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ACTIVATION ANALYSIS METHODS AND APPLICATIONS

Trace element determinations in human cortical and trabecular bones

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Rib bones of Brazilian people were analyzed by neutron activation analysis to evaluate element composition. Freeze-dried cortical and trabecular tissues, separately, and calcinated total rib tissues were analyzed. The concentrations of the Ba, Br, Ca, Cl, Fe, K, Mg, Mn, Na, P, Rb, Sr, and Zn elements were determined. Comparisons between the results obtained in cortical and trabecular bones indicated significant differences in the concentration for several elements. Results obtained in cortical and trabecular bones were also compared with literature values.

Introduction

In the past years, there has been an increasing interest in determining trace elements in biological tissues in order to elucidate the roles they play in human organisms. Trace element determinations have been undertaken in bones because they are deposits of essential and toxic elements and the knowledge of their concentration could be important in the studies on several kinds of diseases.[1–4] Bones are also analyzed to evaluate the effect of elements on the health of populations exposed to toxic elements.

On the other hand, data about trace elements in different compartments of bone are very scarce since it is difficult to obtain representative specimens for chemical analyses. The bones are commonly divided into two compartments: compact (cortical) and trabecular (spongy and porous, cancellous) bone, in dependence of their hardness, porosity, and the content of soft tissue bones. However, not all bones can be strictly classified as compact or trabecular, since some types are intermediate in porosity and difficult to classify. Besides medico-legal implications, collecting samples from humans is, generally, a problem. Cortical and trabecular tissues have been analyzed separately in iliac crest by ARAS et al.[5] and in rib bones by SAMUDRALWAR and ROBERTSON.[6]

In this study, experimental conditions were defined to obtain representative samples of human cortical and trabecular bones and comparisons were made between the results obtained in these different bone subcompartments. These determinations could be important to increase the current knowledge of the elemental composition in each subcompartment of human bones and to establish a baseline of concentration values.

As in a previous work,[7] the certified reference materials NIST 1400 Bone Ash and NIST 1486 Bone Meal were also analyzed for quality control. The relative errors obtained were lower than 12% and the precision of the results was satisfactory for most of the elements with relative standard deviations varying from 3 to 12%.

Experimental

Samples

Animal and human rib bones were analyzed. Bovine rib bones, obtained from a local butchery, were analyzed to establish adequate conditions for the analytical procedures and to evaluate the homogeneity of the samples.

Rib bones from humans were obtained after pathological autopsy of Brazilian people (15 males, 3 females; mean age, 54.9 years) at the Institute of Forensic Medicine of the University of São Paulo. The *causas mortis* of these donors were not due to chronic bone diseases and the Ethics Committee authorized sample collection. The samples were wrapped in polyethylene foils and stored in a freezer until they were treated for the analyses. The ribs were cleaned free of connected soft tissues (periosteum) and cut transversely with a stainless steal saw to obtain about 2 mm thick slices of bone and the samples were then washed with MilliQ water. The trabecular tissues were separated from the cortical ones using a titanium knife whereas the cortical tissues were broken into small pieces. These samples were freeze-dried for the analysis until to obtain constant weight and in this process, mean weight losses of 50.8% and 15.8% were obtained for trabecular and cortical bones, respectively. For total bone analyses, the

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ribs (cortical plus trabecular tissues) were calcinated in a furnace at 800 °C and the mean weight loss of 73% was obtained. The calcinated samples were crushed with an agate or plastic pestle and homogenized. To avoid sample contamination, a series of precautions were taken. Sample handling was performed inside a 100 class laminar-flow hood and a polyethylene spatula was used for their transfer.

Standards

The standards for comparative neutron activation analysis were obtained by pipetting known aliquots of multielemental or single standard solutions onto sheets of Whatman No. 41 filter paper. The standard solutions provided from SpexCertiprep Chemical (USA) were used to obtain the diluted solutions containing one or more elements. The amounts of the elements used for irradiation were (in μg): Ba = 254; Br = 6.0; Ca = 1003; Cl = 100; Fe = 502; K = 1002.0; Mg = 1004.0; Mn = 2.1; Na = 65.0; Rb = 5.0; Sr = 500.0 and Zn = 50.0. In the case of P, 30.0 mg of ammonium dihydrogen phosphate, Puratonic, 99.998% purity from Alfa Aesar (USA) weighed directly in polyethylene vials were utilized. After drying at room temperature in a desiccator, the filters were placed in clean polyethylene bags and irradiated with the samples.

