GLYCOLIPIDOMICS OF HUMAN CEREBELLUM IN DEVELOPMENT AND AGING BY ION MOBILITY TANDEM MASS SPECTROMETRY

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Abstract
In this study ion mobility separation (IMS) mass spectrometry (MS) was for the first time introduced in human cerebellum ganglioside research. The work was focused on a comprehensive mapping and structural characterization of human cerebellar gangliosides and determination of the specific changes induced in their expression by brain development and aging. We have carried out a comparative IMS MS mapping of the native ganglioside mixtures extracted from fetal cerebellum in the second trimester of pregnancy vs. near-term fetus vs. aged cerebellum, followed by IMS CID MS/MS fragmentation analysis.

Introduction
Cerebellum represents a highly specialized region of the brain encompassing approximately 80% of the total brain neurons and regular arrays of neuronal units involved in motor control, learning, cognitive and emotional functions [1, 2]. In recent years, human cerebellum started to be investigated at the molecular level, in order to correlate its functions with the expression of various biomolecules, especially gangliosides, sialylated glycosphingolipids highly expressed in the central nervous system. Among the bioanalytical methods employed so far in the analysis of cerebellar biomolecules, mass spectrometry (MS) provided the most comprehensive information [3].

Experimental
We report here on the first introduction in cerebellum research of ion mobility separation (IMS) mass spectrometry for a systematic mapping of cerebellar gangliosides and determination of the species associated to development and aging. For this purpose, gangliosides extracted from post-mortem tissue biopsies of fetal -in the 15 and 40 gestational weeks (samples 15GW and 40GW)- and 65 years of age (sample 65Y) cerebellum were analyzed by nanoESI IMS MS in the negative ion mode under identical conditions. In the next stage of the research, using CID MS/MS at low and variable collision energy after mobility separation in the transfer cell, were confirmed and characterized the structural conformation of the glycan core and ceramide moiety of three precursor ions that are common in all samples.

Results and discussion
Altogether, no less than 734 molecular ions corresponding to 551 gangliosides were identified by IMS MS separation and screening, which represents almost five times more cerebellar structures than ever reported before. In the absence of specialized software designed for automated interpretation of ganglioside mass spectra, extensive data analysis required manual calculations. As a result, average mass accuracies of 8.358 ppm for C15GW, 7.953 ppm for

C40GW, and 8.456 ppm for C65Y were obtained after assigning all 734 ions. The IMS MS analysis also revealed various intriguing aspects concerning the sialylation and modifications of ganglioside components, encompassing both carbohydrate and non-carbohydrate attachments, across the three extracts. Furthermore, the IMS MS data highlighted substantial deviations in sialylation patterns, as well as a surprisingly diverse array of alterations in the fundamental structure of the glycan core due to fucosylation, *O*-GalNAc attachment, *O*-acetylation, and modifications involving CH_3COO^-. Another significant discovery pertained to the pronounced heterogeneity and substantial differences in the composition and structure of the Cer moieties within the ganglioside species present in C15GW, C40GW, and C65Y. Notably, an unusually high number of compounds exhibiting trihydroxylated sphingoid bases, fatty acid chains with atypical lengths, or a combination of these characteristics were also observed.

The optimized isolation and fragmentation conditions induced efficient ion dissociation with high sequence coverage and a significant number of fragment ions diagnostic for the proposed structures of GD1 (d18:1/18:0), GQ1(t18:18:0) and GQ1(d18:1/18:0). Considering the MS/MS results, which provide evidence on $(Neu5Ac)_2$, the three structural candidates are b-isomers.

Conclusion

The comparative analysis revealed for the first time that: i) 40GW contains the highest number of species (373), followed by 15GW (327) and 65Y (192); ii) fetal cerebellum gangliosidome is characterized by a much higher sialylation degree and species altered by carbohydrate and non-carbohydrate type of modifications than the gangliosidome of aged cerebellum; iii) significant developmentally- and age-regulated changes in the expression and structure of cerebellum gangliosides exist. These variations are to be correlated in the future with the neurological diseases, leading to the discovery of pathways to more effective therapeutic schemes.

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