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Zinc and Cadmium in Cell Fractions of Prostatic Cancer Tissues of Different Histological Grading in Comparsion to BPH and Normal Prostate

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Summary. The concentrations of zinc and cadmium in cellular fractions of normal prostate gland, BPH and prostatic carcinomas of different histological grading were determined by electrothermal atomic absorption spectrometry. We found distinct differences in the content of Zn and Cd in the nuclear fractions of malignant tissues in comparison with BPH and normal prostatic tissues. The highest values of Cd were obtained in the nuclear fractions of poorly-differentiated carcinomas. In these samples we also found a low concentration of zinc. In comparison to this the highest Zn-values were found in the nuclear fraction of the BPH.

Key words: Prostatic carcinomas, Cellular tractions, Zn and Cd concentration.

Introduction

For more than thirty years [2] it has been known that there is an abnormally high Zn concentration in the prostate and in its secretion. In modern literature a very heterogeneous distribution of zinc inside the normal prostate gland is described [16]. It does not parallel the anatomical structure of the prostate.

The accumulation of Zn in prostate cells and the high concentration of this element in its secretion, like other specific functions of the prostate, depend on androgen influences and begin at puberty [24, 27, 30]. A specific zinc-binding protein has been detected in the cytoplasma of the prostate gland [25]. In spite of many investigations during the last few years, the reason for the high Zn level [26] and the mode of action of this element are still unknown [28]. Both the accumulation of zinc in the prostate tissue and the high Zn concentration in the prostatic secretion are reduced by pathological processes in the prostate gland, such as prostatitis and cancer [5, 12, 16].

On the other hand the positive inference of Zn on the structural integrity of the prostatic epithelisis [15, 30] and the ability of Zn to have a stabilising effect on RNA and DNA in other human organs has been discussed. Recently the possibility of a positive correlation between the function of prolactin and zinc accumulation in prostatic cells was suggested [17]. The evidence for a Zn- and testosterone binding protein in the prostate adenoma has also been discussed [14, 25].

In our recent investigations [6] we found a distinct biological antagonistic effect between Zn and Cd in the prostate gland. We measured an increased content of Zn in BPH and a decrease of this element in prostatic cancer. We also found a continuous increase of Cd concentration from the normal prostate via BPH to carcinomas.

Therefore we were interested in obtaining more information about the localisation of zinc and cadmium in cell fractions of normal and of pathologically changed prostate glands. This might be a small step to recognizing the function of these trace elements in prostatic cells.

Materials and Methods

In these investigations whole prostates and pieces after transurethral resection were taken. Tissue was cut into pieces for the preparation of cellular fractions whereas other samples were used for the determination of Cd and Zn without special fractionation. The two normal prostates were taken from men after fatal accidents. Material was heated at 180 °C until a constant weight was obtained. The dried samples were dissolved in nitric acid (Suprapur, Merck) by slowly heating.

Methods of the preparation of cellular fractions have been described previously [3, 4, 8, 10].

The tissue was sliced and then transfered to a special Potter homogenizer made from PTFE. Some millilitres of a Tris-buffer (pH 7.4) were added. (The buffer substances had been analyzed for their content of Cd and Zn). The homogenate was filtered through a nylon gauze (50 μm) into specially cleaned polyethylene tubes. The material which did not pass through the gauze was prepared like normal tissue for atomic absorption measurement. The homogenate was sedimented in a refrigerated centrifuge at 1,000 x g

Table 1. Zn-concentration in cellular fractions of prostatic tissues [Zn-Concentration $\mu g \cdot g^{-1}$ dried sample]

Histology	Number of men	Nuclear fraction	Mitochondrial fraction	Cytosol fraction[a]
Adenoma	9	456 ± 261	221 ± 291	224 ± 140
Carcinoma	5	50	381 ± 146	35 ± 34
Normal prostate	2	250	269	27

[a] see text

Table 2. Cd-concentration in prostatic cell-fractions [Cd-content $\mu g \cdot g^{-1}$ dried samples]

