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Instrumental neutron activation analysis, a technique for measurement of Se, Hg, Fe, Zn, K, Mn, Br, and the Hg:Se ratio in brain tissue samples with results from the Memory and Aging Project (MAP)

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Abstract

An INAA method for measurement of Se, Hg, Fe, Cr, Zn, Mn, K, and Br in autopsy cerebellum, anterior putamen, white matter, mid-frontal cortex, and inferior temporal lobe. Se, Hg, Fe, Cr, and Zn were measured autopsy samples collected from participants of the Memory and Aging Project. The first study examined the association between seafood consumption, brain Hg and Se, Apolipoprotein E (APOE-ε4) status, and brain neuropathology. Following the initial study, the samples were archived. A subsequent method was developed to measure Mn, K, and Br in the archived brain tissue samples.

Introduction

The causes of neurodegenerative diseases are complex and multifactorial. Oxidative stress has been widely studied as a factor in the progression of neurodegenerative diseases [1]. Disruption of trace element homeostasis is observed in the pathogenesis of progressive neurodegenerative diseases, including Alzheimer's (AD), Parkinson (PD) and Lewy Body (LB) diseases [2, 3]. Instrumental neutron activation analysis (INAA) is a useful analytical technique to measure trace elements in autopsy brain samples to study neurological diseases [4–6].

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In this study we report on an INAA method to measure trace elements in autopsy brain samples from the Rush Memory and Aging Project (MAP), an epidemiological neuropathological study involving resident of Chicago, IL [7]. The primary aim of the original study was to determine the association between AD and the levels of Hg, Se, omega-3 fatty acids in brain tissues, and the Apolipoprotein E (APOE-ε4 gene) [8]. The method to measure Se and Hg in brain tissue samples was adapted from work published by Ehmann et al. [4], After the initial study was completed, the brain tissue samples were archived for 4 years. This work describes development and optimization of an INAA method to measure Mn, K, and, Br in the same brain samples already analyzed for Se and Hg. This work highlights the non-destructive advantages of INAA over conventional multi-element techniques such as ICP-MS by allowing re-analysis of archived biological specimens.

The association of Mn, K, and, Br in brain tissue samples with neurodegenerative diseases has not been well studied. Mn is an essential trace element that is a component of the metalloenzyme manganese superoxide dismutase (MnSOD) that works in concert with copper and zinc containing superoxide dismutase (Cu/ZnSOD) to remove the radical super oxide species $O_2^{\cdot-}$ [1]. Overexposure to

Mn causes a parkinsonian-like disease [9]. The element K is regulated by the cellular pump enzyme Na^+/K^+-ATPase. This key enzyme pumps regulates intracellular Na^+ and K^+ concentrations, preventing neuronal excitotoxicity [10]. Depressed Na^+/K^+-ATPase activity and increased levels of cerebellar K^+ have been reported in brain samples of patients with AD [11]. The element Br does not have a known physiological role; however, Br is reported to induce reactive species in the brain such as atomic Br, HOBr, Br_2, and BrCl [3]. Its widespread presence in the environment, from its use in polybrominated diphenyl ethers (PBDEs) in flame retardant materials, is consequently a cause for concern [12].

Experimental

Instrumentation

Samples were irradiated using in the graphite reflector region of the 10 MW research reactor at the University of Missouri-Columbia (MURR). Gamma ray spectroscopy measurements were made using 3 Canberra HPGe detectors with matched relative efficiencies of 37–40% and FWHM of 1.72–1.85 keV at 1.33 MeV. Samples with dead times greater than 20% were recounted.

Materials

Samples and standards were encapsulated in high purity Suprasil™ quartz tubes (ID = 6 mm) purchased from Heraeus (Kleinsotheim, Germany) and precleaned with aqua regia. NIST 1577 bovine liver, NIST 1571 orchard leaves, DOLT-4 dogfish liver, and NCS DC 73347 hair were used as quality control materials. Comparator standards were prepared from commercial ICP-MS standards (High Purity Standards, Charleston, SC). Comparator standards, with the exception of Au and Br, were prepared by freeze drying with centrifugation then sealed. The Au and Br standards were pipetted into the quartz (prepared in 1% v/v NaOH) and sealed without freeze drying.

