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Review

Could a dysfunction of ferritin be a determinant factor in the aetiology of some neurodegenerative diseases?

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ABSTRACT

Background: The concentration of iron in the brain increases with aging. Furthermore, it has also been observed that patients suffering from neurological diseases (e.g. Parkinson, Alzheimer…) accumulate iron in the brain regions affected by the disease. Nevertheless, it is still not clear whether this accumulation is the initial cause or a secondary consequence of the disease. Free iron excess may be an oxidative stress source causing cell damage if it is not correctly stored in ferritin cores as a ferric iron oxide redox-inert form.
Scope: Both, the composition of ferritin cores and their location at subcellular level have been studied using analytical transmission electron microscopy in brain tissues from progressive supranuclear palsy (PSP) and Alzheimer disease (AD) patients.
Major conclusions: Ferritin has been mainly found in oligodendrocytes and in dystrophic myelinated axons from the neuropili in AD. In relation to the biomineralization of iron inside the ferritin shell, several different crystalline structures have been observed in the study of physiological and pathological ferritin. Two cubic mixed ferric–ferrous iron oxides are the major components of pathological ferritins whereas ferrihydrite, a hexagonal ferric iron oxide, is the major component of physiological ferritin. We hypothesize a dysfunction of ferritin in its ferroxidase activity.
General significance: The different mineralization of iron inside ferritin may be related to oxidative stress in olygodendrocites, which could affect myelination processes with the consequent perturbation of information transference.

1. Introduction

1.1. General introduction

Over the last decades, the technological progress in different scientific fields as biochemistry, physics, telecommunications, computers, etc., has enabled significant advances in biomedical areas. A few examples of these spectacular advances are the laser multiple applications in therapy and the increasing number of imaging techniques, as the ultrasonography, the computed axial tomography (CAT), magnetic resonance imaging (MRI) and positron emission tomography (PET), which allow an early and accurate diagnosis of different diseases. Moreover, new challenges have been achieved by bringing together experts from different disciplines such as nanotechnology and medicine. To this effect, the use of a technique such as analytical transmission electron microscopy (ATEM) for tissue analysis has revealed interesting features, invisible under typical analysis techniques in biomedicine.

In particular, this article summarizes and discusses our previous results obtained by ATEM and other physical complementary techniques on the composition and distribution of the ferritin/haemosiderin iron-containing cores in the brain of patients suffering from two neurological diseases: progressive supranuclear palsy (PSP) and Alzheimer's disease (AD). The main aim is to recall the most relevant data from the different experiments in order to discuss all the results as a whole.

PSP is a rare degenerative disease with an incidence rate of about 0.3–1.1 cases per 100,000 persons [1]. This disease is characterised by

Abbreviations: Aβ, Amyloid beta peptide; AD, Alzheimer's disease; ATEM, Analytical transmission electron microscopy; CAT, Computed axial tomography; ED, Electron diffraction; EELS, Electron energy loss spectrometry; END, Electron nanodiffraction; EXAFS, Extended X-ray Absorption Fine Structure; HAADF, High Angle Annular Dark Field; HRTEM, High resolution transmission electron microscopy; MRI, Magnetic resonance imaging; PHF, Paired helical filaments; PET, Positron emission tomography; PSP, Progressive supranuclear palsy; ROI, Regions of interest; SAD, Selected area diffraction; SIMS, Secondary Ion Mass Spectroscopy; SP, Senile (neuritic) plaques; STEM, Scanning Transmission Electron Microscopy; TEM, Transmission electron microscopy; XANES, X-ray Absorption Near Edge Structure; XRD, X-ray diffraction; XRF, X-ray fluorescence

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atrophy, loss of neurons and gliosis in three brain areas: the cortex, the basal ganglia and the brain stem. Neurophatologically, PSP is characterised by the accumulation, in affected neurons, of neurofibrillary tangles, that are aggregates of tau protein after its hyperphosphorylation [2].

In contrast, AD is one of the most common causes of dementia in western societies, being responsible of 60% of all the cases [3]. It was first described by the German physician Alois Alzheimer in 1907 [4] and is characterised by short-term memory loss and a progressive decline of cognitive and motor function. Biochemically, AD is characterised by the accumulation of amyloid-β peptide (Aβ) and tau protein in the frontal and temporal lobes, including the hippocampus. Amyloid-β peptide forms extra-cellular insoluble proteinaceous deposits called senile (neuritic) plaques, while the hyperphosphorylation of tau protein gives rise to bundles of paired helical filaments (PHF) that aggregate to form neurofibrillary tangles [5]. The causes and progression of Alzheimer's disease have not been totally clarified. As this disease does not usually appear in young people, factors associated with the aging process have to be taken into account to understand the aetiology of the disease, for example, oxidative stress, age-related myelin breakdown and its relationship with iron, proteinopathy-induced neuronal senescence or metabolic disorders [6–13]. Genetic factors also count for the few cases of family oriented early-onset of AD and recently three genes related to the disease have been identified [14]. However, the ultimate cause of AD is unknown and current treatment remains symptomatic.

In both PSP and AD, but also in other neurodegenerative diseases as Parkinson's disease, Pick's disease, Huntington's disease, Hallervorden–Spatz syndrome or Friedreich's ataxia, a significantly high accumulation of iron is observed in specific brain regions. Yet, little is known about the chemical speciation and the role of the accumulated iron and hence the interest on the study of this brain element.

