Journal of Alzheimer's Disease 12 (2007) 157–160
IOS Press

Short Communication

About the Presence of Hemosiderin in the Hippocampus of Alzheimer Patients

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Ferritin and Hemosiderin (Hm) are the iron-storing "elements" in cells. Whereas Ferritin is a well-characterized *soluble protein* that has been extensively studied [1–5], the term Hemosiderin does not have the same clarity of meaning for the pathologist, biochemist or electron microscopist. For the pathologist [6], Hm represents iron-containing conglomerates, stainable with Perl's stain. For the biochemist, Hm is a heterogeneous *insoluble compound*, containing iron, proteins, carbohydrates and lipids. For the electron microscopist, Ferritin and Hm are nanoparticles (5–7 nm in diameter), recognizable because of the electron-density of the iron concentrated in their "cores".

Hm has been observed in various tissues and organs, including the liver, spleen, heart, intestines, pancreas and tumors (neuroblastomes), *associated with iron-overload pathologies* such as primary (PH) and secondary (SH) hemochromatosis and transfusional or local bleeding siderosis [6–8]. Hm is considered a product of degradation of Ferritin localized within siderosomes [4,7]. In Ferritin, iron is mainly stored as ferrihydrite, hydrated Fe^{3+} iron oxide nanocrystals [1–3]. In Hm, several different iron oxyhydroxide mineral structures have been identified depending on the disease [9–11].

By employing electron microscopy, Hm can be distinguished from Ferritin when individual particles cannot be resolved or when they come closer than 13 nm (the mean external diameter of one Ferritin protein shell [2]).

We have observed, using electron microscopy, Hm in the hippocampus of AD patients [12,13]. Figures 1 and 2 show two examples of Hm rich-regions: (1) the cytoplasm of oligodentrocytes near the nuclei; in these cells Ferritin is often observed in the nucleoplasm [12, 13] and (2) the oligodendrocyte processes associated with myelinated axons devoid of abnormal accumulations of filaments [13]. These oligodendrocyte processes also contain numerous Ferritin molecules and the myelin sheaths of these axons are frayed.

What could be the role of Hm in oligodendrocytes ?

Let us remind ourselves of *some* of the known roles of iron in the brain.

1. Iron is the most abundant metal in the brain. It participates in the main neuronal processes, including neurotransmitter synthesis and myelination of axons. Oligodendrocytes, the cells directly involved in myelin production and maintenance [14,15], are the predominant iron-containing cells in the brain.
2. Iron overload occurs in certain neurodegenerative diseases including AD [16–18].
3. Iron in the ferrous form is a chemically-irrefutable source of oxidative stress [19] because it is able to catalyze the formation of free radicals via the Fenton reaction [20].
4. The relationship between iron overload, oxidative stress and AD has been widely discussed in recent years [21–25].

Also, let us not forget the events that occur in liver cells during pathological iron overload [8]. An excess of iron leads to:

1. Synthesis of new Ferritin molecules.

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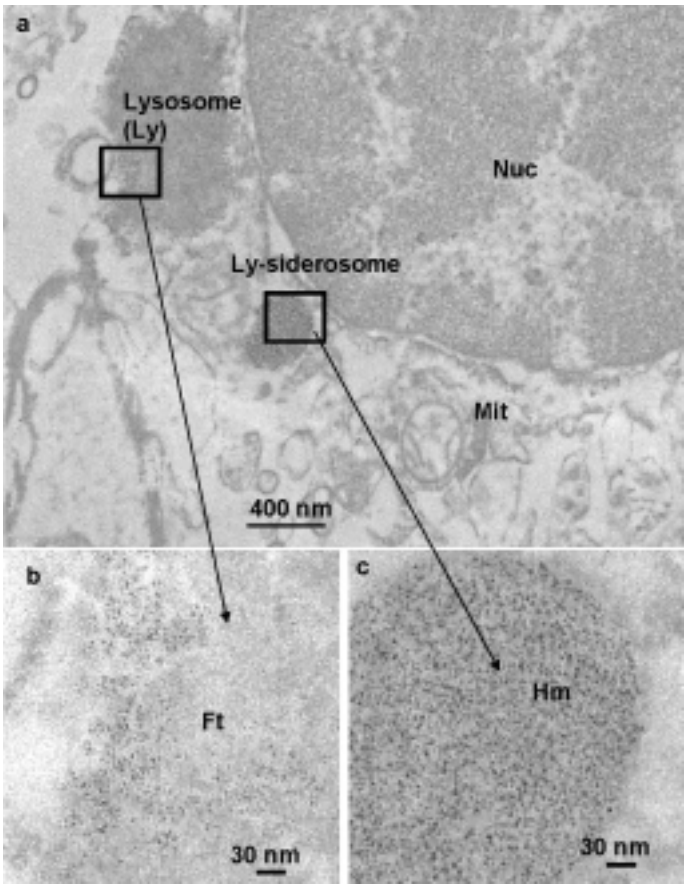

