INAA AND ICP-MS IN THE INVESTIGATION OF CADMIUM/TRACE ELEMENT CONTENT RATIOS IN MALIGNANT PROSTATE GLAND

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Introduction

Prostate cancer (PCa) is the most common non-cutaneous male cancer in most populations. Although the etiology of PCa is unknown, several risk factors including age, diet, and environment (Cd and some other trace element) have been well determined. It is also reported that the risk of having PCa drastically increase with age, being three orders of magnitude higher for the age group 40–79 years than for those younger than 39 years. Trace elements are involved in regulation of cell membrane and cell function, gene regulation, activation or inhibition of enzymatic reactions. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively. Excessive accumulation or an imbalance of the trace elements may disturb the cell functions and may result in cellular degeneration, death or, on the contrary, intensive uncontrolled proliferation, and malignancy.

Some trace elements have been highlighted in the literature in relation to the development of PCa.[1-20] However, questions on the role of trace elements in etiology and pathogenesis of PCa are far from being answered. First of all, it is necessary to establish the normal level and changes of trace element contents in PCa tissue, and relationships of trace elements in norm and disease. To that end, we determined the ratio to Cd of 42 trace element (Ag, Al, Au, B, Be, Bi, Br, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr) in normal and cancerous prostate gland.

All studies were approved by the Ethical Committee of the Medical Radiological Research Center, Obninsk.

Experimental

The 60 patients aged 40-79 years (M±SD 65±10) suffered from PCa (stage T1-T4) were hospitalized in the Urological Department of the Medical Radiological Research Centre (Obninsk, Russia). Transrectal puncture biopsy of suspicious indurated regions of the prostate was performed for every patient, to permit morphological study of prostatic tissue at these sites and to estimate their chemical element contents. In all cases the diagnosis has been confirmed by clinical and morphological results obtained during studies of biopsy and resected materials.

Intact prostates (N) were removed at necropsy from 37 men aged 41-87 who had died suddenly. Their mean age was 55±11 (M±SD) years. The majority of deaths were due to trauma. Tissue samples were collected from the peripheral zone of dorsal and lateral lobes of their prostates, within 2 days of death. A histological examination was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer.[6,7,12,20]

All tissue samples were divided into two portions. One was used for morphological study while the other was intended for trace element analysis. After the samples intended for trace element analysis were weighed, they were freeze-dried and homogenized. The sample weighing about 10 mg (for biopsy materials) and 50-100 mg (for resected materials) was used for trace element measurement by INAA-LLR. The samples for INAA-LLR were wrapped separately in a high-purity aluminum foil washed with double rectified alcohol beforehand and placed in a nitric acid-washed quartz ampoule.

After INAA-LLR investigation, the prostate samples were taken out and used for ICP-MS method. The samples were decomposed in autoclaves; 1.5 mL of concentrated HNO_3 (nitric acid at 65 %, maximum (max) of 0.0000005 % Hg; GR, ISO, Merck) and 0.3 mL of H_2O_2 (pure for analysis) were added to prostate tissue samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Russia) and then heated for 3 h at 160–200 °C. After autoclaving, they were cooled to room temperature and solutions from the decomposed samples were diluted with deionized water (up to 20 mL) and transferred to the plastic measuring bottles. Simultaneously, the same procedure was performed in autoclaves without tissue samples (only HNO_3+H_2O_2+ deionized water), and the resultant solutions were used as control samples.

A vertical channel of a nuclear reactor was applied to determine the trace element mass fractions by INAA-LLR. The quartz ampoule with prostate samples and certified reference materials was soldered, positioned in a transport aluminum container, and exposed to a 24-hour neutron irradiation in a vertical channel with a neutron flux of $1.3 \cdot 10^{13}$ $n \cdot cm^{-2} \cdot s^{-1}$. Ten days after irradiation samples were reweighed and repacked. The samples were measured for period from 10 to 30 days after irradiation. The duration of measurements was from 20 min to 10 hours subject to pulse counting rate. The gamma spectrometer used for INAA-LLR included the 100 cm^3 Ge(Li) detector and on-line computer-based multichannel analyzer. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line. Other details of the INAA-LLR analysis were presented in our previous publication.[7]

