Application of HPLC-Chip/MS to the Study of Brain Lipidomics

Faizy Ahmed[1, 2], Giuseppe Astarita[2], Hua Xiao[2] and Daniele Piomelli[2]

[1]Agilent Technologies, Inc., [2]University of California Irvine, Irvine, 92697 USA

Abstract

Lipids are important effectors of health and disease. Recent development of analytical applications using High Performance Liquid Chromatography coupled with Mass Spectrometry (LC-MS) had an enormous impact on the field of lipidomics, the large-scale study of lipid metabolites. Now it is possible to ask unprecedented questions, such as what is the precise distribution of lipids in the different sub-regions of the brain? Here we present a lipid profiling of the rat hippocampus sub-regions using nano column LC and HPLC-Chip MS. These techniques enabled us to measure lipids in exceedingly small amounts of brain tissue. First, we developed a fast and robust analytical method for the separation and identification of lipids, on a 1.8 µm Zorbax XDB C_{18}, 4.6 mm x 50 mm column, in brain hippocampus tissue obtained using laser micro-dissection. In order to lower the limit of detection, we transferred the method to a 0.5 mm id column (0.5 x 75 mm) enabling us to measure lipids in 1 mm^3 of hippocampus. Next, we lowered the limit of detection to 5 femto moles, with a dynamic range of 5 pico moles, using nano-columns (75 µm x 75 mm) with optimized Agilent Technologies nanoflow LC system coupled to nanospray MS. Finally, we adapted the method to HPLC-Chip/MS further lowering the limit of detection to 2.5 femto moles with a dynamic range of 100 femto moles. It was possible to generate lipid maps of the rat hippocampus analyzing brain regions as small as 50 $µm^3$. In conclusion, in this proof-of-concept study we demonstrated that lipidomic applications using HPLC-Chip/MS could contribute significantly to our understanding of the brain lipid metabolism.

Introduction-Neural Lipidomics

Although lipids constitute more than 50 % dry weight of human brain , their multiple roles are poorly understood. Developments in cell biology, synaptic physiology and pharmacology have shown that lipids not only make up the building blocks of cellular membranes but they can also act as intra-cellular and trans-cellular signaling messengers. Profiling large-scale changes in neural lipid composition and determining the topological distribution of individual lipid species in biological tissues constitutes the charter of Neural Lipidomics.

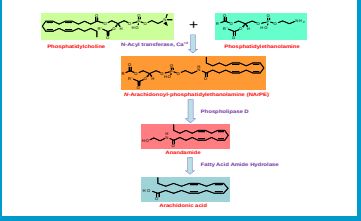
The Challenge

In our laboratory we have been studying the endogenous signaling lipids, which bind and activate the cannabinoid receptors in the brain and peripheral tissues, modulating various physiological functions such as pain, feeding and inflammation. Anandamide is one such signaling lipid produced from endogenous N-arachidonyl phosphatidylethanolamines (NarPEs), its precursor complex glycerophospholipids are derived from an exchange reaction of arachidonyl group between *sn*-1 position of phosphatidylcholine and primary amine of phosphatidylethanolamine catalyzed by the enzyme N-acyltransferase (Scheme 1). The composition of NarPEs species generating anandamide has not yet been determined.

Many current bio-analytical approaches ignore the fine biological borders of brain regions, thus missing on critical information. The challenge, for Neural Lipidomics, is to perform quantitative determinations of neural lipids in vanishingly small amounts of brain tissue, where these molecules are present in extremely small concentrations.

In the current study, we have attempted to develop sensitive HPLC/MS methods for the determination of endocannabinoids and their precursors in the sub-regions of rat brain hippocampus.

Lipid Biosynthetic Pathways



Scheme 1: Biosynthetic pathway for endocannabinoid anandamide

Materials & Methods

LC-MS Analysis of Lipids

Instruments: Agilent 1100 system coupled with an Agilent Quad 1964A-MSD or Agilent Ion Trap XCT equipped with an electrospray ionization interface were used for the initial method development for lipid analysis with Eclipse XDB C_{18}, 1.8 µm 80 Å (4.6 x 50 mm). For high sensitivity lipid analysis an Agilent 1100 Nano-LC coupled with a capillary LC was used with an Eclipse XDB C_{18} (75 µm x 75 mm) as the analytical column and an Eclipse XDB C_8 (0.3 x 35 mm) as an enrichment column. The HPLC-Chip consisted of an Eclipse XDB C_{18} (3.5 µm 80 Å, 75 µm x 150 mm) as the analytical column and Eclipse XDB C_8 (3.5 µm 80 Å, 163 nL) as the enrichment column. Agilent Ion-Trap XCT-Ultra was used as the MSD with the Nano or HPLC-Chip systems. The HPLC-Chip was interfaced with the MSD with the Agilent Technologies Chip-interface.

Columns and Chemicals: LC columns were obtained from Agilent Technologies, Inc. (Wilmington DE) and the HPLC-Chip from Agilent Technologies (Waldbronn, Germany). Chemicals were from Sigma/Aldrich (St. Louis, MO) and Nu-Chek Prep (Elysian, MN).

Lipid Extraction: Frozen brains were weighed and homogenized in methanol (100 mg tissue/mL) containing internal standard. Lipids were extracted with two volumes of chloroform and washed with one volume of water. Organic phases were collected and dried under nitrogen and reconstituted in 0.1 mL of chloroform: methanol (1:4 V: V) and used for LC/MS^n analysis.

