World Journal of Gastrointestinal Pathophysiology

Online Submissions: http://www.wjgnet.com/2150-5330office
wjgp@wjgnet.com
doi:10.4291/wjgp.v3.i6.99

World J Gastrointest Pathophysiol 2012 December 15; 3(6): 99-101
ISSN 2150-5330 (online)

EDITORIAL

Sphingolipids in intestine and liver: How to analyze?

Nikolaus Gassler

Nikolaus Gassler, Institute of Pathology, RWTH Aachen University, Pauwelsstraße 30, 52074 Aachen, Germany
Author contributions: Gassler N solely contributed to this paper.
Correspondence to: Dr. Nikolaus Gassler, MA, Professor, Institute of Pathology, RWTH Aachen University, Pauwelsstraße, 30, 52074 Aachen, Germany. ngassler@ukaachen.de
Telephone: +49-241-8088897 Fax: +49-241-8082439
Received: August 26, 2011 Revised: November 26, 2012
Accepted: December 10, 2012
Published online: December 15, 2012

Abstract

Identification and quantification of lipids, in particular sphingolipids from intestine and liver, using multidimensional mass spectrometry has dramatically improved our understanding of lipid-based molecular pathways and signaling. The editorial gives a short overview about basic technical approaches to characterize lipids from intestine and liver.

Key words: Intestine; Lipids; Liver; Mass spectrometry

Peer reviewers: Murielle Mimeault, PhD, Senior Associate Researcher, Department of Biochemistry and Molecular Biology, College of Medicine, University of Nebraska Medical Center, 985870 Nebraska Medical Center, Omaha, NE 68198-5870, United States; Sharon DeMorrow, PhD, Assistant Professor, Digestive Disease Research Center, Department of Internal Medicine, Scott and White Hospital and Texas A and M Health Science Center, 702 SW H.K Dodgen Loop, Temple, TX 76504, United States

Gassler N. Sphingolipids in intestine and liver: How to analyze? *World J Gastrointest Pathophysiol* 2012; 3(6): 99-101 Available from: URL: http://www.wjgnet.com/2150-5330/full/v3/i6/99.htm DOI: http://dx.doi.org/10.4291/wjgp.v3.i6.99

INTRODUCTION

The molecular class of lipids includes a highly divers group of molecules with hydrophobic or amphiphilic character. Important molecule sub-groups are fatty acids, glycerolipids, and sphingolipids which are widely distributed in intestinal tissues. Lipids are involved in several signaling cascades as pure molecules or protein modifying agents. In addition, lipids are important as structural components and in energy storage.

The family of sphingolipids includes ceramides and sphingosines which are of high importance in cellular signaling and addressing regulation of cellular permeability, cell survival, and transformation. The complex sphingolipids are molecular described and sub-classified by backbones and additional groups including sugars[1,2]. The sphingoid base backbone is available by two biochemical pathways: (1) *de novo* or (2) cleavage of sphingomyelin[3]. The interested reader is referred to more specific articles describing the biochemistry of sphingolipid metabolism in more detail[4].

Sphingolipids are target molecules for coupling of highly diverse sugar residues. The term complex sphingolipids describes this structural feature. Such molecules are abundantly found in liver and intestine and essential for structural integrity, barrier function, and inflammation, where they act as bioactive messengers[5,6]. In the last decade, molecular analysis of lipids from liver tissues has become a very important topic, because the incidence of fatty liver associated disorders, *i.e.*, fibrosis and carcinogenesis, is dramatically increasing.

Standard algorithms are established for extraction of lipids from liver and intestinal tissues[7]. Dissection of target areas from fresh intestinal or liver tissues followed by homogenization without cryo-conservation is essential. Prior to the extraction of lipids for measurements an internal lipid marker must be added. After the extraction step cryo-conservation and storing of the mix is possible.

In following a short overview of mass spectrometry-based lipid analysis and profiling of sphingolipids from intestinal and liver sources is given.

MASS SPECTROMETRY-BASED SPHINGOLIPID ANALYSIS

Lipidomics, the large-scale analysis of cellular lipid path-

ways and networks, is an analytical approach to distinguish different lipid species that are structurally similar and often metabolically interconvertible[5]. In this setting, comprehensive mass spectrometry-based techniques are established for elucidation of physiological properties and functionality of lipids in a single sample[2,6].

