NEUTRON ACTIVATION ANALYSIS OF Br, Ca, K, Mg, Mn, and Na 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.[1-3] BPH is histologically defined as an overgrowth of the epithelial and stromal cells from the transition zone and peri-urethral area of prostate.[4,5] The excessive cell proliferation associated with BPH causes benign prostatic enlargement, bladder outlet obstruction, and lower urinary tract symptoms, which afflict the patients.[3] Incidence of histological BPH could be over 70% at 60 years old and over 90% at 70 years old.[1,6] 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.[7,8]

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.[9] 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. [10,11]

In our previous study a significant positive correlation between age and Ca mass fraction in the prostate was observed.[12,13] High intraprostatic Ca concentrations are probably one of the main factors acting in prostate cell proliferation.[12,13] Moreover, a significant positive correlation was seen between the prostatic Ca and Na contents.[13] Hence it is possible that besides Ca, some other elements also play a role in the pathophysiology of the prostate.

This work had four aims. The first aim was to assess the Br, Ca, K, Mg, Mn, and Na mass fractions in intact prostate of healthy men aged over 40 years using instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR). The second aim was to evaluate the quality of obtained results. The third aim was to compare the levels of chemical elements in the prostate gland of age-matched patients, who had BPH. The final aim was to estimate the intercorrelations of chemical element mass fractions in normal and BPH glands.

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

Experimental

All patients suffered from BPH (n=32, mean age M±SD was 67±6 years, range 56-78) 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 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 were removed at necropsy from 37 men (mean age 55±11 years, range 41-79) who had died suddenly. The majority of deaths were due to trauma. A histological examination in the control group was used to control the age norm conformity, as well as to confirm the absence of microadenomatosis and latent cancer.[14] Tissue samples were divided into two portions. One was used for morphological study while the other was intended for chemical element analysis. After the samples intended for chemical element analysis were weighed, they were freeze-dried and homogenized. The pounded sample weighing about 100 mg was used for chemical element measurement by INAA-SLR.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used.[15] 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 100 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-SLR and the results of quality control were presented in our earlier publications concerning the instrumental neutron activation analysis of human prostate tissue.[12,13]

All prostate samples were prepared in duplicate, and mean values of chemical 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 chemical element mass fractions in normal and BPH prostate tissue. The same programs were used to estimate the Pearson's correlation coefficient between the different chemical 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

Table 1 depicts our data for six chemical elements in ten sub- samples of CRM IAEA H-4 (animal muscle) and the certified values of this material. Good agreement with the certified data of certified reference materials indicate an acceptable accuracy of the results obtained in the study. As was shown by us,[12,15] 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 Br, Ca, K, Mg, Mn, and Na mass fractions analyzed by INAA-SLR with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of chemical elements of the prostate presented in Tables 2–5.

Table 1 INAA-SLR data of chemical element contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg, dry mass basis)

Element	Certified values			This work results
	Mean	95% confidence interval	Type	Mean±SD
Br	4.1	3.5 – 4.7	C	5.0±0.9
Ca	188	163 – 213	C	238±59
K	15800	15300 – 16400	C	16200±3800
Mg	1050	990 – 1110	C	1100±190
Mn	0.52	0.48 – 0.55	N	0.55±0.11
Na	2060	1930 – 2180	C	2190±140

Mean arithmetical mean, *SD* standard deviation, *C*, *N* certified or non-certified values

Table 2 presents basic statistical parameters of the Br, Ca, K, Mg, Mn, and Na mass fraction in normal and BPH prostate tissue. The mass fraction of Br, Ca, K, Mg, Mn, and Na were measured in all, or a major portion of normal and PCa samples.

Table 2. Basic statistical parameters of Br, Ca, K, Mg, Mn, and Na 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
Normal (n=37)	Br	32.9	17.7	3.6	12.5	80.7	28.2	12.6	70.9
	Ca	2280	874	178	1205	4908	2082	1340	4386
	K	11211	2071	414	7100	14328	11399	7100	13998
	Mg	1118	396	76	604	2060	1062	626	1963
	Mn	1.24	0.32	0.07	0.400	1.80	1.30	0.650	1.75
	Na	11100	2159	408	6834	15300	11071	6879	15161
BPH (n=32)	Br	30.4	18.4	3.6	5.50	77.0	25.6	5.75	66.7
	Ca	2030	550	165	1170	2760	1900	1170	2760
	K	14470	2450	740	11680	20520	13550	12030	19740
	Mg	1200	280	80	690	1590	1260	750	1550
	Mn	1.19	0.31	0.09	0.800	1.80	1.20	0.800	1.73
	Na	11610	2880	870	7760	15503	10560	7890	15400

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 Br, Ca, K, Mg, Mn, and Na mass fractions in normal and BPH prostate tissue are presented in Table 3.

Table 3. Comparison of mean values (M±SEM) of Br, Ca, K, Mg, Mn, and Na mass fraction (mg/kg, dry mass basis) in normal and BPH prostate tissue

Element	Prostatic tissue		Ratio	
	Normal 41-87 year n=37	BPH 56-78 year n=32	BPH to Normal	Student's t-test
Br	32.9±3.6	30.4±3.6	0.92	NS
Ca	2280±178	2030±165	0.89	NS
K	11211±414	14470±740	1.29	$p \leq 0.01$
Mg	1118±76	1200±80	1.07	NS
Mn	1.24±0.07	1.19±0.09	0.96	NS
Na	11100±408	11610±870	1.05	NS

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

From Table 3, it is observed that in BPH tissue the K mass fraction ratio is significantly ($p<0.01$) higher than in normal tissue.

