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Trace element concentration differences in regions of human brain by INAA

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Abstract Studies have shown that there is a potential relationship between the levels of trace elements in cerebral tissues and neurological disorders. However, there are few publications available on the elemental composition of these tissues as well as for different regions of the brain. The aim of this study was to investigate trace element differences in various regions of the human brain from an elderly population of normal individuals. Brain samples from 31 individuals of both genders, aged 51–95 years were provided by the Brain Bank of the Brazilian Aging Study Group of the São Paulo University, Medical School. The tissues from the regions of the hippocampus, cerebellum and frontal, parietal, temporal, occipital cortex were dissected using a titanium knife, ground, freeze-dried and then analyzed by instrumental neutron activation analysis (INAA). Samples and element standards were irradiated with a neutron flux at the IEA-R1 nuclear research reactor for Br, Fe, K, Na, Rb, Se and Zn determinations. One-way ANOVA test ($p < 0.05$) was used to compare the results which showed significant differences for several elements among the brain regions. Most of our brain analysis results agreed with the literature data. The results were also submitted for brain region classification by cluster analysis.

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Introduction

In Brazil, as in most developing countries, the increase in life expectancy and of the elderly population has led to a greater number of demented individuals or patients with Alzheimer's disease. As a result, the rate of dementia has become one of the most serious problems facing public health. Various hypotheses have tried to explain the etiology of degenerative diseases including neurotoxicity of trace elements [1–3], genetic defects [4], free radical mediated processes [5, 6] and defects in the metabolism of membrane processes [7] or a combination of these factors. Of these hypotheses, trace element toxicity is one that has gained much attention in the pathogenesis of Alzheimer's disease [8]. Several elements are essential in many biological reactions. However, any variation in their levels may negatively influence cognitive functions. Certain essential elements are required in the brain for development and maintenance of the central nervous system. They also play an important role in neurodegenerative disorders. According to Andrasi et al. [9], neurochemical and neurophysiological evidence indicates that trace elements markedly affect the metabolism and transmitters in the brain.

Recently much attention has been given to the role of trace elements in brain degenerative diseases [3, 10–12]. 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

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understanding the role of elements in nervous system diseases has been hampered by a lack of data. Element concentration ranges in different parts of human brains from a normal population have not yet been defined.

The knowledge of element distribution in normal human brains is of interest for attempting to correlate composition with function as well as for determining baseline normal elemental levels to be used for comparison with diseased tissue concentrations. Due to the relatively limited comprehensive studies available for trace elements present in distinct parts of the human brain, we decided to investigate trace elements in the following regions: hippocampus, cerebellum and frontal, parietal, temporal, occipital cortex.

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, 4, 13–15]. 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.

Experimental

Brain samples

Brain samples of an over 50 year old population (mean age of 77.5 ± 8.8; range, 51–95 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 Clinicas of the São Paulo University, Medical School. Brains were removed during autopsy within 4–20 h after death according to the BBBABSG's protocols described by Grinberg et al. [16]. Cognitive status was evaluated through a collateral source using the Clinical Dementia Rating (CDR) scale. The CDR scores were evaluated according to the criteria presented by Morris [17]. 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 31 individuals included in this study were classified as cognitively normal according to CDR scale.

Sample preparation

Brain samples were dissected from the regions of hippocampus, cerebellum, frontal, parietal, temporal and occipital. They were isolated using a titanium knife, plastic tools and using powder free surgical gloves. Special care was taken to avoid sample contamination with metals during handling. Brain tissues stored in clean polyethylene bags were kept at −80 °C until their transportation to the Neutron Activation Analysis Laboratory of IPEN-CNEN/SP. The dissected brain tissues of each area were rinsed using purified water in order to reduce contamination due to cerebrospinal fluid and blood. They were then homogenized and freeze-dried until constant weight was obtained for the analyses.

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 heat sealed. In these standards, the quantities of each element, in μg (in parentheses) were the following: Br(5.0), Fe(350), K(601.5), Na(100.0), Rb(10.0), Se(8.0) and Zn(35.0).