From the technical point of view, mass spectrometers include an ion source, a mass analyzer (separation of ions by mass to charge ratio), and a detector module. Electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) mass spectrometry are the basic technical systems. MALDI mass spectrometry is a laser-based soft ionization method preferentially used for protein analysis, but useful in lipid research too. A special organic matrix component is mixed-up with the lipid containing sample and irritated with a laser to ionize probe molecules. Because generation of intact molecular ions with MALDI is possible, this technique has been successfully used to analyze complex lipids. Tissue imaging mass spectrometry, another interesting application for MALDI, has the advantage to identify lipid quantities and their distribution and sub-cellular localization in a tissue. In this setting extraction of lipids from their biological sources is not necessary[7,8].

In contrast to MALDI, ESI based technologies are soft ionization methods, where lipid containing solutions continuously infused through a small capillary into an electric field generating very fine charged droplets. The droplets rapidly evaporate and divide into individual charged ions, best analyzed with a triple quadrupole mass analyzer[9-12].

Liquid chromatography-mass spectrometry (LC-MS) and tandem mass spectrometry (LC-MS/MS) are further important technologies and very popular in lipid research[2]. In addition, electron crystallography has been established to study membrane proteins in a lipid-rich environment. The shotgun lipidomics approach, another mass spectrometric technique, works without direct coupling of any chromatography for lipid separation. This infusion-based lipidomic technique allows quantification of a high number of lipid species in small probes from intestine and liver. The approach is hampered by the fact that the method does not distinguish isomeric and isobaric lipid species[13-15].

Concerning sphingolipids, LC-MS- or LC-/MS/MS, shotgun-lipidomics, and MALDI imaging mass spectrometers are established as basic technical approaches. Using LC-MS/MS identification, quantification, and structural analysis of free sphingoid bases/phosphates, ceramides, monohexosylceramides, lactosylceramides, sphingomyelins and other glycosphingolipids are possible[16-19]. Combination of normal-phase HPLC and ESI-MS analysis is another interesting approach to identify sphingolipids in heterogeneous lipid containing solutions and materials[20]. Although direct infusion shotgun lipidomics has some limitations, the method is useful and very efficient to quantify lipid species from any unknown sample including detection of low-abundance sphingolipid metabolites *e.g.*, ceramide-phosphates and sphinganine[21].

CONCLUSION

Lipids and in particular sphingolipids are highly distributed throughout intestinal and liver. They have diverse functions in cellular systems including molecular signaling. In-detail analysis of sphingolipids is assumed as promising for better understanding of intestinal and liver physiology and pathology. At present, several types of mass spectrometry-based measurements are established as powerful tools in lipid analysis. Advantages and disadvantages of the different technologies have to been critically proofed to find the adequate method answering an experimental working hypothesis.

ACKNOWLEDGEMENTS

The comments of Dr. Reinartz A and Dr. Henkel C to improve the manuscript as well as the help of P Akens in typing and proofreading the manuscript are highly acknowledged.

REFERENCES

1 **Fahy E**, Subramaniam S, Brown HA, Glass CK, Merrill AH, Murphy RC, Raetz CR, Russell DW, Seyama Y, Shaw W, Shimizu T, Spener F, van Meer G, VanNieuwenhze MS, White SH, Witztum JL, Dennis EA. A comprehensive classification system for lipids. *J Lipid Res* 2005; **46**: 839-861
2 **Wenk MR**. The emerging field of lipidomics. *Nat Rev Drug Discov* 2005; **4**: 594-610
3 **Pyne S**, Pyne NJ. Sphingosine 1-phosphate signalling in mammalian cells. *Biochem J* 2000; **349**: 385-402
4 **Gault CR**, Obeid LM, Hannun YA. An overview of sphingolipid metabolism: from synthesis to breakdown. *Adv Exp Med Biol* 2010; **688**: 1-23
5 **Han X**. Lipidomics: developments and applications. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009; **877**: 2663
6 **Brown HA**, Murphy RC. Working towards an exegesis for lipids in biology. *Nat Chem Biol* 2009; **5**: 602-606
7 **Garrett TJ**, Dawson WW. Lipid geographical analysis of the primate macula by imaging mass spectrometry. *Methods Mol Biol* 2009; **579**: 247-260
8 **Chen Y**, Allegood J, Liu Y, Wang E, Cachón-Gonzalez B, Cox TM, Merrill AH, Sullards MC. Imaging MALDI mass spectrometry using an oscillating capillary nebulizer matrix coating system and its application to analysis of lipids in brain from a mouse model of Tay-Sachs/Sandhoff disease. *Anal Chem* 2008; **80**: 2780-2788
9 **Liebisch G**, Drobnik W, Reil M, Trümbach B, Arnecke R, Olgemöller B, Roscher A, Schmitz G. Quantitative measurement of different ceramide species from crude cellular extracts by electrospray ionization tandem mass spectrometry (ESI-MS/MS). *J Lipid Res* 1999; **40**: 1539-1546
10 **Fujiwaki T**, Yamaguchi S, Sukegawa K, Taketomi T. Application of delayed extraction matrix-assisted laser desorption ionization time-of-flight mass spectrometry for analysis of sphingolipids in tissues from sphingolipidosis patients. *J Chromatogr B Biomed Sci Appl* 1999; **731**: 45-52
11 **Merrill AH**, Sullards MC, Allegood JC, Kelly S, Wang E. Sphingolipidomics: high-throughput, structure-specific, and quantitative analysis of sphingolipids by liquid chromatography tandem mass spectrometry. *Methods* 2005; **36**: 207-224
12 **Suzuki Y**, Suzuki M, Ito E, Goto-Inoue N, Miseki K, Iida J,

