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The effect of age on Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in pediatric and young adult prostate glands investigated by neutron activation analysis

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ABSTRACT

The effect of age on chemical element mass fractions in intact prostate of 50 apparently healthy 0–30 year old males was investigated by neutron activation analysis with high resolution spectrometry of short-lived radionuclides. Mean values ($M \pm SEM$) for mass fraction ($mg\,kg^{-1}$, dry mass basis) of chemical elements before the time of puberty and in the period of puberty and post-puberty were: Br 46.0 ± 6.7, Ca 1151 ± 140, Cl 14572 ± 700, K 10147 ± 700, Mg 771 ± 131, Mn 2.13 ± 0.25, Na 9880 ± 659 and Br 29.0 ± 4.6, Ca 2049 ± 364, Cl 11518 ± 1121, K 13029 ± 542, Mg 1186 ± 134, Mn 1.74 ± 0.16, Na 9887 ± 716, respectively. A tendency of age-related increase in Ca, K, and Mg mass fraction and of age-related decrease in Br mass fraction was observed in period of life from 0 to 30 years. This new data indicates that of the elements studied, only the Ca, K, and Mg mass fraction in prostate tissue is an androgen-dependent parameter.

1. Introduction

The prostate gland is a key part of the male reproductive system. It produces and excretes much of the liquid portion of semen (about 30–35% of the semen ejaculate). The prostate mixes its fluids with those from the seminal vesicles to transport the sperm made in the testicles.

The prostate of adult male is known to accumulate high levels of zinc. The findings of low zinc level in pediatric prostate warranted the conclusion that androgens are the major factors controlling the accumulation and maintenance of a high content of zinc in the prostate (Hienzsch et al., 1970; Leissner et al., 1980; Zaichick, 2004a; Zaichick and Zaichick, 2013).

In our previous studies the high mass fraction of Zn as well as some other trace elements were observed in prostate tissue of adult males when compared with those in nonprostatic soft tissues of the human body (Zaichick and Zaichick, 2011a–c; Zaichick et al., 2012a,b). However, some questions about the androgen control and the involvement of trace elements in prostatic reproductive function still remain unanswered. One valuable way to elucidate the situation is to compare the values for the prostatic mass fractions of chemical elements in pre-pubertal boys with those during puberty early, post-puberty and young adulthood.

The data on chemical element mass fractions in pediatric prostate is apparently extremely limited (Hienzsch et al., 1970; Leissner et al., 1980). There are few studies regarding chemical element content in prostate of young adult males, using chemical techniques and instrumental methods (Hienzsch et al., 1970; Schneider et al., 1970; Leissner et al., 1980; Tisell et al., 1982; Feustel and Wennrich, 1984; Saltzman et al., 1990; Picurelli et al., 1991; Oldereid et al., 1993; Schöpfer et al., 2010). However, the majority of these data are based on measurements of processed tissue. In many studies tissue samples are ashed before analysis. In other cases, prostate samples are treated with solvents (distilled water, ethanol etc.) and then are dried at high temperature for many hours. There is evidence that certain quantities of chemical elements are lost as a result of such treatment (Zaichick, 2004b). Moreover, only two of these studies employed quality control using certified reference materials (CRM) for determination of the chemical element mass fractions (Oldereid et al., 1993; Schöpfer et al., 2010).

The primary purpose of this study was to determine reference values for the Br, Ca, Cl, K, Mg, Mn, and Na mass fraction in the intact prostate proper of subjects of different age from newborns to young adult males using non-destructive instrumental neutron activation analysis. The second aim was to evaluate the quality of obtained results. The third aim was to compare the Br, Ca, Cl, K, Mg, Mn, and Na mass fractions in pre-pubertal boys (group 1) with those during puberty early, post-puberty and young adulthood (group 2). The final

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aim was to estimate the inter-correlations of chemical elements in normal prostate in the period of life from 0 to 30 years.