Instrumental neutron activation analysis procedure

Aliquots of about 150 mg of dried brain tissue weighed in polyethylene bags were irradiated in the IEA-R1 nuclear research reactor along with the synthetic standards of the elements. Sixteen-hour irradiations with a thermal neutron flux of about 5×10^{12} n cm^{-2} s^{-1} were performed for Br, 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 1,332 keV gamma-ray of ^{60}Co. Samples and standards were each measured at least twice for different decay times. Counting times from 5,400 to 50,000 s 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 the 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. The results agreed 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 [18] were lower than 1 indicating that the results are satisfactory and they are within the ranges of the certified values at a significance level of 32 %.

Results and discussion

Determination of dry to wet (fresh) weight ratios of samples

The loss in the freeze drying process was determined separately for each sample. According to Andrasi et al. [11], water content varies greatly inside the brain and thus, it was necessary to determine the dry mass content for each sample. Table 1 shows the mean values of loss percentages and dry to wet weight ratios obtained for each brain region. Some literature results of element concentrations in brain tissues are given on wet basis. So, for comparison, our results were converted to wet weight basis using the dry to wet ratio values presented in Table 1.

Results of brain tissue analyses

Since some of our experimental data presented outliers (values that are very different from the data values for the majority of the cases in the data set), the normalized residual method [19] was used to obtain a robust averages with minimum interference of outliers. Table 2 shows the results obtained in different regions of brains from normal individuals. These results are the mean ± standard deviation (in dry weight basis of the tissue). The overall values and interregional and interpersonal variability are also presented in Table 2. Interregional variability was characterized as relative standard deviation (RSD) of mean concentrations representative of different brain areas,

Table 1 Mean values for weight loss and dry to wet weight ratio obtained in the freeze drying process (mean and standard deviation)

Brain regions	Weight loss, %	Dry to wet ratio
Hippocampus	80.6 ± 1.7	0.241 ± 0.027
Frontal	80.99 ± 1.2	0.235 ± 0.019
Temporal	80.95 ± 0.96	0.235 ± 0.015
Occipital	79.78 ± 1.50	0.254 ± 0.023
Parietal	80.41 ± 1.40	0.244 ± 0.022
Cerebellum	81.64 ± 1.17	0.225 ± 0.017

Table 2 Mean element concentrations in different regions of brain from normal individuals. Results are given on dry basis in mg kg^{-1}

Brain regions (*n*)*	Br	Fe	K	Na	Rb	Se	Zn
Hippocampus (31)	2.57 ± 1.16 **a**	199.7 ± 37.6 **b**	11,375 ± 1,644 **b**	7,506 ± 1,317 **b**	22.2 ± 5.2 **b c**	0.553 ± 0.087 **b**	66.0 ± 7.6 **a b**
Frontal (31)	3.08 ± 1.18 **a**	242.0 ± 35.2 **c**	12,225 ± 1,302 **c**	7,964 ± 1,685 **b c**	21.2 ± 4.5 **c**	0.607 ± 0.107 **c**	59.6 ± 8.8 **c**
Temporal (18)	2.91 ± 0.80 **a**	242.1 ± 32.0 **d e**	13,343 ± 1,598 **d**	8,199 ± 1,347 **d a b**	21.6 ± 4.1 **d c**	0.628 ± 0.110 **d c**	59.8 ± 7.8 **d b**
Occipital (18)	2.57 ± 0.69 **a**	254.4 ± 37.4 **f**	12,365 ± 1,239 **f c b**	6,020 ± 1,014 **a f**	21.1 ± 3.7 **f c**	0.665 ± 0.084 **f c**	55.9 ± 9.3 **f c**
Parietal (18)	2.79 ± 1.01 **a**	235.6 ± 38.9 **e f**	12,181 ± 1,022 **e c**	6,410 ± 986 a **e**	23.2 ± 5.0 **e c**	0.61± 0.11 **e c**	56.2 ± 8.2 **e d c**
Cerebellum (18)	3.36 ± 0.69 **a**	222.7 ± 91.4 **a c f e d**	15,873 ± 1,415 **a**	6,297 ± 1,085 **a**	24.5 ± 5.0 **a b e**	0.715 ± 0.100 **a**	64.4 ± 4.9 **a**
Overall value	2.88 ± 0.31	232.8 ± 19.2	12,894 ± 1,589	7,066 ± 938	22.4 ± 1.2	0.629 ± 0.056	60.9 ± 5.3
Regional variability, %	10.7	8.2	12.3	13.3	5.4	8.8	8.7
Interpersonal variability, %	32.0	19.5	10.6	17.5	20.4	15.8	12.8