Histology	Number of men	Nuclear fraction	Mitochondrial fraction	Cytosol fraction[a]	separated tissue
Adenoma	9	0.84 ± 0.62	0.85 ± 0.78	0.20 ± 0.15	0.36 ± 0.17
Carcinoma	5	–	4.08 ± 2.05	0.36 ± 0.20	0.40 ± 0.30
poorly diff.	2	12.1	–	–	–
well diff. Adenocarcinoma	3	3.01 ± 2.46	–	–	–
Normal prostate	2	1.36	–	0.08	0.18

[a] see: determination of Zn

for 10 min. The supernatant was decanted from the nuclear fraction. After that, the solution was centrifuged at 10,000 x g for 20 min to isolate the mitochondrial fraction. (All these procedures were carried out at 0 to 4 °C in a cooling room.)

All fractions were transfered into small quartz beakers to be heated at 180 °C until a constant weight was obtained. Then the samples were dissolved in HNO_3.

The atomic absorption measurements were made by using of 10 µl of sample solution. The standard addition technique was used.

All determinations were done with a Jarrell-Ash double-beam atomic absorption spectrometer combined with a Beckmann graphite furnace atomizer. The fractions were also tested histologically.

Results

Zinc Investigations

The Zn-investigations were made with 9 adenomas, 2 normal prostates and 5 carcinomas with different histological grading (see Table 1).

The concentration of Zn (and Cd) in both in the nuclear and in the mitochondrial fractions is based on the weight of the dried materials. The supernatant contains cytosol and the buffer substances. Therefore it is a problem to use the mass of this dried material as a reference. Thus the Zn (and Cd) concentration was based on the mass-value of the nuclear fraction (We chose 100 mg of dried nuclei for comparison.) The results show that in adenofibromyomatosis the Zn-concentration in the nuclear fraction (456 ± 261 $\mu g \cdot g^{-1}$) was distincly higher than in the mitochondrial one (221 ± 291 $\mu g \cdot g^{-1}$). Zn was also present in the supernatant.

The carcinoma material gave a lower Zn concentration in the nuclear fraction than the BPH. The content of this element in the mitochondrial fraction was similiar in all materials. The amount of Zn contained in the supernatant of carcinomatous materials was similiar to that of the normal prostate samples and lower than the values measured in BPH.

We also investigated the Zn concentration of carcinomatous materials with respect to the histological grading. In these preliminary studies we obtained no distinct difference dependent on the histology.

Cadmium Investigation

The Cd analysis was made with the same samples as were used in the Zn investigation.

We obtained distinct differences in the Cd concentration in carcinomia material in comparison with BPH and normal prostates (see Tab. 2).

An extremely high concentration of cadmium was measured in the nuclear fraction of poorly-differentiated carcinoma. The Cd-values in the mitochondrial fraction of these materials were also higher than in BPH but in the same range as in adenocarcinoma. The Cd concentration in the nuclear fraction of well-differentiated adenocarcinoma was also higher than in normal prostate and BPH. We obtained no distinct differences in the Cd content fractions of BPH and normal prostate tissue.

The Cd concentration in the tissue which did not pass through the gauze in the sample homogenization (see Materials and Methods) was distinctly lower than in the cell fractions.

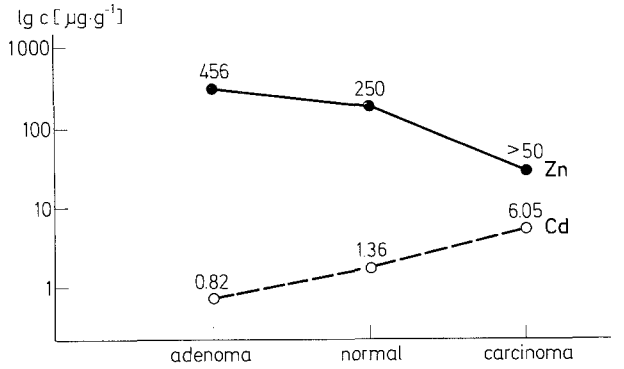

Fig. 1. Zn and Cd concentration in prostatic tissues of different histology [µg/g of dried samples]