An ICP-MS Thermo-Fisher "X-7" Spectrometer (Thermo Electron, USA) was used to determine the content of trace elements by ICP-MS. The element concentrations in aqueous solutions were determined by the quantitative method using multi elemental calibration solutions ICP-MS-68A and ICP-AM-6-A produced by High-Purity Standards (Charleston, SC 29423, USA). Indium was used as an internal standard in all measurements. Information detailing with the ICP-MS method used was presented in our previous publication.[10]

For quality control, ten subsamples of the certified reference materials (CRM) IAEA H-4 Animal muscle and IAEA HH-1 Human hair from the International Atomic Energy Agency (IAEA), and also five sub-samples INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves and INCT-MPH-2Mixed Polish Herbs from the Institute of Nuclear Chemistry and Technology (INCT, Warszawa, Poland) were analyzed simultaneously with the investigated prostate tissue samples. All samples of CRMs were treated in the same way as the prostate samples. Detailed results of this quality assurance program were presented in earlier publications.[7,10]

A dedicated computer program for INAA mode optimization was used.[21] All prostate samples for INAA-LLR were prepared in duplicate and mean values of trace element contents were used in final calculation. For elements investigated by both INAA-LLR and ICP-MS methods the mean of all results was used. Using the Microsoft Office Excel software Cd/trace element contents for each trace element in every sample were calculated. Then arithmetic mean ± standard error of mean was calculated for trace element mass fraction and for ratios of Cd/trace element mass fraction in normal and cancerous prostate. The difference in the results between PCa and N was evaluated by parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test.

Results and discussion

As was shown by us[6,7,9,10], the use of CRM IAEA H-4 Animal muscle, IAEA HH-1 Human hair, INCT-SBF-4 Soya Bean Flour, INCT-TL-1 Tea Leaves, and INCT-MPH-2Mixed Polish Herbs as certified reference materials for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the trace element contents in these CRMs, measured by us using INAA-LLR and ICP-MS methods, with the certified data[6,7,9,10] indicates an acceptable accuracy of the results obtained in the present study.

Table 1 represents mean values ± standard error of mean (M±SEM) of the Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr mass fraction, as well as the ratio to Cd of Ag, Al, Au, B, Be, Bi, Br, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn, and Zr mass fraction in normal and cancerous prostate.

The mean values and standard error of mean (±SEM) were calculated for 43 trace element contents including Cd, as well as for 42 ratios of Cd/trace element mass fractions (Table 1). The mass fraction of Cd and other 42 trace elements were measured in all, or a major portion of normal prostate samples. The masses of PCa samples varied very strong from a few milligrams (sample from needle biopsy material) to 100 mg (sample from resected material). Therefore, in PCa prostates mass fraction ratios to Cd of other trace element content were determined in 11 samples.

The ratios of means and the difference between mean values of the Cd/trace element mass fraction ratios in normal and cancerous prostate are presented in Table 2.From Table 2, it is observed that in cancerous tissue the all Cd/trace element mass fraction ratios investigated in the study are significantly lower, than in normal prostate, with the exception of Cd/Nb, Cd/Sc, and Cd/Zn ratios.

Analysis of the mass fraction ratios for trace element in prostate tissue could become a powerful diagnostic tool[22-26]. To a large extent, the resumption of the search for new methods for early diagnosis of PCa was due to experience gained in a critical assessment of the limited capacity of the prostate specific antigen (PSA) serum test. In addition to the PSA serum test and morphological study of needle-biopsy cores of the prostate, the development of other highly precise testing methods seems to be very useful. Experimental conditions of the present study were approximated to the hospital conditions as closely as possible. In PCa cases we analyzed a part of the material obtained from a puncture transrectal biopsy of the indurated site in the prostate. Therefore, our data allow us to evaluate adequately the importance of Cd/trace element mass fraction ratios for the diagnosis of PCa.

Table 1: Mean values (M±SEM) of the trace element mass fraction (mg/kg, dry mass basis) and the Cd mass fraction/ trace element mass fraction ratios in normal (N), benign hypertrophic (BPH) and cancerous prostate (PCa)

