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Iron levels in the human brain: A post-mortem study of anatomical region differences and age-related changes

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ABSTRACT

The link between brain iron homeostasis and neurodegenerative disease has been the subject of extensive research. There is increasing evidence of iron accumulation during ageing, and altered iron levels in some specific brain regions in neurodegenerative disease patients have been reported.

Using graphite furnace atomic absorption spectrometry after microwave-assisted acid digestion of the samples, iron levels were determined in 14 different areas of the human brain [frontal cortex, superior and middle temporal, caudate nucleus, putamen, globus pallidus, cingulated gyrus, hippocampus, inferior parietal lobule, visual cortex of the occipital lobe, midbrain, pons (locus coeruleus), medulla and cerebellum (dentate nucleus)] of $n = 42$ adult individuals (71 ± 12 years old, range: 53–101 years old) with no known history or evidence of neurodegenerative, neurological or psychiatric disorders.

It was found that the iron distribution in the adult human brain is quite heterogeneous. The highest levels were found in the putamen (mean ± SD, range: 855 ± 295 μg/g, 304–1628 μg/g) and globus pallidus (739 ± 390 μg/g, 225–1870 μg/g), and the lowest levels were observed in the pons (98 ± 43 μg/g, 11–253 μg/g) and medulla (56 ± 25 μg/g, 13–115 μg/g).

Globally, iron levels proved to be age-related. The positive correlation between iron levels and age was most significant in the basal ganglia (caudate nucleus, putamen and globus pallidus).

Compared with the age-matched control group, altered iron levels were observed in specific brain areas of one Parkinson's disease patient (the basal ganglia) and two Alzheimer's disease patients (the hippocampus).

Introduction

According to the US National Institute of Aging and WHO's 2011 "Global Health and Aging" report [1] and as a result of longer life expectancy, chronic and degenerative diseases will become the most prevalent diseases worldwide over the next several decades.

The aetiology of neurodegenerative diseases (NDs) is multifactorial, and it is assumed to involve a complex interaction between (natural) ageing, genetic predisposition and environmental factors [2]. Disturbances in trace element (TE) homeostasis in specific areas of the brain [3,4] have been identified as contributing to the development of many NDs.

Iron (Fe) is the most abundant transition metal in the human body and has many essential functions in the brain and nervous system, such as oxygen transport, mitochondrial respiration, protein and DNA synthesis, myelination, dendrite development and neurotransmitter biosynthesis [5,6]. Interestingly, it has been observed that patients with NDs such as Parkinson's and Alzheimer's disease tend to accumulate Fe in their nervous system [7–12], suggesting a role for this transition metal in these disorders. Fe accumulation leads to excessive production of reactive oxygen species, protein, DNA and phospholipid oxidation and, ultimately, to structural and functional damage [3].

Despite the intensive research that has been conducted on the relationship between TE and ND, the evidence is still fragmentary,

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and the exact role of TE remains poorly understood. Its direct determination in human brain samples from both healthy individuals and patients with ND is crucial for a better understanding of the underlying disease mechanisms, but most of the current information about the relationship between TE and human brain functioning is based on animal studies [13,14] or relies on determinations in cerebrospinal fluid, blood or serum samples [13,15–19]. Studies on TE levels in normal and pathological human brains are scarce, restricted to a few brain areas [20–23] and/or involve a small number of subjects [24,25].

Based on this background, the main goal of the present study was to directly quantify Fe levels in normal human brain tissue, extending the number of brain areas and the number of subjects studied, to evaluate (a) the regional anatomic differences and (b) age-related changes in Fe levels within the brain tissue. This type of information is indispensable for interpreting the data obtained from ND patients.

Materials and methods

Subjects

Brain samples were collected from men ($n = 27$; 67 ± 11 years old) and women ($n = 15$; 77 ± 12 years old) not registered in the Portuguese *National Registry of Refusal to Organ Donation* database and complying with all the current regulations regarding human tissue collection for scientific research purposes.

Samples were obtained from individuals submitted to forensic autopsy exams during the first semester of 2012 at the North Branch (Porto) of the Portuguese National Institute of Legal Medicine (INML). Individuals from each of the following age groups were studied: 50–60 ($n = 10$), 60–70 ($n = 10$), 70–80 ($n = 10$), 80–90 ($n = 9$) and ≥90 ($n = 3$) years old. Inclusion criteria were (a) the absence of a history of known neurodegenerative, neurological or psychiatric disorders, (b) the absence of injuries involving the central nervous system (CNS), and (c) macroscopically normal tissues.