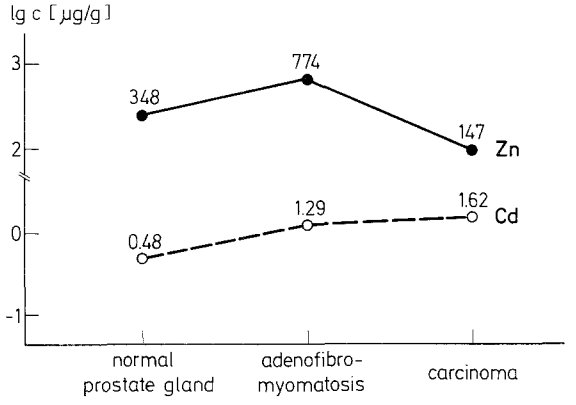

Fig. 2. Concentration of Cd and Zn in nuclear fractions of prostatic tissues

Discussion

Cd has been classified as a non-essential element [20]. It is present in food, tobacco, air and water as a contaminant. The absorption of ingested Cd is rather small, whereas that of inhaled Cd is greater. Smoking can be an important source of Cd exposure. This amount is comparable to the daily gastrointestinal absorption from ingested food [29]. The absorbed Cd is excreted very slowly and it accumulates primarily in the liver, where it is bound to metallothionein, a special Cd-binding protein [23], in the kidney and in other organs. The authors also found a distinct influence of cadmium toxicity on the cellular membranes and ribosomale RNA-synthesis [18, 19] by microscopical investigations with liver cells and endothelial cells from uterine vessels in rats. Other authors [7] have suggested that bile salts represent an endogenous factor which might be involved in preventing extensive Cd-uptake from the intestinal lumen.

Cadmium acts as a competitive inhibitor of zinc and Zn-binding proteins [9, 11, 13] and produces malignant tumours in animals. Partial or total inhibition of zinc-dependent enzymes, like carbonic anhydrase and others, is a possible mechanism for the carcinogenicity of the metal. One of the consequenses of the many similarities between Zn and Cd could be the direct substitution of Zn by Cd in zinc-containing enzymes, e.g. phosphatases, carboxypeptidases, carbonic anhydrase, which normally contain Zn bound to sulphur [23].

In a recent experimental study Aughy et al. [1] showed that prostatic cells are capable of retaining Cd in measurable amounts up to 6 weeks after injection, but that areas with high Zn concentration retain less Cd then areas with low zinc. They also found histological changes suggestive of carcinogenic activity by Cd. In experiments the carcinogenic action of cadmium could be prevented by injection with Zn salt [22]. It has also been demonstrated in several animal experiments that selenium has an antagonistic effect on heavy metals like Cd [20, 21]. Cadmium does change the metabolism, but whether these changes are primary or secondary effects is difficult to say [23]. In our recent paper [6] we found differences in the Cd and Zn concentration in prostatic tissues depending on the histological grading.

Our results show that only in the nuclear fraction were distinct differences obtained, dependant on the histological grading, in both the Cd and Zn concentration (Fig. 1).

The Zn content in the nuclear fraction of carcinoma is distinctly lower than that in BPH and normal prostate (Fig. 2). We also observed that the nuclear fractions of poorly-differentiated carcinomas with their bad clinical prognosis obtained the highest level of Cd. The Cd concentration in the mitochondrial fraction was higher when we investigated carcinoma than in the case of BPH and normal prostates.

The highest Zn concentration in normal prostate tissue was also measured in the nuclear fraction by nuclear activation analysis with ^{65}Zn [25] by others.

All results show that the accumulation of Cd could be one of the factors causing the disturbance in RNA synthesis occuring in carcinogenesis of the prostate. In future work we hope to confirm this with more experimental results.

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