THE EFFECT OF AGE ON THE ZINC CONTENT IN PROSTATE OF HEALTHY MEN INVESTIGATED BY INAA AND ICP-MS

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Introduction

Globally, prostate cancer is the sixth most common cancer, and the third most common cancer in males in Western industrialized countries [1,2]. In North America, it is the most common cancer in males and, except for lung cancer, is the leading cause of death from cancer [3-5]. Although the etiology of prostate cancer is unknown, several risk factors including age and diet (calcium, zinc and some other nutrients) have been well identified [6,7]. It is also reported that the risk of having prostate cancer drastically increase with age, being three orders of magnitude higher for the age group 40–79 years than in those younger than 39 years [7,8].

It is well known that zinc levels in the peripheral zone of dorsal and lateral lobes of the prostate are almost 10 times higher than in other soft tissues [9]. The high content of zinc in the prostate suggests that zinc may play a role in prostate health. Therefore, in investigating the effect of diet on prostate cancer risk, much attention has been paid to dietary and supplemental zinc [10-19], as well as consumption of red meat [20-25], as it is a major source of zinc from food for the residents of the continental countries [26]. It should also be noted that zinc is more bioavailable in red meat and less bioavailable in vegetables [27]. Estimates of per capita zinc intake in many countries showed a positive correlation with mortality from prostatic cancer [10, 11, 18]. A positive association with risk of prostate cancer was also observed in a population-based case-control study in the island of Oahu, Hawaii [12]. Leitzmann et al. [19] examined the association between supplemental zinc intake and prostate cancer risk among 46,974 U.S. men participating in the Health Professionals Follow-Up Study. It was shown that men who consumed more than 100mg/day did have a relative risk of advanced prostate cancer of 2.29–2.37 greater than nonusers. Consumption of red meat has been proposed as a possible risk factor for prostate cancer [21]. Many epidemiological studies that have presented results on this subject showed statistically significant increased risk with increasing meat consumption [20-25]. On the contrary, modest to moderate inverse associations were observed in two case-control studies for dietary zinc [15] and zinc supplement use [17]. Some case-control studies have not observed a cancer protective association for dietary or combined dietary and supplemental zinc intake [13,14,16].

The possibility that zinc adversely affects prostate cancer opposes its possible beneficial effect on the health of elderly individuals [28-32]. It is, therefore, important clarify the role of dietary zinc and supplemental zinc on prostate cancer risk among older men.

At present there are two diametrically opposite points of view on this issue. Proponents of the position that high zinc intakes and, consequently, high intraprostatic zinc concentrations may be positively associated with prostate cancer risk use multiple arguments to support their theory [18,19,33]. For example, zinc enhances the activity of telomerase [34], an enzyme thought to be responsible for unlimited proliferation tumor cells, the activity of which is increased in prostate cancer [35]. Zinc has also been found to antagonize the potential inhibitory effect of bisphosphonates on prostate tumor cell invasion [36]. Excessive intake of zinc has undesirable metabolic effects, such as immune dysfunction [37] and impaired antioxidant defense [38] that are potentially related to prostate cancer. In humans, zinc intake is positively correlated with circulating levels of insulinlike growth factor-I [39] and testosterone [40] that are directly related to prostate carcinogenesis. Much data have been accumulated on both direct and indirect effect of zinc on the DNA, and to its vital role for prostatic cell division [41-43]. All these facts allow it to be assumed that excessive intracellular concentrations of zinc are probably one of the main factors acting at both initiation and promotion stages of prostate carcinogenesis. Thus, zinc supplementation could promote the development of prostate cancer.

Much of the interest in zinc as an agent for prostate cancer treatment and prevention [44-46] is due to studies that have shown a marked reduction in prostate tissue zinc levels in prostate cancer cells versus normal prostate cells [9]. Proponents of this theory think that high cellular zinc accumulation is detrimental to the malignant activities of prostate cancer cells. Due to lifestyle, eating and dietary habits, and physiological effects of aging, the elderly male population is normally predisposed to conditions of zinc deficiency [28-31,47], which can increase their susceptibility to prostate cancer. According to their hypothesis in the absence of zinc supplement cellular zinc uptake will be depressed and zinc levels in prostate normal cells will be reduced [44,46].