Results

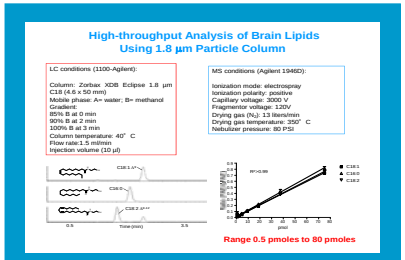


Figure 1: High-throughput analysis of lipids by LC/MS

Results

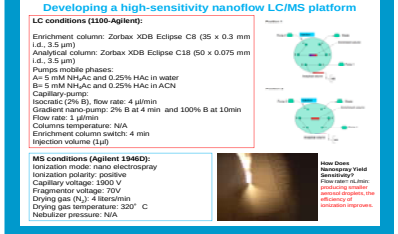


Figure 2 : Nano LC\MS conditions and flow path with the enrichment column

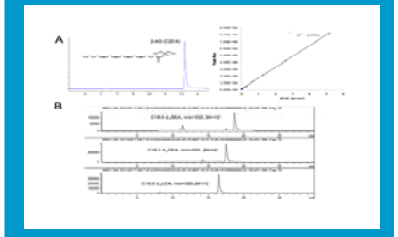


Figure 3 :Nano LC\MS and calibration curve for 2-AG with a range of 5 fmole to 5 pmoles.

Figure 4: Agilent HPLC-Chip/MS system- Integration of enrichment and analytical columns on the HPLC-chip.

Results

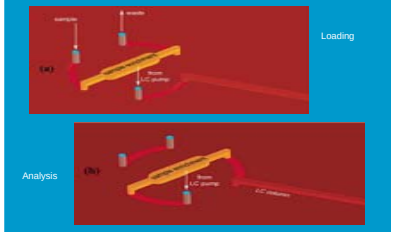


Figure 5: Flow path for on-line concentration and analysis of lipids on HPLC-Chip/MS.

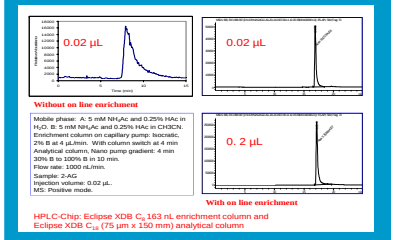


Figure 6: Effects of sample enrichment on HPLC-Chip.

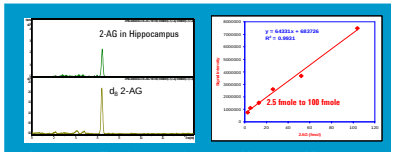


Figure 7A: Calibration curve for 2-AG with HPLC-Chip

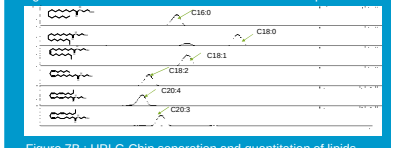


Figure 7B : HPLC-Chip separation and quantitation of lipids.

Results

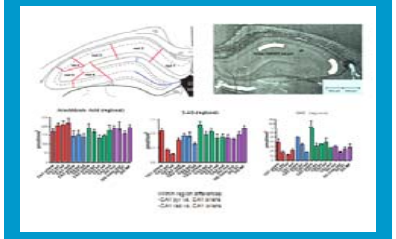


Figure 8: Lipid profiles of sub-regions of rat brain hippocampus.

Conclusions

- In our previous work we showed a the development of a fast HPLC/MS method for the determination of lipids in the rat brain hippocampus (Figure 1).
- Continuing this study, we developed a more sensitive method for lipid analysis using the nano-LC/MS with a detection limit of 5 fmole (Figures 2 and 3).
- The nano-LC has limitations such as, leaks, misalignment of connecting capillaries, clogging of MS spray needles and large extra-column volume, all of which may lead to significant instrument down time as well as peak broadening.
- The HPLC-Chip alleviates these issues by integrating all the functions, such as enrichment column, capillary connections and MS spray needle into a credit card size microfluidic device (Figure 4).
- The integrated on-chip enrichment column allowed the injection of large amounts of sample (200 nL) without losing chromatographic performance (Figure 6).
- HPLC-Chip/MS further improved the sensitivity of detection by two-fold to 2.5 fmole of 2-AG (Figure 7 A and 7B).
- Using these techniques, we were able to determine the differences in lipid levels of the rat brain hippocampus sub-regions in samples as small as 50 $µm^3$ (Figure 8).
- These studies will significantly advance our understanding of the biological role of lipids in the brain in health and disease.

References:

1. Astarita, G and Piomelli, D. Lipidomic analysis of endocannabinoid metabolism in biological samples. J. Chr. B, Epub ahead of print, January 2009.
2. Astarita, G; Ahmed, F and Piomelli, D. Identification of biosynthetic precursors of endocannabinoid anandamide in the rat brain. J. Lipid Res. **49**, 48, 2008.
3. Piomelli, D; Rapaka, R and Astarita, G. A Neuroscientists guide to lipidomics. Nature Reviews/Neuroscience 8, 743, 2007.

Acknowledgements:

The authors thank the Agilent Foundation for a grant in support of these studies.

Agilent Technologies