Brain tissue sample preparation

Autopsied brains analyzed in this work were from 10 deceased participants of the MAP. Five brain regions were analyzed; the cerebellum, anterior putamen, white matter, mid-frontal cortex, and inferior temporal lobe regions. The mean age at death for the sample was 91.5 years. Among the 10 brain cases, 5 had neuropathological-defined AD according to the National Institute on Aging/Reagan Institute of the Alzheimer Association Consensus recommendation for the post mortem diagnosis of Alzheimer's

disease [13]. The brain tissue collection protocol used in MAP cohort is to loosely wrap tissue in a phosphate-buffered saline (PBS) dampened towel to prevent adherence to the container during transport. The brains were then hemisected and one half of each brain was selected for freezing without further wash or rinse. The hemispheres were rapidly frozen and stored in a − 80 °C freezer. The samples selected for this study were dissected using a ceramic blade.

At the MURR, tissue samples were loaded into precleaned quartz vials using acid rinsed PTFE tools. Of the 50 samples (10 brains and 5 regions per brain) the 5 with the highest mass were spilt and analyzed as duplicate pairs. Samples were lyophilized until a stable mass was archived, approximately 38 h. A wet and dry mass was recorded for each sample.

Determination of the mass fraction by INAA

The mass fraction of an element determined by standard comparator INAA is described by:

$$C_x = \left(C_z \cdot \frac{A_{0x}}{A_{0z}} \cdot R_\theta \cdot R_\varphi \cdot R_\sigma \cdot R_{\text{eff}} - B \right) \cdot \frac{m_z}{w \cdot m_x} \qquad (1)$$

where C_x is the mass fraction of the unknown, m_x is the mass of the sample, C_z is the mass fraction of the primary standard, m_z is the mass of the standard, and w is the mass correction factor (wet to dry ratio). The terms A_{0x} and A_{0z} are the decay corrected counting rate for the standard (z) and the unknown (x). The term B is the mass of analyte in the quartz vial blank. The terms $R_\theta, R_\varphi, R_\sigma$, and R_{eff} are the sample to standard ratio of the isotopic abundance (θ), neutron fluence (φ), cross section (σ), and detector efficiency [14]. The term A_o is:

$$A_0 = \frac{N \cdot \lambda \cdot e^{\lambda \cdot t_d}}{1 - e^{-\lambda \cdot t_c}} \qquad (2)$$

where N is the number of counts in the gamma-ray photo peak, λ is the decay constant, t_d is the decay time, and t_c is the count time.

Se, Hg, Fe, Cr, and Zn INAA measurement

A 'long' INAA method, adapted from Ehmann et al., was used to measure Se, Hg, Fe, Cr, and Zn in autopsy brain samples using a 40 h irradiation, 30 day minimum decay time, and 4 h count time [4]. The sample bundles were rotated during irradiation in a thermal neutron flux of 5.5×10^{13} n cm^{-2} s^{-1}. A sample bundle consisted of 50 samples, 5 duplicate sample, 6 comparator standards, 12 quality control materials, and 6 empty quartz vial. A Co/Al flux wire was used to monitor variation in the axial neutron flux (R_φ). Following irradiation, quartz vials were cleaned

in aqua regia and then loaded onto a sample changer for measurement by HPGe detectors. Quartz vials were rotated during the detector count time to minimize geometry differences. The ^{203}Hg contribution to the peak at 279 keV is determined by subtracting the ^{75}Se contribution using the 136/279 keV and 264/279 keV gamma rays measured in single element Se standards. The 136 keV gamma ray from ^{75}Se was corrected for small (< 1%), direct interferences from ^{181}W and ^{181}Hf. Samples with less than 1000 counts in the corrected ^{203}Hg peak at 279 keV were recounted for 6 h.