There are few studies regarding the effect of age on Zn content in prostate, using chemical techniques and instrumental methods [48-51]. However, majority of these data are based on non-intact tissue. In many studies tissue samples are ashed. 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 by these methods some amount of chemical elements is lost upon treatment [52]. Moreover, only one study used a quality control using certified reference material for Zn content [51].

This work had three aims. The first one was to assess the Zn content in intact prostate of healthy men using instrumental neutron activation analysis (INAA) and inductively coupled plasma mass spectrometry (ICP-MS). The second aim was to evaluate the quality of obtained results. The third aim was to compare the contents of Zn in different age groups.

All studies were approved by the Institute of Forensic Medicine, Moscow, and the Medical Radiological Research Center, Obninsk, Ethical Committees.

Experimental

Prostates were removed at necropsy from 64 men (mean age 36.5 years, range 13–60) who had died suddenly. The majority of deaths were due to traumas. Some of deaths were due

to alcohol poisoning and acute illness (cardiac insufficiency, stroke, embolism of pulmonary artery) but without inpatient treatment. Information about chronic alcoholism or other diseases was not available from the medical reports of subjects. All cadavers had undergone routine autopsy at the Institute of Forensic Medicine, Moscow. Tissue samples were collected from the peripheral zone of prostate dorsal and lateral lobes within 2 days of death and then divided into two portions. One of them was used for morphological study while another was intended for chemical element analysis. A histological examination was used to control the age norm conformity as well as the absence of microadenomatosis and latent cancer. After the samples intended for chemical element analysis were weighed, they were transferred to – 20 ºC 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 samples weighing about 50 mg were used for Zn measurement by INAA and ICP-MS. A tool made of titanium and plastic was used for sampling and sample preparation.

A vertical channel of nuclear reactor was applied to determine the Zn content by instrumental neutron activation analysis with high resolution spectrometry of long-lived radionuclides (INAA-LLR). The quartz ampoule with prostate samples, standards, and certified reference materials was soldered, positioned in a transport aluminum container and exposed to a 24-h neutron irradiation in a vertical channel with a neutron flux of 1.3×10^{13} n cm^{-2} 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 h subject to pulse counting rate. The gamma spectrometer included the 100 cm3 Ge(Li) detector and on-line computer-based MCA system. The spectrometer provided a resolution of 1.9 keV on the 60Co 1332 keV line. Details of nuclear reactions, radionuclides, gamma energies, methods of analysis and the results of quality control were presented in our earlier publications concerning the chemical elements of intact human prostate [53].

For ICP-MS analysis 1.5 mL of concentrated HNO_3 (*Nitric acid 65%, max. 0.0000005% Hg, GR, ISO, Merck*) and 0.3 mL of H_2O_2 (*pure for analysis*) were added to tissue samples, placed in one-chamber autoclaves (Ancon-AT2, Ltd., Russia) and then heated for 3 h at 160–200 ºC to decompose. After autoclaves were cooled to room temperature solutions from the decomposed samples were diluted with deionized water (up to 20 mL) and transferred to 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. Sample aliquots were used to determine the content of Zn by ICP-MS using an ICP-MS Thermo-Fisher ‘‘X-7’’ (Thermo Electron, USA). The measurements were made with the spectrometer parameters: RF generator power – 1250 W, nebulizer – PolyCon, spray chamber – cooling 3^{o}C, plasma gas flow rate – 12 L/min, auxiliary flow rate –0.9 L/min, nebuliser flow rate – 0.9 L/min, sample update – 0.8 mL/min, resolution – 0.8M.

The main parameters of mass-spectrum measurements were: detector mode – double (pulse counting and analogous) and scanning mode – Survey Scan and Peak Jumping. The setting for the Survey Scan was: the number of runs – 10, dwell time – 0.6 ms, channels per mass – 10, acquisition duration – 13.2 s. The setting for the Peak Jumping was: sweeps – 25, dwell time – 10 ms, channels per mass – 1, acquisition duration – 34 s.

The Zn contents in aqueous solutions were determined by the quantitative method using calibration solutions (*High Purity Standards, USA*) with 5, 10, and 100 μkg/L. Indium was used as an internal standard in all measurements.

The detection limit (DL) was calculated as: $DL = C_{Zn} + 3 \cdot SD$, where C_{Zn} is a mean value of the Zn concentration for measurements in control samples and SD is a standard deviation of C_{Zn} determination in control samples.

