IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 11, Issue 6 Ver. VI (Nov. - Dec.2016), PP 88-92
www.iosrjournals.org

Recent Analytical Trends in Lipidomics; Techniques and Applications in Clinical Medicine

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Abstract:*Lipidomics is an emerging field of biomedical research which includes complex lipidome analysis. Basically it is the lipidome whichis the comprehensive and quantitative description of a set of lipid species present in an organism. Lipidomics encompasses the comprehensive analysis of molecular lipid species, including their quantitation and metabolic pathways. The wide diversity of native lipids and their modifications make lipidomic analyses challenging and difficult. The method of choice for sensitive detection and quantitation of molecular lipid species is mass spectrometry, either by direct infusion or coupled with liquid chromatography. Although lipidomics allows for high-throughput analysis, low-abundant lipid species are not properly detected. Previous separation of lipid species by liquid chromatography increases ionization efficiency and is better suited for quantifying low abundant and isomeric lipid species. This article reviews the recent trends in this emerging field.*

Keywords:*Lipids, Lipidomics, analytical, chromatography, MALDI-TOF, spectrometry*

I. Introduction

The increase in interest in lipid research is due to the emergence of the term 'lipidomics' some twelve years ago.The major aim of lipidomics is the complete characterization of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation.While achieving this aim is still some way off, recent advances in lipidomics technology and methodology over the past decade has greatly enhanced our understanding of lipids and their functions. Lipids are an extremely diverse class of biomolecules that were originally classified based on a physicalproperty, their insolubility in aqueous solvents. Many of these classes themselves contain subclasses that contain dozens or even hundreds of unique and distinct molecules (Refer Fig.1 and Table 1). This encompasses an enormous number of compounds that exist in a wide range of concentrations in cells, fluidsor tissues and thus represents a significant analytical challenge [1]. Lipidomics is a relatively recent research field that has seen rapid advances in analytical technologies such as mass spectrometry (MS), nuclear magnetic resonance (NMR) spectroscopy, fluorescence spectroscopy, dual polarisation interferometry and computational methods, supported by the recognition of the role of lipids in many metabolic diseases such as obesity, atherosclerosis, stroke, hypertension and diabetes. This rapidly developing field complements the significant progress made in genomics and proteomics, all of which constitute the family of systems biology [2].Lipidomics research involves the identification and quantification of the thousands of cellular lipid molecular species and their interactions with other lipids, proteins, and other metabolites. Investigators in lipidomics are examining the structures, functions, interactions, and dynamics of cellular lipids and the changes that occur during perturbation of the system.

This review will provide a brief overview of the distinctlipid classes and lipidomics techniques currently in use and the challenges they are posing to the scientific researchers.

Mass Spectrometry Based Lipid Analysis

Several modern technologies including mass spectrometry (MS), nuclear magnetic resonance (NMR), fluorescence spectroscopy, column chromatography, and microfluidic devices are now being utilised in lipidomics to identify, quantify, and understand the structure and function of lipids in biological systems [3]. Advancements in mass spectrometric technology have proved highly efficient for the characterization and quantification of molecular lipid species in total lipid extracts. The main advantage of mass spectrometry is its ability to separate and characterize charged ionized analytes according to their mass-to-charge ratios (m/z). It can also provide structural information by fragmenting the lipid ions by collision-induced dissociation (CID). The various techniques used to record these fragmentation reactions are called tandem MS, or MS/MS or MS^n. These qualities lead to unparalleled selectivity, sensitivity and the ability to provide structural information for components in complex mixtures [4]. Another new advancement in MS is the Multi-dimensional mass spectrometry based high through put lipidomics (MDMS-SL) is a further development of high through put lipidomics taking into account the concept of building blocks in lipid structures [5]. MDMS-SL takes advantage of differential intrasource separation properties with various additives like Li+ , NH4 + or Na+ , and

specificfragments for each lipid class. Glycerolipids without class specific fragments are detected by constant neutral losses of fatty acids and inference about the intact lipid is drawn from the combinatorial possibilities of all monitored fatty acid neutral losses [6]. This technique is coupled with the Nanomate® system (Advion Biosciences, NY, USA), the MDMS-SL concept proves to be a powerful high throughput device because of its high degree of automatization and the enhanced sensitivity provided by a nano-ESI source.The MDMS-SL system covers quantitative analysis of different classes of glycerophospholipids, glycerolipids and sphingolipids.The developed MS techniques can be mainly categorized basically into three categories:

a) **Global Lipidomic Evaluation**: This is aimed at identifying and quantifying hundreds to thousands of cellular lipid species via a high throughput investigation basis. Different throughput lipidomics-based platforms have been developed and extensively used to analyze diverse pathways and networks associated with lipid metabolism, trafficking, and homeostasis. Newly emerging mapping techniques play major roles in studying the spatial and temporal relationships of lipids.

