NEUTRON ACTIVATION ANALYSIS OF Ca, Cl, Mg, Na, and P CONTENTS IN THE EWING'S SARCOMA TISSUE

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Introduction

Bone tumors are a heterogeneous group of tumors that all arise from bone tissue, which consists of cartilaginous, osteoid and fibrous tissue, and bone marrow elements. Each tissue can give rise to benign or malignant tumors. The differentiation of benign and malignant intraosseous lesions can often be accomplished by means of conventional roentgenology, CT, and MRI. All of these methods of introscopy are very important, particularly for the assessment of tumor location, form, size, and infiltration of the adjacent tissue. However, the radiographic appearance of many lesions is indeterminate, and final diagnosis must be achieved using biopsy and histopathologic evaluation [1].

It is well known that tissues of human body differ greatly in their contents of chemical elements. Thus, it can be expected that bone tumors of a different origin would have specific elemental composition. In vivo neutron activation analysis (*in vivo* NAA) allows determination of some chemical element contents (Ca, Cl, and Na) in tumor tissue and has a potential to become a useful tool in oncology diagnostics [2-4].

To our knowledge, no data are available about the chemical element contents of bone tumors with respect to different origin of disease. Therefore, we determined the Ca, Cl, Mg, Na, and P contents in the Ewing's sarcoma and intact bone tissue using instrumental neutron-activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR).

Experimental

Samples of the Ewing's sarcoma tissue were obtained from 12 patients (2 females and 10 males from 4 to 24 years old, M=9.8 years). All patients were hospitalized at the Medical Radiological Research Centre. In all cases the diagnosis has been confirmed by clinical and morphological data. The tumor samples for NAA were received from biopsy and resected specimens. The control group consisted of 27 patients with intact bone (7 females and 20 males from 6 to 50 years old) who died from different deceases. The intact cortical bone samples of femur and tibia were collected at the Department of Pathology, Obninsk City Hospital. All bone samples were freeze dried until constant mass was obtained. Then samples were sealed separately in thin polyethylene films washed with acetone and rectified alcohol. The sealed samples were placed in labeled polyethylene ampoules.

To determine contents of the elements by comparison with a known standard, biological synthetic standards (BSS) prepared from phenol-formaldehyde resins were used [5]. In addition to BSS, aliquots of commercial, chemically pure compounds were also used as standards. Ten certified reference material CRM IAEA H-5 (Animal Bone) and standard reference material SRM NIST 1486 (Bone Meal) sub-samples weighing about 50–100 mg were analyzed in the same conditions as bone samples to estimate the precision and accuracy of results.

The contents of Ca, Cl, Mg, Na, and P were determined by instrumental neutron-activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR) using a horizontal channel equipped with the pneumatic rabbit system of the WWR-c research nuclear reactor. The neutron flux in the channel was 1.7×10^{13} n cm^{-2} s^{-1}. Ampoules with bone samples, BSS, intralaboratory-made standards, CRM and SRM were put into polyethylene rabbits and then irradiated separately for 60 s. Copper foils were used to assess neutron flux. The measurement of each sample was made 1 min after irradiation. The duration of the measurements was 10 min. A coaxial 98 cm^3 Ge (Li) detector and a spectrometric unit (NUC 8100), including a PC-coupled multichannel analyzer, were used for measurements. The spectrometric unit provided 2.9 keV resolution at the ^{60}Co 1332 keV line. The information of used nuclear reactions, radionuclides, gamma-energies, and other details of the analysis including the quality control of results were reported by us before [6,7].

A dedicated computer program of NAA mode optimization was used [8]. Using standard 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 were calculated for different chemical element mass fractions. The reliability of difference in the results between intact bone and the Ewing's sarcoma tissue was evaluated by Student's t-test.

Results and discussion

Table 1 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 Ca, Cl, Mg, Na, and P mass fractions in intact cortical bone samples and the Ewing's sarcoma tissue.

The information of the effect of malignant transformation on the chemical element contents in bone tissue is presented in Table 2. From Tables 2, it is observed that in the Ewing's sarcoma tissue the mass fractions of Cl ($p \leq 0.001$) and Na ($p \leq 0.01$) are higher and the mass fractions of Ca and P are lower ($p \leq 0.01$) than in normal tissues. Different directions of changes suggest potential of mass fraction ratios of these elements as the Ewing's sarcoma markers.

Table 3 depicts our data for some ratios of Ca, Cl, Mg, Na, and P mass fractions in intact cortical bone samples and osteogenic sarcoma tissue. It was shown that higher Cl/Na as well as lower Ca/P, Ca/Cl, Ca/Mg, and Ca/Na mass fraction ratios were typical of the Ewing's sarcoma tissue compared with intact cortical bone (Table 3).