Results with same small letters in column indicate that they do not present statistically significant differences ($p < 0.05$, 1-way ANOVA)

* *n* indicates number of samples analyzed

Table 3 Arithmetic mean of element concentrations and literature values. All results are given in mg kg^{-1}

Brain region	Element	Dry weight		Wet weight	
		This study	Reported values	This study	Reported values
	Fe	199.7 ± 37.6	240 ± 20 [20] 216 ± 16 [3] 214 [21]	48.2 ± 10.5	
	K	11,375 ± 1,644	12,500 ± 1,500 [20]	2,747 ± 500	
	Na	7,506 ± 1,317	7,986 ± 1,427 [15]	1,813 ± 376	
Hippocampus	Rb	22.3 ± 5.2	21.6 ± 5.5 [15]		
	Se	0.553 ± 0.087	0.529 ± 0.092 [15]	0.134 ± 0.026	
	Zn	66.97 ± 7.61	72.0 ± 4.8 [3] 67.2 ± 7.3 [15] 75 [21] 86 ± 5 [20] 67.41 [23]	15.9 ± 2.6	15.2–14.1 [22]
	Br	3.08 ± 1.18	3.19 ± 1.38 [15]	0.724 ± 0.283	0.331 ± 0.123 [24] 1.23 ± 0.26 [25]
	Fe	242.0 ± 35.2	183–236 [21] 224.0 ± 41.5 [15]	56.89 ± 9.47	54 ± 9 [24] 50 ± 11 [25]
	K	12,225 ± 1,302	12,030–13,810 [26] 11,585 ± 1,356 [15]	2,874 ± 384	2,474 ± 277 [24] 3,000 ± 640 [25]
Frontal	Na	7,965 ± 1,685	9,230–7,930 [26] 8,670 ± 1,864 [15]	1,872 ± 424	1,732 ± 331 [24] 1,480 ± 280 [25]
	Rb	21.24 ± 4.52	10.5–11.7 [26] 19.4 ± 5.1 [15]	4.99 ± 1.14	2.34 ± 0.93 [24] 2.46 ± 0.65 [25]
	Se	0.607 ± 0.107	0.617 ± 0.131 [15]	0.143 ± 0.028	0.128 ± 0.029 [24] 0.20 ± 0.07 [25]
	Zn	59.60 ± 8.83	68–66 [21] 62.2 ± 11.0 [15]	14.01 ± 2.36	12.5 ± 1.8 [24] 13.9–13.0 [22] 10.0 ± 1.7 [25]
	Fe	242.1 ± 32.0	266 ± 7 [3] 271–280 [21]	57.01 ± 8.33	49 ± 9 [24]
Temporal	K	13,343 ± 1,598		3,142 ± 424	2,935 ± 730 [24]
	Zn	59.76 ± 7.79	61.5 ± 1.6 [3] 65–72 [21]	14.07 ± 2.03	13.1–15.2 [22]
	K	12,365 ± 1,239	13,990 ± 1,530 [26]	3,139 ± 425	
Occipital	Na	6,020 ± 1,014	6,820 ± 390 [26]	1,528 ± 293	
	Rb	21.07 ± 3.70	12.2 ± 5.9 [26]	5.35 ± 1.06	
	Fe	235.6 ± 38.9	272 ± 8 [3] 228–266 [21]	62.06 ± 10.68	
	K	12,181 ± 1,022	11,380 ± 3,860 [26]	2,972 ± 364	
Parietal	Na	6,410 ± 986	8,620 ± 3,907 [26]	1,469 ± 280	
	Rb	23.15 ± 4.96	13.4 ± 5.6 [26]	5.65 ± 1.31	
	Zn	56.20 ± 13.71	56.7 ± 1.2 [3] 64–68 [21]	13.71 ± 2.34	14.7–12.2 [22]
	Fe	222.7 ± 50.13	297 ± 28 [3] 266 [21]	50.13 ± 20.94	
Cerebellum	Zn	64.36 ± 4.92	67.7 ± 2.1 [3] 70 [21]	14.49 ± 1.58	

