EDXRF DETERMINATION OF TRACE ELEMENT CONTENTS IN BENIGN PROSTATIC HYPERTROPHIC TISSUE

S. Zaichick[1,2], V. Zaichick[1]

[1] *Medical Radiological Research Centre of the Russian Ministry of Health, Koroleva str., 4, Obninsk, 249020, Russia, e-mail: vezai@obninsk.com*

[2] *Current address: Northwestern University, Chicago, IL, 60611, USA*

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 Zn mass fraction in the prostate was observed.[12,13] High intraprostatic zinc concentrations are probably one of the main factors acting in prostate cell proliferation.[12,13] A strongly pronounced tendency of age-related exponential increase in Zn mass fraction as well an increase in Zn/Fe, Zn/Rb, and Zn/Sr ratios in prostate was recently demonstrated by us.[14] Moreover, a significant positive correlation was seen between the prostatic zinc and iron contents, and between the prostatic zinc and bromine contents.[14] Hence it is possible that besides Zn, such trace elements as Br, Fe, Rb, and Sr also play a role in the pathophysiology of the prostate.

This work had three aims. The first was to assess the Br, Fe, Rb, Sr, and Zn contents in intact prostate of healthy men aged over 40 years using radionuclide-induced (^{109}Cd) energy dispersive X-ray fluorescent (EDXRF) analysis. The second aim was to compare the levels of trace elements in the prostate gland of age-matched patients, who had BPH, and the third was to calculate the ratios of Zn/trace element content 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=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 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 8 mg was applied to the piece of Scotch tape serving as an adhesive fixing backing.

To determine the mass fractions of the elements by comparison with a known standard, aliquots of commercial, chemically pure compounds were used. The microliter standards were placed on disks made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in a vacuum. Ten subsamples of the Certified Reference Material (CRM) IAEA H-4 (animal muscle) weighing about 8 mg were analyzed to estimate the precision and accuracy of results. The CRM IAEA H-4 subsamples were prepared in the same way as the samples of dry homogenized prostate tissue.

Details of the relevant facility for EDXRF, source with ^{109}Cd radionuclide, methods of analysis and the results of quality control were presented in our earlier publications concerning the EDXRF analysis of human prostate tissue.[14,15]

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 inter-correlations of chemical element contents. The reliability of difference in the results between the two groups of prostate tissues was evaluated by Student's *t*-test.

Results and discussion

Table 1 depicts our data for 5 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,[14,15] 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, Fe, Rb, Sr, and Zn mass fractions analyzed by EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicates an acceptable accuracy of the results obtained in the study of trace elements of the prostate presented in Tables 2–5.

Table 1. EDXRF data Br, Fe, Rb, Sr, and Zn mass fraction in the IAEA H-4 (animal muscle) reference material compared to certified values (mg·kg^{-1}, 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±1.2
Fe	49	47 - 51	C	48±9
Rb	18	17 - 20	C	22±4
Sr	0.1	-	N	<1
Zn	86	83 - 90	C	90±5

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

Table 2 presents basic statistical parameters of the Br, Fe, Rb, Sr, Zn mass fraction and the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr mass fraction ratios in normal and BPH prostate tissue. The mass fraction of Br, Fe, Rb, Sr, and Zn were measured in all, or a major portion of normal and PCa samples.

Table 2. Basic statistical parameters of Br, Fe, Rb, Sr, and Zn mass fraction (mg·kg^{-1}, dry mass basis) and the Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr mass fraction ratios in normal and BPH prostate tissue

Tissue	Element	Mean	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Normal (n=37)	Br	40.6	30.3	5.6	5.0	143	34.0	5.63	109
	Fe	118	41.3	7.5	44	244	112	57.1	203
	Rb	16.3	6.51	1.1	6.3	31.0	15.6	7.46	31.0
	Sr	2.5	2.1	0.4	0.95	9.7	1.5	0.98	7.3
	Zn	1154	723	119	229	3513	961	233	2637
	Zn/Br	39.1	32.3	6.2	2.44	116	31.0	5.03	107
	Zn/Fe	11.2	7.4	1.3	1.70	28.3	9.58	1.95	26.0
	Zn/Rb	71.7	49.8	9.0	14.3	196	62.9	16.6	188
	Zn/Sr	534	382	83	23.6	1463	509	48.2	1326
BPH (n=43)	Br	30.0	19.1	3.6	5.5	77.0	25.6	5.77	68.9
	Fe	126	92.3	15	19	405	98	35.2	379
	Rb	14.9	5.82	0.98	4.9	31.9	14.6	5.58	25.2
	Sr	3.8	2.6	0.6	0.2	10.9	3.4	0.52	9.7
	Zn	1073	543	74	314	2515	975	333	2454
	Zn/Br	68.8	60.8	11.5	10.4	241	44.8	11.2	231
	Zn/Fe	14.0	11.0	1.8	1.01	52.9	13.0	1.80	39.4
	Zn/Rb	87.4	54.9	9.3	24.8	298	71.0	26.2	210
	Zn/Sr	423	311	74	78.1	1158	348	88.2	1047

