Analysis of Trace Elements in the Central Nerve Tissues with Inductively Coupled Plasma-Mass Spectrometry

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EJIMA, A., WATANABE, C., KOYAMA, H. and SATOH, H. *Analysis of Trace Elements in the Central Nerve Tissues with Inductively Coupled Plasma-Mass Spectrometry*. Tohoku J. Exp. Med., 1996, **178** (1), 1-10 — To investigate the roles of various elements in neurological disorders, multi-element analysis of limited quantities of samples in the central nervous system is required. Inductively coupled plasma-mass spectrometry (ICP-MS) analysis suits this requirement, but spectral and non-spectral interferences are inevitable. We studied correction methods for the non-spectral interferences by analyzing signals of 21 elements in various concentrations of HNO₃ as well as 5 major elements (Na, K, P, Ca, and Cl). Using internal standards, the interferences caused by the major elements were corrected, but the interferences caused by HNO₃ were impossible to correct for the elements with high ionization potentials. Thus, we decided to use a standard addition method to correct these interferences. The spectral interferences on Mn and Fe rising from HNO₃ or major elements were compared with the signals of samples. Although the interferences on Mn were negligible, those on Fe were considerable and careful blank subtraction were needed. We measured concentrations of elements in the spinal cord of 4 controls and a patient with amyotrophic lateral sclerosis (ALS) using the standard addition method. The concentrations of Mn, Se, Fe, and Zn in the controls were nearly the same as previously reported values, whereas Mn concentrations in the ALS patient were higher. ——— inductively coupled plasma-mass spectrometry; spectral interferences; non-spectral interferences; spinal cord; amyotrophic lateral sclerosis

Deficiencies or excesses of trace elements or the presence of toxic elements in the central nervous system has been reported in some neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS). In ALS, motor neurons selectively degenerate, mainly in the anterior horn and the lateral funiculus of the spinal cord. Then, the quantification of the

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elements that is possibly relate to the pathogenesis of ALS in these regions is important. However, multi-element quantitative analysis have not been reported in the spinal cord divided into these affected regions and the other regions. One of the reasons is that such highly sensitive analytical methods have not been widely used that is enough to quantify many elements in limited quantities of samples available from such selected regions.

Inductively coupled plasma-mass spectrometry (ICP-MS) is a highly sensitive multi-element analytical method, thus, is suitable for analysis of elements in such small samples. However, ICP-MS analysis is seriously affected by matrix interferences such as spectral overlapping (spectral interferences) and matrix-induced signal intensity changes (non-spectral interferences). In the present study, we evaluated the influence of these matrix interferences on a multi-element analysis of the central nerve tissue. Then, we demonstrate the analytical results of the brain and spinal cord tissues with ICP-MS.

Non-spectral interferences

Non-spectral interferences are usually corrected by internal standardization or by the standard addition method. Internal standardization requires less time and sample amounts than does the standard addition method, but it does not always appropriately correct the interferences (Moens et al. 1994). The standard addition method, which is considered to be more accurate and reliable, is used when internal standardization is not appropriate (Beauchemin et al. 1988; Goossens et al. 1993).

In the analysis of elements in wet digested biological tissues, two major interferences were considered. One is the interferences by elements abundant in tissue samples, such as Na, K, P, or Cl. The other is interferences by HNO_3 , which is used for wet digestion although it causes less spectral interferences than HClO_4 or H_2SO_4 does (Tan and Horlick 1986; Beauchemin et al. 1988). The concentration of HNO_3 in each sample after the digestion is various and is difficult to estimate.

