Sphingolipids in intestine and liver: How to analyze?

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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.

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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.
ways and networks, is an analytical approach to distin-
guish different lipid species that are structurally similar
and often metabolically interconvertible[9]. In this setting,
comprehensive mass spectrometry-based techniques are
established for elucidation of physiological properties
and functionality of lipids in a single sample[12,14].

From the technical point of view, mass spectrom-
eters 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 imag-
ing 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[9,10].

In contrast to MALDI, ESI based technologies are
soft ionization methods, where lipid containing solu-
tions 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[11-12].

Liquid chromatography-mass spectrometry (LC-MS)
and tandem mass spectrometry (LC-MS/MS) are fur-
ther important technologies and very popular in lipid
research[13]. In addition, electron crystallography has been
established to study membrane proteins in a lipid-rich
environment. The shotgun lipomics approach, another
mass spectrometric technique, works without direct cou-
pling of any chromatography for lipid separation. This
infusion-based lipidic 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 iso-
baric lipid species[13-14].

Concerning sphingolipids, LC-MS- or LC-/MS/MS,
shotgun-lipidomics, and MALDI imaging mass spectrom-
eters are established as basic technical approaches. Using
LC-MS/MS identification, quantification, and structural
analysis of free sphingoid bases/phosphates, ceramides,
monohexosylceramides, lactosylceramides, sphingomy-
elines and other glycosphingolipids are possible[15-16].
Combination of normal-phase HPLC and ESI-MS analysis
is another interesting approach to identify sphingolipids in
heterogeneous lipid containing solutions and materials[17].
Although direct infusion shotgun lipidomics has some
limitations, the method is useful and very efficient to
quantify lipid species from any unknown sample includ-
ing detection of low-abundance sphingolipid metabolites
e.g., ceramide-phosphates and sphinganine[20].

CONCLUSION

Lipids and in particular sphingolipids are highly distrib-
uted throughout intestinal and liver. They have diverse
functions in cellular systems including molecular signal-
ing. 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 disad-
vantages of the different technologies have to been criti-
cally proofed to find the adequate method answering an
experimental working hypothesis.

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