Mass fraction			Ratio		
Symbol	N	PCa	Symbol	N	PCa
Ag	0.038±0.006	0.252±0.030	Cd/Ag	69.5±21.0	1.06±0.22
Al	34.2±3.5	328±73	Cd/Al	0.0437±0.0076	0.0013±0.0005
Au	0.0041±0.0008	0.0297±0.0056	Cd/Au	496±120	12.2±2.9
B	1.04±0.18	12.6±3,7	Cd/B	1.79±0.37	0.0309±0.0051
Be	0.00094±0.00007	0.0137±0.0022	Cd/Be	1384±188	28.8±5.9
Bi	0.029±0.011	1.75±0.27	Cd/Bi	285±87	0.153±0.030
Br	27.9±2.9	99.9±8.9	Cd/Br	0.0528±0.0086	0.00388±0.00085
Cd	1.12±0.13	0.425±0.099	Cd/Cd	1.00	1.00
Ce	0.0309±0.0050	0.101±0.013	Cd/Ce	65.4±16.9	4.02±1.01
Co	0.0467±0.0064	0.0336±0.0040	Cd/Co	31.4±4.5	9.83±1.90
Cr	0.56±0.08	2.34±0.32	Cd/Cr	5.91±2.78	0.138±0.037
Cs	0.0339±0.0033	0.0389±0.0039	Cd/Cs	43.8±7.2	14.9±5.1
Dy	0.00293±0.00049	0.00771±0.00110	Cd/Dy	724±162	74.2±24.6
Er	0.00148±0.00023	0.00297±0.00038	Cd/Er	1219±235	151±45
Fe	111±9	165±15	Cd/Fe	0.0106±0.0013	0.00344±0.00097
Gd	0.00290±0.00041	0.00945±0.00173	Cd/Gd	654±151	37.1±8.5
Hg	0.052±0.008	0.122±0.019	Cd/Hg	34.9±6.2	2.56±1.01
Ho	0.00057±0.00008	0.00178±0.00022	Cd/Ho	3100±584	196±35
La	0.080±0.020	0.969±0.537	Cd/La	55.8±11.9	1.30±0.50
Li	0.0419±0.0055	0.251±0.054	Cd/Li	39.1±7.4	1.62±0.53
Mn	1.34±0.08	6.99±1.35	Cd/Mn	0.967±0.139	0.108±0.034
Mo	0.282±0.038	0.298±0.035	Cd/Mo	5.69±1.05	2.10±1.15
Nb	0.0054±0.0012	0.0052±0.0002	Cd/Nb	475±109	148±92
Nd	0.0137±0.0021	0.0413±0.0065	Cd/Nd	146±38	10.0±2.4
Ni	3.10±0.51	6.96±1.04	Cd/Ni	0.810±0.213	0.0555±0.0128
Pb	2.39±0.56	1.81±0.35	Cd/Pb	1.98±0.68	0.209±0.034
Pr	0.00353±0.00053	0.00973±0.00174	Cd/Pr	565±134	34.7±7.6
Rb	13.3±0.9	8.71±0.66	Cd/Rb	0.101±0.014	0.0473±0.0085
Sb	0.043±0.006	0.490±0.059	Cd/Sb	58.2±12.8	1.62±0.33
Sc	0.0294±0.0053	0.0116±0.0015	Cd/Sc	62.3±16.9	40.2±17.1
Se	0.75±0.05	0.56±0.08	Cd/Se	1.63±0.23	0.601±0.112
Sm	0.0027±0.0004	0.0095±0.0029	Cd/Sm	773±182	95.9±30.1
Sn	0.32±0.06	1.28±0.24	Cd/Sn	9.45±2.14	0.430±0.155
Tb	0.00039±0.00006	0.00089±0.00012	Cd/Tb	6314±1729	361±84
Th	0.0033±0.0007	0.0495±0.0123	Cd/Th	727±145	9.41±3.81
Ti*	2.82±0.64	8.60±2.20	Cd/Ti*	0.848±0.184	0.0938±0.0438
Tl	0.0014±0.0001	0.0219±0.0056	Cd/Tl	1134±195	13.2±2.2
Tm	0.00024±0.00003	0.00054±0.00011	Cd/Tm	6818±1129	439±80
U	0.0070±0.0021	0.0068±0.0013	Cd/U	659±150	81.5±20.0
Y	0.0187±0.0043	0.0340±0.0038	Cd/Y	340±204	15.5±4.9
Yb	0.00141±0.00025	0.00174±0.00039	Cd/Yb	1787±537	314±113
Zn	1031±129	136±10	Cd/Zn	0.00200±0.00045	0.00295±0.00034
Zr	0.036±0.006	2.13±0.89	Cd/Zr	55.1±10.7	0.454±0.133

M – arithmetic mean, SEM – standard error of mean, * Titanium tools were used for sampling and sample preparation.

