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Zinc and Cadmium Analysis in Human Prostate Neoplasms

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ABSTRACT

The objective of this study was to test the hypothesis that prostatic cancer is associated with the changes of zinc (Zn) and cadmium (Cd) concentration. Normal prostate, benign prostatic hyperplasia (BPH), and prostatic carcinoma (PCA) were analyzed for Zn and Cd by atomic absorption spectrometry. Cd level was measured using a graphite furnace and Zn level was measured by flame mode. Metal content was assessed in whole tissues and in nuclear, plasma membrane, and cytosolic fractions. An increase of Zn content in BPH, but a decrease in PCA as compared to normal tissue, was observed. Cd concentration appeared to be higher in BPH and PCA than in normal tissue. No correlation between Zn and Cd level was found in BPH specimens obtained from the same patients. Probability values of $p \leq 0.05$ were considered to indicate significant differences. Obtained results seem to support the hypothesis of Cd carcinogenicity and preventing function of Zn in prostatic cancer. Plasma membrane fraction corresponding to lysosomal, mitochondrial, and microsomal subcellular compartments are probably critical in Zn and Cd participation in human prostate neoplasms.

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Index Entries: Zinc; cadmium; human; benign prostatic hyperplasia; prostatic carcinoma.

INTRODUCTION

A very common pathology in the aging human male is the abnormal growth of the prostate gland, as reflected in the incidence of benign prostatic hyperplasia (BPH) and prostatic carcinoma (PCA). Prostate cancer is now the second leading cause of cancer-related death in men. Despite the magnitude of morbidity and mortality associated with this disease, very little is known regarding the mechanisms involved in prostate tumorigenesis. A variety of growth factors, steroidal hormones, proteases, and other factors are involved in normal prostatic morphogenesis and function, but their role in BPH and PCA remains poorly understood *(1–5)*.

Occupational and environmental studies suggest a potential role of cadmium (Cd) in the prostate cancer etiology. Cd seems to be implicated in the increase of the incidence of prostate and other cancers in men exposed to high levels of this metal or its compounds *(6–8)*. Cd is also a known carcinogen for several tissues in animals, e.g., prostate tumors, sarcomas, and lung cancer *(9–11)*.

Zinc (Zn) is relatively nontoxic and plays a very important role in human metabolism. The largest concentration of Zn has been found in liver, kidney, retina, prostate, and muscle. Prostatic androgen metabolism is modified by the intracellular concentration of Zn. This trace element at high and low tissue concentration inhibits the transformation of testosterone to dihydrotestosterone *(12,13)*.

Studies on Cd and Zn reciprocal effects seem to suggest that Zn prevents the degenerative effect of Cd and that this process is a complex phenomenon *(11)*.

The aim of this work was to compare Zn and Cd concentrations in normal prostate tissue, as a control, and in two neoplastic tissues, BPH and PCA, with respect to total tissue and its subcellular fractions. This comparison may deliver successive proofs to support either the same or different BPH and PCA genesis. Moreover, analysis of Zn and Cd subcellular localization in neoplastic cells may be helpful in the evaluation of biological effect of these trace elements.

MATERIALS AND METHODS

Eleven normal prostate samples, 16 BPH, and 7 PCA (4 in stage C and 3 in stage D) were collected for this study. Tissue specimens were collected from nonsmoking patients undergoing open prostatectomy (PCA) or transurethal resection (BPH) of the prostate. The latter were obtained from the peripheral and transition zone of lateral lobes. Specimens obtained from patients after open prostatectomy were cut out of the peripheral zone

of the right lobe. Normal prostatic samples were collected during autopsies from the same area as PCA. The report on pathological evaluation was obtained for each sample. Tissues were kept in polyethylene tubes at –70°C until use. The samples were digested with concentrated nitric acid and then analyzed for Zn and Cd contents. The Zn concentration was measured by flame atomic absorption spectrometry (Varian Spectr AA 20, Melbourne, Australia), whereas Cd was determined by graphite furnace atomic absorption spectrometry (Perkin-Elmer Model 4800, Norwalk, CT). Concentration of Zn and Cd was determined in whole normal and in neoplastic prostate tissues, as well as in subcellular fractions.

Subcellular Fractionation

Cell homogenates in 0.25 *M* sucrose, 3 m*M* $CaCl_2$, 0.8 m*M* KH_2PO_4, pH 6.8, were centrifuged at 1000*g* for 10 min to collect nuclei, and the resulting supernatants were centrifuged again under the same conditions to remove any remaining nuclei. Supernatants after second centrifugation were spun down at 100,000*g* for 1 h to pellet plasma membranes representing crude lysosomal, mitochondrial, and microsomal fraction. The final supernatants corresponding to soluble part of the cell were called cytosol fraction. Nuclei were purified with additional treatment with 0.5% Triton X-100 to remove membrane ghosts. The nuclear pellet was finally purified by centrifugation at 40,000*g* through 2.2 *M* sucrose.