Fig. 1. AD hippocampus. TEM image of an ultra-thin section of an oligodendrocyte, lightly stained with uranyl acetate. The nucleus (Nuc), a mitochondrion (Mit), a Ferritin containing lysosome (Ly) and an Hm containing Ly-siderosome can be observed. (b) Detail of Ferritin in Ly. (c) Detail of Hm in Ly-siderosome. For the preparation of samples see [13].

2. Iron sequestration in the iron-rich Ferritin cores until the core capacity is saturated, (about 4500 at iron/core).
3. The formation of clusters of Ferritin in lysosomes.
4. The formation of Hm via the digestion of the Ferritin protein shell by the lysosomes-enzymes (the lysosome is now called *siderosome*). In iron-overloaded liver, the Hm shell consists predominantly of denatured heavy H-Ferritin subunit [26].

It has been proposed that the *formation* of Hm represents a biological protection mechanism, in that it decreases the ability of iron to promote oxygen radical reactions [7]. In fact, cells containing Hm-filled siderosomes can continue to function normally for variable periods of time [7]. Nevertheless, if the iron-storing capacity of both Ferritin and Hm molecules is strained, the amount of free iron can be seen to increase with the risk of cellular damage by oxidative stress.

The presence of Hm in AD hippocampal oligodendrocytes would offer "direct proof" that these cells are supporting important iron overload.

However, not just the "quantity" of the stored iron molecules must be taken into account, but also the "quality" of the iron compound itself. In normal conditions iron is stored mainly in the Ferritin cores in a non-toxic *ferric* form (nanocrystals of ferrihydrite, hematite and other minor phases) [1–3,12]. Spectroscopic investigation has shown a similar composition of Ferritin cores and animal and normal human Hm cores, but a different composition of human primary (PH) and secondary (SH) hemochromatosis Hm cores [9–11]. The Hm isolated from SH shows a microcrystalline goethite structure and the Hm cores obtained in the case of PH are formed by a mixture of two phases: a minor ferrihydrite phase and a major, poorly crystallized phase with reticular distances of 0.249, 0.212 and 0.153 nm. In our study with ATEM [12]

