UNCOVERING GANGLIOSIDE BIOMARKERS IN HUMAN EPILEPSY BY ION MOBILITY MS/MS

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Abstract
In the present study we introduced ion mobility separation mass spectrometry (IMS MS) for a comprehensive analysis of the changes in ganglioside pattern in human adult hippocampus affected by temporal lobe epilepsy (TLE) *vs.* normal hippocampus and discovery of disease-associated species.

Introduction
Epilepsy represents a neurological disorder manifested by recurrent seizures caused by damaged brain cells generating abnormal electrical signals. TLE is the most common type of epilepsy and over the years was approached by imaging, electroencephalographic recording, histological and molecular methods, however it still remained poorly understood [1]. Gangliosides (GGs) are implicated in modulation of neuronal excitability via interactions with ion transport systems, which is altered during epileptic seizures [2]. Therefore, here, we comparatively analyze the gangliosidome of normal and epileptic hippocampus using the most advanced MS technique.

Experimental
GG were extracted from the hippocampus of a 28 y.o. female affected by temporal lobe epilepsy (sample 28TLE-HS) and from a normal hippocampus of a 63 y.o. male, used as the control (sample 63CTRL). IMS MS experiments were conducted on a Synapt G2S (Waters) equipped with a nanoESI source at 1.3 kV ESI and 25 V cone potentials, IMS gas flow 90 $mL \cdot min^{-1}$, IMS wave velocity 650 $m \cdot s^{-1}$; IMS wave height 40 V. MS/MS experiments were performed by CID using collision energies within (30-50) eV.

Results and discussion
IMS MS analysis conducted under identical parameters for both 28TLE-HS and 63CTRL hippocampal tissue, revealed significant differences in GG profiles between the two samples. A total of 217 ions corresponding to 192 GGs were identified in 28TLE-HS, compared to 156 ions assigned to 137 distinct species in the healthy brain. Most structures were polysialylated and showed variations not only in sialylation degree and glycan core composition but also in ceramide structure. Additional modifications such as fucosylation, *O*-acetylation, and GalNAc or CH_3COO^-- attachments were detected. The comparison revealed marked dissimilarities in GG expression and structure between epileptic and normal tissues. Notably, the TLE-associated gangliosidome exhibited higher overall sialylation, with a predominance of penta-, hepta-, and octasialylated species. This could reflect a compensatory mechanism or result from altered neuronal excitability in the diseased hippocampus. Although di- and trisialylated gangliosides

were more present in TLE sample, GT species were more abundant in CTRL (31.8%) than in TLE (28.2%). Another notable finding is the elevated GD3 expression in 28TLE-HS (6.3%) *vs.* 63CTRL (2.9%), supporting its association with neuronal death in epilepsy and its potential role in TLE-HS pathogenesis and progression. Modified gangliosides also differed in expression pattern, for instance, *O*-acetylated structures were underrepresented in TLE, potentially indicating tissue deterioration as a consequence of this disease. Further, structural characterization was achieved via CID MS/MS analysis under varying collision energies in the transfer cell. These optimized conditions produced relevant fragment ions, crucial for precise structural elucidation and isomer identification.

Conclusion

IMS MS and CID MS/MS discovered a higher number of GG structures associated to TLE than reported before, differences in their expression in the hippocampus affected by epilepsy *vs.* normal hippocampus and indicated the sialylation and *O*-acetylation of the species as general markers of this pathology of the temporal lobe.

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