The influence of autolysis on the analysis of iron distribution in human brain

Summary

Gerd Leitinger, Gottfried Schatz Research Center, Cell Biology, Histology and Embryology, Medical University of Graz, Austria

Supervisor:	Prof. Dr. Gerd Leitinger
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between February 04, 2019 00:00 and March 31, 2019 23:59 (Europe/Zurich)

Description

Background:

During the first four decades of human life, Iron is known to accumulate in the human brain (1). Moreover, the iron is unevenly distributed between different brain areas (e.g. (2)). Excess iron has been implicated in neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, or multiple sclerosis, but little is known about the role iron plays in the etiology of these diseases (e.g. (3)).

Iron is stored in ferritin particles, and, using analytical electron microscopy, we can visualise the iron core of these particles directly in thin sections of the human brain. We can also identify ferritin within the sections using an immunogold method, and combine both methods to give proof that it is ferritins that are loaded with iron. Using an unbiased sampling protocol (4), we have thus obtained data on the ferritin iron distribution in several different brain areas (unpublished data). But these samples are prone to degradation by autolysis, as the *post mortem* interval is up to 24 hours, which could introduce changes in the ferritin iron distribution. During the *post mortem* interval the oxygen supply of the cells stops, and lack of oxygen could induce autophagy (5). Autophagy via lysosomes is a major way of ferritin degradation (6), so ferritins may become degraded *post mortem*.

As we now have access to fresh tissue samples as soon as they were removed from Neurosurgery, we will be able to determine the ferritin iron distribution in fresh tissue samples that have not undergone autolysis. Moreover, the fresh tissue samples will give us the opportunity of establishing tissue cultures, with direct access to interconnected neurons and glial cells for manipulation. Once these cultures have been established, the effect of iron chelation agents on the iron distribution can be tested.

Hypothesis and Objectives:

1. Ferritin is degraded during autolysis, leading to a loss of ferritin iron as compared to fresh brain tissue. To test this hypothesis, the ferritin iron distribution in freshly obtained tissue will be compared to the distribution in tissue with defined *post mortem* times.
2. Iron overload and iron chelation in the culture medium lead to changes in the iron level in glial cells and neurons. This hypothesis will be tested by applying iron overload or iron chelation to brain tissue cultures.
3. Autophagy is one mechanism of ferritin degradation during autolysis. This will be tested with electron microscopy, both in tissue and in tissue cultures.

Methodology:

The PhD candidate will collect human brain samples from Pathology and Neurosurgery, process these samples for electron microscopy, and perform analytical electron microscopy to determine the ferritin iron distribution. This will be complemented by Immunogold procedures that enable us to localise proteins at electron microscopic resolution, and with a range of molecular biological methods, such as Western Blotting, and isolating ferritin for examining its iron load (chelation of iron or iron overload), and tissue cultures.

References:

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