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, Fe, Rb, Sr, Zn mass fractions and the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr mass fraction ratios in normal and BPH prostate tissue are presented in Table 3.

Table 3. Comparison of mean values (M±SEM) of Br, Fe, Rb, Sr, and Zn mass fraction ($mg \cdot kg^{-1}$, dry mass basis) and the Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr mass fraction ratios in normal and BPH prostate tissue

Element	Prostatic tissue		Ratio	
	Normal 41-87 year n=37	BPH 38-83 year n=43	BPH to Normal	Student's (t-test) *p*≤
Br	40.6±5.6	30.0±3.6	0.75	NS
Fe	118±8	126±15	1.07	NS
Rb	16.3±1.1	14.9±1.0	0.91	NS
Sr	2.5±0.4	3.8±0.6	1.52	NS
Zn	1154±119	1073±74	0.93	NS
Zn/Br	39.1±6.2	68.8±11.5	1.76	≤0.05
Zn/Fe	11.2±1.3	14.0±1.8	1.25	NS
Zn/Rb	71.7±9.0	87.4±9.3	1.22	NS
Zn/Sr	534±83	423±74	0.79	NS

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

From Table 3, it is observed that in BPH tissue the Zn/Br mass fraction ratio is significantly ($p<0.05$) 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, Fe, Rb, Sr, and Zn mass fractions in normal and BPH prostate tissues

Prostate tissue	Element	Br	Fe	Rb	Sr	Zn
Normal	Br	**1.00**	-0,109	-0,086	0,235	-0,110
	Fe		**1.00**	-0,062	-0,052	0,115
	Rb			**1.00**	0,105	0,076
	Sr				**1.00**	-0,421[a]
	Zn					**1.00**
BPH	Br	**1.00**	-0,017	-0,029	0,292[a]	0,123
	Fe		**1.00**	0,173	0,116	-0,249[a]
	Rb			**1.00**	0,131	0,306[b]
	Sr				**1.00**	-0,153
	Zn					**1.00**

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

The negative inter-element correlation of Zn mass fractions with Sr ($p<0.05$) mass fraction 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 trace elements are significantly altered in BPH tissue as compared to their relationships in normal prostate tissue. Zn mass fractions have only positive correlation with Rb ($p<0.01$) and negative correlation with Fe ($p<0.05$) in BPH prostate tissue.

The comparison of our results with published data for Br, Fe, Rb, Sr, and Zn mass fraction in normal and BPH prostate tissue is shown in Table 5.

Table 5. Median, minimum and maximum value of means of Br, Fe, Rb, Sr, and Zn mass fraction in normal and cancerous prostate tissue according to data from the literature in comparison with our results (mg·kg^{-1}, dry mass basis)

Prostate 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)**
Normal	Br	14.5 (2)	12±8 (4) [16]	17 (12) [17]	41±30
	Fe	150 (14)	5.7±0.1 (5) [18]	1040±65 (10) [19]	118±41
	Rb	34.5(3)	4.7 (9) [20]	58±33 (4) [21]	16±7
	Sr	0.94 (4)	0.75±0.75 (48) [22]	1.4 (12) [17]	2.5±2.1
	Zn[a]	1058 (5)	160±20 (11) [23]	1305 (10) [24]	1154±723
BPH	Br	12 (1)	12±8 (27) [25]	12±8 (27) [25]	30±19
	Fe	150 (7)	5.9±0.4 (8) [18]	1345±95 (27) [25]	126±92
	Rb	-	-	-	15±6
	Sr	-	-	-	3.8±2.6
	Zn	773 (31)	55±25 (23) [26]	3800±65 (10) [27]	1073±543

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 trace 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.[15] Values obtained for Br, Fe, Rb, Sr, and Zn mass fractions (Table 5) agree well with median of mean values cited by other researches for the human prostate.[16-27] 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 (80%) [27] and ash (1% on wet mass basis)[28] contents in the prostate of adult men.