b) **Targeted Lipidomic Evaluation**: Identification of one or a few lipid classes of interest. LC-MS and LC-MS/MS based methods have been extensively utilized for this purpose. Nevertheless, mass spectrometry suffers limitations in its ability to distinguish between the many structural isomers commonly found among lipids. Specifically, it cannot properly determine: (i) the site of fatty acid attachment on glycerol backbones of glycerolipids and glycerophospholipids, (ii) the positions of double bonds, and (iii) the stereochemistry of double bonds.

c) Novel Lipid Discovery: It is directed towards the discovery of novel lipid classes and molecular species. Methodology using LC coupled with MS plays an essential role in this area through different enrichment technologies.The mass spectrometry techniques described above provide an unprecedented level of lipid molecular analysis.

High performance liquid chromatography (HPLC) techniques in Lipidomics:

There are several forms of HPLC techniques now used in lipidomics experiments.In an application note published by Eric Verrett and Derrick Hillbeck [7] described the analysis of a mixture of twenty five fatty acids using a reversed-phase HPLC method with a 1.9 µm column and evaporative light scattering detection. However, more recently faster technologies with better resolution have been developed including high throughput HPLC-MS and GC-MS systems. Establishing good separation, both analyte from analyte and analyte from matrix components is key to the success of these analyses. The Thermo Scientific Hypersil GOLD range of HPLC columns was developed to give reproducible and reliable chromatographic analysis with excellent peak shape. Greater resolution can be achieved through increased efficiency when using 2 µm particle size products. This application note demonstrates the effective separation of a number of typical compounds in the field lipidomic analysis and their detection at low nanogram levels using a light scattering detector, the SEDEX LT-ELSD™

Off late several new forms of HPLC techniques have been employed to detect lipids. For e.g.,theNormal phase HPLC (NPLC) separates lipids based on their polar head groups, while reverse phase HPLC (RPLC) separates lipids based on their acyl chains structure. Recent advancements in stationary phase technology have led to the introduction of ultrahigh performance liquid chromatography (UHPLC or UPLC),which provides increased resolution and much faster analysis times. The power of reverse phase UPLC-MS is demonstrated in a recent study where 182 eicosanoids were monitored in human plasma samples in just a matter of minutes [8]. Meikle and coworkers have also utilised HPLC-MS to identify and measure specific plasma lipids that are markers of coronary artery disease risk [9].

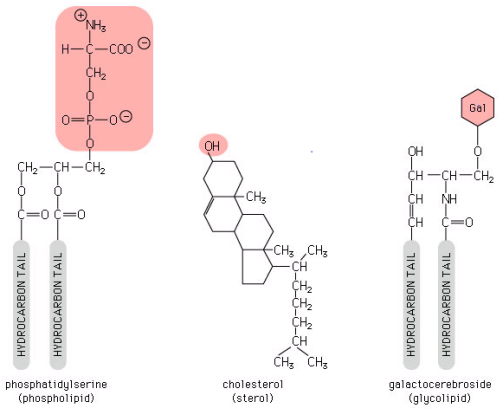

Fig.1: Chemical Structure of some common lipids
Source: Courtesy; Hierarchy of Life (http://xarquon.jcu.cz/edu).

Table 1: Different classes of Lipids

Glycerophospholipids	Phosphatidic acid Phosphatidylcholine Phosphatidylethanolamine Phosphatidylglycerol Phosphatidylserine Phosphatidylinositol Cardiolipin
Sphingolipids	Sphingoid bases Ceramides Phosphosphingolipids Sphingomyelins Glycosphingolipids
Sterol Lipids	Sterols Steroids Secosteriods Bile acids
Glycerolipids	Monoacylglycerols Diacylglycerols Triacylglycerols
Fattyacyls	Fatty acids and conjugates Octadecanoids Eicosanoids Docosonoids Fatty alcohols Fatty aldehydes Fatty amides Hydrocarbons
Prenol Lipids	Isoprenoids Quinones and hydroquinones Polyprenols
Saccharolipids	Acylaminosugars Acylaminosugar glycans Acyltrehaloses Acyltrehalose glycans
Polyketides	Aromatic polyketides Macrolide polyketides

Imaging Mass Spectrometry of Lipids

An area of specific interest in lipid mass spectrometry in recent years has been imaging mass spectrometry (IMS).The most common technique used for IMS is matrix assisted laser desorption/ionisation (MALDI) MS. In these experiments, a tissue slice is coated with a matrix (to absorb most of the photon energy) and is then rastered with a laser to create lipidions that are analysed by the mass spectrometer. The mass spectral data can then be correlated to a specific site on the tissue. By tracking the abundance of a specific lipid ion, a map can be obtained of its distribution and relative abundance within a tissue [10]. An interesting example of IMS of lipids and shows how the distribution of a sphingomyelin in the human ocular lens changes with aging [11].

