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Studies of Cd, Zn and Cu Levels in Human Kidney Tumours and Normal Kidney

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Summary. The distribution of Cd, Zn and Cu was analyzed in nuclear, mitochondrial and cytosol cellular fractions of renal tumours and normal surrounding kidney tissues by electrothermal atomic absorption spectrometry (AAS). The normal kidney tissues were separated into cortical and medullary parts. A significant decrease of the Cd-levels was found in all cellular fractions from normal kidney to tumour tissues (hypernephroma). The Zn values varied strongly. We noted a slight decrease of Zn levels in renal tumour. The Cu concentration in the nucleus of kidney tumours was significantly increased.

Key words: Renal tumours, Normal renal tissues cellular fractions, Cd, Zn, Cu concentration.

Introduction

The knowledge of the importance of trace elements in medical research has increased in the last few years. Intensive studies on the influence of these elements, especially Cd, on the metabolism of cells have been made with reference to the aetiology of cancer. Based on our results of the interaction between cadmium and zinc in different prostatic tissues, which showed an antogonistical biological effect in the prostatic cell [3–5] we studied the influence of the heavy metals in other urological tumour tissues. The accumulation of Cd in kidney, the known interaction with Zn [10] and also with Cu [8] suggested a possible carcinogenic effect of Cd in kidney tissues. We studied the interaction of Cd, Zn and Cu in human renal cancer and normal renal cellular fractions.

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Materials and Methods

Sections of human kidney tumours and surrounding normal kidney tissues were used. The normal kidney tissue was separated into cortex and medulla. All samples were examined histologically.

The cellular fractions were prepared in the following manner. The tissues were sliced and then transferred into a special Potter homogenizer made from PTFE. A few millilitres of Tris-HCl buffer were added. The homogenate was filtered through a nylon gauze (150 μm). The homogenate was then sedimented in a refrigerated centrifuge at 10,000 x g for 10 min. The supernatant was decanted from the nuclear fraction. The solution was centrifuged at 100,000 x g for 1 h to isolate the mitochondrial fraction together with microsomes from the cytosol.

All fractions were transferred into small quartz beakers to be heated at 180 °C until a constant weight was obtained. Then the samples were dissolved in suprapure nitric acid.

All sample solutions were analyzed by flameless atomic absorption spectrometry (Jarrell Ash 811). The standard addition technique was used for the determination of Cd, Zn and Cu.

The concentration values were based on the weight of the dried samples for both the nuclear and the mitochondrial fractions. The concentration in the cytosol fraction was based on the mass of the nucleus of the separated sample – 100 mg (dried nucleus) were taken for comparison.

Results

The concentration of Cd, Zn and Cu was measured in the medulla, the cortex and in tumour tissues. The results were compared by the univariate variance analysis. We obtained significant differences (error probability 0.01) in the Cd values in the cortical and medullary tissues and also between cortical and tumour tissue. The Cd concentration in kidney cortex was higher than in both medullary and in tumour tissues. But there was no significant difference in the Cd values between medulla and tumour. We did not obtain significant differences in the concentration of Zn and Cu in kidney medulla, cortex and tumour. Therefore the elements were determined in the cellular fractions of different renal tissues.

Table 1. Cd concentration in cellular fractions of kidney

Sample	*n*	Nuclear fraction mg/kg	Mitochondrial fraction (dry weight)	Cytosol[a]
Medulla	8	5.28 ± 3.15 (1.1 – 9.0)	4.61 ± 6.10 (0.5 – 17.9)	2.26 ± 1.84 (0.57 – 6.41)
Cortex	8	10.11 ± 10.88 (0.7 – 32.9)	12.26 ± 13.31 (0.26 – 36.23)	9.30 ± 11.47 (0.25 – 32.92)
Tumour	8	1.71 ± 1.55 (0.3 – 5.1)	1.50 ± 1.69 (0.19 – 5.64)	1.40 ± 1.26 (0.37 – 4.56)