Protein was estimated using bovine serum albumin (BSA) as standard by means of the modified Lowry procedure *(14)*.

All chemicals used in experiments were of the highest purity and were purchased from Sigma (St. Louis, MO) and Merck (Darmstadt, Germany).

The statistical package program was used for data analysis. Means were compared using the Students *t*-test and results were expressed as mean ± SD. Probability value of $p < 0.05$ was considered to be significant.

RESULTS

As shown in Table 1, evident boundary was found between three analyzed tissues. Concentration of Zn (mg/g dry wt) was higher in BPH (0.28 ± 0.02) than in normal prostatic tissue (0.16 ± 0.02) and PCA (0.09 ± 0.12). A different situation was observed in the case of Cd concentration [μg/g dry wt]. The highest level was found in PCA (0.73 ± 0.12), compared to BPH (0.64 ± 0.21) and normal tissue (0.40 ± 0.10). However, difference between PCA and BPH appeared to be of no significance. Paired analysis of Zn and Cd measurement in normal and BPH tissues obtained from the same patients has been illustrated in Fig. 1. In all 11 analyzed specimens, Zn and Cd concentrations were higher in BPH than in normal tissue. This observation proves that results presented in Table 1 have not been the consequence of mathematical cal-

Table 1
Zinc and Cadmium Level in Whole Prostatic Tissues

Zn concentration $\bar{X}$ ± SD, mg/g dry wt		
Normal (n=11)	BPH (n=16)	PCA (n=7)
0.16 ± 0.02	0.28 ± 0.02	0.09 ± 0.12
Cd concentration $\bar{X}$ ± SD, μg/g dry tissue		
Normal (n=11)	BPH (n=16)	PCA (n=7)
0.40 ± 0.10	0.64 ± 0.21	0.73 ± 0.12

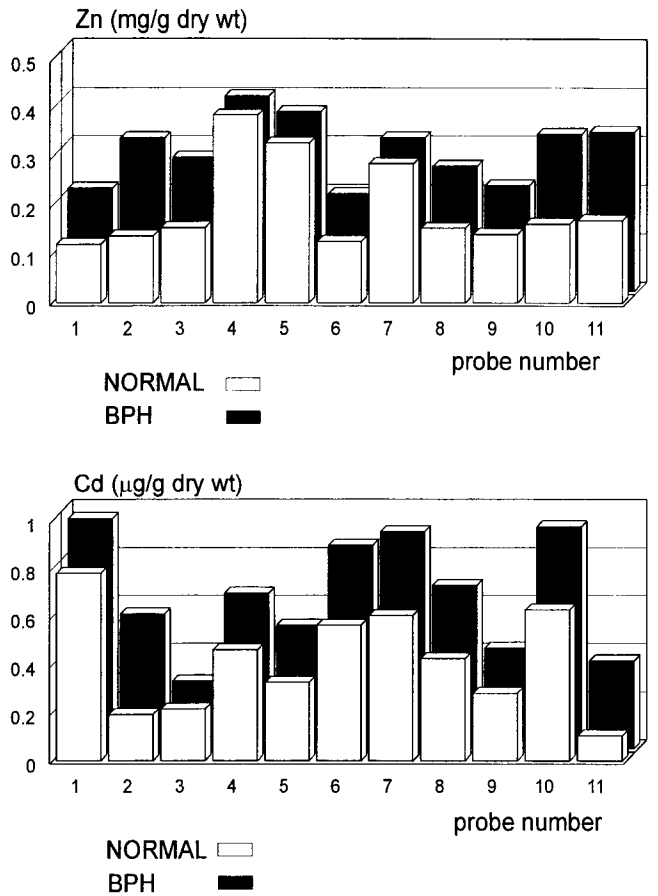

Fig. 1. Paired comparison of zinc and cadmium concentration in normal prostatic tissue and BPH.

Table 2
Zinc Level (μg/mg protein) in Subcellular Fractions of Prostatic Tissues ($\bar{X}$ ± SD)

Normal (n=11)	BPH (n=16)	PCA (n=7)
nuclear fraction		
2.8 ± 0.65	3.7 ± 0.45	2.7 ± 0.92
plasma membrane fraction		
3.5 ± 0.61	5.3 ± 0.39	0.45 ± 0.15
cytosol fraction		
1.3 ± 0.27	0.74 ± 0.11	0.43 ± 0.09

Table 3
Cadmium Level (μg/mg Protein) in Subcellular Fractions of Prostatic Tissues ($\bar{X}$ ± SD)

Normal (n=11)	BPH (n=16)	PCA (n=7)
nuclear fraction		
0.031 ± 0.003	0.042 ± 0.010	0.036 ± 0.009
plasma membrane fraction		
0.008 ± 0.002	0.030 ± 0.006	0.109 ± 0.013
cytosol fraction		
0.027 ± 0.010	0.016 ± 0.002	0.014 ± 0.004

culations. No correlation between Zn and Cd concentrations in all tissues, and the patients' age and PSA level, was observed.

