Mapping of gangliosides from defined regions of human brain by nanoelectrospray ionization quadrupole time-of-flight mass spectrometry

Alina F. Serb[1,2], Florian Harja[3], Željka Vukelić[4], Nicolae Dinca[3], Eugen Sisu[1] and Alina D. Zamfir[2,3]

[1]University of Medicine and Pharmacy, Timisoara, Romania; [2]Mass Spectrometry Division, National Institute for Research and Development in Electrochemistry and Condensed Matter, Timisoara; Romania; [3] Mass Spectrometry Laboratory, Department of Chemistr and Biology, Aurel Vlaicu University of Arad, Romania; [4]University of Zagreb Medical School, Zagreb, Croatia

INTRODUCTION

Gangliosides represent a family of complex glycosphingolipids characterized by the presence of one or more sialic acid units in an oligosaccharide chain attached to a hydrophobic ceramide anchor. Glycolipids of this type are found ubiquitously in the outer monolayer of the membrane bilayer surface of all animal tissues and are present at the highest concentration in neuronal and glial cells.

In the past decade fast atom bombardment (FAB) mass spectrometry (MS) was applied as the first MS method for human brain ganglioside analysis [1]. Recently, modern approaches based on matrix assisted laser/desorption ionization (MALDI) MS [2] and electrospray (ESI) MS [3] were introduced to enhance precise analysis of complex human brain ganglioside mixtures at high sensitivity [2, 3]. Differences in ganglioside composition and quantity in different anatomic regions of the brain have been so far demonstrated by thin layer chromatographic (TLC) as well as immunochemical and immunohistochemical methods. The experimental results obtained using these methods are, however, able to provide only data upon the major components present in complex mixtures.

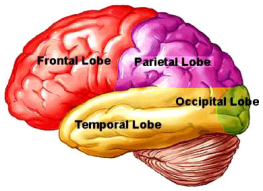

Figure 1. Lobes of the human brain

In this study, a comparative assay upon ganglioside expression followed by structural analysis of individual species in three different normal adult (42 y) human brain regions: frontal, parietal and occipital lobes was carried out by nanoESI quadrupole orthogonal acceleration time-of-flight mass spectrometry.

EXPERIMENTAL

1. Mass spectrometry

Nano electrospray mass spectrometry was performed on a hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (QTOF Micro Quattro Ultima, Waters Micromass, Manchester, U.K.). All mass spectra were acquired in the negative ion mode, previously shown to be best suited for glycosphingolipid screening by mass spectrometric methods [1]. nanoESI parameters were optimized to ensure a proper ionization and transfer into MS of the ganglioside components.

2. Ganglioside sample

The native mixtures of gangliosides were extracted from the following regions of the same normal adult brain: frontal, parietal and occipital. The brain originated from an adult (42 y) subject deceased in a traffic accident. The morphoanatomic and histopathologic examinations indicated a normal tissue without any neurological ailment, or malformation. The ganglioside mixtures were extracted following identical procedures/conditions and purified in our laboratories as described in detail previously [5]. For nano ESI QTOF MS and MS/MS analysis each ganglioside mixture was dissolved in pure methanol (MeOH) to the final concentration of 2-3 pmol/μL.

All three samples were analyzed under the same instrumental conditions: ESI capillary voltage, cone potential, desolvation gas flow rate, desolvation temperature, acquisition time.

References

[1] Peter-Katalinic, J. and Egge, H. Methods Enzymol. 193, 713-733, 1990.
[2] Ivleva, V.B., Elkin,Y.N., Budnik, B. A., Moyer, S.C., O'Connor P.B. and Costello C.E. Anal Chem. 76, 6484-6491, 2004.
[3] Vukelic, Ž., Kalanj-Bognar, S., Froesch, M., Bindila, L., Radic, B., Allen, M., Peter-Katalinic, J. and Zamfir A.D. Glycobiology (in press)
[4] Vukelic, Ž., Zarei, M., Peter-Katalinic, J. and Zamfir A.D. J. Chromatogr. A 20, 238-245, 2006.
[5] Vukelic, Z., Metelmann, W., Muthing, J., Kos, M. and Peter-Katalinic, J. Biol Chem. 382, 259-274, 2001.