Fig.1 shows the individual data sets for Ca and Cl mass fractions (g/kg, dry mass basis) and Ca/Cl and Cl/Na mass fraction ratios in all samples of intact bone (1) and the Ewing's sarcoma tissue (2). These element mass fractions and ratios were chosen among others because it is possible to determine these parameters by *in vivo* NAA [2-4]. Using ratios of chemical elements mass fractions instead of the absolute values of mass fraction is better for

making a specific diagnosis because of at least two reasons: 1) relations of elements do not depend on the moisture content (water) in the tissue; 2) defining relations of elements is more convenient for *in vivo* analysis.

Table 1. Some statistical parameters of Ca, Cl, Mg, Na, and P mass fractions in intact cortical bone and the Ewing's sarcoma tissue (g/kg, dry mass basis)

Tissue	Element	M	SD	SEM	Min	Max	Med	P0.025	P0.975
Intact	Ca	222	43.6	9.3	166	369	212	174	317
cortical	Cl	1.52	1.42	0.30	0.40	6.80	1.10	0.455	5.04
bone	Mg	2.94	0.79	0.17	0.90	5.04	3.00	1.51	4.36
n=27	Na	6.40	1.74	0.36	3.80	11.7	6.00	4.41	10.9
	P	112	29.5	6.1	66.0	174	107	66.1	168
The Ewing's	Ca	81.3	19.0	5.5	51.0	123	79.5	54.9	116
sarcoma	Cl	13.0	3.0	0.87	8.10	17.8	12.9	8.35	17.6
n=12	Mg	2.38	0.54	0.15	1.40	3.40	2.35	1.51	3.29
	Na	10.4	3.8	1.1	4.30	19.8	9.09	5.32	18.0
	P	84.2	25.5	7.4	41.6	138	83.5	46.7	134

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.

Table 2. Comparison between mean values (M±SEM) of Ca, Cl, Mg, Na, and P mass fraction in intact cortical bone and the Ewing's sarcoma tissue (g/kg, dry mass basis)

Element	Intact cortical bone (I) n=27	Ewing's sarcoma (II) n=12	Ratio II to I *p* (Student's *t*-test)
Ca	222±9	81.3±5.5	0.37[a]
Cl	1.52±0.30	13.0±0.87	8.55[b]
Mg	2.45±0.37	2.38±0.15	0.97
Na	6.40±0.36	10.4±1.1	1.63[a]
P	112±6	84.2±7.4	0.75[a]

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

Table 3. Comparison between mean values (M±SEM) of Ca/P, Ca/Mg, Ca/Na, Cl/Ca, and Cl/Na mass fraction ratios in intact cortical bone and osteogenic sarcoma tissue

Element	Intact cortical bone (I) n=27	Ewing's sarcoma (II) n=12	Ratio II to I *p* (Student's *t*-test)
Ca/P	2.06±0.07	1.05±0.10	0.51[b]
(Ca/Cl)·0.01	2.22±0.25	0.069±0.009	0.031[b]
(Ca/Mg)·0.01	0.81±0.07	0.35±0.03	0.43[b]
(Ca/Na)·0.01	0.36±0.02	0.087±0.010	0.24[b]
Cl/Na	0.22±0.03	1.42±0.24	6.45[b]

M - arithmetic mean, SEM – standard error of mean, n – number of samples, [b] – $p \leq 0.001$.