Mn and K INAA measurement

Following measurement of Se, Hg, Fe, Cr, and Zn the samples were archived. After 4 years a decision was made to re-measure the samples for Mn, K and Br. An INAA method was developed to measure Mn, K, and Br using the MURR pneumatic tube system. For Mn and K determination, a total of 7 quartz vials were loaded into a single high density polyethylene rabbit. An analysis batch consisted of 3 rabbits irradiated sequentially for 20 s each. Each rabbit contained Mn and K comparator standards, blank quartz vials, samples, and quality control material NIST SRM 1577 bovine liver. The samples were clustered in the rabbit as closely as possible to minimize neutron flux variation in the rabbit. Following irradiation, the samples were decayed for 1 h and counted for 10 min with an HPGe detector. The neutron flux variation was measured across all sample positions by irradiating 13 identical Mn and K standards in a single rabbit. The coefficients of variation (CV) of the measured activities were of 4 and 4%, respectively. Based on this result, a correction was not made for positional flux variation in the rabbit. Br was measured in a separate INAA method with a 15 min pneumatic tube irradiation, 2.5 day decay, and 2 h count time. Br concentrations were measured using Au as a standard comparator. The flux variation and Au/Br ratio was measured in a single using a set of 13 standards containing Au and Br. The measured specific activity of the Au/Br ratio was 0.0218 ± 0.0003.

Results and discussion

The empty quartz vials used for the analytical method blank determination in the original study were used for the subsequent method blank determination of Mn, K, and Br. The quartz vial method blank did not have measurable levels of Se, Hg, Fe, Mn, K, or Br but it did contain Zn and Cr. The samples were corrected for the quartz vial blank (B) using Eq. 1. The sample correction for the Zn in the quartz vials was insignificant compared to the level of Zn in the samples. However, the sample correction for the Cr

in the quartz vials was significant. The average mass of Cr measured in 5 blank quartz vials was 0.7 ± 0.7 ng and the mass of Cr in the samples ranged from 1 ng to 60 ng. The LODs for Se, Hg, Cr, Fe, and Zn were 0.03, 0.02, 0.06, 3, and 0.2 $\mu g \ g^{-1}$, respectively.

The challenge to develop the INAA method for K and Mn was measurement of ^{56}Mn (half-life = 2.58 h) over the ^{31}S activity (half-life = 2.62 h) from the quartz vials. Additional neutron capture reactions originating from the quartz vial that add to the total activity include; ^{30}Si(n,α)^{27}Mg, ^{29}Si(n,p)^{29}Al, and ^{28}Si(n,p)^{28}Al. The ^{27}Mg ($t_{1/2}$ = 9.45 min) produces gamma emissions at 843.8 keV which can interfere with the 846.7 keV emission of ^{56}Mn. The irradiation and decay times for Mn and K were optimized using NIST SRM 1577 bovine liver sealed in quartz vials. Irradiation times of 20, 30, and 60 s and decay times of 1–6 h were evaluated and resulted in Mn levels that were in reasonable agreement (10.2, 9.7 and 10.7 $\mu g \ g^{-1}$) with the certified value of 10.3 ± 1 $\mu g \ g^{-1}$. A 20 s irradiation time and 1 -3 h decay time was chosen to limit production of ^{31}Si and allow short live radionuclides to decay. The LODs for Mn and K were 0.03 and 103.5 $\mu g \ g^{-1}$, respectively. The Br was measured in the samples at least 60 days after the Mn and K measurements were completed. The Br LOD was 0.06 $\mu g \ g^{-1}$.

The combined results of Se, Hg, Cr, Fe, Zn, Mn, K, and Br measured in the quality control materials are reported in Table 1. With the exception of Cr in NIST 1577 bovine liver and Cr in NCS DC 73347 human hair, the trace elements measured in the quality control materials are in good agreement with the certified values. The Cr levels in NIST 1571 orchard leaves and DOLT-4 dogfish liver were in good agreement with the accepted values. The discrepancy observed in the Cr measured in NIST 1577 bovine liver likely reflects that the value is near the method LOD. The discrepancy measured for Cr in NCS DC 73347 is not understood.

The 25th, 50th, and 75th percentile of Se, Hg, Cr, Fe, Zn, Mn, K, and Br levels in wet weight measured in anterior putamen (n = 10), cerebellum (n = 10), mid-frontal lobe (n = 10), inferior temporal (n = 10) and white matter (n = 10) are reported in Table 2. A wet to dry ratio was measured for each sample. The mean wet to dry ratios for anterior putamen, cerebellum, mid-frontal lobe inferior temporal, and white matter were 5.3 ± 1.8, 6.0 ± 0.5, 4.5 ± 1.2, 4.9 ± 0.8 and 2.6 ± 1.2, respectively.

