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APPLIED METHODOLOGY

Assessment of trace elements in human brain using inductively coupled plasma mass spectrometry

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ABSTRACT

Recent brain research reveals a major role of trace elements in various diseases such as multiple sclerosis, Alzheimer's and Wilson's disease. The majority of published tissue concentrations dates back decades, and was assessed with various methods. Little is known about hemispherical differences, the correlation of trace elements or age-dependent changes in the human brain. Thus, the aim of this study was to examine trace element concentrations in different human brain regions after whole brain formalin fixation.

549 samples of 13 brain regions were investigated in 11 deceased subjects without known history of brain pathology. Regional wet-to-dry mass ratios and concentrations of iron, copper, magnesium, manganese, calcium and zinc were determined using inductively coupled plasma mass spectrometry.

Cortical gray matter revealed higher water content (wet-to-dry mass ratios 5.84–6.40) than white matter regions (wet-to-dry mass ratios 2.95–3.05). Element concentrations displayed specific regional differences. Good linear correlation of concentrations between elements was found for iron/copper as well as for manganese/magnesium (Spearman's rank correlation coefficient 0.74 and 0.65, respectively). Significant inter-hemispherical differences were found for copper in occipital white matter, for magnesium and calcium in putamen and for iron and copper in temporal white matter. An age dependent increase was seen in cortical gray matter for calcium, for magnesium in all regions except in cortical gray matter, for copper in substantia nigra and for zinc in occipital cortex.

The presented trace element concentrations can serve as a fundamental basis for further brain research. Wet-to-dry mass ratios allow a comparison with reference data from other studies.

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Introduction

In the aging brain the abnormal accumulation of reactive iron, copper and zinc elicit oxidative stress and macromolecular damage in Alzheimer's and Parkinson's disease, and may play a major role in the pathophysiology of multiple sclerosis by promoting the generation of reactive oxygen species [1–3]. It is likely that defects in a divalent-cation transporter (DCT1) which transfers iron and divalent metal ions including zinc, copper and manganese [4] contribute to the etiology of neurodegenerative diseases and may lead to neuropsychological disturbance [5]. An imbalance of the expression of DCT1 in combination with iron deficiency is linked to restless legs syndrome [6], whereas brain iron accumulation has been found in the process of normal aging [7].

Distributions of trace elements show a wide individual variation in different types of human tissue [8–11] as well as substantial variability in different brain regions. Authors of studies other than Hallgren and Sourander [7] included only small numbers of individuals (see references in Table 1). Additionally, the availability of analytical methods and technical developments resulted in considerable methodological differences and varying accuracy of reference values. Inter-study comparability is also hindered indicating solely the dry tissue concentration but not the dry-to-wet mass ratio [20].

As non-invasive assessment of trace element concentrations is increasingly feasible, e.g., the quantification of vascular calcifications by computed tomography [21,22] or the determination of iron levels in the brain by magnetic resonance imaging [23], accurate

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Table 1
Overview of related literature (in chronological order).

Publication	Elements	Regions	Method	Brains (*n*)
Cumings, 1948 [12]	Fe, Cu	GP, Put, NC, Th, CGM, CWM[a]	Spectrophotometry	6
Hallgren and Sourander, 1958 [7]	Fe	GP, Put, NC, Th, RN, SN, OGM, FGM, FWM[a]	Spectrophotometry	81
Warren et al., 1960 [13]	Cu	GP, Put, NC, Th, SN, RN, FGM, OGM, CC, Pons, FWM, TWM, OWM[a]	Spectrophotometry	9
Harrison et al., 1968 [14]	Fe, Cu, Zn, Mg	GP, Put, NC, Th, FGM, CC, FWM[a]	AAS	36
Hock et al., 1975 [15]	Fe, Zn[b]	Put, NC, Th, CC[a]	INAA	13
Smeyers-Verbeke et al., 1976 [16]	Mn	Th, FGM, OGM, CC[a]	AAS	10
Goldberg and Allen, 1981 [17]	Fe, Cu, Ca, Mn	GP, Put, NC, SN[a]	AAS	3
Markesbery et al., 1984 [18]	Mn	GP, Put, NC, Th, SN[a]	INAA	33[c]
Dexter et al., 1991 [19]	Fe, Cu, Zn, Mn	GP, Put, NC, SN[a]	ICP-MS	34[d]
Maeda et al., 1997 [5]	Fe, Cu, Zn, Mg, Ca, Mn	GP, Put, FWM	ICP-AES	4

GP, globus pallidus; Put, putamen; NC, nucleus caudatus; Th, thalamus; CGM, cortical gray matter; CWM, cortical white matter, RN, red nucleus; SN, substantia nigra; OGM, occipital gray matter; FGM, frontal gray matter; FWM, frontal white matter; CC, corpus callosum; TWM, temporal white matter; OWM, occipital white matter; AAS, atomic absorption spectrometry; INAA, instrumental neutron activation analysis; ICP-MS, inductively coupled plasma mass spectrometry; ICP-AES, inductively coupled atomic emission spectrometry.