Table 2 shows Zn content in different subcellular fractions of normal and neoplastic tissues. No significant differences in the case of nuclear fraction were found. In plasma membranes representing lysosomal, mitochondrial, and microsomal fractions, Zn level in normal tissue, BPH, and PCA was 3.5 ± 0.61; 5.3 ± 0.39; and 0.45 ± 0.15 μg/mg protein, respectively. In cytosol fraction, Zn content (μg/mg protein) decreased gradually from 1.3 ± 0.27 in normal tissue to 0.43 ± 0.09 in PCA. It is noteworthy that the highest differences in Zn concentration were observed in plasma membrane fraction.

Table 3 presents the Cd level analysis. Cd concentration in nuclear fraction did not vary significantly in normal, BPH, and PCA tissues. Similar to Zn, the highest changes in Cd concentration between normal,

BPH, and PCA specimens were observed in plasma membrane fraction. This fraction seems to be critical in respect of Zn and Cd participation in human prostate neoplasms. Generally, BPH was characterized by an elevated Zn level, whereas PCA by a diminished Zn level. On the other hand, both BPH and PCA were characterized by an increased concentration of Cd. The Cd level in membrane fraction of normal tissue BPH, and PCA was 0.008 ± 0.002; 0.030 ± 0.006; and 0.109 ± 0.013 μg/mg protein, respectively. In cytosol fraction, the Cd level dropped down in BPH and PCA when compared to normal tissue. The Cd content in normal tissue was 0.027 ± 0.010, whereas in BPH it was 0.016 ± 0.002, and in PCA, 0.014 ± 0.004. The difference between BPH and PCA was not found to be statistically significant.

DISCUSSION

The etiology and pathogenesis of BPH still present unresolved questions, and although a number of hypotheses have been developed, most still await experimental validation. BPH was regarded as

1. a kind of adenoma,
2. a stromal disease,
3. the result of either hormonal imbalance (altered estrogen/testosterone ratio), or
4. testosterone, dihydrotestosterone, or estrogen stimulation, either perinatal or presenescent. Prostate cancer appears to be androgen-dependent during the early stages of oncogenesis as initial stimulation of prostatic growth is mediated by androgens *(5)*.

Two different models can be postulated for the progression of normal prostatic epithelial cells to either BPH or PCA. The first model predicts that the early events for progression from either normal to BPH or normal to PCA are similar. The second model predicts that progression for BPH and PCA undergo different processes *(15)*.

There is considerable evidence to show that Zn has the ability to reduce the toxicity of Cd in rats. It is suggested that Zn protects against Cd-induced PCA. However, the mechanism of this process is not clear *(11)*.

Results presented in this report have indicated the probability of Cd-Zn interaction, but only in PCA. It is possible that Zn plays role in cancer prevention and also reduces Cd carcinogenic effect. In BPH, Cd-Zn interaction is questionable and there are no studies suggesting that these trace elements may be involved in this neoplasm. Obtained data seem to indicate that the progression from normal prostatic tissue to BPH or PCA is not the same process.

The absorption of Zn^{2+} occurs mainly in the small intestine and particularly in the jejunum. Zn^{2+} is absorbed at the level of the intestinal

epithelial cells, possibly in the form of complexes with amino acids, citrate, etc. Inside, it then combines with metalloenzymes, membrane proteins, and metallothionein. One of the main biochemical roles of Zn is its influence on the activity of over 300 enzymes. Zn can be essential for the structure, regulation, and/or catalytic activity of an enzyme. Zn^{2+} occurs in enzymes that realize the synthesis and metabolism of DNA and RNA, affects the metabolism and synthesis of proteins, and occurs in biologically active proteins such as growth factors. Zn stabilizes plasma and subcellular membranes as well as nucleic acids, microtubules, and lysosomes. It is necessary for various physiological functions including the growth and multiplication of cells.

Antagonistic effects of Zn and Cd in prostate carcinogenesis may be the result of chemical similarity in many respects of Zn and Cd. It seems to be possible that Zn and Cd can contribute to the same biological systems and thus alter biochemical and physiological effects. From the present data, no mechanism of the protective effect of Zn and the carcinogenic effect of Cd can be suggested. Our investigations, however, provide a basis for further research; it seems necessary to examine more closely plasma membrane fraction and proteins participating in the binding of Zn and Cd in prostate neoplasms.

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