NEUTRON ACTIVATION ANALYSIS OF Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, AND Zn CONTENTS IN BENIGN PROSTATIC HYPERTROPHIC TISSUE

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Introduction

Benign prostatic hyperplasia (BPH) assigns most of men after the age of fifty and represents the most common urologic disease among elderly males.[1] BPH is histologically defined as an overgrowth of the epithelial and stromal cells from the transition zone and peri-urethral area of prostate.[2] The excessive cell proliferation associated with BPH causes benign prostatic enlargement, bladder outlet obstruction, and lower urinary tract symptoms, which afflict the patients.[1] Incidence of histological BPH could be over 70% at 60 years old and over 90% at 70 years old.[3] To date, we still have no precise knowledge of the biochemical, cellular and molecular processes underlying the pathogenesis of BPH. Although the influence of androgens and estrogens has been demonstrated, hormonal factors alone may not fully explain BPH development.[4]

Trace elements have essential physiological functions such as maintenance and regulation of cell function, gene regulation, activation or inhibition of enzymatic reactions, and regulation of membrane function. Essential or toxic (mutagenic, carcinogenic) properties of trace elements depend on tissue-specific need or tolerance, respectively.[5] 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 proliferation. [6]

In our previous study a significant positive correlation between age and Zn mass fraction in the prostate was observed.[7] Moreover, it was shown that the levels of Zn and some other trace elements in prostate tissue are the androgen-dependent parameters.[8-13] The contents of these trace elements in prostate tissue jump up after puberty and continue to increase during the lifespan especially after the fourth decade.[7, 14-18] Hence it is possible that besides Zn, some other trace elements also play a role in the pathophysiology of the prostate.

This work had four aims. The first aim was to assess the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions in intact prostate of healthy men aged over 40 years (control group) and BPH glands using instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). The second aim was to evaluate the quality of obtained results. The third aim was to compare the levels of trace elements in the BPH prostates and in normal glands of age-matched patients from the control group. The final aim was to estimate the intercorrelations of trace element mass fractions in normal and BPH glands.

All studies were approved by the Ethical Committees of the Medical Radiological Research Centre, Obninsk.

Experimental part

All patients suffered from BPH (n=43, mean age M±SD was 66±8 years, range 38-83) were hospitalized in the Urological Department of the Medical Radiological Research Centre. 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 trace 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 were removed at necropsy from 37 men (mean age 55±11 years, range 41-87) who had died suddenly. The majority of deaths were due to trauma. A histological examination in this group was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer.[19] 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 pounded sample weighing about 50 mg was used for trace element measurement by INAA-LLR.

To determine contents of the trace elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used.[20] In addition to SSB, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material (CRM) IAEA H-4 (animal muscle) sub-samples weighing about 50 mg were treated and analyzed in the same way as prostate samples to estimate the precision and accuracy of results.

Details of the relevant facility for INAA-LLR and the results of quality control were presented in our earlier publications concerning the instrumental neutron activation analysis of human prostate tissue of health subjects.[15]

A dedicated computer program for INAA mode optimization was used.[21] All prostate samples were prepared in duplicate, and mean values of trace element mass fractions were used in final calculation. Using the Microsoft Office Excel programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels was calculated for trace element mass fractions in normal and BPH prostate tissue. The same programs were used to estimate the Pearson's correlation coefficient between the different trace elements. The reliability of difference in the results between the normal and hypertrophic prostate tissues was evaluated by Student's *t*-test.

Results and discussion

As was shown by us,[16,20] the use of CRM IAEA H-4 as a CRM for the analysis of samples of prostate tissue can be seen as quite acceptable. Good agreement of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions analyzed by INAA-LLR with the certified data of CRM IAEA H-4 indicates an acceptable accuracy of the results obtained in the study of chemical elements of the prostate presented in Tables 1–4.

Table 1 presents basic statistical parameters of the Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in normal and BPH prostate tissue. The mass fraction of these elements were measured in all, or a major portion of normal and BPH samples.

Table 1. Basic statistical parameters of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal and BPH prostate tissue

Tissue	Element	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
BPH	Ag	0.036	0.029	0.007	0.00709	0.132	0.0288	0.0118	0.104
n=43	Co	0.075	0.044	0.011	0.0246	0.170	0.0591	0.0286	0.163
	Cr	1.59	1.99	0.48	0.046	9.01	1.08	0.300	6.31
	Fe	141	42	10	76.0	247	137	79.1	229
	Hg	0.231	0.151	0.035	0.0527	0.520	0.179	0.0568	0.518
	Rb	15.6	4.6	1.1	7.50	23.1	14.9	7.59	22.9
	Sb	0.262	0.282	0.067	0.0131	1.04	0.168	0.0395	1.01
	Sc	0.0257	0.0156	0.0040	0.00390	0.0543	0.0213	0.00509	0.0536
	Se	1.363	0.282	0.065	0.614	1.77	1.42	0.740	1.73
	Zn	1207	648	149	241	2325	1260	294	2232
Normal	Ag	0.048	0.046	0.009	0.010	0.223	0.0344	0.010	0.156
n=37	Co	0.045	0.024	0.004	0.0165	0.106	0.040	0.0169	0.0988
	Cr	0.532	0.422	0.081	0.0300	1.81	0.392	0.0300	1.54
	Fe	111	51	9	35.0	267	100	35.8	221
	Hg	0.056	0.057	0.011	0.0077	0.242	0.0320	0.0114	0.216
	Rb	12.7	5.0	0.9	4.70	25.3	12.0	5.69	24.6
	Sb	0.0445	0.0374	0.0067	0.00460	0.158	0.0380	0.00865	0.154
	Sc	0.0294	0.0236	0.0053	0.00460	0.0771	0.0200	0.00660	0.0768
	Se	0.696	0.240	0.044	0.318	1.43	0.691	0.346	1.15
	Zn	1002	664	109	231	3513	915	252	2185

