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Determination of the Distribution of Zinc and Cadmium in Cellular Fractions of BPH, Normal Prostate and Prostatic Cancers of Different Histologies by Atomic and Laser Absorption Spectrometry in Tissue Slices

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Summary. The distribution of Zinc (Zn) and Cadmium (Cd) was analyzed in nuclear, mitochondrial and cytosol cellular fractions of prostatic carcinoma of different histological grading, BPH and normal prostate by electrothermal atomic absorption spectrometry (AAS). Distinct differences in Zn and Cd concentration in carcinomatous material in comparison with BPH and normal prostates was found. The highest concentration of Cd and the lowest level of Zn were found in poorly differentiated carcinomas. The results in cellular fractions were compared with investigations on Zn and Cd distribution in prostatic slices by laser AAS.

Key words: Prostatic carcinoma, Cellular fractions, Zn and Cd concentration, Zn and Cd distribution in prostatic slices by laser AAS.

Introduction

The influence of trace elements on the growth and the action of the prostate has been of interest for investigators during the last few years. The very high Zn in levels in the human prostate are today generally considered to be a special function of the prostate, influenced by androgens. Both the accumulation of Zn in prostatic tissue and the high concentration in prostatic secretion are reduced by pathological processes in the prostate gland, such as prostatitis and cancer [2, 4, 5].

In spite of many investigations the reason for the high Zn concentration [8] and the function of this element are still unknown.

It has been found that Zn is not disease-specific. The Zn contents of carcinomatous and hyperplastic prostates are shifted significantly in opposite directions, whereas the normal prostate has medium Zn levels [7].

In a series of autopsies in man a high Zn concentration was found in the dorsal lobes of the prostate [12].

In our recent investigations [2] we found a distinct antagonistic biological effect between Zn and Cd in the human prostate.

A direct action of Cd on the prostate gland was observed in cadmium treated cultures on rat prostate in the direction of the degradation of stromal elements. Cd was detected mainly in the nuclei of the necrotic epithelial cells and in the rapidly growing basal cells [1].

The role of Cd in carcinogenesis and its interaction with Zn inside the prostate is complicated. Zn can obviate some of the effects of Cd in experimental animals [9].

The aim of our investigations was to analyze the distribution of Zn and Cd in cellular fractions of different prostatic tissues. We also want to carry out experiments with laser evaporation of tissue material and the determination of trace elements in such vaporized samples by flameless atomic absorption spectrometry in order to obtain information on the distribution of these elements in prostatic slices. These investigations are based on experiments for the determination of Cd in human kidney tissues by laser AAS [10].

Materials and Methods

In these investigations whole prostates and pieces after transuretral resection were taken. Some samples were cut into pieces for the preparation of cellular fractions whereas others were freeze-cut for the preparation of slices for laser-AAS.

The cellular fractions were prepared in the following manner. The tissues were sliced and than transferred into a special Potter homogenizer made from PTFE. Some 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. After that the solution was centrifuged at 100,000 x g for 1 h to isolate the mitochondrial fraction together with microsomes from the cytosol.

In the first figure we can see the test of the purity of the nuclear fraction. 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.