The intra-sample variation of the brain tissues was measured by preparation of 5 duplicate samples. The duplicates included 2 inferior temporal, 2 anterior putamen, and 1 mid frontal lobe sample and the results are summarized in supplemental data Table 2. The CVs for elements in the duplicate samples are often higher than the standard deviation of the replicates reported in Table 1 suggesting

Table 1 The concentration of Se, Hg, Cr, Fe, Zn, Se, Mn, K, and Br measured in the SRMs

	NIST SRM 1577 bovine liver		NIST SRM 1571 orchard leaves		NCS DC 73347 hair		DOLT-4 dogfish liver	
	Measured value	Accepted value	Measured value	Accepted value	Measured value	Accepted value	Measured value	Accepted value
Hg	0.0156 ± 0.006	0.016 ± 0.02	0.126 ± 0.004	0.155 ± 0.015	0.42 ± 0.01	0.36 ± 0.08	2.8 ± 0.1	2.58 ± 0.22
Cr	0.053 ± 0.04	0.088 ± 0.012	2.22 ± 0.16	2.6 ± 0.3	0.18 ± 0.03	0.37 ± 0.06	1.4 ± 0.4	1.4*
Fe	257 ± 9	268 ± 8	258 ± 16	300 ± 20	49 ± 2	54 ± 10	1800 ± 100	1833 ± 75
Zn	137 ± 1	130 ± 13	24.1 ± 0.1	25 ± 3	182 ± 4	190 ± 9	118 ± 2	116 ± 6
Se	1.18 ± 0.001	1.1 ± 0.1	0.11 ± 0.04	0.08 ± 0.02	0.62 ± 0.04	0.60 ± 0.04	8.8 ± 0.1	8.3 ± 1.3
Mn	10.2 ± 0.3	10.3 ± 1						
K	1.02 ± 0.05 wt%	0.97 ± 0.06 wt%						
Br					0.40 ± 0.03	0.36*		

All concentration values are reported in µg g^{-1} except K which is reported in wt%. The uncertainty is the standard deviation of replicate measurements. For Se, Hg, Cr, Fe, and Zn ($n = 3$). For Mn, K, and Br ($n = 10$). Blank values were not measured

*Informational value

Table 2 Dry weight concentrations of trace elements measured in anterior putamen (AP), cerebellum (CB), mid-frontal lobe (MFL), inferior temporal (IT) and white matter (WM)

	Percentile	Se (µg g^{-1})	Hg (µg g^{-1})	Hg:Se (molar ratio)	Cr (µg g^{-1})	Fe (µg g^{-1})	Zn (µg g^{-1})	Mn (µg g^{-1})	K (wt%)	Br (µg g^{-1})
AP	25%	1.18	0.014	0.006	0.15	687	58	2.5	1.3	4.3
	50%	1.28	0.027	0.008	0.34	723	72	3.0	1.8	5.7
	75%	1.39	0.034	0.011	0.47	1052	74	3.2	2.0	12.4
CB	25%	1.10	0.035	0.013	0.27	198	72	2.1	1.4	3.3
	50%	1.16	0.058	0.020	0.61	230	75	2.2	1.6	4.9
	75%	1.23	0.095	0.035	1.17	317	79	2.4	1.8	15.5
IT	25%	0.9	0.016	0.006	0.33	202	74	1.2	1.4	2.7
	50%	1.1	0.032	0.013	0.56	231	81	1.3	1.5	3.9
	75%	1.3	0.061	0.020	0.73	259	90	1.6	1.6	10.7
MFL	25%	0.8	0.022	0.009	0.29	216	56	1.3	1.1	3.0
	50%	1.1	0.032	0.011	0.45	227	61	1.4	1.3	5.1
	75%	1.3	0.040	0.016	0.70	251	72	1.6	1.4	10.5
WM	25%	0.4	0.005	0.007	0.12	102	17	0.6	0.5	1.8
	50%	0.5	0.011	0.010	0.13	120	21	0.7	0.7	2.3
	75%	0.5	0.018	0.013	0.23	151	26	1.1	0.9	4.1