RESULTS

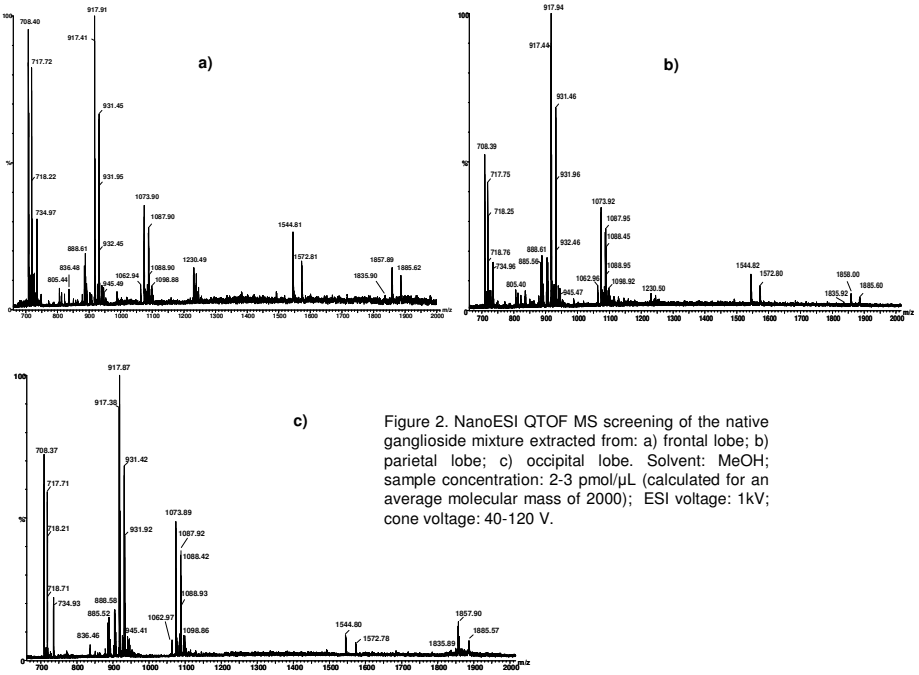

Figure 2. NanoESI QTOF MS screening of the native ganglioside mixture extracted from: a) frontal lobe; b) parietal lobe; c) occipital lobe. Solvent: MeOH; sample concentration: 2-3 pmol/μL (calculated for an average molecular mass of 2000); ESI voltage: 1kV; cone voltage: 40-120 V.

Table 1. Comparative assignment of the molecular ions detected in the three different lobes by nanoESI QTOF MS

