Available online at www.sciencedirect.com
ScienceDirect
Neuroscience Letters 418 (2007) 72–76
Neuroscience Letters
www.elsevier.com/locate/neulet

Iron, copper, and iron regulatory protein 2 in Alzheimer's disease and related dementias

Shino Magaki [a], Ravi Raghavan [b], Claudius Mueller [a], Kerby C. Oberg [c], Harry V. Vinters [d], Wolff M. Kirsch [a,*]

[a] *Center for Neurosurgery Research, Loma Linda University, Loma Linda, CA, USA*
[b] *Neuropathology Section, Department of Pathology, Loma Linda University, Loma Linda, CA, USA*
[c] *Department of Pathology and Human Anatomy, Loma Linda University, Loma Linda, CA, USA*
[d] *Section of Neuropathology, Department of Neurology, University of California, Los Angeles, CA, USA*

Received 19 December 2006; received in revised form 27 February 2007; accepted 28 February 2007

Abstract

Accumulating evidence implicates a role for altered iron and copper metabolism in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD). However, imbalances in the levels of the various forms of iron at different stages of AD have not been examined. In this pilot study we extracted and measured the levels of loosely bound, non-heme and total iron and copper in the frontal cortex and hippocampus of patients with mild–moderate AD ($n = 3$), severe AD ($n = 8$) and dementia with Lewy bodies (DLB, $n = 6$), using graphite furnace atomic absorption spectrometry (GFAAS). Additionally, the expression of iron regulatory protein 2 (IRP2) was examined in relation to the pathological hallmarks of AD and DLB, amyloid plaques, neurofibrillary tangles (NFT), and Lewy bodies, by immunohistochemistry. We found significantly decreased loosely bound iron in the hippocampal white matter of mild–moderate and severe AD patients and a trend towards increased non-heme iron in the hippocampal gray matter of severe AD patients. Furthermore, decreased levels of total copper were seen in severe AD and DLB frontal cortex compared to controls, suggesting an imbalance in brain metal levels in both AD and DLB. The decrease in loosely bound iron in mild–moderate AD patients may be associated with myelin breakdown seen in the beginning stages of AD and implicates that iron dysregulation is an early event in AD pathogenesis.

Keywords: Alzheimer's disease; Dementia with Lewy bodies; Loosely bound iron; Non-heme iron

A dysregulation of brain metals, especially iron and copper, has been implicated in the pathogenesis of Alzheimer's disease (AD) [15,22,27]. Brain iron is important in neural development and function, as neurons and glial cells require iron for electron transport, myelination of axons, and as a cofactor for enzymes involved in the synthesis of neurotransmitters [14]. However, while iron deficiency impairs cell growth, iron overload can cause cellular damage. Thus, the maintenance of iron homeostasis is critical for the cell. Intracellular iron is tightly regulated by the iron regulatory proteins, IRP1 and IRP2, which post-transcriptionally regulate the expression of proteins involved in iron homeostasis, such as the transferrin receptor and ferritin, in response to intracellular "free" iron concentrations [29].

Redox active iron and copper, capable of generating reactive oxygen species (ROS), have been found associated with amyloid plaques and neurofibrillary tangles (NFT), pathological hallmarks of AD [19,27]. These and other transition metals were also demonstrated to mediate β-amyloid aggregation and neurotoxicity [15,24]. Furthermore, alterations in the localization of IRP2, but not IRP1, have been reported in AD [26]. However, little is known about the relative distribution of the different pools of iron, copper, and IRP2 expression at different stages of the disease. To address this question, we measured the levels of (1) loosely bound, non-heme, and total iron, (2) copper, and (3) examined IRP2 expression in the brains of patients with mild–moderate AD and severe AD. In addition, we evaluated cases of dementia with Lewy bodies (DLB) to determine if changes in iron levels and IRP2 expression are specific to AD or involved in both AD and DLB.

* Corresponding author at: Loma Linda University, Coleman Pavilion, 1175 Campus Street, Suite 11113, Loma Linda, CA 92350, USA.
Tel.: +1 909 558 7070; fax: +1 909 558 0472.
E-mail address: wkirsch@llu.edu (W.M. Kirsch).

0304-3940/$ – see front matter © 2007 Published by Elsevier Ireland Ltd.
doi:10.1016/j.neulet.2007.02.077

Table 1
Antibodies used for immunohistochemistry

Antibody	Antigen	Species	Type	Clone	Source	Dilution
IRP2	138–200aa of IRP2	Mouse	Monoclonal	4G11	This study	1:100
PHF-tau	PHF-tau	Mouse	Monoclonal	AT8	Pierce	1:40
Aβ40	C-terminal 7aa from Aβ40	Rabbit	Polyclonal	NA[a]	Chemicon	1:100
Aβ42	C-terminal 6aa from Aβ42	Rabbit	Polyclonal	NA[a]	Chemicon	1:100
α-Synuclein	Lewy bodies from patients with DLB	Mouse	Monoclonal	LB509	Zymed	1:50

[a] Not applicable.