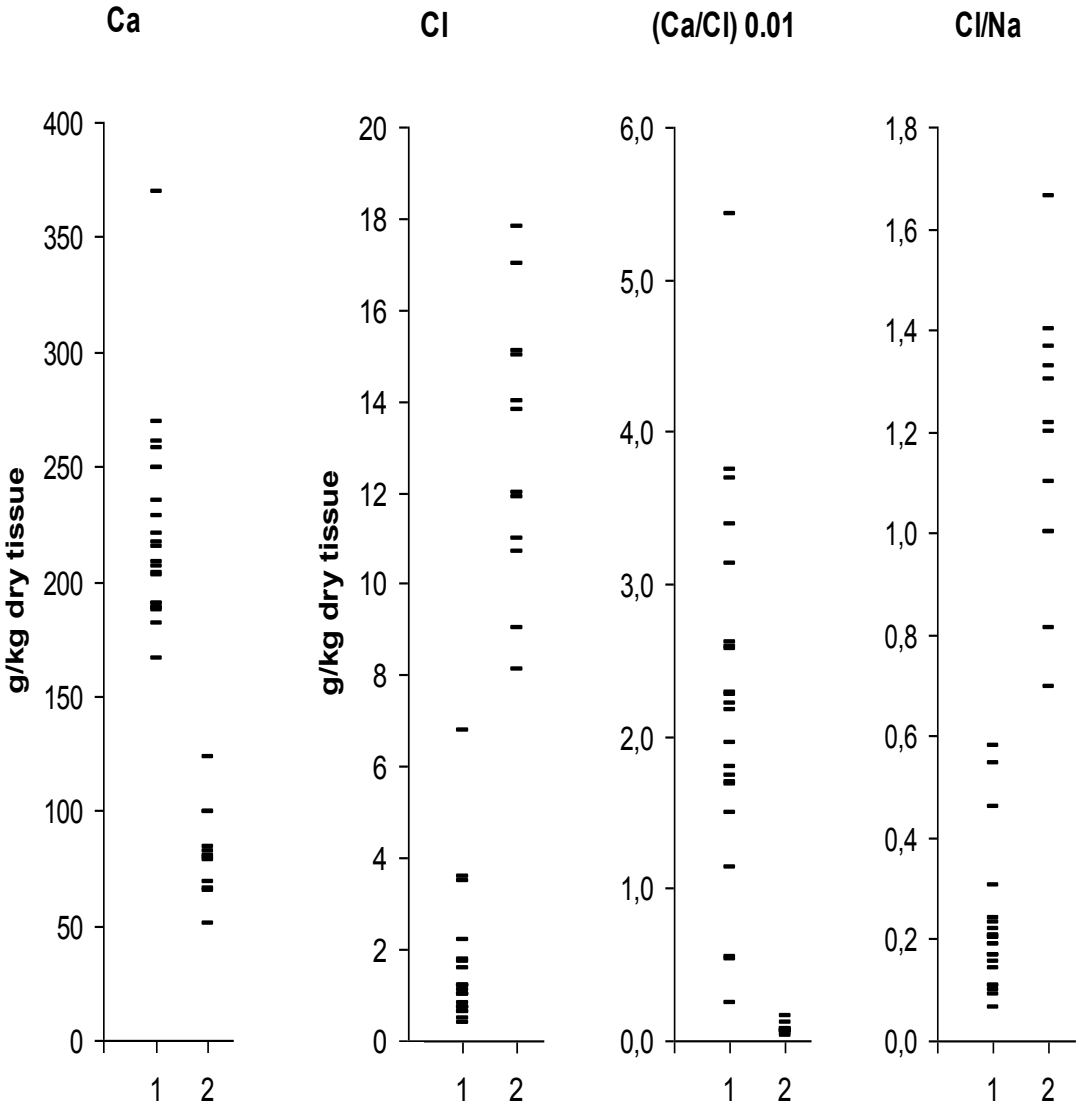

Fig. 1. Individual data sets for Ca and Cl mass fractions (g/kg, dry mass basis) and Ca/Cl and Cl/Na mass fraction ratios in all samples of intact bone (1) and the Ewing's sarcoma tissue (2).

As evident from the individual data sets all parameters chosen in this work as tumor markers are very informative for the differential diagnostics between intact bone and the Ewing's sarcoma. For example, if Ca mass fraction level of 135 g/kg (M-2SD) was assumed to be a lower limit of intact bone tissue (Fig.1), the results of estimation "the Ewing's sarcoma or intact bone" are the following:

Sensitivity = {True Positives (TP)/[TP + False Negatives (FN)]} ·100% = 100-8%;
Specificity = {True Negatives (TN)/[TN + False Positives (FP)]} ·100% = 100-4%;
Accuracy = [(TP+TN)/(TP+FP+TN+FN)] ·100% = 100-2%.

The confidential intervals of these calculations with taking account of the number of the examined samples were taken from the Statistical tables by Genes [9]. In other words, if the analysis showed that Ca mass fraction does not higher 135 g/kg in tissue of bone examined site, one can diagnose a Ewing's sarcoma with accuracy of 100-2%. Using Ca -test makes it possible to find the 100-8% the Ewing's sarcoma cases (sensitivity).

Conclusions

INAA-SLR is the adequate analytical tools for the non-destructive determination of Ca, Cl, Mg, Na, and P contents in the human bone samples and samples of intraosseous lesions weighing about 50 mg. It needs no more than 15 min (1 min irradiation by neutrons + 1 min exposure + 10 min spectrometric measurement) for analysis. It was found that in the Ewing's sarcoma tissue the mass fractions of Cl and Na are significantly higher and the mass fraction of Ca and P is lower than in normal tissues. Moreover, it was shown that higher Cl/Ca and Cl/Na mass fraction ratios as well as lower Ca/P, Ca/Mg, and Ca/Na mass fraction ratios were typical of the Ewing's sarcoma tissue compared with intact cortical bone. Differences between mass fraction of Ca and Cl, and between ratios of Ca/Cl and Cl/Na can be used as an additional test for differential diagnosis of normal bone and the Ewing's sarcoma.

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