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

2. Material and methods

2.1. Samples

Samples of the human prostate were obtained from randomly selected autopsy specimens of 50 males (European–Caucasian) aged 0 to 30 years. Age ranges for subjects were divided into two groups, with group 1, 0–13 years (3.3 ± 0.09 years, M ± SEM, n=29), and group 2, 14–30 years (24.4 ± 1.0 years, M ± SEM, n=21. These age groups were selected to reflect the situation before puberty (group 1 – infant, childhood, and peripubertal periods) and during and after puberty (group 2 – adolescent and young adult periods). The available clinical data were reviewed for each subject. None of the subjects had a history of an intersex condition, endocrine disorder, neoplasm or other chronic disease that would affect the normal development of the prostate. None of the subjects was receiving medications known to affect prostate morphology and prostatic chemical element content. The typical causes of death in most of these patients included sudden infant death syndrome, acute pulmonary etiologies, and trauma. All prostate glands were divided (with an anterior–posterior cross-section) into two portions using a titanium scalpel. One tissue portion was reviewed by an anatomical pathologist while the other was used for the chemical element content determination. Only posterior part of the prostate, including the transitional, central, and peripheral zones, was investigated. A histological examination was used to control the age norm conformity as well as the absence of any microadenomatosis and/or latent cancer.

2.2. Sample preparation

After the samples intended for chemical element analysis were weighed, they were transferred to −20 °C and stored until the day of transportation in the Medical Radiological Research Center (MRRC), Obninsk. In the MRRC all samples were freeze-dried and homogenized. The pounded sample weighing about 100 mg was used for chemical element measurement by instrumental neutron activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR). The samples for INAA-SLR were sealed separately in thin polyethylene films washed with acetone and rectified alcohol beforehand. The sealed samples were placed in labeled polyethylene ampoules.

2.3. Standards and certified reference materials

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used (Mosulishvili et al., 1975). Corrected certified values of BSS element contents were reported by us before (Zaichick, 1995). In addition to BSS, 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 conditions that prostate samples to estimate the precision and accuracy of results.

2.4. Instrumentation and methods

A horizontal channel equipped with the pneumatic rabbit system of the WWR-C research nuclear reactor was applied to determine the mass fraction of Br, Ca, Cl, K, Mg, Mn, and Na by INAA-SLR. The neutron flux in the channel was 1.7×10^{13} n cm^{-2} s^{-1}. Ampoules with prostate samples, SSB, intralaboratory-made standards, and CRM were put into polyethylene rabbits and then irradiated separately for 180 s. Copper foils were used to assess neutron flux.

The measurement of each sample was made twice, 1 and 120 min after irradiation. The duration of the first and second measurements was 10 and 20 min, respectively. The gamma spectrometer included the 100 cm^3 Ge(Li) detector and on-line computer-based MCA system. The spectrometer provided a resolution of 1.9 keV on the ^{60}Co 1332 keV line.

2.5. Computer programs and statistic

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. The reliability of difference in the results between all age groups was evaluated by parametric Student′s t-test and Pearson′s chi-squared test. For the construction of "age—chemical element mass fraction" diagrams and the estimation of the Pearson correlation coefficient between different chemical elements the Microsoft Office Excel programs were also used.

3. Results

The information of used nuclear reactions, radionuclides, gamma-energies, and other details of the analysis is presented in Table 1.

Table 1
Radionuclides, some of their characteristics and conditions of analysis used for INAA-SLR prostate samples and certified reference materials.

Element	Nuclear reaction	Radionuclide	Half-life	γ-Energy used in measurement (keV)	Conditions of analysis[a]
Br	^{81}Br(n, γ)	^{82}Br	1.47 d	619, 776, 1044, 1317	B
Ca	^{48}Ca(n, γ)	^{49}Ca	8.75 m	3085	A
Cl	^{37}Cl(n, γ)	^{38}Cl	37.3 m	1642, 2166	A
K	^{41}K(n, γ)	^{42}K	12.4 h	1524	B
Mg	^{26}Mg(n, γ)	^{27}Mg	9.46 m	844, 1014	A
Mn	^{55}Mn(n, γ)	^{56}Mn	2.58 h	847	B
Na	^{23}Na(n, γ)	^{24}Na	15.0 h	1369, 2754	B

(A) 3 m, 1 m, 10 m; sample-detector distance, 10 cm; detector shielding, 5–10 cm lead.
(B) 3 m, 120 m, 20 m; sample-detector distance, 0 cm; detector shielding, 5–10 cm lead.

[a] Irradiation time, decay, and measurement