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TRACE ELEMENT DETERMINATIONS IN BRAIN TISSUES FROM NORMAL AND CLINICALLY DEMENTED INDIVIDUALS

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ABSTRACT

Studies on trace element levels in human brains under normal and pathological conditions have indicated a possible correlation between some trace element concentrations and neurodegenerative diseases. In this study, analysis of brain tissues was carried out to investigate if there are any differences in elemental concentrations between brain tissues from a normal population above 50 years of age presenting Clinical Dementia Rating (CDR) equal to zero (CDR=0) and that cognitively affected population (CDR=3). The tissues were dissected, ground, freeze-dried and then analyzed by instrumental neutron activation analysis. Samples and elemental standards were irradiated in a neutron flux at the IEA-R1 nuclear research reactor for Br, Fe, K, Na, Rb, Se and Zn determinations. The induced gamma ray activities were measured using a hyperpure Ge detector coupled to a gamma ray spectrometer. The one-way ANOVA test ($p < 0.05$) was used to compare the results. All the elements determined in the hippocampus brain region presented differences between the groups presenting CDR=0 and CDR=3. In the case of frontal region only the elements Na, Rb and Zn showed differences between these two groups. These findings proved the correlation between elemental levels present in brain tissues neurodegenerative diseases. Biological standard reference materials SRM 1566b Oyster Tissue and SRM 1577b Bovine Liver analyzed for quality control indicated good accuracy and precision of the results.

1. INTRODUCTION

Brain disorders are among the most serious health problems facing modern society. These disorders become more common with the advancing age and recently the neurodegenerative disease is an important problem of public health with increasing life expectancy of the elderly population.

Various hypotheses have tried to explain the cause of degenerative diseases including neurotoxicity of trace elements [1-3], genetic defects [4], free radical mediated processes [5, 6] defects in the metabolism of membrane processes [7] or a combination of these factors. Among these hypotheses, the toxicity of chemical elements is one that has received much attention in the pathogenesis of Alzheimer disease [8]. Several elements are essential in many

biological reactions for brain development and maintenance of the central nervous system. However some variation in their levels can negatively affect cognitive function. According to Andrasi et al [9], neurochemical and neurophysiological evidence indicates that trace elements markedly affect the metabolism of transmitters in the brain.

Studies have shown that there is a potential relationship between the levels of trace elements in cerebral tissues and neurological disorders but there are still few publications available concerning the elemental composition of this tissue as well as for different brain regions. Progress in understanding the role of elements in nervous system diseases has been hampered by a lack of data.

The aim of this study was to investigate if there are any differences in elemental concentrations between brain tissues from a normal population presenting Clinical Dementia Rating (CDR) equal to zero (CDR=0) and that cognitively affected population (CDR=3)[10].

In this study instrumental neutron activation analysis (INAA) was applied for trace element determinations in brain tissues. This method has been applied in the analysis of brain tissues in several studies [3, 9, 11 - 13]. INAA is known as a highly sensitive, precise and accurate analytical technique and very suitable for trace element determinations in biological tissues due to its multielemental nature and sample treatment without dissolution and cross-contamination.

2. MATERIALS AND METHODS

2.1. Brain Tissue Samples

Brain samples from a population above 50 years of age (mean age of 82 ± 10 years) of both genders were provided by the Brain Bank of the Brazilian Aging Study Group (BBBABSG) of the São Paulo University, Medical School. This study was approved by the Ethics Committee of the Hospital das Clinics of the São Paulo University Medical School. Brains were removed during autopsy within 4-20 hours after death according to the BBBABSG´s protocols described by Grinberg et al [14]. Cognitive status was evaluated through a collateral source using the Clinical Dementia Rating scale (CDR). The CDR scores were evaluated according to the criteria presented by Morris [10]. A CDR of 0 (zero) indicates no cognitive impairment and CDRs of 0.5, 1, 2 and 3 indicate questionable, mild, moderate and severe dementia, respectively. All the individuals included in this study were classified as cognitively normal or with severe dementia according to CDR scale.

The slices of brain tissues were dissected from the hippocampal region and frontal lobe that are among the cerebral regions affected by neuron loss and neurodegenerative diseases [15]. They were isolated using a titanium knife and plastic tools. Care was taken to avoid sample contamination. The brain tissues placed in clean polyethylene bags were kept at - 80 ^{o}C until its transportation to the Neutron Activation Analysis Laboratory of IPEN-CNEN/SP.