The effect of HNO_3 has rarely been concerned about in the analysis of biological samples. From our results of experiments on an Elan 5000 ICP-MS (Perkin-Elmer Sciex, Toronto, Canada), the interferences from HNO_3 were difficult to be corrected by internal standardization. We examined whether interferences from HNO_3 on the 14 target elements (Al, As, Cd, Co, Cu, Fe, Hg, Mg, Mn, Mo, Pb, Se, V, Zn) were corrected by the 7 possible internal standard elements (Eu, Ga, Rh, Sr, Ti, Tl, and Y). The ion counts of the constant concentrations of those elements were measured as the concentrations of HNO_3 (Ultrapur, Cica-MERCK, Kanto Chemical, Tokyo) were varied. As the HNO_3 concentrations increased, the changes in the signals of the most elements including 7 potential internal standards were within 10% between 0.05 M and 0.8 M of HNO_3 compared with the signals at 0.05 M. However, the signals of As, Cd, Hg,

Fig. 1. The effect of HNO₃ concentrations on the signals of ⁷⁵As (×), ¹¹⁴Cd (●), ²⁰²Hg (◇), ⁸²Se (△) and ⁶⁷Zn (▼). HNO₃ concentrations: 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 mol/liter.

Se and Zn, which have high first ionization potentials (8.99–10.44 eV), decreased remarkably as the concentrations of HNO₃ increased (Fig. 1). The HNO₃ concentration in each sample after HNO₃-digestion is difficult to estimate, though it should be less than 0.5 M in our usual way of sample preparation. Therefore, we concluded that measurements of As, Cd, Hg, Se and Zn would not be corrected with the internal standards used in this analysis.

On the other hand, the interferences by major elements have been demonstrated and internal standardization have been used for the correction (Olivares and Houk 1986; Gerotto et al. 1995). In our experiments, the interferences by major elements were also considered to be corrected by appropriate internal standards.

Fig. 2. The effect of the major elements on the signals of ⁶⁷Zn (×), ⁶⁹Ga (—), ⁸⁹Y (+), ⁹⁸Mo (▲), ²⁰⁵Tl (□), and ²⁰⁸Pb (●). The concentrations of major elements were adjusted to simulate fresh tissue diluted to 0.05, 0.1, 0.25, 0.5, 1, 2, 4, and 8% w/v. P, K, Na, Cl, and Ca are at the concentrations of 4.0, 3.0, 1.6, 1.3, and 0.2 mg/g in the fresh tissue, respectively.

We measured the same elements as the experiments above, with various concentrations of major elements. The concentrations of major elements were so adjusted that they simulate diluted biological tissues in the assumption that the concentrations of P, K, Na, Cl, and Ca are 4.0, 3.0, 1.6, 1.3, and 0.2 mg/g fresh tissue, respectively. Fig. 2 shows typical examples of the signal suppression of the target elements and internal standards. For most target elements, the changes in the signals corrected by the internal standards with the similar mass number as the target elements. Although the signal suppression of Al and Mg were not corrected by internal standards with the close mass number analyzed, they were corrected with Rh under the concentrations of major elements corresponding to 1%w/v tissue.

From the above results, for the correction of the non-spectral interferences from HNO_3 , we decided to use the method of standard addition.

Spectral interferences

Most of biologically important elements are between 40 and 80 in mass number, where many polyatomic species containing Ar, H, O or tissue major elements overlap. Thus, spectral interferences are serious problems for some of such elements when they have no isotope free from interferences, and it is difficult to apply a mathematical correction method using the natural abundance of the

Fig. 3. The effect of HNO_3 concentrations on the background signals of (A) m/z 54 and (B) m/z 55.

isotopes (Goosens et al. 1994). Another correction method, a simulated blank subtraction (Vanhoe et al. 1989), is applied when the concentrations of interfering element is known. We examined spectral interferences about Fe and Mn, both are important for the central nervous system.

In the analysis of Fe in the central nerve tissues, spectral interferences were not negligible for all 4 stable isotopes (mass 54, 56, 57, and 58). ^{56}Fe is the most abundant (91.7%) but it is severely interfered with by $^{40}\text{Ar}^{16}\text{O}$ and was impossible to analyze. Thus, we determined the other isotopes by comparing the signals of the blank solution that does not contain Fe (background signals) to those of 100 $\mu\text{g/liter}$ of Fe. This Fe concentration is the lowest level in the sample solution of the central nerve tissue diluted to 1%w/v.