Table 2: Ratio of means and the difference between mean values of the Cd mass fraction/ trace element mass fraction ratios in normal (N) and cancerous prostate (PCa)

Symbol	Ratio PCa/N	$p\leq$, t-test	p, U-test
Cd/Ag	0.015	**0.0034**	**≤0.01**
Cd/Al	0.029	**0.00001**	**≤0.01**
Cd/Au	0.025	**0.00069**	**≤0.01**
Cd/B	0.017	**0.00009**	**≤0.01**
Cd/Be	0.021	**0.00001**	**≤0.01**
Cd/Bi	0.001	**0.0032**	**≤0.01**
Cd/Br	0.073	**0.00002**	**≤0.01**
Cd/Ce	0.061	**0.0014**	**≤0.01**
Cd/Co	0.313	**0.00014**	**≤0.01**
Cd/Cr	0.023	0.052	**≤0.01**
Cd/Cs	0.340	**0.0024**	**≤0.01**
Cd/Dy	0.102	**0.00059**	**≤0.01**
Cd/Er	0.124	**0.00016**	**≤0.01**
Cd/Fe	0.325	**0.00011**	**≤0.01**
Cd/Gd	0.057	**0.00046**	**≤0.01**
Cd/Hg	0.073	**0.00003**	**≤0.01**
Cd/Ho	0.063	**0.00005**	**≤0.01**
Cd/La	0.023	**0.00011**	**≤0.01**
Cd/Li	0.041	**0.00006**	**≤0.01**
Cd/Mn	0.112	**0.00001**	**≤0.01**
Cd/Mo	0.369	**0.039**	>0.05
Cd/Nb	0.312	0.067	>0.05
Cd/Nd	0.068	**0.0017**	**≤0.01**
Cd/Ni	0.069	**0.0020**	**≤0.01**
Cd/Pb	0.106	**0.016**	**≤0.01**
Cd/Pr	0.061	**0.00065**	**≤0.01**
Cd/Rb	0.468	**0.0030**	**≤0.01**
Cd/Sb	0.028	**0.00022**	**≤0.01**
Cd/Sc	0.645	0.391	>0.05
Cd/Se	0.369	**0.00044**	**≤0.01**
Cd/Sm	0.124	**0.0013**	**≤0.01**
Cd/Sn	0.046	**0.00036**	**≤0.01**
Cd/Tb	0.057	**0.0023**	**≤0.01**
Cd/Th	0.013	**0.00001**	**≤0.01**
Cd/Ti*	0.111	**0.00058**	**≤0.01**
Cd/Tl	0.012	**0.00001**	**≤0.01**
Cd/Tm	0.064	**0.00002**	**≤0.05**
Cd/U	0.124	**0.00089**	**≤0.01**
Cd/Y	0.046	0.126	**≤0.05**
Cd/Yb	0.176	**0.013**	**≤0.05**
Cd/Zn	1.48	0.104	>0.05
Cd/Zr	0.008	**0.00004**	**≤0.01**

t-test - Student's *t*-test, U-test - Wilcoxon-Mann-Whitney *U*-test, ***Bold*** significant differences

Conclusions

The combination of nondestructive INAA-LLR and destructive ICP-MS methods is satisfactory analytical tool for the precise determination of 43 trace element mass fractions in the tissue samples of normal and carcinomatous prostate glands. The sequential application of these methods allowed precise quantitative determinations of mean mass fraction of Ag, Al, Au, B, Be, Bi, Br, Cd, Ce, Co, Cr, Cs, Dy, Er, Fe, Gd, Hg, Ho, La, Li, Mn, Mo, Nb, Nd, Ni, Pb, Pr, Rb, Sb, Sc, Se, Sm, Sn, Tb, Th, Ti, Tl, Tm, U, Y, Yb, Zn and Zr. It was observed that the ratio to Cd of Ag, Al, Au, B, Be, Bi, Br, Ce, Cr, Cs, Dy, Er, Gd, Ho, La, Li, Mn, Nd, Ni, Pb, Pr, Sb, Sm, Sn, Tb, Th, Ti, Tl, U, Y, Yb, and Zr mass fraction were significantly lower in cancerous tissues than in normal prostate. Further studies on larger number of samples are required to confirm our findings and to investigate the impact of the trace element relationships on prostate cancer etiology.

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