Iron is the most abundant metal in the brain, however it should be mentioned that it also has distinct regional and cellular distributions [15]. In addition, it has been observed that the concentration of brain iron increases with aging [16]. On one hand, this element is necessary in the main neuronal processes, including neurotransmitter synthesis and myelination of axons. But, on the other hand, free iron is extremely harmful as it generates oxidative stress, a hallmark in aging diseases [17–25]. In fact, previous studies have shown an increased activity of antioxidant proteins in the brain of AD patients [26], especially the hippocampus and amygdale, where iron is accumulated.

As free iron is a source of oxidative stress, in physiological conditions it is usually linked to proteins as, for example, ferritin or transferrin. Moreover, particularly in the brain, large amounts of iron are sequestered in neuromelanin granules [16].

The use of ATEM for the study of brain iron is especially interesting to characterise the biomineral structure of brain ferritin cores and also to localize this protein and its degradation product, haemosiderin, at subcelullar level. Hence, our work has been focused on these two species: ferritin and haemosiderin.

Here, we summarize various studies on the composition of ferritin cores from the brain of PSP and AD patients (named "pathological ferritin") compared to physiological ferritins (horse spleen and human liver and brain), using analytical TEM methods such as high resolution transmission electron microscopy (HRTEM), electron nanodiffraction (END) and electron energy loss spectrometry (EELS), which allow the identification of the mineral phases of the individual ferritin cores. These techniques, which are frequently used for nanoanalysis in materials science, are rarely used in life sciences. The analysis of the core of "pathological" ferritins showed significant differences in the mineral composition compared to the physiological ones [27,28]. A special care should be taken in order to prevent transformations of the crystalline structure of the cores that have been recently reported [29] after electron beam irradiation. Therefore the protocols used to avoid these transformations have been described in detail.

In this article, we also describe the "in situ" visualization and distribution of the ferritin cores in sections of the hippocampus and the caudate nucleus of AD and PSP patients respectively [28,30]. Transmission electron microscopy (TEM) was used for the observation of unstained or lightly stained brain sections, allowing the differentiation of the ferritin cores from metal precipitates coming from conventional staining. Both ferritin and haemosiderin were visualized in damaged brain regions of PSP and AD patients. Besides, ferritin was found to be particularly abundant in neuropili regions, associated to dystrophic myelinated axons.

1.2. Ferritin description

As one of our main interests was the study of the composition of the ferritin cores, a description of this protein is provided as follows.

Ferritin, the main molecule involved in iron storage, is a globular protein of about 12–14 nm diameter with a central cavity — the core — of about 5–7 nm diameter where iron is stored in redox-inert forms as hydrated ferric iron oxide nanocrystals.

The protein shell, apoferritin, is composed of 24 sub-units of two different sizes: a heavy polypeptide Ft-H (MW$\approx$21 kD) and a light polypeptide Ft-L (MW$\approx$19 kD). The Ft-H and Ft-L subunits have different physiological roles. Ft-H has a ferroxidase center that catalyses Fe^{2+} oxidation and is predominant in organs requiring iron detoxification and cellular protection, such as heart and brain. The Ft-L subunit is involved in the nucleation and stabilisation of nanocrystals [31] and it is predominant in liver and spleen. Massover [32] and more recently by Chasteen and Harrison [33] revised the mechanisms of ferritin uptake and oxidation of ferrous ion (Fe^{2+}), as well as the nucleation and growth of iron oxide nanocrystals in the cores, and the reduction and release of iron from the molecule.

Ferritin has been identified at a histological level in pathological brains by means of immunohistological methods [15,34]. Using biochemical methods it has been observed that the increase in both Ft-H and Ft-L subunits that takes place during aging, fails to occur in Alzheimer's disease [35].

Identification of the mineral phase in the ferritin cores was performed mainly by X-ray diffraction (XRD) and electron diffraction (ED). Maxima position in the diffraction patterns allowed the determination of the lattice spacing. It should be mentioned that the ferritin core does not match with most common iron oxides or oxyhydroxides, i.e. goethite, hematite and magnetite. Therefore, different models that differ from lattice constants, atomic position, Fe coordination number and chemical composition have been proposed to resolve the ferritin core structure (for a review, see [32]). Towe and Bradley proposed that the mineral that composes ferritin cores was similar to ferrihydrite [36], and this compound was similar to hematite in structure. This was the most commonly accepted model at the time, although Harrison et al. [37] and Brady et al. [38] proposed other alternatives. Two decades later, ferrihydrite nanoparticles raised industrial interest mainly for its catalyst properties due to the high surface to volume ratio and the lack of agglomeration. This last characteristic makes its structural analysis cumbersome. In fact, none of the structure models at that time were generally accepted because the scarcity of XRD maxima made it difficult to determine its actual structure by this technique [39]. Other techniques such as Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy, X-ray Absorption Near Edge Structure (XANES) spectroscopy, Mössbauer spectroscopy, isotopic exchange and structural modelling of nanodiffraction patterns [40–43] were then used to analyze the ferrihydrite structure and as a result new models were proposed [44,45]. Although significant progress was made in the study of the ferrihydrite structure, none of these models are still irrefutable and the ferrihydrite structure is still being investigated [46–48]. As a result, the new data on the structure of ferrihydrite demanded a revision of the structure of ferritin cores that had been