Samples from two individuals with documented Alzheimer's disease (women, 73 and 85 years old) and one with Parkinson's disease (woman, 91 years old) were also collected.

Sample collection

Samples were collected by the pathologists at the INML following a standard protocol.

To prevent sample contamination, all materials in contact with the samples, including the stainless steel tools used by the pathologists, were previously decontaminated with a 5% (v/v) nitric acid solution prepared from concentrated (≥69% w/w) HNO_3 (Sigma–Aldrich, Germany) and thoroughly rinsed with ultrapure water (resistivity 18.2 MΩ cm at 25 °C) produced by a Milli-Q water purification system (Millipore, USA).

After removing the brain from the cranium, the contaminating blood was washed away with ultrapure water. The meninges were removed with plastic tweezers, and the brain was washed again with ultrapure water to minimise sample contamination with blood or cerebrospinal fluid.

The brain areas studied were defined based on Paine and Lowe's recommendations for regions to be sampled when a neurodegenerative disease is suspected [26]. Using decontaminated plastic knives, tissue fragments (approximately 1 cm^3) were collected from the following brain areas: the frontal cortex (1); superior (2A) and middle (2B) temporal gyri; basal ganglia, including the caudate nucleus (3A), putamen (3B) and globus pallidus (3 C); cingulated gyrus (4); hippocampus (5); inferior parietal lobule (6); visual cortex of the occipital lobe (7); midbrain, including the *substantia nigra* at the level of the third nerve (8); pons (locus coeruleus) (9); medulla (10); and cerebellum (dentate nucleus) (11). Samples were stored in decontaminated polypropylene tubes (Sarstedt, Germany) at −4 °C until analysis.

Table 1
Instrument settings and graphite furnace programme for Fe determination.

Parameter	Value
Wavelength	248.3 nm
Slit width	0.2 nm
Lamp current	30 mA
Inert gas	Argon
Flow rate	250 mL/min (0 at the atomisation step)
Background correction	Longitudinal Zeeman-effect
Sample injection volume	15 μL
Matrix modifier injection volume	5 μL
Measurement mode	Integrated absorbance (A s)
Integration time	5 s
Baseline offset correction (BOC)	2 s

Step	Temp. (°C)	Ramp (s)	Hold (s)
Graphite furnace program			
Dry1	110	1	30
Dry2	130	15	30
Pyrolysis	1400	10	20
Atomisation	2100	0	5
Cleaning	2450	1	3

Injection temperature: 20 °C.

Sample pre-treatment

After defrosting, the brain samples were thoroughly washed with ultrapure water and placed in a dry oven (Raypa, Spain) at 110 °C until constant weight (*ca.* 24 h). Dried samples (*ca.* 100–500 mg) were weighed directly in the microwave digestion vessels, which had been previously decontaminated with 10% (v/v) HNO_3 and thoroughly rinsed with ultrapure water. Samples were digested using 2.5 mL of concentrated (≥65% w/w) HNO_3 (*Trace*SELECT®, Fluka, France) and 1.0 mL of ≥30% (v/v) H_2O_2 solution (*Trace*SELECT®, Fluka, Germany). The sample digestion was performed in an MLS 1200 Mega microwave oven from Milestone (Italy) equipped with an HPR 1000/10 rotor using the following power (W)/time (min) programme: 250/1, 0/2, 250/5, 400/5 and 600/5. After cooling, sample solutions were made up to 50 mL with ultrapure water and stored in closed propylene tubes at 4 °C until analysis.

Iron determination

Fe determination was performed using a PerkinElmer (Germany) model 4100 ZL atomic absorption spectrometer (longitudinal Zeeman background correction) equipped with a transversely heated graphite atomiser (THGA), end-capped graphite tubes with an integrated L'vov platform (PerkinElmer Part No. B3 000653) and an AS-70 autosampler. An Intensitron™ (PerkinElmer) hollow cathode lamp was used as a light source ($\lambda = 248.3$ nm). Argon of 99.9999% purity (Gasin, Portugal) was used as an inert gas. A commercial magnesium nitrate solution (Ref. 63043; Fluka, Switzerland) was used to prepare the matrix modifier solution [5 μL = 15 μg $Mg(NO_3)_2$]. The instrumental conditions and graphite furnace programme used are summarised in Table 1.

The sample digests were diluted (100-fold) with 0.2% (v/v) HNO_3 solution before injection into the graphite tube. Sample solutions were measured in duplicate. For results with RSD ≥10%, an additional two injections were performed.