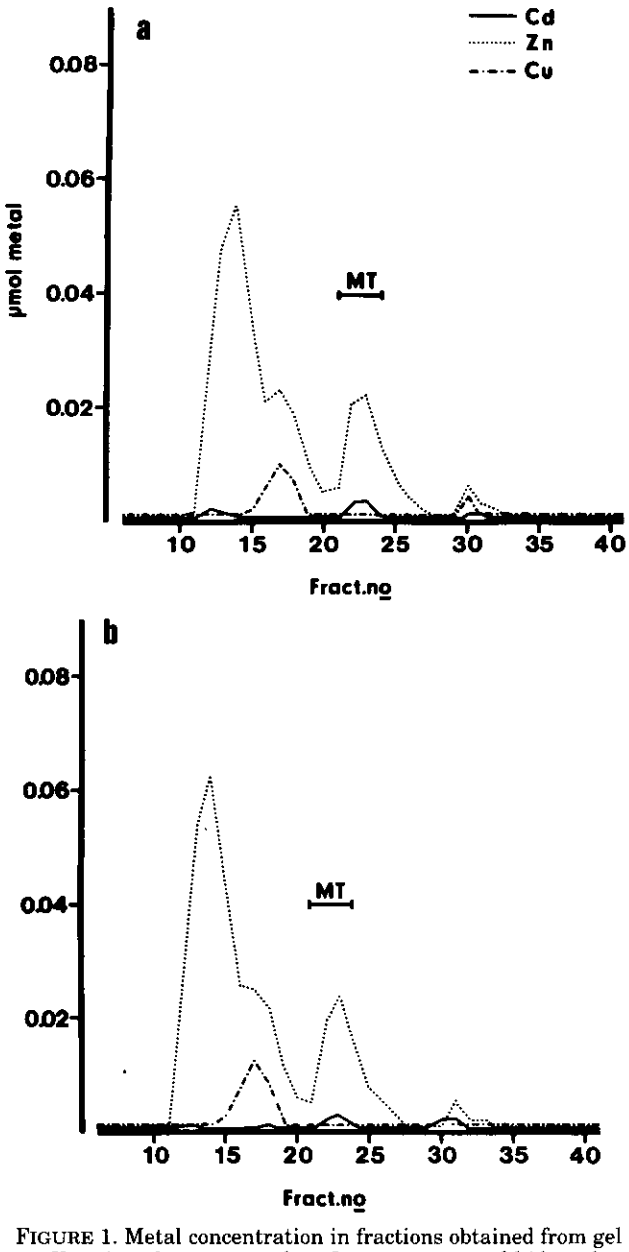

FIGURE 1. Metal concentration in fractions obtained from gel filtration chromatography of supernatants of kidney homogenates using Sephadex G-75. Results from (*a*) fresh frozen and (*b*) preserved kidneys from a control rabbit.

