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Determination of trace elements in human brain tissues using neutron activation analysis

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Neutron activation analysis was applied to assess trace element concentrations in brain tissues from normal ($n = 21$) and demented individuals ($n = 21$) of both genders aged more than 50 years. Concentrations of the elements Br, Fe, K, Na, Rb, Se and Zn were determined. Comparisons were made between the results obtained for the hippocampus and frontal cortex tissues, as well as, those obtained in brains of normal and demented individuals. Certified reference materials, NIST 1566b Oyster Tissue and NIST 1577b Bovine Liver were analyzed for quality of the analytical results.

Introduction

During the last two decades, many attempts have been made to investigate the correlation between trace element concentrations in human tissues and different kinds of diseases. Analyses of cerebral tissues have also been performed in order to elucidate about neurodegenerative diseases resulting from the gradual and progressive loss of neural cells which leads to the dysfunction of the nervous system. Trace elements may influence in the cognitive functions as they are involved in metabolic processes and redox reactions.[1]

Several studies have been presented concerning the effect of trace elements in neurodegenerative diseases. DEIBEL et al.[2] reported about imbalances in levels of Cu, Fe and Zn in brains tissues that may increase oxidative stress and enhancing neurodegeneration in Alzheimer's disease. MAYNARD et al.[3] presented a review of metals that play a role in the pathogenesis of Alzheimer's disease and discussed about metal brain homeostasis factor in beta amyloid accumulation and amyloid formation. BUSH[4] suggested that Alzheimer's disease is caused by abnormal interactions of amyloid beta with cortical metal ions even though this protein may also participate in normal metal homeostasis. On the other hand, according to KONOHA et al.[5] zinc plays an important role in normal functions since its deficiency impairs brain development and capacities of learning and memory. However, zinc can influence the homeostasis of other elements and accelerate the aggregation of amyloid beta and contributes to the pathogenesis of Alzheimer's disease.

Despite intensive research efforts on neurodegenerative diseases the effect of trace elements in cognitive functions is not well known. In this context, the determination of reliable data of trace elements in cerebral tissues can contribute to the understanding on the role of elements in the researches related to cerebral diseases.

The objective of this initial study was to evaluate the differences if any, in elemental concentrations of the hippocampus and frontal cortex areas of the brain and to compare results obtained in brain tissue analysis of normal individuals and those with severe cognitive decline individuals.

Experimental

Brain tissues and treatment for the analysis

Brain samples of an over 50 year old population of both genders were provided from the Brain Bank of the Brazilian Aging Study Group (BBBABSG). Brains were removed at autopsy within 4–20 hours after death according to the BBBABSG´s protocols described by GRINBERG et al.[6] Severity of the cognitive impairment was assessed with the Clinical Dementia Rating scale (CDR). The CDR scores were evaluated through a collateral source according to the criteria presented by MORRIS.[7] 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. Brain samples of two groups of individuals of CDRs 0 (normal) and 3 (demented) were selected for the analyses. The slices of brain tissues were dissected from the hippocampus and frontal cortex of the left hemisphere. Hippocampus and frontal cortex are among the cerebral regions affected by neuron loss and neurodegenerative diseases.[8] 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 °C until its transportation to the Neutron Activation Analysis Laboratory of IPEN-CNEN/SP. The brain tissues of

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each area were homogenized and freeze-dried for the analysis until its constant weight was obtained. In this process, mean weight loss of 81.1±2.7% was obtained considering all samples analyzed. However in the hippocampus tissue we found slightly higher weight loss for demented individuals ($p = 0.05$). Mean loss of 82.1±3.2% was obtained for the case of demented individuals and of 80.1±1.6 for normal group. According to ANDRASI et al.,[9] water content changes greatly inside the brain. A significantly high content of water has been also found in some Alzheimer brain parts.[10]

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 pipetors 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).

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 with a thermal neutron flux of about $5 \cdot 10^{12}$ 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 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 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.

Results and discussion

Table 1 presents the results obtained from the analyses of certified reference materials along with their certified values.[11,12] The results agree with certified values presenting relative errors lower than 10.0%. They also presented good precision with relative standard deviations varying from 0.1 to 10.7%. The standardized difference or *Z*-score values[13] obtained are presented in Table 1 and were $|Z\text{-score}|<1$, indicating that the results are satisfactory and agree with the certified values.

Tables 2 and 3 show the results obtained in brain tissues from normal and demented individuals, respectively. Concentrations values in these tables are given for dry weight. Comparisons made between the results for hippocampus and frontal tissues of the normal group (Table 2) indicated significant difference for Se (*t*-test, $p = 0.05$). Higher Se concentrations (617±131 μg·kg^{-1}) were obtained in frontal cortex area than in hippocampus (529±92 μg·kg^{-1}). In these case of demented individuals, brain tissues from frontal cortex area presented higher K concentrations, but for Zn were lower than those found in hippocampus region. Differences in the elemental concentrations between hemispheres and several compartments of brain have been also reported.[14,15] Concentrations of Br, Fe, Na, Rb presented no significant difference between the two brain parts analyzed in this study for demented and normal groups.