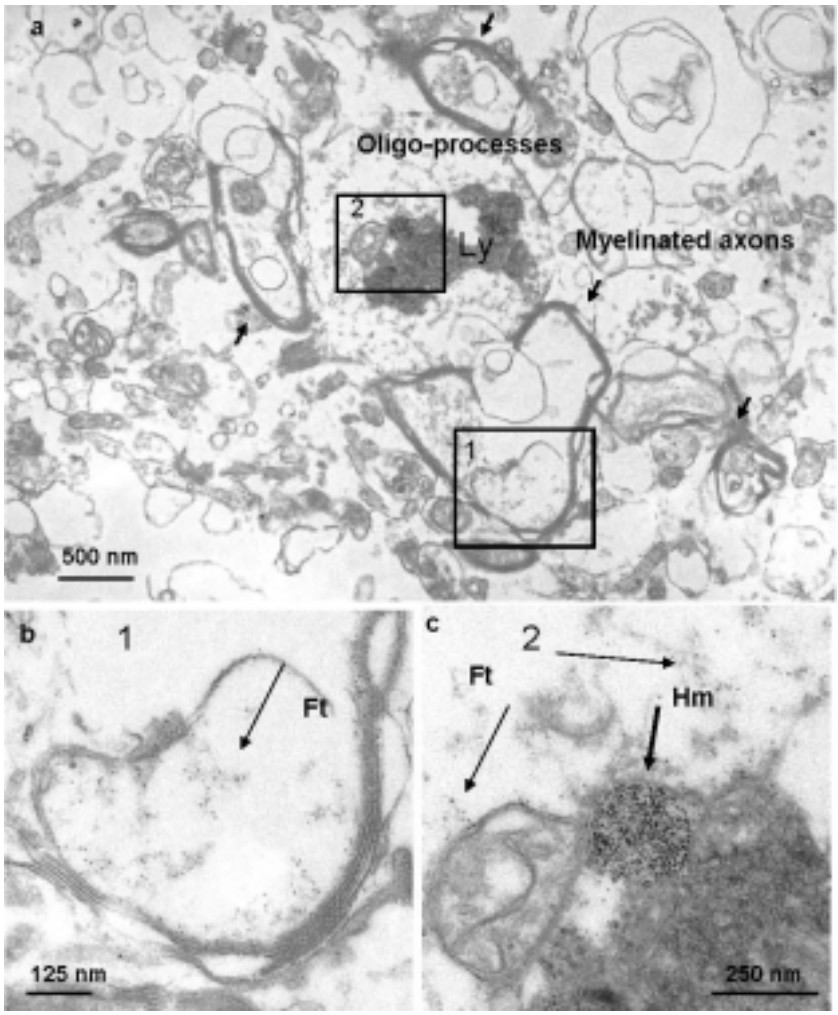

Fig. 2. AD hippocampus. TEM image of an ultra-thin section of a neuropili region, lightly stained with uranyl acetate. (a) Several myelinate axons with frayed myelin sheaths can be observed around a oligodendrocyte processes. (b) Ferritin can be observed in the framed region 1. (c) Ferritin and Hm can be observed in the framed region 2. For the preparation of samples see [13].

we have shown the similarity between the composition of certain cores of "isolated pathological Ferritin" with those of primary hemochromatosis Hm cores; the mineral composition with fcc structure ($a = 0.43$ nm; $d_{111} = 0.248$ nm, $d_{200} = 0.215$ nm, $d_{220} = 0.152$ nm) corresponds to wüstite, a *ferrous* iron compound. This result leads us to suspect the presence of Hm in AD [12], data confirmed later by "in situ" ultrastructural observations [13].

Additionally, we also found a higher concentration of magnetite nanocrystals, a mixed *ferric-ferrous* mineral, in pathological Ferritin cores [12]. The presence of biogenic ferromagnetic magnetite/maghemite crystals in the *extracts* and in the *sections* from healthy and pathological brains has been described since 1992 [27, 28]. The possible relationship [29] between these biogenic *magnetite/maghemite crystals*, observed in the brain *extracts* and in the *sections* of the human AD brain, and the *magnetite nanocrystals* present in some Ferritin cores in AD has previously been discussed [12].

These ferrous iron-containing cores of Ferritin and Hm can also be a source of free ferrous iron.

Microscopic observations are static observations and do not permit data to be gathered on dynamic (or functional) behaviors. Nevertheless, the systematic presence of Hm in *oligodendrocytes*, together with a high concentration of ferrous iron-rich cores, would be correlated with the degradation of the myelin sheets. A possible relationship between oxidative stress and demyelinating disorder in oligodendrocytes has been

pointed out [15].

In summary, in addition to the dysfunction of certain proteins in neurodegenerative diseases, particularly APP and tau proteins in AD, *a dysfunction of Ferritin and Hm iron-storing molecules could be associated with AD*. The dysfunction could be related to a defect in the iron oxidation in the cores, probably a defect in the ferroxidase activity of the heavy H-subunit. In fact, this dysfunction of iron-storing proteins would be a *normal aging event* [14,30]. As Smith has pointed out [19], oxidative stress precedes both amyloid-β and tau phosphorylation and the subsequent dynamic behavior of these proteins would indicate their antioxidant proprieties. That is a neuronal response to oxidative stress. *The oligodendrocyte response to oxidative stress derived from the excess of free ferrous iron would be the lipid damage and demyelination with the consequent perturbation of information transfer* (*memory functions in the hippocampus of AD patients*).

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