In BPH tissues our results were comparable with published data for Fe and Zn mass fractions and 2.5 times higher for Br (Table 5). No published data referring to Rb and Sr mass fractions of BPH prostate tissue were found. No published data referring to mass fraction ratios of Br, Fe, Rb, Sr, and Zn or inter correlations of these element mass fractions in normal and BPH tissues of the human prostate gland were also found.

Compared to other soft tissues, the human prostate has higher levels of Zn and some other trace elements.[29] These data suggests that these elements could be involved in functional features of BPH tissue. BPH transformation is accompanied by high level of Zn. Therefore, it is plausible that the reason for the emergence and development of BPH is associated with abnormally high concentration of Zn in the prostate tissue of older men.

Conclusions

In this work, trace elemental analysis was carried out in the tissue samples of normal and BPH prostates using EDXRF. It was shown that EDXRF is an adequate analytical tool for the non-destructive determination of Br, Fe, Rb, Sr, and Zn mass fraction in the tissue samples of human prostate, including needle-biopsy cores. The analysis Zn mass fraction only requires no more than 20 min. The Zn/Br mass fraction ratio was significantly higher in BPH tissues than in normal tissues. In our opinion, the abnormal high level of Zn 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.

References

1. H.B. Carter, D.S. Coffey, *Prostate* **1990**, *16*, 39
2. W.M. Garraway et al., *Lancet* **1991**, *338*, 469.
3. A.L. Burnett, A.J. Wein, *J. Urol.* **2006**, *175*, S19.
4. E. Shapiro, V. Hartanto, H. Lepor, *J. Urol.* **1992**, 147, 1167.
5. G. Robert et al., *Prostate*. **2009**, 69, 1774
6. E.M. Gong, G.S. Gerber, *Am. J. Chin. Med.* **2004**, *32*, 331.
7. K.L. Lee, D.M. Peehl, *J. Urol.* **2004**, *172*, 1784.
8. W. Li et al., *The American Journal of Pathology*, **2007**, *171*, 1189.
9. V. Zaichick, *J. Radioanal. Nucl. Chem*. **2006**, *269*, 303.
10. A. Ektessabi et al., *X-Ray Spectrom.* **2001**, *30*, 44.
11. S. Yoshida, A. Ektessabi, S. Fujisawa, *J. Synchrotron Radiat*. **2001**, *8*, 998.
12. V. Zaichick, S. Zaichick, *Mengen und Spurenelemente. 19. Arbeitstagung*, Friedrich-Schiller-Universitat: Jena, **1999**, p.104.
13. V. Zaichick, *J. Radioanal. Nucl. Chem*. **2004**, *262*, 229.
14. S. Zaichick, V. Zaichick, *X-Ray Spectrom*, **2011**, *40*, 464.
15. S. Zaichick, V. Zaichick, *X-Ray Spectrom.* **2010**, *39*, 83.
16. H. Kubo et al., *Medical Physics* **1976**, *3*, 204.
17. A. Forssen, *Annales medicinae Experimentalis et Biologie (Finland)* **1972**, *50*, 99.
18. H. Sangen, *Jap. J. Urol.* **1967**, *58*, 1146.
19. A. Jafa et al, *Indian J. Cancer* **1980**, *17*, 34.
20. S.R. Stitch, *Biochem. J.* **1957**, *67*, 97.
21. S.D. Soman et al., *Health Phys*. **1970**, *19*, 641.
22. I.H. Tipton, M.J. Cook, *Health Phys*. **1963**, *9*, 103.
23. M. Brys et al., *Biol. Trace Element Res.* **1997**, *59*, 145.
24. C. Lindholmer, H. Glaumann, *Andrologie (Berlin)* **1972**, *4*, 231.
25. G.J. Naga Raju et al., *Eur. J. Cancer Prev.* **2007,** *16*, 108.
26. A.R. Kiziler et al., *Trace Elements and Electrolytes* **2010**, *2*, 65.
27. F. Györkey et al., *Cancer Res*. **1967**, *27*, 1349.
28. B.E. Saltzman et al., *Inviron. Res.* **1990**, *52*, 126.
29. S. Zaichick et al., *Biol. Trace Elem. Res*. **2012**, *149*, 171.