Postmortem tissue samples from the frontal cortex and hippocampus of non-demented elderly controls ($n = 6$, mean age = 9 72 ± 11 (S.D.), range = 56–86 years, M/F = 4/2, postmortem interval (PMI) = 21 ± 7), clinically and histopathologically confirmed cases of mild–moderate AD ($n = 3$, mean age = 83 ± 10, range = 73–92 years, M/F = 1/2, PMI = 11 ± 11), severe AD ($n = 8$, mean age = 78 ± 12, range = 59–91 years, M/F = 2/6, PMI = 21 ± 6), using the CERAD/NIA and Braak/Braak staging criteria, and DLB ($n = 6$, mean age = 78 ± 5, range = 71–85, M/F = 2/4, PMI = 16 ± 9) were obtained from the Neuropathology & Molecular Genetics Core of the UCLA Alzheimer's Disease Research Center.

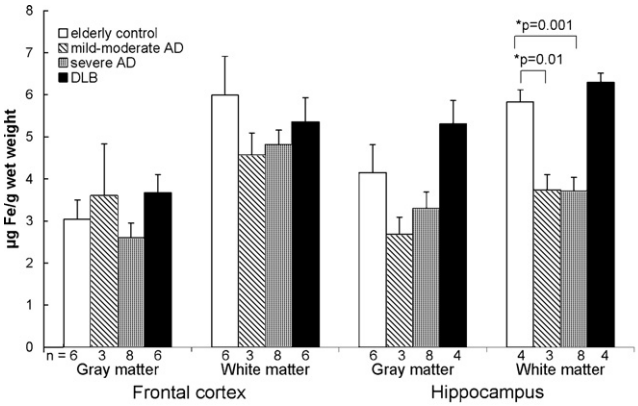
We dissected frozen brain samples into gray and white matter and extracted loosely bound and non-heme iron according to the method of Nelson et al. [21] with slight modifications. The hippocampus consisted of a 6–8 mm tissue block from its mid-portion, sampled at approximately the level (coronal) of the lateral geniculate nucleus, and included the hippocampus proper, parahippocampal gyrus and entorhinal cortex. Small wedges of white matter were dissected out from areas around the temporal horn and within the parahippocampal gyrus in a caudo-rostral direction. Regions of the frontal cortex that were sampled correspond to Brodmann areas 10 and 11. For loosely bound iron, tissues were homogenized in 180 μl of 0.5 mM EDTA, and samples were centrifuged at 13,000 × g for 10 min, after which 34 μl of 20% trichloroacetic acid (TCA)/0.5 mM EDTA was added to 120 μl of the supernatant. Then samples were vortexed, centrifuged again at 13,000 × g for 10 min, and the supernatant collected and stored at −20 °C. For non-heme iron, tissues were homogenized in 360 μl of 6% TCA/0.5 mM EDTA and incubated at 90 °C for 30 min. Then 0.7 ml of 0.5 mM EDTA was added, samples were centrifuged at 13,000 × g for 10 min, and the supernatant was collected and stored at −20 °C. For total iron and copper, tissues were wet ashed according to the method of Maynard et al. [20]. All metals were measured in duplicate by graphite furnace atomic absorption spectrometry (GFAAS) with a SpectrAA 220Z (Varian, Victoria, Australia).

Immunohistochemistry was done using the BioGenex Autostainer system (San Ramon, CA) with 6 μm paraffin-embedded tissue sections from the hippocampus and frontal cortex. Tissue sections were deparaffinized by two 15 min incubations in EZ-DeWax solution followed by antigen retrieval using the Antigen Retrieval Citra Plus solution, both from BioGenex, according to the manufacturer's instructions. Endogenous peroxidase activity was inhibited by two 15 min incubations in 3% hydrogen peroxide in 10% methanol, and non-specific protein binding was blocked with Power Block (Biogenex). Antibodies used are listed in Table 1. The anti-IRP2 antibody is now available from Santa Cruz Biotechnology (Santa Cruz, CA). After 30 min incubations with the respective antibodies, sections were washed with PBS and incubated for 20 min with biotinylated secondary antibodies, followed by HRP-conjugated streptavidin. Immunoreactivity was revealed using AEC as the chromogen.

Data are presented as means ± S.E.M. Statistical analysis was performed using one-way ANOVA followed by the Tukey post hoc test with SPSS 12.0.1 software. Corrections for age were done using ANCOVA analysis. $P < 0.05$ was considered significant.

Levels of loosely bound iron were similar between the frontal cortex and hippocampus although concentrations in the white matter tended to be higher than in the gray matter in both regions in all groups (Fig. 1). We found no differences in the levels of loosely bound iron in the frontal cortex between elderly controls, mild–moderate AD, severe AD, and DLB. However, there was a significant decrease of loosely bound iron in the hippocampal white matter in mild–moderate and severe AD brains compared to controls (3.7 ± 0.4 and 3.7 ± 0.3, respectively, versus 5.2 ± 0.6 μg Fe/g wet weight) which remained significant when corrected for age in severe AD but was reduced to a trend in mild–moderate AD.

Non-heme and total iron were also consistently higher in the white matter compared to the gray matter with slightly higher levels in the frontal white matter compared to that of the hip-



*When corrected for age differences between groups, the decrease in loosely bound iron in the hippocampal white matter in severe AD remained significant ($p = 0.033$) whereas that for mild-moderate AD was reduced to a trend ($p = 0.052$).

Fig. 1. Loosely bound iron was decreased in the hippocampal white matter of mild–moderate and severe AD brains compared to controls. Data are presented as mean (μg Fe/g wet weight) ± S.E.M. and n values for each group are shown below the bars.