Type of Molecular Ion	m/z (monoisotopic)		Assigned structure	Frontal lobe	Parietal lobe	Occipital lobe
	Detected	Calculated				
$[M+2Na-4H]^{2-}$ $[M-H]^-$	611.40 1179.70	611.35 1179.74	GM3 (d18:1/18:0)	+	+	+
$[M-H]^-$	1382.68	1382.72	GM2 (d18:1/18:0)	+	+	+
$[M-2H]^{2-}$ $[M+Na-2H]^-$	734.96 1492.78	734.91 1492.81	GD3 (d18:1/18:0)	+	+	+
$[M-2H]^{2-}$	748.97	748.93	GD3 (d18:1/20:0)	+	+	+
$[M-H]^-$	1518.51	1518.55	GM1, nLM1 and /or LM1 (d18:0/16:0)	+	+	+
$[M-2H]^{2-}$ $[M-H]^-$	771.98 1544.80	771.93 1544.85	GM1, nLM1 and /or LM1 (d18:1/18:0)	+	+	+
$[M-2H]^{2-}$ $[M-H]^-$	786.00 1572.80	785.92 1572.85	GM1, nLM1 and /or LM1 (d18:1/20:0)	+	+	+
$[M-2H]^{2-}$	836.46	836.45	GD2 (d18:1/18:0)	+	+	+
$[M-2H]^{2-}$	850.47	850.47	GD2 (d18:1/20:0)	+	+	+
$[M-2H]^{2-}$ $[M+Na-3H]^{2-}$ $[M-H]^-$ $[M+Na-2H]^-$	917.44 928.45 1835.92 1858.00	917.48 928.47 1835.96 1857.95	GD1, nLD1 and /or LD1 (d18:1/18:0)	+	+	+
$[M-2H]^{2-}$	926.44	926.48	GD1, nLD1 and /or LD1 (t18:0/18:0)	+	+	+
$[M-2H]^{2-}$	924.44	924.49	GD1, nLD1 and /or LD1 (d18:1/19:0)	+	+	+
$[M-2H]^{2-}$ $[M+Na-3H]^{2-}$ $[M-H]^-$	931.46 942.44 1885.60	931.49 942.48 1885.68	GD1, nLD1 and /or LD1 (d18:1/20:0)	+	+	+
$[M-2H]^{2-}$	940.49	940.50	GD1, nLD1 and /or LD1 (t18:0/20:0)	+	+	+
$[M-2H]^{2-}$	938.44	938.50	GD1, nLD1 and /or LD1 (d18:1/21:0)	+	+	+
$[M-2H]^{2-}$	945.47	945.51	GD1, nLD1 and /or LD1 (d18:1/22:0)	+	+	+
$[M-2H]^{2-}$	954.46	954.51	GD1, nLD1 and /or LD1 (t18:0/22:0)	+	+	+
$[M-2H]^{2-}$	952.47	952.52	GD1, nLD1 and /or LD1 (d18:1/23:0)	+	+	+
$[M-2H]^{2-}$	958.46*	958.52	GD1, nLD1 and /or LD1 (d18:1/24:1)	+	+	+
$[M-2H]^{2-}$	966.46	966.53	GD1, nLD1 and /or LD1 (d18:1/25:0) or (d20:1/23:0)	+	+	+
$[M-2H]^{2-}$	**988.40**	**988.49**	**Fuc-GD1 (d18:1/18:2)**	+	+	-
$[M-2H]^{2-}$	**990.40**	**990.51**	**Fuc-GD1 (d18:1/18:0)**	+	+	-
$[M-2H]^{2-}$	**999.46***	**999.51**	**Fuc-GD1 (t18:0/18:0)**	+	+	-
$[M-2H]^{2-}$	**1002.48**	**1002.51**	**Fuc-GD1 (d18:1/20:2)**	+	+	-
$[M-2H]^{2-}$	**1004.49**	**1004.52**	**Fuc-GD1 (d18:1/20:0)**	+	+	-
$[M-2H]^{2-}$	**1013.49***	**1013.53**	**Fuc-GD1 (t18:0/20:0)**	+	+	-

Table 1 (continued)