Procedure for the analysis of bone samples

About 150 mg of sample weighed and heat-sealed in polyethylene bags were irradiated at the IEA-R1 nuclear reactor. Four-minute irradiations under a thermal neutron flux of $4.5 \cdot 10^{11}$ n·cm^{-2}·s^{-1} were used for Ba, Ca, Cl, K, Mg, Mn, Na, P and Sr determinations. Sixteen-hour irradiations under thermal neutron flux of 10^{12} n·cm^{-2}·s^{-2} were used for Ba, Br, Ca, Fe, Rb, Sr and Zn determinations. After adequate decay times, the irradiated samples and standards were measured using an HPGe detector (Model GX2020) coupled to an integrated signal processor (Model 1510), both from Canberra. The resolution (FWHM) of the system was 0.90 keV for 122 keV gamma-ray peak of ^{57}Co and 1.87 keV for 1332 keV gamma-ray of ^{60}Co. For P analyses, ^{32}P beta activity was measured using a Geiger-Muller detector. A preliminary experiment was carried out to assure that in the counting of ^{32}P no interference of other activated radionuclides was present in the samples. The samples were measured for different decay times in order to verify the half-life in ^{32}P identification.

In all the analyses, the radioisotopes measured were identified according to their half-lives and gamma-ray energies. The elemental concentrations were calculated by comparative method.

Results and discussion

The homogeneity of the sample obtained for the analyses was examined by analyzing each subcompartment of a bovine rib bone sample in replicates (about 120 mg). These results, summarized in Table 1, show a relative standard deviation lower than 13% demonstrating a good homogeneity. These findings indicate that the procedure adopted for bone preparation and homogenization was appropriate.

Arithmetic mean values, standard deviations and concentration ranges of the elements determined in cortical and trabecular tissues are presented in Tables 2 and 3, respectively. In these tables, literature values are also given for comparison. Ca and P were found at the percentage levels, Ba, Br, Cl, Fe, K, Mg, Na, Rb, Sr and Zn at μg·g^{-1} levels and Mn at μg·kg^{-1} levels. Ba , Mn and Rb were not detected in cortical bones. As can be seen, the data of some elements showed considerable intersubject variability. These results indicated that biological factors such as gender, weight, height, age as well as health status and nutritional habits might be responsible for the variations in elemental composition of bones. As human bone collection was difficult, a limited number of samples were analyzed.

Table 1. Element concentrations (in μg·g^{-1} dry weight, unless otherwise indicated) obtained in replicate analyses of a bovine rib bone sample

Element	Freeze dried cortical tissue		Freeze dried trabecular tissue		Calcinated total bone	
	Mean ± s[a]	S_R,[b] %	Mean ± s	S_R, %	Mean ± s	S_R, %
Ba	128 ± 2	1.5	118.4 ± 4.1	3.5	476 ± 19	4.0
Ca, %	21.8 ± 1.2	5.5	15.2 ± 1.6	10.5	33.8 ± 2.5	7.4
Cl	227 ± 3	1.3	816 ± 85	10.4	239 ± 3	1.2
Fe	Not detected		121 ± 6	4.9	56 ± 4	7.1
K	815 ± 7	0.9	1589 ± 181	11.4	857 ± 45	5.2
Mg	4097 ± 79	1.9	3333 ± 404	12.1	6335 ± 206	3.2
Na	5601 ± 116	2.1	4233 ± 286	6.7	8686 ± 554	6.4
P, %	7.0 ± 0.6	8.5	7.6 ± 0.6	7.9	27.7 ± 3.6	13.0
Sr	308.9 ± 9.1	2.9	242.3 ± 19.8	8.2	284 ± 13	4.6
Zn	53.6 ± 0.4	0.7	70.3 ± 2.3	3.3	96.4 ± 5.3	5.5

[a] Arithmetic mean and standard deviation of *n* determinations and *n* varied from 3 to 9.
[b] Relative standard deviation.

Table 2. Elemental concentrations (in $\mu g \cdot g^{-1}$ dry weight, unless otherwise indicated) obtained in freeze dried human cortical rib bones

Element	This work (ages between 30–80)		Reference 6
	Mean ± s (*n*)[a]	Range	(ages between 60–82)
Br	0.67 ± 0.25 (18)	0.29 – 1.12	4.1 ± 4.0
Ca, %	22.2 ± 2.6 (18)	16.9 – 26.7	21.0 ± 4.0
Cl	538 ± 162 (18)	322 – 941	–
K	572 ± 205 (14)	237 – 956	–
Mg	2379 ± 314 (18)	1951 – 3147	2600 ± 400
Na	5342 ± 496 (18)	4554 – 6172	5400 ± 1000
P, %	10.0 ± 2.8 (18)	4.49 – 16.9	8.8 ± 2.2
Sr	112 ± 37 (18)	49.8 – 184.6	62 ± 18
Zn	114 ± 16 (18)	85.0 – 140.8	180 ± 44

[a] Arithmetic mean and standard deviation.
n: Number of determinations.

