Iron visualisation in the human brain with electron microscopy

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Iron is involved in various forms and mechanisms in humans from oxygen transport in erythrocytes to synthesis of neurotransmitters like dopamine. The most iron which is not bound in haemoglobin but contained in ferritin, the highly conserved main iron storage protein. Ferritin is known to be in liver and spleen but also in heart and brain. It is known that the nonheme iron amount in the brain increases in the first decades of life. Iron in the brain is very unequally distributed and in certain brain areas, the basal ganglia, the iron amount is even higher than in the liver. Until now little is known about the functional importance of iron in the human brain or the mechanism of iron accumulation during aging. But Iron in the brain has been associated with many inflammatory and neurodegenerative diseases like Alzheimer's disease or multiple sclerosis. It may promote the production of oxygen free radicals which could lead to the death of neuronal cells.

In rat brain it has been shown that oligodendrocytes store a large amount of ferritin iron as well as astrocytes, certain microglial cells and larger neurons but these findings yet need to be verified in human tissue. Furthermore, the size of the ferritin core varies with brain region but it is not known whether iron accumulates by an increased amount of ferritin molecule or by an increased filling of the cores of existing molecules.

Therefore it is important to study the mechanism of iron accumulation in different brain areas and we aim to elucidate total ferritin iron content and its cellular and subcellular distribution in areas with high and low iron content in human samples. With a comprehensive approach we aim to combine findings from quantitative magnetic resonance imaging, mass spectrometry, analytical electron microscopy and immunochemical tests of samples taken from various areas of the brain.

Challenging in human post-mortem brain studies is the considerable delay from time of death of the deceased until fixation of the tissue samples, especially in comparison to freshly fixed animal brain samples, due to ethical and logistical reasons. The degeneration and dismantling of the ultrastructure of post-mortem brain is very fast. Hence, the preservation of the remaining ultrastructure is crucial. We established a new hybrid-freeze method which combines the advantages of classical aldehyde fixation with high pressure freezing in combination with freeze substitution.

We confirm the visual findings of the ferritin particles with analytical electron microscopic techniques which detect the iron within the protein shell. With energy loss and energy filtered transmission electron microscopy (EELS/EFTEM) in combination with Energy-dispersive X-ray spectroscopy (EDX) we are able to analyse this element directly in the ultrastructure of the brain.