$[M-2H]^{2-}$	1018.99	1019.02	GalNAc- GD1 (d18:1/18:0)	+	+	+
$[M-2H]^{2-}$	1032.93*	1033.03	GalNAc- GD1 (d18:1/20:0)	+	+	+
$[M-3H]^{3-}$ $[M-2H]^{2-}$ $[M+Na-3H]^{2-}$ $[M+2Na-4H]^{2-}$	708.39 1062.96 1073.92 1084.93	708.35 1063.03 1074.02 1085.01	GT1 (d18:1/18:0)	+	+	+
$[M-3H]^{3-}$ $[M+Na-3H]^{2-}$	714.41 1082.92	714.35 1083.02	GT1 (t18:0/18:0)	+	+	+
$[M-3H]^{3-}$ $[M-2H]^{2-}$ $[M+Na-3H]^{2-}$ $[M+2Na-4H]^{2-}$	717.75 1076.97 1087.95 1098.92	717.69 1077.04 1088.03 1099.02	GT1 (d18:1/20:0)	+	+	+
$[M-3H]^{3-}$ $[M+Na-3H]^{2-}$	723.75 1096.93	723.70 1097.04	GT1 (t18:0/20:0)	+	+	+
$[M+Na-3H]^{2-}$	1094.95*	1095.04	GT1 (d18:1/21:0)	+	+	+
$[M-3H]^{3-}$ $[M+Na-3H]^{2-}$	727.08 1101.92	727.04 1102.05	GT1 (d18:1/22:0)	+	+	+
$[M+Na-3H]^{2-}$	1106.92*	1109.06	GT1 (d18:1/23:0)	+	+	+
$[M+Na-3H]^{2-}$	1114.96	1115.06	GT1 (d18:1/24:1)	+	+	+
$[M-3H]^{3-}$	**722.39**	**722.35**	**O-Ac-GT1 (d18:1/18:0)**	+	+	-
$[M-3H]^{3-}$	**731.74**	**731.70**	**O-Ac-GT1 (d18:1/20:0)**	+	+	-
$[M-2H]^{2-}$	**1138.95**	**1129.05**	**Fuc-GT1 (d18:1/17:0)**	-	+	-
$[M-2H]^{2-}$	**1144.89**	**1145.06**	**Fuc-GT1 (t18:0/18:0)**	-	+	-
$[M-2H]^{2-}$	**1159.89**	**1159.08**	**Fuc-GT1 (t18:0/20:0)**	-	+	-
$[M-3H]^{3-}$ **$[M+Na-4H]^{2-}$** **$[M+2Na-4H]^{2-}$** **$[M+3Na-5H]^{2-}$**	**805.40** **812.73** **1230.50** **1241.48**	**805.38** **812.71** **1230.56** **1241.55**	**GQ1 (d18:1/18:0)**	+	+	-
$[M-3H]^{3-}$ **$[M+Na-4H]^{2-}$** **$[M+2Na-4H]^{2-}$**	**814.74** **822.07** **1244.49**	**814.72** **822.05** **1244.57**	**GQ1 (d18:1/20:0)**	+	+	-
$[M-3H]^{3-}$ **$[M+Na-4H]^{2-}$**	**819.38*** **826.73***	**819.38** **826.71**	**O-Ac-GQ1 (d18:1/18:0)**	-	+	-

d – dihydroxy sphingoid base; t – trihydroxy sphingoid base; * low intensity ions; + the structure is present; - the structure is not present; red highlight – structures differently expressed

DISCUSSION

☑ **This work is a part of an extensive research program endeavouring a systematic brain ganglioside mapping by mass spectrometry. The final goal is to identify biomarkers valuable in early diagnosis and therapy of human brain diseases.**

☑ **By nanoESI QTOF MS screening of the three ganglioside mixtures, carried out under the same instrumental and solution conditions, in total 43 structures differing in glycan and/or ceramide constitution could be detected and identified.**

☑ **14 polysialylated structures and minor species modified by *O*-acetylation and fucosylation were found differently expressed in the three lobes. Of highest biological significance is that the parietal lobe showed the maximum diversity of ganglioside structures expressed, while the occipital region the less. The occipital was also the sole region showing no evidence upon the presence of *O*-acetylated and fucosylated species or components exceeding the sialylation degree 3.**

☑ **These results demonstrate the topospecificity of brain ganglioside composition on one side and corroborate the data upon previously explored brain regions [4] on the other.**

Acknowledgements

We are grateful to BET2 Software Königsbrunn, Germany for project assistance through CSI-Diff-MS (Chemical Structure Identification Based on Differential Mass Spectra) v. 2.5.1. software developed within the collaboration with N.D. The financial support was provided by the Romanian Ministry of Education and Research through the "Research of Excellence" grants No. 14/2005 and 98/2006 to A.D.Z.