[a] Additional regions analyzed.
[b] Additional elements analyzed.
[c] Additionally, individuals with Alzheimer's disease were analyzed.
[d] Additionally, individuals with Parkinson's disease were analyzed.

data of regional element concentrations is essential for validation and inter-study comparability.

Thus, the aim of this study was to provide the basis for such validation and to provide further insight into the regional distribution of several trace elements in the brain. Wet tissue concentrations of iron, calcium, copper, zinc, magnesium and manganese from 13 regions of the human brain were determined using inductively coupled plasma mass spectrometry, and hemispherical differences, inter-element correlation and age dependence were investigated.

Materials and methods

Subjects

Eleven deceased human subjects (9 males, median age 61 years, range 48–81 years; 2 females, 75 and 80 years), were included in this study. Corpses were transferred to the Institute of Forensic Medicine with an autopsy request from the local health authority or the public prosecution service. Subjects complied with the following inclusion criteria: post-mortem interval less than 72 h, no signs of decomposition, no known history of neurological disorder, and absence of head trauma. Next of kin or the prosecution authority, respectively, gave their informed consent in a telephone call which was voice recorded. Table 2 provides an overview of the subjects and their causes of death. The study was approved by the responsible Institutional Review Board of the Local Medical University.

Sample preparation and chemical analysis

Brains were extracted during autopsy within 72 h after death, and main supplying arteries (*Arteria basilaris*, *Arteriae carotides internae*) were ligated to prevent blood discharge. Entire brains were fixed in a 4% phosphate buffered formaldehyde solution (pH 7.0 ± 0.5; Carl Roth GmbH) for 30–143 days (median 58 days). After 3 days formalin solution was renewed and brains were stored for at least further 27 days to ensure sufficient fixation. Potential contamination of the trace element measurements was quantified by measuring the iron concentration of two batches of the fixation solution both before and after use, i.e., after the first three days and after the entire duration of fixation. Fixed brains were dissected into transversal slices with a thickness of 10 mm using a ceramic knife to avoid contamination of the samples from metallic abrasion. Tissue specimens (0.011–3.370 g wet mass) were taken for all brains at identical anatomical locations. The following 13 regions covering both hemispheres were sampled: globus pallidus, putamen, caudate nucleus, thalamus, body of corpus callosum, pons, red nucleus, substantia nigra, occipital and frontal cortex as well as frontal, temporal and occipital white matter. Depending on the size of anatomical regions specimens were further divided in up to 3 subunits to evaluate intra-regional homogeneity of concentrations. Tissue specimens were weighed with a digital scale (Mettler Toledo Int. Inc.), placed in a sterile 90 mL polypropylene container (Carl Roth) and frozen at −12.0 °C. Specimens were freeze-dried in a Gamma 1-16 LSC freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH). Freeze-dried samples were weighed to 0.1 mg and mineralized with nitric acid in a microwave-heated autoclave (UltraCLAVE III; MLS Mikrowellen-Labor Systeme GmbH). The concentration of iron (Fe), copper (Cu), zinc (Zn), magnesium (Mg), calcium (Ca) and manganese (Mn) in the brain tissue samples was determined using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500ce; Agilent Technologies) at the following mass-to-charge ratios: Fe $m/z = 56$, Ca $m/z = 43$, Cu $m/z = 65$, Zn $m/z = 66$, Mg $m/z = 24$ and Mn $m/z = 55$. Mg, Ca and Mn concentrations were only determined for 3 male (56, 64 and 69 years) and 2 female (75 and 80 years) subjects. To reduce polyatomic interferences at the mass-to-charge ratio of 56 (e.g., $^{40}Ar^{16}O^{+}$), helium was added as a collision gas at a flow rate of 5.3 mL/min. All concentrations were expressed on a wet mass basis in mg/kg. Accuracy of the determined concentrations was confirmed with the certified material bovine muscle (RM 8414; NIST).

Table 2
Age, sex, and cause of death of the examined subjects.

Subject	Age	Sex	Cause of death
1	61	M	Pulmonary edema
2	58	M	Intestinal bleeding
3	81	M	Pulmonary edema
4	48	M	Myocardial infarction
5	62	M	Heart failure
6	60	M	Myocardial infarction
7	64	M	Heart failure
8	56	M	Intestinal bleeding
9	69	M	Heart failure, pulmonary edema
10	80	F	Heart failure
11	75	F	Suffocation

Statistical analysis

Mean values and standard deviations of wet-to-dry mass ratios, water content and element concentrations were calculated. The water content was calculated using the formula

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