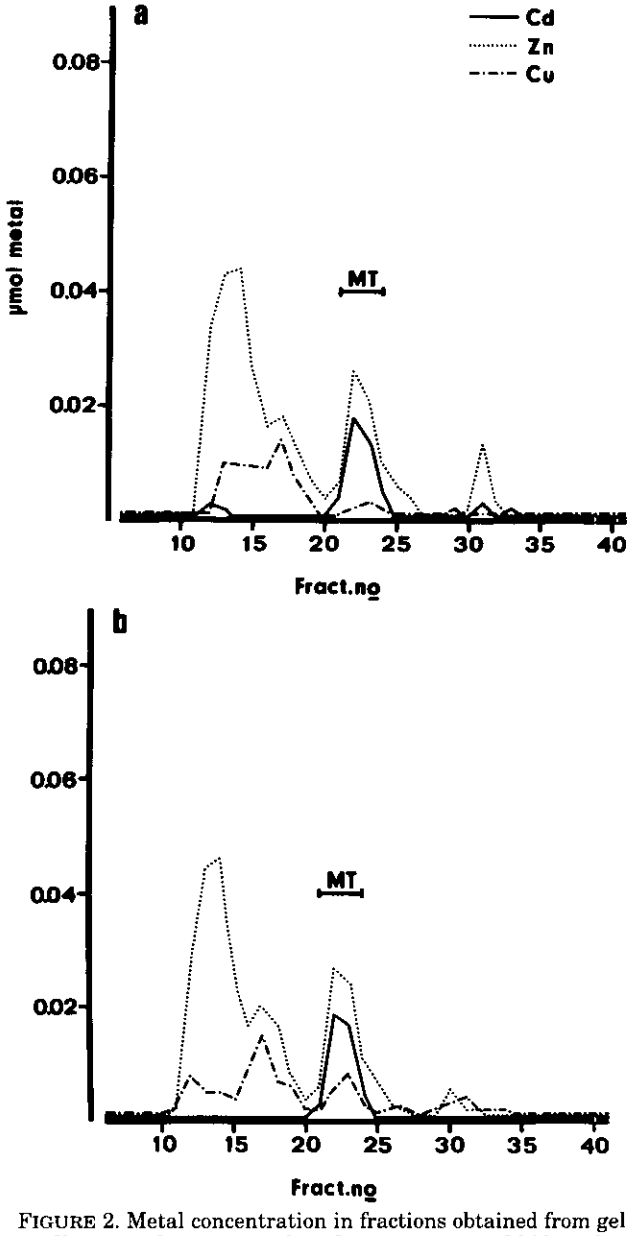

FIGURE 2. Metal concentration in fractions obtained from gel filtration chromatography of supernatants of kidney homogenates using Sephadex G-75. Results from (*a*) fresh frozen and (*b*) preserved kidneys from a rabbit given 2.7 µmole Cd/kg.

tions were almost identical in fresh frozen and preserved kidneys. Figures 1–3 present elution profiles for cadmium, zinc and copper from frozen and preserved kidneys of three animals with cadmium concentrations of 0.01, 0.06 and 0.27 mmole Cd/kg in the cortex.

In conclusion, the present work provides no evidence of any major metallothionein degradation or release of zinc or cadmium from metallothionein as a result of 24-hr storage in Sachs' solution at 4°C. It is, however, possible that a degradation of metallothionein may have taken place if the storage time had been longer, the temperature elevated or the cadmium concentrations in kidneys higher. Furthermore, this experimental set-up cannot exclude minor release of metals from metallothionein during storage. Another interesting question is what happens during and after the actual transplantation.

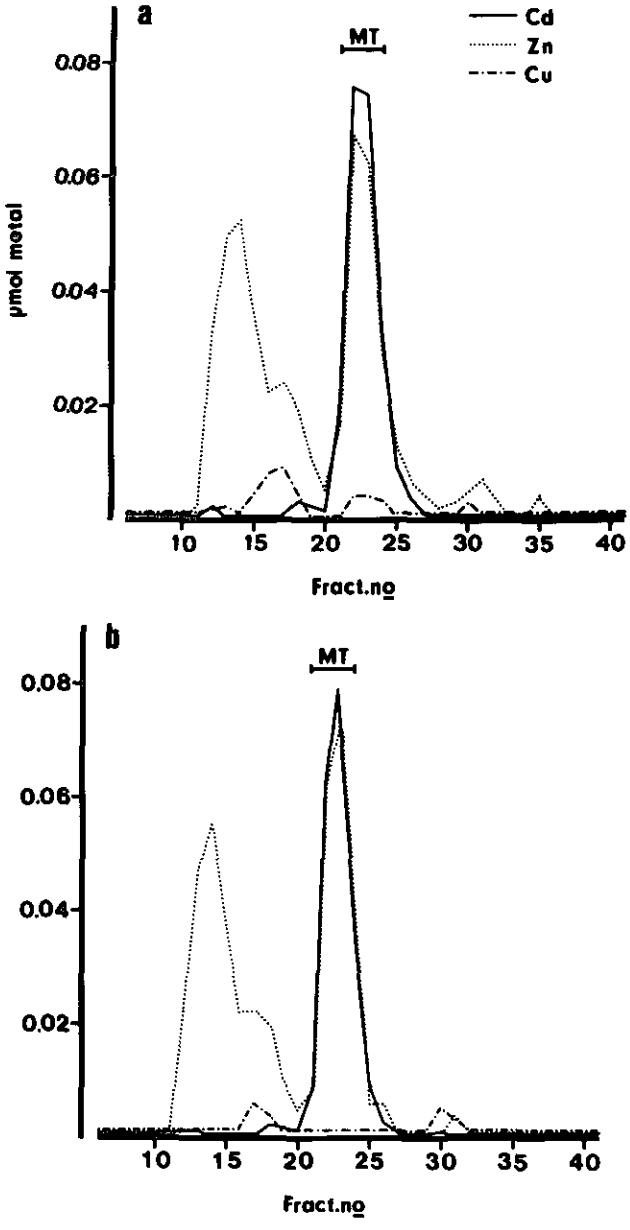

FIGURE 3. Metal concentration in fractions obtained from gel filtration chromatography of supernatants of kidney homogenates using Sephadex G-75. Results from (*a*) fresh frozen and (*b*) preserved kidneys from a rabbit given a total dose of 13.3 µmole Cd/kg.

Grants from the Karolinska Institute are gratefully acknowledged.

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