Table 4 contains results of inter-element correlation calculations (values of r – coefficient of correlation) including all chemical elements identified in this work.

Table 4. Coefficient of correlation between Br, Ca, K, Mg, Mn, and Na mass fractions in normal and BPH prostate tissues

Prostate tissue	Element	Br	Ca	K	Mg	Mn	Na
Normal	Br	**1.00**	0.248	-0.342[a]	0.169	-0.105	-0.074
	Ca	0.248	**1.00**	0.263	0.216	-0.367[a]	-0.127
	K	-0.342[a]	0.263	**1.00**	-0.364[a]	-0.171	-0.126
	Mg	0.169	0.216	-0.364[a]	**1.00**	-0.064	0.417[b]
	Mn	-0.105	-0.367[a]	-0.171	-0.064	**1.00**	0.392[a]
	Na	-0.074	-0.127	-0.126	0.417[b]	0.392[a]	**1.00**
BPH	Br	**1.00**	-0.350[a]	-0.157	-0.282	0.294	0.050
	Ca	-0.350[a]	**1.00**	0.013	0.569[b]	0.096	-0.251
	K	-0.157	0.013	**1.00**	0.552[b]	0.058	0.255
	Mg	-0.282	0.569[b]	0.552[b]	**1.00**	-0.172	0.378[a]
	Mn	0.294	0.096	0.058	-0.172	**1.00**	-0.213
	Na	0.050	-0.251	0.255	0.378[a]	-0.213	**1.00**

Statistically significant: [a] - $p \leq 0.05$, [b] - $p \leq 0.01$.

The negative inter-element correlation of Ca mass fraction with Mn mass fraction ($p<0.05$) was only found in healthy prostate of men in their 40's, which indicate a possible antagonism between these elements under the normal function of the gland. Inter-element correlations between chemical elements are significantly altered in BPH tissue as compared to their relationships in normal prostate tissue. For example, Ca mass fractions have positive correlation with Mg ($p<0.01$) and negative correlation with Br ($p<0.05$) in BPH prostate tissue.

The comparison of our results with published data for Br, Ca, K, Mg, Mn, and Na mass fraction in normal and BPH prostate tissue is shown in Table 5.

Table 5. Median, minimum and maximum value of means of Br, Ca, K, Mg, Mn, and Na mass fraction in normal and cancerous prostate tissue according to data from the literature in comparison with our results (mg/kg, dry mass basis)

Tissue	Element	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(n)**
Norm	Br	27.0 (4)	14±9 (4)[16]	35.5±30.2 (12)[17]	32.9±17.7
	Ca	1800 (17)	427±117 (21)[18]	7500±12300 (57)[19]	2280±874
	K	11600 (15)	4360±70 (27)[20]	13000±660 (16)[21]	11211±2071
	Mg	1029 (16)	498±172 (13)[19]	2056±476 (21)[18]	1118±396
	Mn	1.52 (15)	<0.47 (12)[22]	106±18 (5)[23]	1.24±0.32
	Na	9250 (11)	23±26 (13)[19]	11700±3000 (4)[24]	11100±2159
BPH	Br	23.3 (2)	21.2±11.2 (27)[20]	25.3±15.3 (9)[25]	30.4±18.4
	Ca	3600 (6)	1000 (34)[26]	5900±3800 (9)[25]	2030±550
	K	8700 (5)	1190±110 (27)[20]	13000±660 (16)[21]	14470±2450
	Mg	965 (7)	566±130 (25)[27]	1560±50 (10)[28]	1200±280
	Mn	11 (7)	10.6±1.2 (2)[29]	27.0±15.3 (27)[20]	1.19±0.31
	Na	7800 (1)	7800 (34)[26]	7800 (34)[26]	11610±2880

M arithmetic mean, *SD* standard deviation, *(n)** number of all references, *(n)*** number of samples, *Zn*[a] zinc mass fraction in peripheral zone of lateral and dorsal lobes.

The results for all chemical element contents in the prostates of the control group (mean age 55±11 years, range 41-79) are in accordance with our earlier findings in prostates of apparently healthy men aged 41-60.[12] Values obtained for Br, Ca, K, Mg, Mn, and Na mass fractions (Table 5) agree well with median of mean values cited by other researches for the human prostate.[16-29] This data also includes samples obtained from patients who died from different diseases. A number of values for chemical 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%) [30] and ash (1% on wet mass basis)[31] contents in the prostate of adult men.

In normal prostatic tissues our results were comparable with published data (Table 5). The means of Br, Ca, K, Mg, and Na mass fractions obtained for BPH tissue, as shown in Table 5, agree well with published data also, but the mean of Mn is one order of magnitude lower. No published data referring to inter correlations of these element mass fractions in normal and BPH tissues of the human prostate gland were found.

Compared to other soft tissues, the human prostate has higher levels of Ca and some other chemical elements.[32,33] These data suggests that these elements could be involved in functional features of BPH tissue. BPH transformation is accompanied by high level of Ca. Therefore, it is plausible that the reason for the emergence and development of BPH is associated with abnormally high concentration of Ca and some other chemical 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-SLR. It was shown that INAA-SLR is an adequate analytical tool for the non-destructive determination of Br, Ca, K, Mg, Mn, and Na mass fraction in the tissue samples of human prostate, including needle-biopsy cores. The K mass fraction was significantly higher in BPH tissues than in normal tissues. It is well known that K is mainly intracellular electrolyte. Thus, the abnormal high level of K in prostate tissue could be a consequence of BPH transformation.

Acknowledgments

We are grateful to Dr Tatyana Sviridova, Medical Radiological Research Center, Obninsk, and Prof. A.A. Zhavoronkov, Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow, for supplying prostate samples.

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