M arithmetic mean, *SD* standard deviation, *SEM* standard error of mean, *Min* minimum value, *Max* maximum value, *Per.0.025* percentile with 0.025 level, *Per.0.975* percentile with 0.975 level

The ratios of means and the reliability of difference between mean values of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions in normal and BPH prostate tissue are presented in Table 2.

From Table 2, it is observed that in BPH tissue the Co, Cr, Fe, Hg, Rb, Sb, and Se mass fraction ratio is significantly higher than in normal tissue.

Table 3 contains results of inter-element correlation calculations (values of r – coefficient of correlation) including all trace elements identified in this work.

In control group of males a statistically significant ($p \leq 0.01$) direct correlation was found, for example, between the prostatic Zn and Se ($r = 0.44$), Zn and Sc ($r = 0.67$), and Zn and Hg ($r = 0.51$), between the prostatic Se and Fe ($r = 0.60$), Se and Hg ($r = 0.51$), Se and Rb ($r = 0.52$), and Se and Sc ($r = 0.70$), between the prostatic Fe and Co ($r = 0.59$), and so on. If some positive correlations between the trace elements were predictable (e.g., Fe–Co), the interpretation of other observed relationships requires further study for a more complete understanding. In hyperplastic prostates many significant correlations between trace elements found in the control group are no longer evident, for example, correlations for some pairs with Se, including pair Zn and Se, etc. (Table 3). Thus, if we accept the levels and relationships of trace element mass fraction in prostate glands of males in the control group as a norm, we have to conclude that with a hyperplastic transformation the levels and relationships of trace elements in prostate significantly changed.

Table 2. Comparison of mean values (M±SEM) of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction (mg/kg, dry mass basis) in normal and BPH prostate tissue

Element	Prostatic tissue		Ratio	
	Normal (n=37)	BPH (n=43)	BPH to Normal	Student's t-test
Ag	0.048±0.009	0.036±0.007	0.75	NS
Co	0.045±0.004	0.075±0.011	1.67	$p \leq 0.05$
Cr	0.53±0.08	1.59±0.48	3.00	$p \leq 0.05$
Fe	111±9	141±10	1.27	$p \leq 0.05$
Hg	0.056±0.011	0.231±0.035	4.13	$p \leq 0.001$
Rb	12.7±0.9	15.6±1.1	1.23	$p \leq 0.05$
Sb	0.045±0.007	0.262±0.067	5.82	$p \leq 0.01$
Sc	0.0294±0.0053	0.0257±0.0050	0.87	NS
Se	0.696±0.044	1.363±0.065	1.96	$p \leq 0.001$
Zn	1002±109	1207±149	1.20	NS

M arithmetic mean, *SEM* standard error of mean, *NS* not significant difference

Table 3 Intercorrelations of pairs of the trace element mass fractions in BPH and normal prostate glands of adults (*r* – coefficient of correlation)

Tissue	Element	Co	Cr	Fe	Hg	Rb	Sb	Sc	Se	Zn
BPH	Ag	0.40	-0.22	-0.13	**0.55**	-0.13	-0.22	**0.64**	-0.47	**0.49**
n=43	Co	-	-0.11	0.25	0.21	0.21	0.33	**0.48**	0.31	**0.60**
	Cr	-0.11	-	**0.69**	0.37	**-0.45**	-0.05	0.03	-0.03	-0.16
	Fe	0.25	**0.69**	-	0.12	-0.28	0.26	0.16	0.10	-0.13
	Hg	0.21	0.37	0.12	-	-0.06	-0.02	**0.71**	-0.24	**0.52**
	Rb	0.21	**-0.45**	-0.28	-0.06	-	0.19	0.14	0.31	0.37
	Sb	0.33	-0.05	0.26	-0.02	0.19	-	0.18	0.21	0.07
	Sc	**0.48**	0.03	0.16	**0.71**	0.14	0.18	-	-0.30	**0.76**
	Se	0.31	-0.03	0.10	-0.24	0.31	0.21	-0.30	-	0.18
	Zn	**0.60**	-0.16	-0.13	**0.52**	0.37	0.07	**0.76**	0.18	-
Normal	Ag	-0.15	-0.24	-0.26	-0.06	-0.18	0.28	-0.02	-0.29	0.12
n=37	Co	-	0.26	**0.59**	0.21	**0.56**	0.30	**0.57**	0.29	0.21
	Cr	0.26	-	0.40	**0.49**	0.44	0.18	0.11	0.26	0.08
	Fe	**0.59**	0.40	-	**0.51**	**0.66**	0.26	**0.70**	**0.60**	0.23
	Hg	0.21	**0.49**	**0.51**	-	0.42	0.01	**0.62**	**0.51**	**0.51**
	Rb	**0.56**	0.44	**0.66**	0.42	-	0.39	**0.59**	**0.52**	0.28
	Sb	0.30	0.18	0.26	0.01	0.39	-	-0.02	-0.03	-0.07
	Sc	**0.57**	0.11	**0.70**	**0.62**	**0.59**	-0.02	-	**0.70**	**0.67**
	Se	0.29	0.26	**0.60**	**0.51**	**0.52**	-0.03	**0.70**	-	**0.44**
	Zn	0.21	0.08	0.23	**0.51**	0.28	-0.07	**0.67**	**0.44**	-