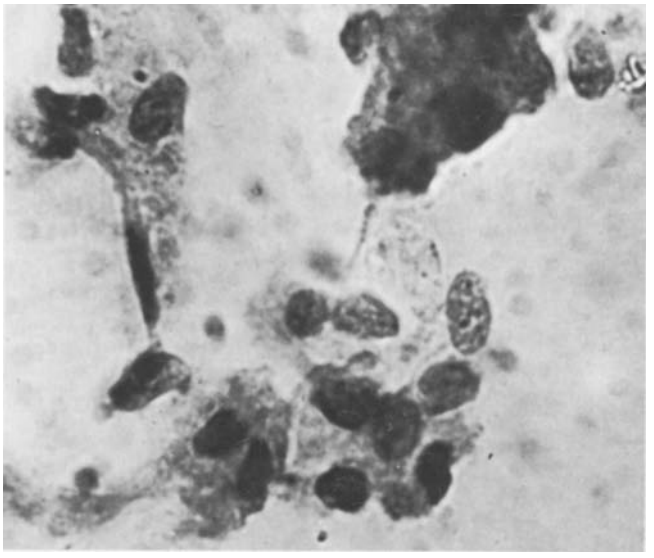

Fig. 1. Nuclear fraction

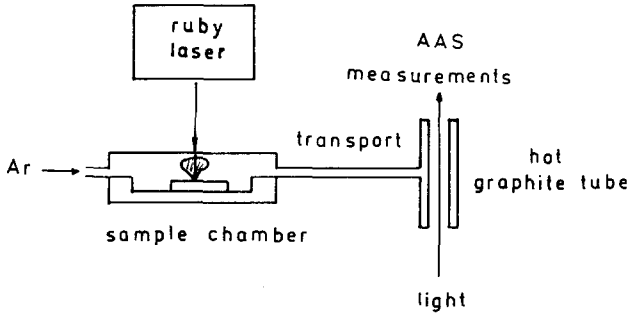

Fig. 2. Scheme of laser AAS

The standard addition technique was used for the flameless atomic absorption measurement of Cd and Zn in such prepared samples. The determination of Zn and Cd in slices of tissues was done with a laboratory-constructed laser-atomic absorption spectrometer. The scheme of this devices is shown in Fig. 2.

A quartz plate with a freeze-dried slide of prostatic tissue without other preparation to avoid contamination with Cd and Zn was placed in a plexiglass chamber. Special areas of the slice were chosen by the laser microscope. Then these special areas having a diameter up to 50 μm were evaporated. The evaporated material was transferred by an argon stream into the hot graphite furnace for the simultaneous determination of Cd and Zn using a double-beam AAS device. The concentration was determined by analyzing standard materials.

Results

Zn Investigations

The Zn investigations were made in 4 normal prostates of men with a mean age of 32 years, 13 adenomas and 12 carcinomas of different histological grading (see Table 1).

The values in the cytosol fractions are based on 100 mg dried nuclei for comparison. In the nuclear fraction the highest Zn level was found in BPH. In comparison to this the poorly differentiated carcinoma had the lowest Zn concentration in this fraction.

The Zn concentrations of the tissues of adenocarcinomas were distinctly higher then those of the normal prostates.

In the mitochondrial fraction only the Zn concentration was distinctly lower in normal prostates.

The reason for the surprisingly high Zn level in the mitochondrial fraction of the poorly differentiated carcinoma is not yet clear. We have to investigate more material having this histology to confirm this result.

Cd Investigations

The results in the determination of Cd are given in the following table (see Table 2). For these investigations the same samples were chosen as were used in the Zn determination.

Discussion

We obtained distinct differences in the Cd concentration in carcinomatous material in comparison with BPH and normal prostates. In agreement with our recent investigations in cellular fractions, we found the highest concentration of Cd in the poorly differentiated carcinoma. This phenomenon was observed in all tissue fractions. The Cd concentration both in the nuclear and in the mitochondrial fraction was also higher than in BPH and normal prostates.

Table 1. Zn concentration in cellular fractions

Histology	*n*	Nuclear fraction	Mitochondrial fraction	Cytosol
		[μg/g of dried samples]		
Normal prostate	4	290 ± 360	99 ± 147	14 ± 11
Adenoma	13	698 ± 633	325 ± 308	280 ± 230
Carcinoma poorly diff.	3	50 ± 11	532 ± 28	6 ± 5
Adenocarcinoma	9	503 ± 317	341 ± 279	145 ± 148

Table 2. Cd concentration in cellular fractions

Histology	*n*	Nuclear fraction	Mitochondrial fraction	Cytosol
		[$\mu g \cdot g^{-1}$ of dried samples]		
Normal prostate	4	0.70 ± 0.70	0.03 ± 0.01	0.06 ± 0.04
BPH	13	0.98 ± 0.86	0.93 ± 0.65	0.14 ± 0.12
Carcinoma poorly diff	3	8.8 ± 5.9	9.6 ± 8.4	0.45 ± 0.13
Adenocarcinoma	9	1.4 ± 1.2	1.7 ± 2.0	0.07 ± 0.08

Table 3. Laser measurement of the Zn distribution in prostatic slices

Histology	Laser craters *n*	Mean value of Zn conc. without areas with high Zn conc.	Special areas with high Zn conc.
Adenocarcinoma	47	2.8 ± 0.9 pg[a]	
	7	–	6 pg 3 times 8 pg 9 pg 10 pg 15 pg
Carcinoma poorly diff.	18	1.0 ± 0.3 pg	
	–	–	–
BPH	33	1.7 ± 0.5 pg	
	4	–	5 pg 6 pg 2 times 12 pg

[a] pg (Piko Gram)