The brain tissues of each area were homogenized and freeze-dried for the analysis until its constant weight was obtained. In this process, in the hippocampal tissues, mean weight losses of 80.3 ± 2.6 and 81.8 ± 2.9 % were obtained for the population groups presenting CDR=0 and CDR=3, respectively. In the frontal lobe tissues, mean weight losses of 81.1 ± 1.4 and

81.3 ± 1.9 % were obtained for the group of CDR=3 and CDR=0, respectively. According to Andrasi et al[16], water content changes greatly inside brain.

2.2. Analysis of Brain Samples

2.2.1. Preparation of synthetic standards of elements

Synthetic standards were prepared by pipetting 50 μL of the elemental standard solutions onto sheets of Whatman No. 40 filter paper. These solutions containing one or more elements were prepared using certified standard solutions provided by Spex Certiprep Chemical, USA. All the pippetors and volumetric flasks were calibrated before use. These filter sheets were dried at room temperature inside a desiccator and then placed into clean polyethylene bags and sealed. In these standards the quantities of each element, in μg (in parentheses) were the following: Br(5.0),Fe(350), Na(100.0), K(601.5), Rb(10.0), Se(8.0) and Zn(35.0).

2.2.2. Neutron activation analysis procedure

Aliquots of about 150 mg of brain tissue weighed in polyethylene bags were irradiated in the IEA-R1 nuclear reactor along with the synthetic standards of the elements. Sixteen-hour irradiations in a thermal neutron flux of about 5 x 10^{12} n cm^{-2} s^{-1} were performed for Br, Cu, Fe, K, Na, Rb, Se and Zn determinations. After adequate decay times, the irradiated samples and standards were measured by a Model GX2020 hyperpure Ge detector coupled to Model 1510 Integrated Signal Processor, 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 peak of ^{60}Co. Samples and standards were each measured at least twice for different decay times. Counting times from 200 to 50,000 seconds were used, depending on the half-lives or activities of the radioisotopes considered. The radioisotopes measured were identified according to their half-lives and gamma- ray energies. The concentrations of elements were calculated by a comparative method. Radioisotopes used in analyses were: ^{82}Br, ^{59}Fe, ^{42}K, ^{24}Na, ^{86}Rb, ^{76}Se and ^{65}Zn.

Quality control of analytical results was performed by analyzing certified reference materials (CRMs) NIST 1566b Oyster Tissue and NIST 1577b Bovine Liver provided by the National Institute of Standards and Technology (NIST), USA. Since there are no certified brain tissue reference materials these types of matrices were analyzed. These reference materials were analyzed by applying the same experimental conditions used for brain analyses and were evaluated on a dry weight basis, as recommended in their certificates.

3. RESULTS AND DISCUSSION

3.1. Quality Control of Results

Table 1 presents the results obtained from the analyses of certified reference materials along with their certified values [17, 18]. The results agree with certified values presenting relative errors lower than 7.3 %. They also presented good precision with relative standard deviations varying from 2.4 to 9.1 %. The standardized difference or Z-score values [19] obtained are presented in Table 1 and were |Z-score| < 1, indicating that the results are satisfactory and agree with the certified values.

Table 1: Concentrations of elements obtained in the certified reference materials NIST 1566b Oyster Tissue and NIST 1577b Bovine Liver

Elements	NIST 1566b Oyster Tissue				
	This study				Values of the certificate [17]
	M ±SD[a]	RSD[b], %	Er[c], %	Z-score	
Br	50.9 ± 3.8	7.5	-	-	-
Fe	198.3 ± 5.7	2.9	3.6	-0.84	205.8 ± 6.8
K	6435 ± 318	4.9	1.3	-0.26	6520 ± 90
Na	3277 ± 212	6.5	0.59	-0.89	3297 ± 53
Rb	3.16 ± 0.12	3.9	3.1	-0.53	3.262 ± 0.145
Se	2.03 ± 0.13	6.5	1.4	0.15	2.06 ± 0.15
Zn	1381 ± 33	2.4	3.0	-0.76	1424 ± 46
Elements	NIST 1577b Bovine Liver				
	This study				Values of the certificate [18]
	M ± SD	RSD. %	Er. %	Z score	
Br	10.9 ± 0.7	6.4			(9.7)[d]
Fe	185.1 ± 8.7	4.7	0.60	0.06	184 ± 15
K	10243 ± 681	6.6	3.1	0.45	9940 ± 20
Na	2480 ± 189	7.6	2.5	0.30	2420 ± 60
Rb	12.7 ± 0.6	4.9	7.3	-0.79	13.7 ± 1.1
Se	0.75 ± 0.07	9.1	2.7	0.22	0.73 ± 0.06
Zn	122.7 ± 5.6	4.5	3.4	-0.25	127 ± 16

a. M ± SD = Mean and standard deviation at the least six determinations; b. RSD = Relative standard deviation; c. Er = Relative Error; d = Numbers in parenthesis are informative values.