Table 3. Elemental concentrations (in $\mu g \cdot g^{-1}$ dry weight, unless otherwise indicated) obtained in freeze dried human trabecular rib bones

Element	This work (ages between 30–80)		Reference 6
	Mean ± s (*n*)[a]	Range	(ages between 60–82)
Ba	12.4 ± 8.4 (10)	4.3 – 30.6	19 ± 7
Br	1.71 ± 0.65 (18)	0.74 – 3.40	1.4 ± 0.6
Ca, %	7.95 ± 2.6 (18)	3.3 – 12.4	19.0 ± 2.2
Cl	1749 ± 678 (18)	895 – 3362	–
Fe	377 ± 189 (18)	146 – 907	77 ± 46
K	2623 ± 557 (18)	1400 – 3595	–
Mg	1183 ± 418 (18)	620 – 1925	2700 ± 400
Mn, $\mu g \cdot kg^{-1}$	216 ± 127 (13)	85 – 498	1010 ± 400
Na	3223 ± 793 (18)	2088 – 4791	5400 ± 600
P, %	3.38 ± 1.97 (18)	1.22 – 9.20	9.6 ± 1.3
Rb	6.58 ± 2.58 (18)	2.47 – 11.8	2.1 ± 3.1
Sr	40.5 ± 15.1 (16)	15.4 – 67.7	58 ± 17
Zn	70.0 ± 21.2 (18)	30.9 – 121.5	144 ± 17

[a] Arithmetic mean and standard deviation.
n: Number of determinations.

Element concentrations found in cortical rib tissues (Table 2) were also compared with the data published by SAMUDRALWAR and ROBERTSON.[6] The results obtained for Ca, Mg, Na and P were very close or showed the same magnitude as the literature values. In the case of trabecular bones (Table 3) only the concentrations of Ba, Br, Na and Sr were similar to the published data.[6]

The statistical *t*-test[8] applied to the results showed significant difference between the concentrations obtained for cortical and trabecular tissues ($p = 0.05$). Ca, Mg, Na, P, Sr and Zn presented higher concentrations in cortical tissues than those in trabecular ones. Concentrations of Br, Cl and K presented in cortical tissues were slightly lower in comparison with those obtained in trabecular ones.

Results of Table 4, obtained from total calcinated rib bones, were higher than those obtained from freeze-dried cortical and trabecular bones because of the different procedure used for sample drying. The calcination is an easy process for bone treatment, but losses of Br and Cl can occur. Fe detected in the calcinated trabecular bone is probably originated from the blood that was not removed.

Table 4. Elemental concentrations (in $\mu g \cdot g^{-1}$ dry weight basis, unless otherwise indicated) in calcinated total rib bones

Element	Total rib bone (ages between 30–80)	
	Mean ± s (*n*)[a]	Range
Ca, %	34.7 ± 3.2 (18)	30.1 – 41.0
Cl	458 ± 163 (18)	258 – 816
Fe	842 ± 213 (13)	224 – 917
K	1707 ± 495 (17)	1028 – 2757
Mg	4184 ± 683 (18)	2886 – 5321
Mn, $\mu g \cdot kg^{-1}$	308 ± 119 (6)	148 – 499
Na	8844 ± 860 (13)	6734 – 10046
P, %	17.9 ± 4.5 (18)	10.2 – 26.4
Sr	176 ± 68 (18)	87 – 345
Zn	220 ± 40 (13)	154 – 278

[a] Arithmetic mean and standard deviation.
n: Number of determinations.

Conclusions

In this study, a bone sample treatment for elemental analysis was evaluated. Preliminary results showed that there are differences in trace element concentrations between cortical and trabecular bones. Therefore, the selective analysis of these tissues may be useful in understanding the role of trace element in human bone. Up to now, there are few data of elemental composition in cortical and in trabecular bones.

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References

1. P. C. D'HAESE, H. COUTTENYE, L. V. LAMBERTS, M. M. ELSEVIERS, W. G. GOODMAN, I. SCHOOTEN, W. E. CARRERA, M. E. DE BROE, Clin. Chem., 45 (1999) 1548.
2. J. YOSHINAGA, T. SUZUKI, M. MORITA, M. HAYAKAWA, Sci. Total Environ., 162 (1995) 239.
3. H. W. KUO, S .M. KUO, C. H. CHOU, T. C. LEE, Sci. Total Environ., 255 (2000) 45.
4. R. M. PARR, G. V. IYENGAR, The role of minerals and trace elements in osteoporosis, in: Proc. 5th Conf. of International Society for Trace Element Research in Humans, Vol. 1, M. ABDULLA, M. BOST, S. GAMON, P. ARNAUD, G. CHAZOT (Eds), 1999, p. 248.
5. N. K. ARAS, G. YIMAZ, F. KORKUSUZ, I. OLMEZ, B. SEPICI, F. EKSIOGLU, P. BODE, J. Radional. Nucl. Chem., 244 (2000) 185.
6. D. L. SAMUDRALWAR, J. D. ROBERTSON, J. Radional. Nucl. Chem., 169 (1993) 259.
7. M. SAIKI, M. K. TAKATA, S. KRAMARSKI, A. BORELLI, Biol. Trace Elem. Res., 71–72 (1999) 41.
8. S. VIEIRA, Introdução à biostatística, 3. ed, Campus, Rio de Janeiro, 1980.