that biological variation of the element is higher than the analytical uncertainty. The levels of Se, Hg, Cr, Fe, Zn, Mn, K, and Br reported in Table 2 are within the broad ranges reported by Ehmann et al. [4]. Similar concentrations have been reported for Se, Hg, Fe, Zn, K, and Br in the amygdala, piriform cortex, and, olefactory bulb [15]. In two studies of people from Sao Paulo, Brazil the dry weight K levels in the hippocampus and frontal cortex ranged from 0.37 to 14.9 wt% and the Br levels ranged from 1.39 to 6.55 µg g^{-1} [16]. In another study of the same Brazilian population, dry weight K levels ranged from 11.4 to 15.9 wt% and Br levels ranged from 2.57 to 3.66 µg g^{-1} in tissue collected from the hippocampus, cerebellum, frontal, parietal, temporal, and occipital cortex [17]. In brain samples from the MRC Alzheimer's Disease Brain Bank in London, the wet weight Br and K levels in normal brain were 1.23 ± 0.26 µg g^{-1} and 0.33 ± 0.06 wt%, respectively [18]. The Br and K levels in AD cases were 1.52 ± 0.39 µg g^{-1} and 0.21 ± 0.06 wt%, respectively. In the present study, the K and Br concentrations reported in Table 2 and supplementary data table ST1 were consistent

with levels reported in Sao Paulo, Brazil and London, England.

The Hg to Se molar ratio is reported in Table 2. There are few studies in the literature that report the Hg:Se molar ratio in autopsy brains samples. Methylmercury (MeHg) is a neurotoxin that is transported through the blood brain barrier while Se is a known antagonist of MeHg toxicity. One hypothesis is that optimal Se levels result in increased antioxidant capacity, reducing the toxicity of MeHg [19]. Another hypothesis is that MeHg irreversibly binds with selenoenzymes leading to localized deficiencies in selenoenzyme activity if Se levels are low [20, 21]. In an autopsy study examining mercury miners and non-exposed controls The Hg:Se molar ratio in cerebellum from the miners was 0.33 ± 0.21 and from the controls was 0.019 ± 0.015 [22]. In this study, the median Hg:Se molar ratio of 0.02 in cerebellum is consistent with non-occupationally exposed controls in a previous study [22].

The INAA method for brain tissue compares favorably with convention multi-element analysis using ICP-MS. In one ICP-MS analysis of brain tissue with microwave digestion pretreatment the authors report LOD values for Hg, Fe, Zn, and Mn of 0.006, 2, 0.006, and 0.008 $\mu g\ g^{-1}$ [23]. The LODs for the INAA methods used in this study were sufficient to measure Hg, Fe, Zn, and Mn in every sample. The elements Se and Cr are challenging to measure by ICP-MS because of formation of the isobaric interferences Ar_2^+ and ArC^+ polyatomic species in the Ar plasma. The INAA method reported in this paper is particularly valuable for measurement of Br in tissue samples. The typical sample pretreatment for ICP-MS analysis is acid digestion at high temperatures. Under these conditions, Br is volatilized and lost from the sample.

The methods described in this paper have been used to re-analyze 692 samples from the MAP study to measure Mn, K, and Br. These results will be combined with other participant data including clinical diagnosis of dementia and AD, pathological featrues of dementia, AD, and parkinsons disease, dietary information, occupation, and genetic testing. The combined results have be used to study the association of Mn, K, and Br with dementia, AD, or Parkinsons disease in the MAP study.

Conclusion

This work highlights several advantages of INAA for biological specimens in epidemiology studies. The non-destructive INAA technique affords the possibility of reanalyzing archived biological specimens for additional trace elements. In this work, a high throughput INAA method for measurement of Se, Hg, Cr, Fe, Zn, S, Mn, K and Br in human brain tissue samples is reported. The accuracy of the method was demonstrated by analysis of standard reference materials. A set of 50 brain tissue samples from 10 individuals in the MAP study were analyzed. The levels of trace elements in these tissues fall within the expected range reported in the literature. The unique advantages of INAA allowed re-analysis of the invaluable MAP study samples four years after the original measurement of Se, Hg, Fe, Zn, and Cr. INAA is still a relaven analytical tool for biological samples, particularly for Se, Hg, Br and other elements which are challenging to measure by conventional multi-element analysis methods.

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