^{54}Fe is interfered with by $^{40}\text{Ar}^{14}\text{N}$ generated from HNO_3 as the concentrations of HNO_3 increased, as shown in Fig. 3A. The contribution of the signals of the background to that of the 100 $\mu\text{g/liter}$ of Fe (60,000 counts/sec) varied from 1% to 50% between 0.05 and 0.5 M of HNO_3 . This background changes dependent on HNO_3 concentrations were too large to correct because the concentration of HNO_3 in each sample after HNO_3 -digestion is difficult to estimate.

^{57}Fe and ^{58}Fe are interfered with by ArO or ArOH . These interfering signals are large (approximately 50% of the ion counts of the those of the 100 $\mu\text{g/liter}$ of Fe), but their noise-to-signal ratios were relatively small ($< 2\%$) when the noise was defined as SD of the background signals in one measurement. Further, their background signals were relatively constant (within 10% compared to the signals in the salt-free 0.1 M HNO_3 solution) even when the concentrations of HNO_3 (0.1–0.8 M) or major element (corresponding to 0–1%w/v tissue) were changed. Thus the background subtraction of 0.1 M HNO_3 for each sample was considered to be practically useful.

In Fe analysis, the background drift with time was sometimes serious, but it was solved by running the blank (0.1 M HNO_3) repeatedly (twice or 3 times per hour) through analysis. To assess the accuracy of this correction, we analyzed bovine liver standard reference material NIST 1577a (National Institute of Standards and Technology, Gaithersburg, MD, USA) by the standard addition method at 15 min and 120 min after the beginning of analysis. The measured concentrations were in the same relative order as those in the central nerve tissues. Their standard addition lines showed good linearity and the analytical results fell within the certified values (Fig. 4). Thus this correction was considered to be accurate. Another report analyzing Fe in the biological samples using ^{57}Fe resulted successfully (Vanhoe et al. 1989). However, care needs to be taken when Fe is analyzed at lower concentrations.

Mn has only one stable isotope, ^{55}Mn , which is potentially interfered with by polyatomic species containing N, K, or Cl ($^{40}\text{Ar}^{15}\text{N}$, $^{40}\text{Ar}^{14}\text{N}^1\text{H}$, $^{39}\text{K}^{16}\text{O}$, and $^{37}\text{Cl}^{18}\text{O}$). Different from Fe, the interfering signals on ^{55}Mn were small. The background ion counts at m/z 55 (ArN , ArNH) increased when HNO_3 concentra-

Fig. 4. The standard addition lines of Fe in the samples of NIST bovine liver, the human brain and the spinal cord. NIST1 (□) was measured at 15 min, NIST2 (◆) was measured at 120 min after the beginning of analysis. The samples of brain (●) was obtained from the frontal cortex, and the spinal cord (○) was obtained from the anterior horn. Standard added were 100, 200, and 500 $\mu\text{g/liter}$ for NIST, and 200 and 500 $\mu\text{g/liter}$ for the brain and the spinal cord samples.

tions increased. But they were negligible compared to the signals of 1.5 $\mu\text{g/liter}$ of Mn, the lowest level in the sample solution of the central nerve tissues diluted to 1% w/v (15,000 counts/sec) (Fig. 3B). The background signals at m/z 55 from major element solutions correspond to 1% w/v of tissue, were estimated as much as 3% of those of samples, which was a little larger than HNO_3 . This background signal, calculated to be a maximum of 40 ng/liter in the final solution, may not solely originate from polyatomic species. That is, the background signal may possibly contain a trace level of Mn contamination in the salt solutions. In any case, the extent of the overestimation was estimated at no more than 3% of the sample signals and is therefore not considered to be a problem in the analysis of biological tissues.

The accuracy of these methods we decided to apply, the standard addition method and the background correction, were confirmed by measuring NIST 1577a bovine liver and IAEA (International Atomic Energy Agency, Vienna, Austria) MA-A-2 fish flesh. The concentrations of Mg, Fe, Zn, Cu, Mn, Se, Cd, V, Co, and Mo in NIST bovine liver and Hg and As in IAEA fish flesh fell within the certified values.