Statistically significant values with $p \leq 0.01$ are in **bold**.

The comparison of our results with published data for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in normal and BPH prostate tissue is shown in Table 4.

Table 4. Median, minimum and maximum value of means of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction in normal and BPH prostate tissue according to data from the literature in comparison with our results (mg/kg, dry mass basis)

Tissue	El	Published data [Reference]			This work result
		Median of means (n)*	Minimum of means M or M±SD (n)**	Maximum of means M or M±SD (n)**	M±SD
BPH	Ag	0.035 (1)	0.035±0.027 (43)[22]	0.035±0.027 (43)[22]	0.036±0.029
	Co	8.5 (2)	0.072±0.042 (43)[22]	19.0±1.5 (43)[23]	0.075±0.044
	Cr	6.5 (3)	1.07±0.53 (43)[22]	191±17 (27) [24]	1.59±1.99
	Fe	150 (13)	5.9±0.4 (8) [25]	1345±95 (27) [24]	141±42
	Hg	0.23 (1)	0.23±0.014 (43)[22]	0.23±0.014 (43)[22]	0.23±0.15
	Rb	14.8 (1)	14.8±4.6 (43)[22]	14.8±4.6 (43)[22]	15.6±4.6
	Sb	0.163 (1)	0.163±0.113 (43)[22]	0.163±0.113 (43)[22]	0.262±0.282
	Sc	0.026 (1)	0.026±0.016 (43)[22]	0.026±0.016 (43)[22]	0.026±0.016
	Se	0.99 (11)	0.76±0.37 (10)[26]	11.5±6.0 (27)[24]	1.36±0.28
	Zn	725 (40)	55±25 (23)[27]	3800±65 (10)[28]	1207±648
Norm	Ag	0.055 (9)	0.028±0.019 (10)[29]	0.24 (7)[30]	0.048±0.046
	Co	0.036 (10)	0.022±0.010 (16)[10]	12 (9)[31]	0.045±0.024
	Cr	0.51 (13)	0.042 (50)[32]	29.4±5.9 (5)[33]	0.53±0.42
	Fe	118 (30)	5.7±0.1 (5)[25]	1224±76 (10)[34]	111±51
	Hg	0.037 (8)	0.024±0.014 (16)[10]	0.65±0.58 (5)[35]	0.056±0.057
	Rb	14.2 (13)	4.7 (9)[31]	68.2±38.8 (4)[36]	12.7±5.0
	Sb	0.046 (8)	0.039±0.026 (10)[29]	0.42±0.56 (7)[35]	0.045±0.037
	Sc	0.014 (6)	0.009±0.005 (16)[10]	0.031±0.025 (7)[37]	0.029±0.024
	Se	0.73 (20)	0.32 (129)[38]	18.8±2.4 (27)[24]	0.70±0.24
	Zn	525 (71)	111 (-)[39]	3218±41 (10)[34]	1002±664

El Element, *M* arithmetic mean, *SD* standard deviation, *(n)** number of all references, *(n)*** number of samples

Values obtained for Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fractions (Table 4) agree well with median of mean values reported for the normal and BPH human prostate.[16, 22-39] This data also includes samples obtained from patients who died from different diseases. A number of values for trace element mass fractions were not expressed on a dry weight basis in the cited literature. Therefore, we calculated these values using published data for water (83%) [40] and ash (1% on wet mass basis)[41] contents in the prostate of adult men.

BPH transformation is accompanied by high level of Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn. Therefore, it is plausible that the reason for the emergence and development of BPH is associated with abnormally high content of these trace elements in the prostate tissue of older men.

Conclusions

In this work, elemental analysis was carried out in the tissue samples of normal and BPH prostates using INAA-LLR. It was shown that INAA-LLR is an adequate analytical tool for the non-destructive determination of Ag, Co, Cr, Fe, Hg, Rb, Sb, Sc, Se, and Zn mass fraction

in the tissue samples of human prostate, including needle-biopsy cores. It was found that BPH transformation is accompanied by high level of Co, Cr, Fe, Hg, Rb, Sb, Se, and Zn.

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