EVALUATION OF NORMAL CONCENTRATIONS OF TRACE ELEMENTS IN HUMAN TISSUES BY NEUTRON ACTIVATION ANALYSIS

Mitiko Saiki
Radiochemistry Division
P.O. Box 11 049
CEP 05422-970
São Paulo, SP, Brazil
(55)(11)8169288

Sizue O. Rogero
Radiobiology Division
P.O. Box 11 049
CEP 05422-970
São Paulo, SP, Brazil
(55)(11)8169229

Lucina J. Arauz
Radiochemistry Division
P.O. Box 11 049
CEP 05422-970
São Paulo, SP, Brazil
(55)(11)8169183

Amilton R. Aguiar
Radiochemistry Division
P.O. Box 11 049
CEP 05422-970
São Paulo, SP, Brazil
(55)(11)8169174

ABSTRACT

The purpose of this work was to obtain normal range values for trace elements in head hair, fingernails, lungs and bones from adult and healthy individuals residing in São Paulo, Brazil. The analytical method applied in these analyses was the instrumental neutron activation analysis and the preparation procedures of these biological tissues for the analyses are described here. Normal concentrations of trace elements obtained were compared to the values reported in the literature.

I. INTRODUCTION

There is considerable interest in analyzing trace elements in biological tissues for a variety of reasons, including their roles in health, nutrition and environmental pollution

Analysis of trace elements in human hair has become popular due to its property of retaining trace elements and to the ease for sample collections. Hair tissue also presents higher concentrations of elements than body fluids like blood and urine, and this led to the use of hair in clinical studies; it has been recognized as an indicator of exposure to many metals.[1]

Fingernail clippings have been analyzed to study the fibrosis cystic disease and to evaluate the nutritional status of individuals. For example, at the Missouri University Research Reactor Center, USA, human nails have been analyzed in order to test hypotheses formulated around dietary intake of certain nutrients, or lack thereof, and disease outcomes.[2] Also the Zn levels found in nail samples from hypertensive and total hypertensive individuals and patients suffering coronary heart diseases have shown to be higher than those obtained from control group.[3] The multivariate statistical treatment of Ca, Cl, K, Mg, Se and Zn results obtained in nail tissues has shown that esophageal cancer patients are distinct from normal individuals.[4] Nail samples are very stable and present advantages for analyses. They can be easily collected and stored, and due to their high composition of keratin, nails can accumulate a number of elements in very high concentrations.

Lung samples have been analyzed in order to study lung diseases. Epidemiological studies[5] and the review of several papers[6] have also shown that various inhaled particles or gases are capable of producing harmful health effects to the population. Therefore lungs are considered to be a critical organ.

Human bone samples have been analyzed in order to examine normal bone metabolism, composition and bone diseases. The control of the mineral content of bones can be a good indicator to detect different diseases, as for instance the osteoporosis. Osteoporosis is one of the primary metabolic and degenerative bone diseases characterized by low mineral bone mass despite the remaining bone being normal. Also analyses of trace elements in bones are very scarce because they are biological samples that present difficulties for obtaining a representative specimen. In the case of humans, collections of samples generally present problems because of medico-legal implications.

The first step to be considered in the trace element analyses of all these biological tissues is to establish their normal values by analyzing specimens collected from healthy people. Several papers have published the baseline elemental concentrations for distinct populations of the world, since differences in local factors of the environment, dietary habits, social class, race, age, sex, etc, can be reflected in the elemental concentrations of human tissues .

In this work, the normal concentration values for trace elements were obtained in samples of head hair, fingernails, lungs and bones collected from adult and healthy individuals of control groups residing in São Paulo, Brazil. For the Brazilian population, these values are not available for many elements.

Normal concentrations of trace elements in hair and nail samples have been evaluated in order to use these data for routine clinical diagnosis of toxic and essential elements in the body, mainly in the orthomolecular medicine field. Hair analysis is very popular and the clinical testing is based on the comparison of the test with normal ranges also called reference values.

Nail samples have been analyzed in this work in order to study the correlation between trace element concentrations and cystic fibrosis disease.

The elemental composition of the lungs from healthy populations composed of non-smokers was obtained in order to study the effect of tobacco smoke on the lungs.

Concentrations of trace elements in bones (ribs) were evaluated in order to provide a preliminary analysis in normal bones and for further use of these data in the study of bone diseases.

With the increasing knowledge of the role of elements in living systems, several analytical methods have been improved in order to obtain low elemental concentrations present in this kind of materials. The analytical method utilized in our analyses was the instrumental neutron activation analysis. Neutron activation analysis is one of the most attractive and promising methods for determining many biologically interesting elements, due to its non- destructive and multi elemental capability, high sensitivity, precision and accuracy.

II. EXPERIMENTAL

A. Sample Preparation

Precautions were taken during the preparation steps of the samples to avoid contamination and they were carried out inside a class 100 laminar flow hood.