Analysis of brain and spinal cord tissues

We analyzed the concentrations of 16 elements (Mg, Fe, Zn, Cu, Al, Cr, Mn, Se, Hg, Cd, Mo, V, As, Co, Ni, and Pb) in 4 regions (anterior horn, posterior horn, lateral funiculus, and posterior funiculus) in the human spinal cord, and 3 regions

TABLE 1. *The control group-samples of human central nerve tissues*

Age (years)	Sex	Cause of death	Other findings
Subjects for brain samples			
0	M	Sudden infantile death syndrome	
28	F	Mecahnical asphyxia	
30	M	Congestive heart failure	Gall bladder stones
34	F	Unknown	Basedow's disease
44	M	Traffic accident	Liver chirrhosis
51	M	Gas intoxication	
55	M	Traffic accident	
55	M	Traumatic shock	Old subdural hematoma
62	M	Traffic accident	Alcohol drinking
Subjects for spinal cord samples			
58	M	Rupture of abdominal aorta	
67	M	Acute myocardial infarction	
77	M	Sepsis	Bedridden for 3 years
89	M	Burn	

TABLE 2. *Element concentrations in the spinal cord of the control group^a*

	Anterior horn (<i>n</i> = 4)	Posterior horn (<i>n</i> = 4)	Lateral funiculus (<i>n</i> = 4)	Posterior funiculus (<i>n</i> = 4)
	mean $\pm$ s.d.	mean $\pm$ s.d.	mean $\pm$ s.d.	mean $\pm$ s.d.
Mg	86.84 $\pm$ 7.82	82.49 $\pm$ 7.37	76.02 $\pm$ 7.20	80.24 $\pm$ 6.92
Fe	15.83 $\pm$ 2.50	12.48 $\pm$ 1.62	7.12 $\pm$ 1.43	8.36 $\pm$ 4.75
Zn	9.06 $\pm$ 0.91	8.28 $\pm$ 1.21	5.51 $\pm$ 1.26	5.95 $\pm$ 0.66
Cu	3.35 $\pm$ 1.22	2.28 $\pm$ 0.87	0.77 $\pm$ 0.11	0.98 $\pm$ 0.33
Al	0.95 $\pm$ 0.07	1.00 $\pm$ 0.08	1.09 $\pm$ 0.20	1.15 $\pm$ 0.35
Cr	0.07 $\pm$ 0.01	0.06 $\pm$ 0.02	0.06 $\pm$ 0.01	0.06 $\pm$ 0.01
Mn	0.38 $\pm$ 0.04	0.30 $\pm$ 0.05	0.24 $\pm$ 0.05	0.22 $\pm$ 0.07
Se	0.143 $\pm$ 0.040	0.146 $\pm$ 0.025	0.119 $\pm$ 0.033	0.131 $\pm$ 0.038
Hg	0.113 $\pm$ 0.061	0.089 $\pm$ 0.023	0.079 $\pm$ 0.023	0.111 $\pm$ 0.019
Cd	0.134 $\pm$ 0.040	0.129 $\pm$ 0.039	0.072 $\pm$ 0.010	0.094 $\pm$ 0.039
Mo	0.019 $\pm$ 0.005	0.017 $\pm$ 0.007	0.012 $\pm$ 0.004	0.013 $\pm$ 0.004
V	0.015 $\pm$ 0.004	0.012 $\pm$ 0.005	0.007 $\pm$ 0.002	0.008 $\pm$ 0.004
As	0.0150 $\pm$ 0.0085	0.0119 $\pm$ 0.0064	0.0138 $\pm$ 0.0083	0.0151 $\pm$ 0.0115
Co	0.0031 $\pm$ 0.0003	0.0030 $\pm$ 0.0003	0.0018 $\pm$ 0.0004	0.0022 $\pm$ 0.0004

^aAll values are expressed in $\mu\text{g/g}$ fresh weight.

(frontal cortex, frontal white matter, and cerebellum) of the human brain. The specimens were obtained postmortem from 1 patient who had died of familial ALS (male, 40 years old) (Ejima et al. 1995), and 13 subjects (4 for the spinal cord and 9 for the brain) who had died of non-neurological diseases or accidents (Table 1). All specimens were not fixed but stored at -70°C until analysis.