Table 1. Elemental concentrations of in certified reference materials NIST 1566b Oyster Tissue and NIST 1577b Bovine Liver (in mg·kg^{-1})

Element	NIST 1566b Oyster Tissue				
	This work				Values of certificate[11]
	Mean ± SD	RSD, %	Er, %	Z_{score}	
Br	50.9 ± 3.8	7.5	–	–	–
Fe	196.4 ± 7.1	3.6	4.6	–0.97	205.8 ± 6.8
K	6428 ± 346	5.4	1.4	–0.26	6520 ± 90
Na	3164 ± 146	4.6	4.0	–0.86	3297 ± 53
Rb	3.151 ± 0.132	4.2	3.4	–0.56	3.262 ± 0.145
Se	2.08 ± 0.02	0.1	0.1	0.13	2.06 ± 0.15
Zn	1373 ± 41	3.0	3.6	–0.83	1424 ± 46
Element	NIST 1577b Bovine Liver				
	This work				Values of certificate[12]
	Mean ± SD	RSD, %	Er, %	Z_{score}	
Br	10.9 ± 0.7	6.4			(9.7)
Fe	181.7 ± 8.1	4.5	1.3	–0.13	184 ± 15
K	10151 ± 1084	10.7	2.1	0.19	9940 ± 20
Na	2257 ± 185	8.2	6.7	–0.84	2420 ± 60
Rb	12.30 ± 0.91	7.4	10.0	–0.97	13.7 ± 1.1
Se	0.749 ± 0.0315	4.1	2.6	0.28	0.73 ± 0.06
Zn	120.7 ± 6.6	5.5	5.0	–0.36	127 ± 16

Mean ± SD = Arithmetic mean and standard deviation.
RSD = Relative standard deviation.
Er = Relative error.

Table 2. Elemental concentrations for hippocampus and frontal cortex of normal individuals

Element	Hippocampus[a]			Frontal cortex[a]		
	Mean ± SD[b]	Median	Range	Mean ± SD	Median	Range
Br, mg·kg^{-1}	2.80 ± 1.30	2.55	1.39–6.03	3.19 ± 1.38	2.98	1.68–6.55
Fe, mg·kg^{-1}	202.4 ± 38.2[d]	194.0	129.1–315.1	224.0 ± 41.5	221.2	168.7–318.2
K, mg·kg^{-1}	12014 ± 1391[d]	12118	8621–14062	11585 ± 1356	11867	8621–14034
Na, mg·kg^{-1}	7986 ± 1427[d]	7973	5595–10736	8670 ± 1864[e]	8279	5595–15452
Rb, mg·kg^{-1}	21.6 ± 5.5[d]	20.5	10.1–33.2	19.4 ± 5.1[e]	19.4	10.1–32.2
Se, µg·kg^{-1}	529 ± 92[c,d]	537	347–714	617 ± 131[c]	621	347–960
Zn, mg·kg^{-1}	67.2 ± 7.3[d]	66.5	55.8–82.3	62.2 ± 11.0	59.8	46.5–92.1

[a] Brain tissues from individuals aged 51 to 94 years (mean = 79 years).
[b] Arithmetic mean and standard deviation.
[c] Significant difference between hippocampus and frontal cortex of normal individuals (p=0.05).
[d] Significant difference between hippocampus of normal and demented individuals (p=0.05).
[e] Significant difference between frontal cortex of normal and demented individuals (p=0.05).

Table 3. Elemental concentrations for hippocampus and frontal cortex from demented individuals

Element	Hippocampus[a]			Frontal cortex[a]		
	Mean ± SD[b]	Median	Range	Mean ± SD	Median	Range
Br, mg·kg^{-1}	3.25 ± 1.46	2.86	1.54–7.00	3.254 ± 1.46	2.86	1.61–7.78
Fe, mg·kg^{-1}	234.2 ± 58.6[d]	224.1	147.6–365.9	235.7 ± 41.2	229.8	181.7–327.9
K, mg·kg^{-1}	9719 ± 1348[d]	9482	6753–12512	10889 ± 1660	10631	8336–14890
Na, mg·kg^{-1}	11555 ± 2772[d]	11460	7392–18503	10634 ± 1534[e]	10333	7373–13169
Rb, mg·kg^{-1}	14.1 ± 5.9[d]	15.8	1.9–23.7	15.1 ± 6.6[e]	16.4	1.7–32.2
Se, µg·kg^{-1}	603 ± 130[d]	617	303–810	619 ± 91	615	450–849
Zn, mg·kg^{-1}	76.9 ± 16.4[d]	74.9	46.3–120.7	66.8 ± 8.7	66.7	49.4–90.9

[a] Brain tissues from individuals aged 51 to 98 years (mean = 80 years).
[b] Arithmetic mean and standard deviation.
[d] Significant difference between hippocampus of normal and demented individuals (p=0.05).
[e] Significant difference between frontal cortex of normal and demented individuals (p=0.05).

A comparative study based on normal and demented individuals was also carried out using the results of Tables 2 and 3. Significantly higher concentrations for Fe, Na, Se and Zn were found in the hippocampus of demented individuals than those presented by the normal group ($p = 0.05$) in the corresponding brain parts. On the other hand, the hippocampus of the demented group presented lower K and Rb concentrations. According to HEBBRECHT et al.,[16] Fe is involved in brain degenerative process initiated by 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.[16] DEIBEL at al.[2] also showed that 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.[2] When this comparison was made for the frontal cortex area only Na and Rb presented difference between normal and demented individuals. 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 cortex tissue. However, the hippocampus of demented group presented higher concentrations of Se than those of the normal group. 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.[16] 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.,[17] 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 cortex tissues in the demented group than those from normal group ($p = 0.05$).

Bromine concentrations in normal and demented individuals, as well as, in hippocampus and frontal cortex area of corresponding groups were compared. This element showed no significant differences between groups and the two parts of the brain ($p = 0.05$).

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 for a large number of normal and demented individuals and for more elements.

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