Yamazaki Y, Yamada M, Suzuki A. Convenient structural analysis of glycosphingolipids using MALDI-QIT-TOF mass spectrometry with increased laser power and cooling gas flow. *J Biochem* 2006; **139**: 771-777

13 **Watson AD**. Thematic review series: systems biology approaches to metabolic and cardiovascular disorders. Lipidomics: a global approach to lipid analysis in biological systems. *J Lipid Res* 2006; **47**: 2101-2111

14 **Albert CJ**, Anbukumar DS, Monda JK, Eckelkamp JT, Ford DA. Myocardial lipidomics. Developments in myocardial nuclear lipidomics. *Front Biosci* 2007; **12**: 2750-2760

15 **Gross RW**, Han X. Shotgun lipidomics of neutral lipids as an enabling technology for elucidation of lipid-related diseases. *Am J Physiol Endocrinol Metab* 2009; **297**: E297-E303

16 **Colsch B**, Afonso C, Popa I, Portoukalian J, Fournier F, Tabet JC, Baumann N. Characterization of the ceramide moieties of sphingoglycolipids from mouse brain by ESI-MS/MS: identification of ceramides containing sphingadienine. *J Lipid Res* 2004; **45**: 281-286

17 **Masukawa Y**, Tsujimura H. Highly sensitive determination of diverse ceramides in human hair using reversed-phase high-performance liquid chromatography-electrospray ionization mass spectrometry. *Lipids* 2007; **42**: 275-290

18 **Berdyshev EV**, Gorshkova IA, Garcia JG, Natarajan V, Hubbard WC. Quantitative analysis of sphingoid base-1-phosphates as bisacetylated derivatives by liquid chromatography-tandem mass spectrometry. *Anal Biochem* 2005; **339**: 129-136

19 **Shaner RL**, Allegood JC, Park H, Wang E, Kelly S, Haynes CA, Sullards MC, Merrill AH. Quantitative analysis of sphingolipids for lipidomics using triple quadrupole and quadrupole linear ion trap mass spectrometers. *J Lipid Res* 2009; **50**: 1692-1707

20 **Quehenberger O**, Armando AM, Brown AH, Milne SB, Myers DS, Merrill AH, Bandyopadhyay S, Jones KN, Kelly S, Shaner RL, Sullards CM, Wang E, Murphy RC, Barkley RM, Leiker TJ, Raetz CR, Guan Z, Laird GM, Six DA, Russell DW, McDonald JG, Subramaniam S, Fahy E, Dennis EA. Lipidomics reveals a remarkable diversity of lipids in human plasma. *J Lipid Res* 2010; **51**: 3299-3305

21 **Zeng Y**, Cheng H, Jiang X, Han X. Endosomes and lysosomes play distinct roles in sulfatide-induced neuroblastoma apoptosis: potential mechanisms contributing to abnormal sulfatide metabolism in related neuronal diseases. *Biochem J* 2008; **410**: 81-92

S- Editor Cheng JX **L- Editor** A **E- Editor** Zhang DN