NEUTRON ACTIVATION ANALYSIS OF Ca, Cl, Mg, Na, and P CONTENTS IN THE BENIGN GIANT CELL TUMOR OF BONE

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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 imaging methods 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.

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.[1,2]

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 benign giant cell tumor of bone and intact bone tissue using instrumental neutron-activation analysis with high resolution spectrometry of short-lived radionuclides (INAA-SLR).

Experimental

Samples of benign giant cell tumor of bone were obtained from 13 patients (4 females and 9 males aged 7-47 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 aged 6-50 years) 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.[3] Corrected certified values of BSS element contents were reported by us before.[4] 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 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.[5]

A dedicated computer program of NAA mode optimization was used.[6] 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 were calculated for different chemical element mass fractions. The reliability of difference in the results between intact bone and benign giant cell tumor of bone 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 and benign giant cell tumor of bone samples.

The information of the effect of neoplasm transformation on the chemical element contents in bone tissue is presented in Table 2. From Tables 2, it is observed that in benign giant cell tumor of bone tissue the mass fractions of Ca ($p \leq 0.001$) and P ($p \leq 0.01$) are lower and, in contrast, the mass fraction of Cl ($p \leq 0.001$) is higher, than in normal tissues. Different directions of Ca and Cl content changes suggest potential of mass fraction ratios as benign giant cell tumor of bone markers.

Table 3 depicts our data for some ratios of Ca, Cl, Mg, Na, and P mass fractions in intact cortical bone and benign giant cell tumor of bone samples. It was shown that higher Cl/Ca and Cl/Na mass fraction ratios and also lower Ca/Na mass fraction ratio were typical of benign giant cell tumor of bone compared with intact cortical bone (Table 3).

Figs.1 depicts individual data sets for Ca and Cl mass fractions ($g\cdot kg^{-1}$, dry weight basis) and Cl/Ca and Cl/Na mass fraction ratios in all samples of intact bone (1) and benign giant cell tumor of bone (2). The Cl/Ca and Cl/Na ratios were chosen among others because it is possible to determine these ratios by *in vivo* NAA.[1] 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 benign giant cell tumor of bone ($g \cdot kg^{-1}$, dry weight 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
Benign	Ca	156	59	16	50	255	160	59.3	246
giant	Cl	4.85	2.88	0.80	0.40	11.0	5.01	0.80	10.0
cell	Mg	2.43	0.72	0.20	1.20	3.88	2.50	1.32	3.62
tumor	Na	6.73	1.94	0.54	2.20	9.90	6.50	2.98	9.66
n=13	P	79.1	26.1	7.25	28.7	108	78.0	30.3	108

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 benign giant cell tumor of bone ($g \cdot kg^{-1}$, dry weight basis)

Element	Intact cortical bone (I) n=27	Benign giant cell tumor (II) n=13	Ratio II to I *p* (Student's *t*-test)
Ca	222±9	156±16	0.70^{b}
Cl	1.52±0.30	4.85±0.80	3.19^{b}
Mg	2.45±0.37	2.43±0.20	1.00
Na	6.40±0.36	6.73±0.54	1.05
P	112±6	79.1±7.3	0.71^{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 benign giant cell tumor of bone

Element	Intact cortical bone (I) n=27	Benign giant cell tumor (II) n=13	Ratio II to I *p* (Student's *t*-test)
Ca/P	2.06±0.07	2.00±0.12	0.97
(Ca/Mg)·0.01	0.81±0.07	0.66±0.06	0.81
(Ca/Na)·0.01	0.36±0.02	0.25±0.03	0.69^{a}
(Cl/Ca)·100	0.59±0.10	2.43±0.49	4.12^{b}
Cl/Na	0.22±0.03	0.67±0.09	3.04^{b}

M – arithmetic mean, SEM – standard error of mean, n – number of samples,
^{a} – $p \leq 0.01$, ^{b} – $p \leq 0.001$