Uncertainties of Zn determination in prostate samples by ICP-MS expressed as mean relative standard deviation (±RSD) in repeatability study did not exceed 10%.

Ten certified reference material (CRM) IAEA H-4 Animal muscle [54] and IAEA HH-1 Human hair [55] sub-samples and three certified reference material INCT-SBF-4 Soya bean flour of the Institute of Nuclear Chemistry and Technology (Poland) sub-samples weighing about 50–100 mg were treated and analyzed in the same conditions as that of the prostate samples to estimate the precision and accuracy of results. The CRM IAEA H-4 Animal muscle and IAEA HH-1 Human hair sub-samples were analyzed by INAA-LLR. The CRM INCT-SBF-4 Soya bean flour sub-samples were analyzed by ICP-MS.

All prostate samples were prepared in duplicate and mean value of Zn content was used in final calculation. Using standard programs, the summary of statistics, arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, and percentiles with 0.025 and 0.975 levels were calculated for Zn content. The reliability of difference in the results between three age groups was evaluated by Student's t-test.

Results and discussion

Table 1 depicts our data for Zn mass fractions in sub-samples of certified reference material and the certified values of these materials. Good agreement with the certified data of certified reference materials indicate an acceptable accuracy of the results obtained in the study of Zn mass fraction in the prostate tissue.

Table 1. INAA-LLR and ICP-MS data of Zn content (M±SD) in Certified Reference Materials compared to certified values (mg/kg on dry weight basis)

Certified	INAA-LLR		ICP-MS	
Reference Material	Certificate	This work result	Certificate	This work result
IAEA H-4 Animal muscle	86.3±11.5[a]	91±2	–	–
IAEA HH-1 Human hair	174±9[a]	173±17	–	–
INCT-SBF-4 Soya bean flour	–	–	52.3[a] ±1.3	54.8±6.6

Mean – arithmetical mean, SD – standard deviation, a – certified values

Fig. 1 shows the individual data for Zn mass fraction obtained by INAA (A) and ICP-MS (B) in all samples of prostate tissue, and exponential lines of trend with age.

Table 2 represents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Zn content in intact prostate of apparently healthy men obtained by INAA-LLR, ICP-MS, and INAA-LLR + ICP-MS (together). The coefficient of correlation between INAA-LLR and ICP-MS data was $r = 0.993$.

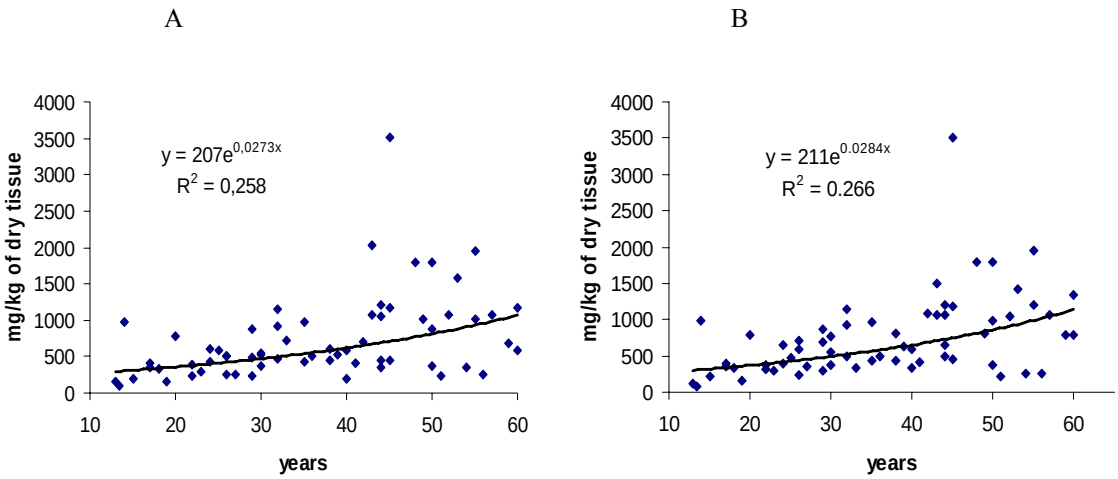

Fig. 1. Individual data for Zn mass fraction obtained by INAA (A) and ICP-MS (B) in all samples of prostate tissue, and exponential lines of trend with age.