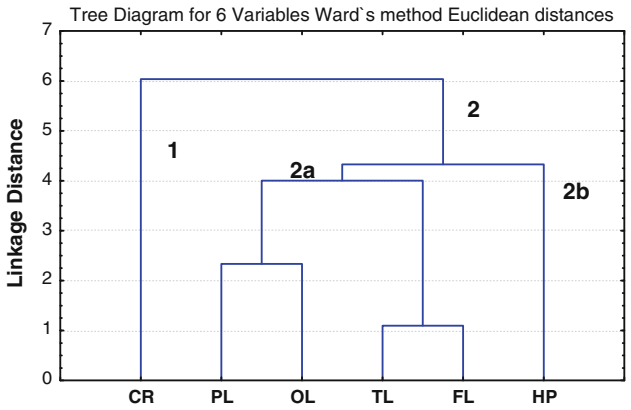

Fig. 1 Dendrogram obtained in cluster analysis for different brain regions. *CR* cerebellum, *PL* parietal, *OL* occipital, *TL* temporal, *FL* frontal, *HP* hippocampus

while interpersonal variability was calculated as the mean of RSDs representative of different individuals. These variations depended on the element. Table 2 indicates that Rb is distributed more homogeneously in the brain than other elements. The more heterogeneously distributed elements are K and Na. Interpersonal variability results demonstrate the following sequence for the elements: Br > Rb > Fe > Na > Se > Zn > K.

Comparisons of the element concentrations from different brain regions were carried out by applying one-way ANOVA test ($p < 0.05$). In the columns of Table 2, results followed with same small letters indicate that this element concentration does not present significant difference between brain regions. There were no significant differences between Br concentrations found in different regions of brain. Se concentrations from hippocampus and cerebellum tissues presented significant differences when compared with those found in tissues from other brain regions. Element K was found at the highest concentration in brains varying from 1.14 to 1.58 %. Results of K found in cerebellum and hippocampus region tissues also showed significant differences when compared with those found in other regions. As can be seen in Table 2, these comparisons showed significant differences of trace element concentrations among distinct regions of the brain.

We compared our results with literature values available (Table 3) and they are in good agreement in many cases. Unfortunately, literature data for some elements determined in this study are not reported and comparisons with our results were not possible.

Hierarchical cluster analysis was applied to group the brain regions into cluster of similarities. The method of squared Euclidian Distance was utilized to determine proximities within brain regions as clusters with relatively homogeneity. The results of this analysis are illustrated with the dendrogram presented in Fig. 1. The obtained dendrogram revealed two main groups or clusters (1 and 2). The cerebellum region formed group 1 separated from the other brain regions. Group 2 is formed by subgroups (2a) and (2b). It is interesting to note that the regions of the cortex (parietal, occipital, temporal and frontal) are in the same subgroup (2a). The hippocampus formed subgroup (2b). These results suggest relationship between element profile of a brain region and its function or morphology. According to Duflow et al. [27] the brain structures with a same physiological function or morphologically similar regions conglomerate in one same subgroup.

Conclusions

Concentrations of Br, Fe, K, Na, Rb, Se and Zn were determined by instrumental neutron activation analysis in six brain regions of normal individuals aged from 51 to 95 years old. These determinations for different brain regions from normal individuals can contribute to the study of elements involved in neurodegenerative disorders. Furthermore, it was verified that very little data of brain element concentrations are available for normal elderly populations. This study showed that the distribution of elements in normal brains is heterogeneous for the elements determined in this study. These findings demonstrate the importance in selecting a specific brain region to study the effect of the trace element composition on neurological disorders.

Comparisons of our results with reported data for normal individuals showed good agreement for most of elements. Some elements showed wide interregional and interpersonal variability in concentration. The cluster analysis using element concentration data grouped the brain regions in different clusters probably due to their distinct functional activities or morphology.

Our results encourage us to continue research to obtain additional data, which can be used as baseline values for normal brains and to further understand the effect of element concentrations in cerebral tissues in neurodegenerative disorders.

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