Human head hairs were collected from healthy people (25 males and 10 females) composed mainly of university students residing in São Paulo, Brazil. Strands of hair were cut close to the scalp of the occipital region, and their lengths did not exceed 5 cm. About 500 mg of each sample were collected and placed in polyethylene bags. In the laboratory, hair filaments were cut using a stainless steel scissors in lengths smaller than 2 mm. Each hair sample was placed in a beaker and washed four times using each following solutions: non-ionic detergent 2% Triton X100, acetone and distilled water. Then the hair samples were placed on Whatman filters and dried at room temperature.

The fingernail clippings from twenty healthy individuals were trimmed using stainless steel scissors or nail clippers. These samples were cleaned by stirring with a 2% Triton X100 solution and then, by washing with acetone p.a and distilled water. Samples were placed on filter paper and air dried at room temperature.

Lung samples were collected from autopsies of seven non smoker individuals at the Institute of Forensic Medicine of the São Paulo University. The samples were collected within 6-12h *post morten,* they were stored in liquid nitrogen and then sent to the laboratory. In the laboratory lung tissues were freeze dried, homogenized and sterilized by irradiation in a ^{60}Co source.

Samples of ribs were obtained from autopsies at the Institute of Forensic Medicine of Mogi das Cruzes in the State of São Paulo from six accident victims. The ribs were cut longitudinally with a titanium knife and the marrow was scooped out. The muscle tissue from the cortical surface was removed and the small pieces of cortical bones were washed with distilled water and freeze-dried for analysis.

B. NEUTRON ACTIVATION ANALYSIS

About 30 - 150 mg of biological samples and synthetic standards of elements were encapsulated in polyethylene envelopes and irradiated together in the IEA-R1 swimming pool type reactor. Irradiations of five minutes under a thermal neutron flux of 3.7 10^{11} n cm^{-2} s^{-1} were performed for the determination of Al, Cl, K, Mg, Mn, Na and P and irradiations of one hour under thermal neutron flux of 10^{12} n cm^{-2} s^{-1} for As, Cu, Hg, K and Na determinations. Longer irradiations of 16 hours under neutron flux of 10^{12} n cm^{-2} s^{-1} were for Br, Ca, Cd, Cr, Co, Fe, K, Na, Rb, Sb, Sc, Se, Sr, Th and Zn analyses. The gamma ray activities of irradiated samples and standards were measured using a hyperpure Ge detector with a resolution (FWHM) of 80 keV for 121.97 keV of ^{57}Co photopeak and 1.80 keV for 1332.5 keV of ^{60}Co photopeak, for appropriate decay times. P was analyzed by measuring beta activities of ^{32}P in a Geiger Muller detector. The gamma spectra were processed using the

VISPECT computer program and the elemental concentrations were calculated by the comparative method.

III. RESULTS AND DISCUSSION

Normal concentrations of trace elements found for hair and nail samples from control groups are presented respectively in Table 1 and 2, together with literature values. For some elements, these results exhibit inter subject variability indicating that more data is required to clearly establish normal range values. This variability can be attributed to the variations in nutritional conditions.

Table 1. Elemental concentrations in hair samples (in μg/g, dry weight, unless otherwise indicated

Element	This work		Ref (7)
	$X_G \times\div s_G$	Range	
Al	14.0 ×÷ 1.8	1.6 - 37.4	2.9 - 82.5
As (μg/kg)	22.2 ×÷ 1.8	6.7 - 126	0.4[a]
Br	2.5 ×÷ 3.1	0.42 - 85.4	
Ca	470 ×÷ 2.1	118 - 1788	204 - 712
Cd (μg/kg)	193 ×÷ 2.6	43.6 - 1220	1.6[a]
Cl	248 ×÷ 2.9	40.7 - 1339	
Co (μg/kg)	32.4 ×÷ 2.5	8.1 - 325	
Cr (μg/kg)	172.9 ×÷ 1.8	68.2 - 753	1030 - 3230
Cu	16.4 ×÷ 2.1	4.0 - 56.1	17 - 67
Fe	14.5 ×÷ 1.5	7.2 - 36.8	21 - 50
Hg	1.05 ×÷ 2.51	0.08 - 4.75	3.0[a]
K	3.76 ×÷ 2.3	0.53 - 25.7	42 - 430
Mg	57.1 ×÷ 2.4	7.7 - 267	29 - 137
Mn	0.39 ×÷ 2.2	0.1 - 2.5	0.62 - 1.97
Na	4.27 ×÷ 1.8	1.50 - 29.7	150 - 350
Sb (μg/kg	30.5 ×÷ 2.9	3.1 - 848	
Sc (μg/kg)	1.92 ×÷ 1.6	1.18 - 5.70	
Se (μg/kg)	351 ×÷ 2.3	9.1 - 869	80 - 640
Zn	159 ×÷ 1.3	106 - 264	104 - 288

$X_G \times\div s_G$ - geometric mean and stardard deviation
a - Normally tolerated limit value.[7]

Table 2. Elemental concentration in fingernails (in μg/g, dry weight, unless otherwise indicated)