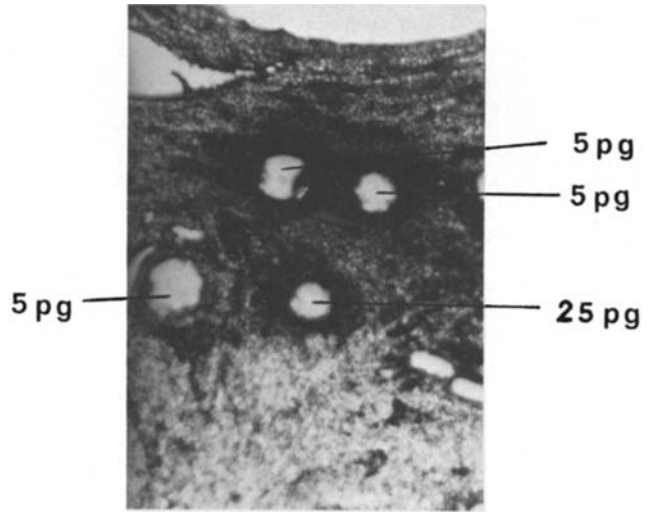

Fig. 3. Laser AAS-craters in tissues of BPH

Cd has been implicated in certain prostatic diseases and it competes with Zn histologically [1, 2]. Cd has been shown to affect prostatic growth by competition with Zn in the testes and in the prostate.

Chandler and Timms [1] found, using subcellular analysis of cells by x-ray micro-analysis, that in rats the highest Zn concentration is obtained in basal cell nucleoli in stromal elastin for cultures without Cd.

The degradation of stromal elements observed in Cd treated cultures may play an important part in the failure to maintain epithelial and basal cell growth even under the influence of androgen. They supposed a direct action of Cd on the prostate gland. The studies of Habib [3] using Cd showed that this metal had a similar distribution throughout the prostate gland to that of Zn.

In this connection it was interesting for us to compare our results in cellular fractions with the investigations of the Zn and Cd distribution within prostatic tissue slices by laser AAS. The tissue slices were prepared by the method of Sumi [11] to form stable coloured derivates between Cd and Zn respectively and Benzothiazolnaphtol. Thus it is possible to see areas with a high concentration of these elements with the laser microscope. The results obtained are shown in Table 3.

The results of laser Zn AAS determination in adenocarcinoma, poorly differentiated carcinoma and BPH in a slice of a thickness of 40 micrometers are compared in Table 3. For example in adenocarcinoma 47 laser craters with a diameter between 35 and 40 μm are evaporated by laser radiation one after another. The investigations were carried out without choice of special histological structures.

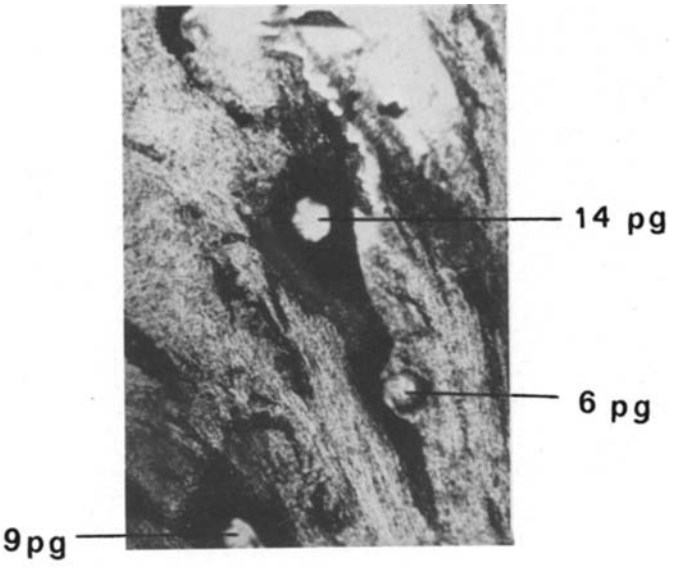

Fig. 4. Laser AAS-craters in tissues of adenocarcinoma

The mean values of Zn concentration in these tissues of different histology show no distinct differences. Nevertheless we have seen in slices of adenocarcinoma and BPH areas with distinct higher Zn concentration.

Examples of the changeable Zn distribution are shown in Figs. 3 and 4.

In Fig. 3 we see a tissue slice of BPH with three laser craters in the stroma with a concentration of 5 pg Zn. Only in the right in an area with proliferating cells do we see an abnormal concentration of 25 pg. In the slice of a well differentiated adenocarcinoma (Fig. 4) we measured a distinctly higher Zn concentration of 14 pg in the laser crater in the neighbourhood of glands.

In these preliminary studies it was not possible to confirm the results of Morita [6] in which the localisation of Zn in the secretory vacuoles of the prostate was reported. In our next investigations with laser AAS it will be possible to have more information for the selection of specially interesting areas of the prostatic tissue slices of different histological grading.

In agreement with the results obtained by de Voogt [13] in the investigation of prostatic slices using PIXE, we also only found Cd levels near the detection limit of our method. Therefore it is at present not possible to give specific information on the distribution of Cd in these slices.

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