Table 2. Some statistical parameters of Zn content (mg/kg on dry weight basis) in the prostate tissue of healthy men obtained by INAA-LLR, ICP-MS, and INAA-LLR + ICP-MS (together)

Method	M	SD	SEM	Min	Max	Med	P0.025	P0.975
INAA-LLR	722	576	72	90.0	3513	533	152	1987
ICP-MS	746	556	69	71.6	3510	619	145	1865
INAA + ICP-MS	734	562	50	80,8	3512	568	148	1865

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

To estimate the effect of age on Zn content in prostate (Table 3) we examined three age groups: the first comprised a younger group with ages from 13 to 20 years (mean age 16.3 years, n=9), the second comprised men with ages ranging from 21 to 40 years (mean age 30.4 years, n=28) and the last one comprised older persons with ages ranging from41 to 60 years (mean age 49.6 years, n=27). The values of Zn content obtained by INAA-LLR, ICP-MS, and INAA-LLR + ICP-MS (together) were used in calculation.

Table 3. Effect of age on mean values (M±SEM) of Zn contents ($mg \cdot kg^{-1}$, dry weight basis)

Method	Age groups			Ratios, *p* (Student's *t*-test)		
	Group 1 13-20 year n=9	Group 2 21-40 year n=28	Group 3 41-60 year n=27	2 to 1	3 to 1	3 to 2
INAA-LLR	384±102	521±44	1045±140	1.36	2.72[b]	2.01[b]
ICP-MS	382±103	557±44	1150±203	1.46	3.01[a]	2.06[a]
INAA+ ICP-MS	383±73	539±30	1097±120	1.41	2.86[b]	2.04[b]

M - arithmetic mean, SEM – standard error of mean, [a] – $p \leq 0.01$, [b] – $p \leq 0.001$

A statistically significant tendency of age-related increase in Zn mass fraction was observed in prostate (Table 3). For example, in prostate of 50 years old men the mean Zn mass fraction was 2.86 times greater than in prostate of 16 years old persons, respectively. This result is in accordance with earlier findings in human prostate [48-51]. For example, Heinzsch et al. [48] and Leissner et al. [49] found that zinc content in whole adult normal prostate was higher after the age of 30 by approximately 1.9 and 1.5 times, respectively, in spite of similar prostatic weight. In the study of Tisell et al. [50], men 50–69 years of age had higher zinc mass fractions in their dorsal and lateral prostatic lobes than had men 20–29 years of age by 1.6 and 1.7 times, respectively. In accordance with Oldereid et al. [51], the mean Zn mass fraction in prostate of 60 years old men was 3 times greater than in prostate of 20 year old subjects.

As we have found, the age-dependent increases of the Zn mass fractions followed an exponential better than a linear trend (Fig. 1). The finding, that age-dependent changes in the mass fraction of Zn in human prostate best fit exponential curve, was previously published [51].

The obtained values of Zn mass fraction, as shown in Table 4, agree well with median of means cited by other researchers for the human prostate, including samples received from persons who died from different diseases. A number of values for chemical element mass fractions were not expressed on a dry weight basis by the authors of the cited references. However, we calculated these values using published data for water—80% [56] and ash—1% on wet weight basis [57] contents in prostate of adult men.

Table 4. Median, minimum and maximum value of means of Zn contents (mg/kg on dry weight basis) in intact prostate of adult males according to data from the literature in comparison with our results

Published data [Reference]			This work results
Median of means, (n)*	Minimum of means, (n)**	Maximum of means, (n)**	M±SD n=64
482 (48)	111 (-) [58]	2735 (10) [59]	734±562

M - arithmetic mean; SD – standard deviation; (n)* – number of all references; (n)** - number of samples.

All the deceased were citizens of Moscow. None of those who died a sudden death had suffered from any systematic or chronic disorders before. The normal state of prostates was confirmed by morphological study. Thus, our data for Zn mass fractions in intact human prostate may serve as indicative normal values for urban population of the Russian Central European region.

Conclusions

INAA-LLR and ICP-MS are the adequate analytical tools for the non-destructive determination of Zn content in the tissue samples of human prostate. From the time of puberty and up to 60 years the mass fraction of Zn in prostate tissue exponentially increases almost 3

times. Thus, the data do not support the hypothesis of age-related deficiency of zinc in prostate tissue and the need for zinc supplementation for the correction of this deficit.

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