3.1 Elemental Concentrations in Human Brain Tissues

Comparisons of the element concentrations between two population groups were carried out using the data of Table 2 and by applying one-way ANOVA test ($p < 0.05$). In the columns of Table 2, in the column of each brain region, the results in italic bold indicate that they do not present statistically significant difference. The elements Br, Fe, K, Na, Rb, Se and Zn determined in the hippocampus brain region presented differences between the groups presenting CDR=0 and CDR=3. In the case of frontal region only the elements Na, Rb and Zn showed differences between these two groups. These findings indicate the correlation between elemental levels present in brain tissues neurodegenerative diseases.

According to Hebbrecht et al [20], Fe is involved in brain degenerative process initiated by

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Table 2: Mean element concentrations in brain tissues from hippocampus and frontal regions obtained for population groups presenting CDR=3 and CDR=0. Results are given on dry basis in mg kg^{-1}

CDR (n)[a]	Hippocampus						
	Br	Fe	K	Na	Rb	Se	Zn
3 (37)	3.4 ± 1.6	222 ± 56	9683 ± 1639	10644 ± 3176	14.9 ± 5.9	0.590±0.136	73,6 ± 17,1
0 (35)	2.6 ± 1.3	198 ± 44	11225 ± 1940	7241 ± 1722	21.9 ± 5.3	0.520±0.094	63.2 ± 12.8
CDR (n)	Frontal						
	Br	Fe	K	Na	Rb	Se	Zn
3 (37)	***3.5± 1.6***[b]	***240 ± 39***	***11159 ± 1870***	9749± 2076	14.9± 5.9	***0.610 ± 0.101***	61.1 ± 8.3
0 (35)	***3.0 ± 1.1***	***236 ± 36***	***11782± 1318***	8249 ± 1739	20.1 ± 4.4	***0.643 ± 0.153***	57.3 ± 9.0

a. n is number of individuals

b. In column of each brain region, the results in italic bold indicate that they do not present statistically significant difference ($p<0.05$, 1-way ANOVA)

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oxygen free radicals stimulated by increasing Fe levels in the brain. The excess of oxygen free radicals severely damages cell membranes in the aging brain, as can be derived from the K and Rb concentration decrease [20]. Deibel at al [3] also showed a significant high Zn concentration in the hippocampus, in agreement with our present study. The increase of Zn probably plays a role in senile plaque formation in Alzheimer's diseases [3]. When this comparison is made for the frontal area, our result also showed higher concentration of Zn in the group of CDR=3 than that obtained for CDR=0. In the case of Se there was no significance difference between the normal and demented group for the concentration of this element in the frontal tissue. However the hippocampus of CDR=3 group presented higher concentrations of Se than those of the normal group (CDR=0). It is known that one of the role of Se is in enzyme glutathiose peroxidase that catalyses hydroperoxides and reduces these harmful oxidizing agents in the brain tissue [20]. The increase of Se in the hippocampus of demented group may be due to the body's cellular reaction against oxidative stress.

According to Belavari et al [11], Na levels in every brain part are significantly higher in Alzheimer patients while Rb levels are lower or equal to the control. Our results agree with these authors. We found significantly higher concentrations of Na and lower Rb in hippocampus and frontal tissues in the population group of CDR=3 than those from normal group of CDR=0.

Bromine concentrations showed significant differences between the groups only in the hippocampal tissue.

4. CONCLUSIONS

This study confirmed that neutron activation analysis can be used as an accurate and precise method for human brain analysis. The application of this method for elemental determinations in brain tissues can help physicians to better understand the role of trace elements in cerebral diseases. The findings of this study showed that Fe and Zn found in high concentrations in the hippocampus can be involved in neurodegenerative diseases. Preliminary data obtained for trace element concentrations encourage further analysis of tissues from other areas of brain as well as a large number of samples and for more elements.

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