Element	This work		Ref (8)
	$X_G \times\div s_G$	Range	
Al	52.1 ×÷2.2	7.6 - 203	130 - 930
As (μg/kg)	73.2 ×÷1.9	36 - 403	200 - 3000
Br	4.4 ×÷4.4	0.73 - 78.2	9
Ca	680 ×÷1.6	257 - 2120	370 - 3400
Cd (μg/kg)	229.2 ×÷2.1	41 - 995	80 - 3400
Cl	536.3 ×÷1.9	121 - 1424	1000 - 3600
Co (μg/kg)	41.3 ×÷2.0	11 - 132	55
Cr (μg/kg)	417 ×÷1.6	197 - 962	5500?
Cu	5.8 ×÷ 2.1	1.7 - 15	18
Fe	20.1 ×÷1.7	11 - 47	27 - 350
K	94.6 ×÷2.5	20 - 278	360 - 2800
Mg	90.6 ×÷ 1.8	40 - 517	16 - 120
Mn (μg/g)	0.47 ×÷2.0	0.15 - 3.17	0.04 - 2.10
Na	340.9 ×÷2.4	36 - 1424	330 - 3000
Se (μg/kg)	694.6 ×÷1.2	473 - 927	750 - 8000
Zn	118.5 ×÷1.2	82 - 193	70 - 300

The comparison of the data indicated that our results of hair and nail analyses are within the range reported in the literature[7,8] for most of elements. The discrepancy obtained for K and Na concentrations in hair and nail samples can be partially attributed to the differences in the washing procedures used by different investigators. In the case of nail samples, the limiting factor in obtaining an accurate elemental composition is believed to be environmental contamination.[9] Therefore a revision of normal range values is of great interest for using these data for diagnostic purposes.

In Table 3 are presented the results obtained for lung tissue and compared with the data published by Vanoeteren at al.[10] It can be seen from this table that some elements exhibit considerable intersubject variability. However, our results are within the range reported in the literature, except for the element Cl.

Results of normal concentrations of elements in cortical bones (ribs) are presented in Table 4 with

published values. For most elements our results are the same magnitude of the published data In the case of Br and Cl analyses in bones, the drying process can be affecting the results. Bone samples are generally calcinated for analysis. Our bone samples were freeze dried because loss of Br and Cl was verified during the calcination process.

Also bone analyses presented difficulties in the determination of several elements by instrumental neutron activation analysis, due to the high activity of ^{32}P and ^{24}Na produced by irradiation. These radionuclides masked the low activities of other radioisotopes formed in the irradiation of bones.

Results obtained in this work confirmed that instrumental neutron activation analysis can contribute to get normal range values for trace elements in biological tissues and, also for routine clinical diagnosis of toxic and essential elements in the body. The quality (precision and accuracy) of the results was also previously controlled by analyzing certified reference materials.

Table 3. Elemental concentrations in lung tissues (in μg/g, dry weight, unless otherwise indicated)

Element	This work		Ref (10)
	$X_G \times\div s_G$	Range	
Br	27.3 ×÷1.5	17 - 46	2 -120
Cl (%)	1.3 ×÷1.3	0.99 - 1.95	0.5 - 1.1
Co (μg/kg)	180 ×÷1.8	120 - 540	
Cr (μg/kg)	1400 ×÷1.8	650 - 3400	10 - 2500
Fe	2015 ×÷1.6	828 - 3181	200 - 2500
K (%)	0.925 ×÷1.2	0.86 - 1.18	0.25 - 1.0
Mn	1.25 ×÷1.24	1.0 - 1.63	0.05 - 1.50
Na (%)	0.98 ×÷1.4	0.63 - 1.65	0.5 - 1.5
Rb	31.3 ×÷1.2	26 - 39	2.5 - 50
Sb (μg/kg)	284 ×÷1.2	200 - 360	10 - 500
Sc (μg/kg)	25 ×÷2.0	14 - 80	0.5 - 35
Se	0.61 ×÷1.2	0.50 - 0.86	0.250 - 25
Th (μg/kg)	32 ×÷1.4	20 - 50	5 - 100
Zn	71 ×÷1.3	58 - 113	5 -150

Table 4. Elemental concentrations in cortical bone (in μg/g, dry weight, unless otherwise indicated)

Element	This work		Ref (11)
	$X_M \pm s_M$	Range	$X_M \pm s_M$
Br	0.83 ± 0.23	0.55 - 1.27	4.1 ± 4.0
Ca (%)	20.5 ± 0.8	19.4 - 21.7	20.0 ± 4.1
Cl	547 ± 183	217 - 716	
Fe	18.7 ± 14.6	3.4 - 44.4	23 ± 11
K	843 ± 200	529 - 1182	
Mg (%)	0.30 ± 0.04	0.23 - 0.37	0.26 ± 0.04
Na (%)	0.46 ± 0.07	0.37 - 0.56	0.54 ± 0.10
P (%)	9.47 ± 1.5	7.4 - 12.1	8.8 ± 2.2
Rb	1.33 ± 0.43	0.84 -2.10	2.1 ± 3.0
Sr	100.4 ± 9.9	84 - 113	62 ± 18
Zn	91.1 ± 14.3	78 - 120	180 ± 44

$X_M \pm s_M$ - Arithmetic mean and standard deviation

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