[a] see text

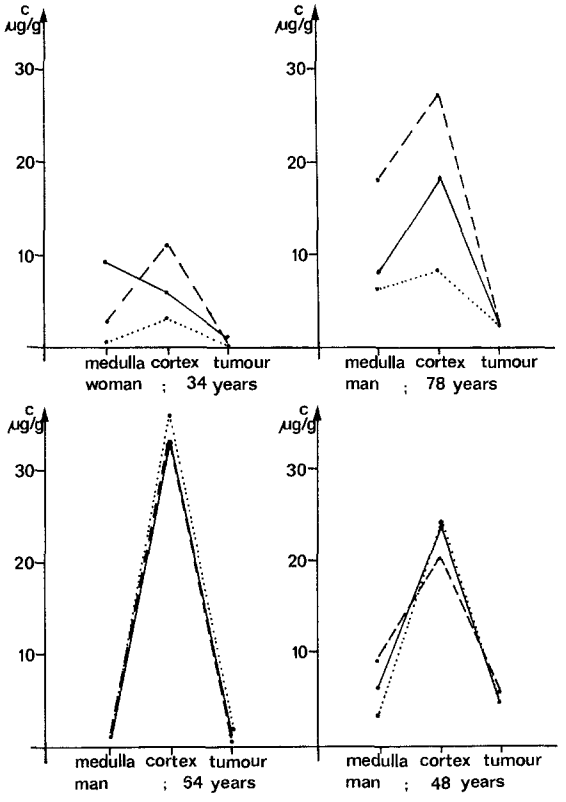

Fig. 1. Cd concentration in cellular fractions of kidney

Cd-Investigations

The results of the Cd determinations in the different cellular fractions of human kidney tissues are shown in Table 1. The highest values we found in the nuclear, the mitochondrial and the cytosol fractions of the tissues of kidney cortex. The graphical display of the Cd-values showed distinct differences between the concentration in all groups. In tumours we found the lowest Cd level in all cellular fractions. The Cd concentration obtained was the highest in all fractions of cortex. The variances were equal in our studies. Therefore an a.t.-test was done. Because we had only 8 cases in each group the differences of the mean values dominated in the statistical calculations. Thus we only obtained significant differences between:

- nucleus of tumour and cortex tissues (critical error probability 2.8%)
- mitochondria of tumour and cortex tissues (critical error probability 3.0%)
- cytosol of tumour and cortex tissues (critical error probability 4.4%)

The critical error probabilities for the separation of all fractions of tumour and medulla tissues was lower than 5%.

The high variance of the results in all groups of samples is connected with the origin of the human tissue samples (e.g. age, Cd-exposition, smoking). Thus the differences in the different tissues were monitored by the individual sample values (Fig. 1).

A rising Cd content from medulla to cortex in normal kidneys and a decrease of the Cd levels in tumour tissues in the cellular fractions were definite.

Zn-Investigations

Our studies with Zn in the same human renal tissues demonstrated no significant differences between the cellular fractions of the normal kidney and of the renal tumours (see Table 2). We obtained no distinct differences in the mean values of the Zn concentration in the different cellular fractions of renal medulla and cortex by a large spread of the Zn values.

In the majority of our patients we found a decrease in the Zn-levels both in nuclear and cytosol fractions from normal kidney to renal tumours (Fig. 2).

Cu-Investigations

The results obtained in the determination of copper are shown in Table 3. Between the normal kidney (medulla and cortex) and the renal clear cell tumours we found differences in the Cu-levels in the nuclear fraction.

Table 2. Zn concentration in cellular fractions of kidney

Sample	*n*	Nuclear fraction mg/kg	Mitochondrial fraction (dry weight)	Cytosol[a]
Medulla	8	1,483 ± 1,055 (288 – 3,342)	1,006 ± 955 (42 – 2,602)	190 ± 53 (118 – 359)
Cortex	8	1,127 ± 387 (564 – 1,793)	750 ± 589 (79 – 1,728)	434 ± 248 (152 – 901)
Tumour	8	1,173 ± 792 (163 – 2,591)	794 ± 497 (144 – 1,809)	186 ± 141 (41 – 496)

[a] see text

Table 3. Cu concentration in cellular fractions of kidney

Sample	*n*	Nuclear fraction mg/kg	Mitochondrial fraction (dry weight)	Cytosol[a]
Medulla	8	0.34 ± 0.18 (0.22 – 0.64)	0.13 ± 0.13 (0 – 0.37)	0.01 ± 0.01 (0 – 0.03)
Cortex	8	0.24 ± 0.15 (0.03 – 0.49)	0.17 ± 0.13 (0.01 – 0.37)	0.01 ± 0.01 (0 – 0.03)
Tumour	8	0.59 ± 0.69 (0.01 – 2.01)	0.28 ± 0.32 (0 – 1.04)	0.01 ± 0.01 (0 – 0.03)