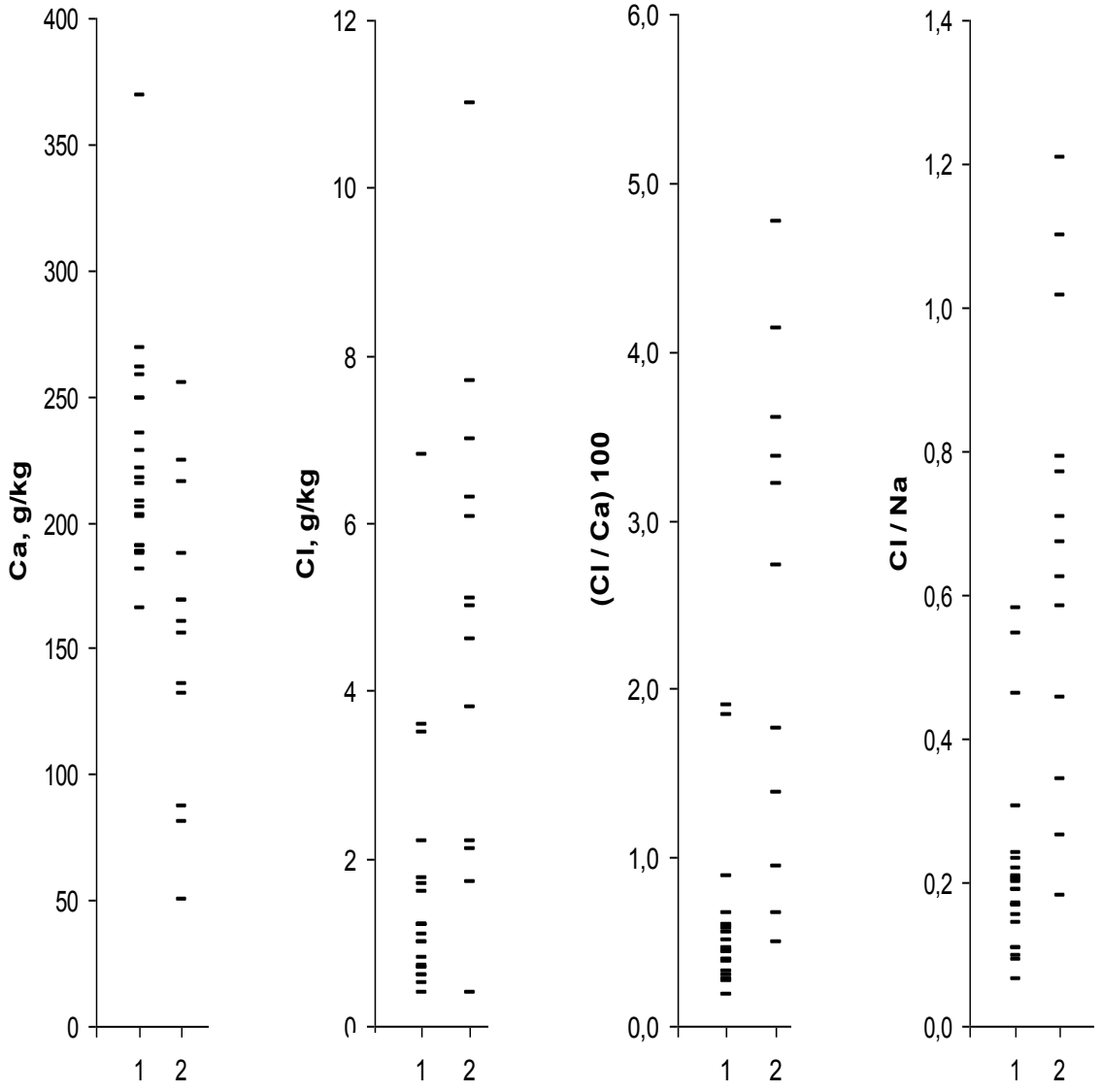

Fig. 1. Individual data sets for Ca and Cl mass fractions ($g \cdot kg^{-1}$, dry weight basis) and Cl/Ca and Cl/Na mass fraction ratios in all samples of intact bone (1) and benign giant cell tumor of bone (2).

As is evident from individual data sets, the (Cl/Ca)×100 ratio is the most informative for a differential diagnosis. If 1.4 is the value assumed to be the upper limit (M+2SD) for an intact bone tissue (Fig.1) and an estimation is made for "benign giant cell tumor of bone or intact bone tissue", the following values are obtained:

Sensitivity = {True Positives (TP)/[TP + False Negatives (FN)]} ·100% = 77±12%
Specificity = {True Negatives (TN)/[TN + False Positives (FP)]} ·100% = 91±6%
Accuracy = [(TP+TN)/(TP+FP+TN+FN)] ·100% = 86±6%

The confidential intervals of these calculations were obtained from the Statistical tables of Genes[7] and take into account of the number of samples examined. In other words, if the analysis shows that the (Cl/Ca)×100 ratio is higher 1.4 for a specific sample of bone tissue, one would classify this sample as a benign giant cell tumor of bone with accuracy of 86±6%. Using the Ca mass fraction as a test makes it possible to identify 77±12% of the benign giant cell tumor of bone samples correctly (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 benign giant cell tumor of bone samples the mass fractions of Ca and P are significantly lower and, in contrast, the mass fraction of Cl is significantly higher, than in normal bone tissues. Moreover, it was shown that higher Cl/Ca and Cl/Na mass fraction ratios were typical of benign giant cell tumor of bone compared with intact cortical bone. Differences between Cl/Ca ratio can be used as an additional test for differential diagnosis of normal bone and benign giant cell tumor of bone.

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