For dissecting and handling samples a pair of tweezers and a knife made of ceramic, which had been immersed in 1N HNO_3 and rinsed with pure water, were used. The samples of 7 regions (30–90 mg wet weight) were digested in a microwave oven with 0.5 ml of concentrated HNO_3 (60%w/v) (Mizushima et al. 1996). The final solutions resulted in 0.3–0.9%w/v and the standard addition method adding spikes at two concentrations (3-point calibration) was used.

Tables 2 and 3 show the analytical results of the spinal cord and the brain of the controls. Among the analyzed elements, Ni and Pb in the spinal cord were near or under the detection limit (not shown in Table 2). The concentrations of the most elements in the brain analyzed were compatible with those previously reported. The element concentrations in the spinal cord, which have been reported by few authors, the concentrations of Mn, Se, Zn, and Fe in the spinal cord were compatible with those previously reported for Mn (Miyata et al. 1983; Khare et al.

TABLE 3. *Element concentrations in the brain of the control group^a*

	Frontal cortex (<i>n</i> = 8)	Frontal white matter (<i>n</i> = 7)	Cerebellum (<i>n</i> = 9)
	mean $\pm$ S.D.	mean $\pm$ S.D.	mean $\pm$ S.D.
Mg	85.20 $\pm$ 9.92	110.57 $\pm$ 7.15	94.01 $\pm$ 14.29
Fe	33.82 $\pm$ 13.78	56.22 $\pm$ 11.52	29.82 $\pm$ 7.83
Zn	10.45 $\pm$ 1.55	9.42 $\pm$ 3.09	10.54 $\pm$ 1.22
Cu	3.64 $\pm$ 1.44	4.33 $\pm$ 1.14	4.51 $\pm$ 1.98
Al	1.08 $\pm$ 0.24	1.34 $\pm$ 0.11	1.16 $\pm$ 0.18
Cr	0.473 $\pm$ 0.573	0.312 $\pm$ 0.409	0.262 $\pm$ 0.013
Mn	0.207 $\pm$ 0.060	0.278 $\pm$ 0.105	0.308 $\pm$ 0.110
Se	0.173 $\pm$ 0.033	0.199 $\pm$ 0.030	0.175 $\pm$ 0.031
Hg	0.049 $\pm$ 0.037	0.051 $\pm$ 0.042	0.056 $\pm$ 0.043
Cd	0.040 $\pm$ 0.027 ^b	0.054 $\pm$ 0.032	0.043 $\pm$ 0.027 ^b
Pb	0.028 $\pm$ 0.029	0.036 $\pm$ 0.048	0.018 $\pm$ 0.001
Mo	0.023 $\pm$ 0.002	0.015 $\pm$ 0.004	0.012 $\pm$ 0.004
V	0.019 $\pm$ 0.009	0.020 $\pm$ 0.005	0.015 $\pm$ 0.004
As	0.0086 $\pm$ 0.0018	0.0109 $\pm$ 0.0012	0.0071 $\pm$ 0.0014
Ni	0.0061 $\pm$ 0.0006	0.0069 $\pm$ 0.0044	0.0059 $\pm$ 0.0003
Co	0.0023 $\pm$ 0.0007	0.0031 $\pm$ 0.0005	0.0028 $\pm$ 0.0009

^aAll values are expressed in $\mu\text{g/g}$ fresh weight.

^bOne third of the detection limit value was used as the concentrations of sample solution in one subject, whose Cd concentrations were under detection limit.

1990; Kihira et al. 1990; Mitchell et al. 1991), Se, Zn, and Fe (Khare et al. 1990; Mitchell et al. 1991) in the spinal cord. Most of the above studies used neutron activation analysis (NAA), while Kihira et al. (1990) used ICP-AES. Co concentrations measured in the present study were lower than those obtained by Khare et al. (1990) with NAA.

The concentrations of Mn were higher in the patient (0.9–1.4 $\mu\text{g/g}$ wet weight) than in the controls (0.2–0.3 $\mu\text{g/g}$ wet weight).

In the analysis of human samples, the concentrations of Mn were higher in the ALS patient than in the controls, which could not be explained by interferences. Further accumulation of data is needed to know the normal level of trace elements, and patients level as well, in the spinal cord, and to investigate the mechanism and cause of the disease.

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