[a] see text

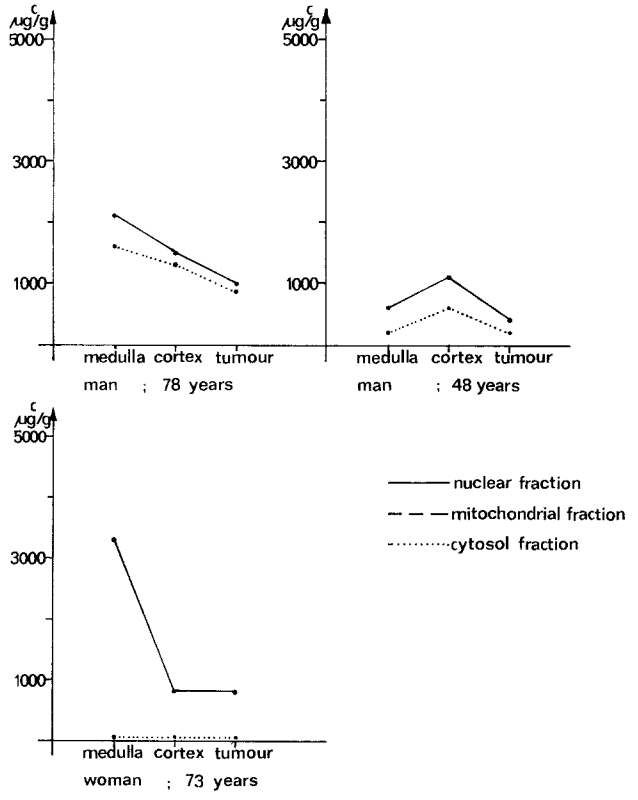

Fig. 2. Zn concentration in cellular fractions of kidney

It seems that copper is enriched in the nuclear fraction of renal tumour tissues. In the mitochondrial fraction of tumours we noted higher concentrations of copper.

The Cu-levels in the mitochondrial fraction vary strongly.

Discussion

Our investigations show a decrease of the Cd concentration in all cellular fractions from normal kidney to renal clear tumours (hypernephroma) in both men and women of different ages. In recent studies Hienzsch et al. [6] found a significantly lower Cd concentration in unseparated kidney tissues with both stones and tumours compared with healthy organs. In the kidney Cd accumulates selectively in the renal cortex [11, 13, 14]. Our investigations show similar relationships in the increase of the cadmium values from medulla to cortex in all cellular fractions. It seems that the Cd-binding metallothioneins [1, 9] exist in all the investigated cellular fractions.

The accumulation of Cd in the normal kidney tissue and the decrease of Cd levels in cellular fractions of tumour tissues presumably have no direct carcinogenic effect in renal tumours.

Cadmium and zinc are closely related elements which share many chemical properties. Since Zn is found in a sig-

nificant quantity in the kidney, which also concentrates Cd, this organ might be a potential target for any effects from lifelong cadmium exposure [7]. The effects of zinc on renal function were, therefore, compared to those of cadmium. The Zn-values in both the nuclear and mitochondrial fractions are much higher than in unseparated kidney tissues [12]. It seems that Zn is strongly bonded in the nucleus and the mitochondria both in normal kidney and in hypernephroma. A significant decrease in the Zn concentration from unseparated tissues of normal kidneys to clear cell cancer was described in 3 cases. We could not confirm this statement. We did not find significant differences in the Zn concentration between normal kidney and renal tumour.

We also found that the Zn levels vary strongly in the different cellular fractions of medulla, cortex and tumour in individuals (see Fig. 2). We observed differences in the relation of the zinc values in the cellular fractions of the different renal tissues for several patients. Zn and Cd exist in a ratio which varies between 100:1 and 1000:1 in cellular fractions of the renal tissues. Assuming that Cd and Zn are bound in a molar ratio of 1:1 to a special metallothionein in kidney [1] the decrease of the Cd-level in all cellular fractions of renal tumour tissues compared with normal ones effects only a small decrease in the Zn concentration in the same fractions. It seems that the Zn content in both the nuclear and the cytosol fractions is decreased in tumour tissues compared with normal kidney. Results of Sakamoto et al. [12] demonstrate that cadmium greatly accelerates the accumulation of zinc and also of copper in kidney cortex. Cu also accelerates the accumulation of Cd in renal tissues. It was indicated that Cd and Zn as mercaptide forming agents antagonize the copper metabolism by displacing copper from sulfhydryl binding sites on metallothioneins [2].

Our investigations show an increase of Cu (not statistically significant) in the nuclear fraction of renal tumour tissues compared with normal kidney.

Therefore it is possible that the increased copper values are associated with the growth of renal tumours. Loss of various enzymatic activities and changes in different properties of proteins and also changes in lipid peroxidation in the kidney could be caused by elevated levels of intracellular copper, also found in cancerous lesions.

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