

Ultrastructural and chemical analysis of human Locus coeruleus using correlative microscopy and mass spectrometry

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Background and aims:

Among the characteristic hallmarks observed in the human brain during aging is the neuronal accumulation of neuromelanin (NM) pigment and iron in substantia nigra (SN) and locus coeruleus (LC), the two brain areas mainly targeted in Alzheimers and Parkinsons disease (PD).

The neuromelanin pigment accumulates inside the catecholaminergic neurons of SN and LC and there particularly within specific organelles (with an average size of 1.5 µm), called neuromelanin organelles. Within these organelles, neuromelanin clusters with lipid and protein-based partitions of less than 100 nm each.

Several spectroscopic studies investigated the role of neuromelanin pigments and metal dyshomeostasis in the SN during aging [1] and a found a correlation of both with disease progression. However, conducting chemical analysis on LC proves to be challenging due to the small size of the tissue. It would necessitate a substantial number of specimens which are difficult to collect. Moreover, only few studies attempted high lateral resolution subcellular (re)distribution analyses of iron storage in brain tissue, e.g. for SN [2] in order to find out where exactly the metal is stored on a subcellular basis.

Therefore, the aim of this work is to develop a workflow to study the ultrastructural metal distributions in NM organelles of LC on individual human tissue sections using novel high resolution analytical approaches based on secondary ion mass spectrometry (SIMS) performed on Focused Ion Beam instruments (FIB-SIMS)..o Chemical analysis were corroborated by CLEM (Abbrev.) to correlate the finding with the cellular and molecular changes that take place in relation to PD.

Methods:

A total of 11 human LC tissues were collected from elderly (n=9) and PD (n=2) specimen (60-80 years old) and were either formalin fixed paraffin embedded (FFPE), Epon-embedded or just as fresh frozen. Tissue sections were investigated by light, fluorescence and electron microscopy for high resolution imaging and immunohistochemistry, respectively. The standard chemical analysis was performed by analytical electron microscopy and mass spectrometric imaging (MSI), using the CAMECA NanoSIMS 50 with the ability for isotopic identification and highest sensitivity and mass resolution and a lateral resolution down to 50-

100 nm. Sub organellar distribution of metals in NM organelles was addressed on selected samples using FIB-SIMS with lateral resolution < 20 nm for SIMS and < 1nm for secondary electron (SE) imaging developed at LIST. A combined “TEM like” ultrastructural investigation together with SIMS is possible on the so-called npSCOPE, an in house novel cryoFIB-SIMS platform with SE, SIMS and scanning transmission ion microscopy (STIM) detectors [2]. Taking the advantage of the cryo-chamber of the npSCOPE, it can also be used to perform respective analyses on frozen-hydrated brain samples and thereby minimizing preparation artefacts.

Results:

A semi-quantitative SIMS approach was implemented here where a single tissue slice was imaged first using CLEM to identify the region of interest in LC tissues of both elderly control and PD subjects. Quantitative EDX maps and qualitative SIMS maps were acquired for regions of interest. Counts/ pixel ratios were calculated to get a semi-quantitative data set that shows different signal abundances for SIMS. Respective data on healthy elderly specimen showed that the melanic moiety of the NM organelle is composed by a mixture of eumelanin/ pheomelanin units with a sulfur/nitrogen ratio of (XXX), as identified by the sulphur signal which derives from the benzothiazines of the pheomelanin part of the NM. NM organelles showed increased signal for iron, calcium, and aluminium, in comparison to the lipid moiety of the NM organelles or the cytoplasmic surrounding areas. Interestingly, copper and zinc signal were at or below the detection limit, which is totally contrast to the isolated NM analysed using the Electron Paramagnetic resonance spectroscopy (EPR) that showed an actual accumulation of these two elements [1].

The higher spatial resolution of LIST’s FIB-SIMS instrument in comparison to the nano-SIMS could more precisely identify the localization of these metals and non-metals in different sub-organellar compartments of the NM organelle.

In addition, PO₂ signal which is an indicator of phosphorus rich areas like myelin sheath, nucleus and lipids was also weak in the lipid moiety and lipid bodies of the NM organelle, which might strongly agree with the fact that the lipid portion of NM is mainly composed of dolichols, and this is to be proved by lipidomic profiling. Another method of molecular profiling is done by IHC, in order to identify the differential expression of the markers related to inflammation, PD and iron storage proteins, and in order to identify the regions that showed, by our elemental analysis, to be rich in iron but are not a NM organelle, who could be glial cells or ferritin rich areas.

Conclusion:

We analysed the sub-cellular localization of certain marker elements for subcellular structures (PO for myelin), NM organelle compartments (based on Cl, S, CN, O signal distribution) in addition to the metal loading into these compartments in elderly controls and PD patients using routine and novel high-resolution chemical and structural imaging techniques in the NM in addition to its molecular proteomic and lipidomic profiling. Continued work will be done to identify more the significant differences in LC tissues of PD patients compared to healthy controls at the molecular and elemental levels, with investigations on fresh frozen samples for a better close to native analysis of PD pathophysiology also including molecular MSI, also done at high resolution, like MALDI and TOF-SIMS.

Keywords:

Correlative electron microscopy, SIMS, Parkinson

Reference:

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