Nuclear Magnetic Resonance: Measurementof Lipoprotein Particle Size

High-density lipoprotein-cholesterol (HDL-C) and total cholesterol are routinely measured for cardiovascular (CVD) risk assessment and for monitoring a patient's response to lipid-lowering therapy. Low-density lipoprotein cholesterol (LDL-C) is not always measured directly but often calculated according to the Friedewald formula. Nuclear Magnetic Resonance (NMR) provides a rapid method for distinguishing and quantifying a wider range of lipoprotein subclasses [12]. On exposure to a magnetic field, distinct lipoprotein subclasses emit a unique signal that is directly proportional to their concentration. In a population-based study of 9399 Finnish men, concentrations of lipoprotein subclasses and lipid composition were altered in subjects with impaired glucose tolerance. Mackey and co-workersreported a stronger association of HDL particle number with CVDs than lipoprotein-cholesterol content. Furthermore, HDL particle number measured by NMR has been implicated as a superior biomarker for residual vascular risk after statin therapy when compared with HDL-C or ApoA-I.Other applications of NMR in lipid research include studies of the structures and cellular functions of lipids, interaction with proteins, peptides, and small molecules, as well as diagnostic strategies by magnetic resonance spectroscopy and magnetic resonance imaging (MRI) [13]. NMR provides a useful tool for lipid structure analysis in both solution and solid state; however, it lacks sensitivity and requires more sample when compared with MS.

Q-TOF and orbitrap mass spectrometry:

For a targeted lipidome analysis approach, a triple quadrupole (QqQ) TOF MS a novel instrument is now being employed. The first quadrupole is used for precursor ion selection, which is then fragmented in the second quadrupole, and the third quadrupole is used to monitor selected fragment ions. Neutral loss (NL) is a fragmentation behaviour of ionized lipids, which allows the set up specific precursor ions and neutral loss scans using QqQ MS [14]. NL scan significantly reduced chemical noise, decrease the number of scans required for structure characterization and hence increase detection sensitivity. Multiple reaction monitoring (MRM) has also been developed to measure lipids with known precursor ion and product ions. MRM MS increases detection sensitivity which is possibly even better than NL and allows detection and quantitation of lipids at very low levels in biological samples, as has been demonstrated for eicosanoids [15]. Selected ion monitoring (SIM) scans unique fragment ions for particular lipid (e.g. m/z 87 for a saturated fatty acid), and the SIM method can be applied in both GC/EI-MS and ESI-QTOF MS with a 10-fold increase in sensitivity as compared to a full scan mode setting on the same instrument . On the other hand, ion trap (Orbitrap) mass spectrometry is a commonly used technique in Lipidomics. Mass (m/z) of lipid species in a given sample can be used for profiling analysis even without lipid species identification (top-down lipidomics). Moreover, with the development of tandem MS (MS^n), such as Q-TOF and Orbitrap LCMS/ MS, selected precursor ions can be fragmented in second MS (MS/ MS) which can aid with lipid identification and quantitation [16]. Indeed, many lipidome profile analyses have used this approach successfully to profile and identify lipids (bottom-up lipidomics). However, the development of high resolution mass spectrometer in earlier part of this centuryi.e in particular new time-of-flight (TOF) along with Orbitrap instruments, make the isobaric distinguishing practicable due to sub-isotope resolution. As an example, phosphatidylcholine (PC) 36:1 and phosphatidylserine (PS) 36:2 are isobaric, but the exact masses differ by 0.0726 Da, which can be distinguished by LTQ Orbitrap MS at the resolution of 100,000.

Clinical application of lipidomics techniques:

To date, few articles applied lipidomics to large patient cohorts. Thus, we have included articles in this review that not only deal with lipidomics but also with measurements related to lipids. Lipid profiling has shown promising results in diabetes mellitus,CVDs, metabolic disorders, dietary habits, and obesity, as well as in determining response to drug therapy. Most studies analyzed either blood or tissue samples i.e, diabetic retinopathy, atherosclerotic plaques, muscle,or adipose tissues.Predominant methods of choice are LC-MS/MS and high through out put lipidomics [17-18]. ELISAs have been used for detection of oxidized lipoproteins and NMR for profiling of lipoprotein subclasses. Lipidomics studies have demonstrated that there were major glycerophospholipids, and sphingolipid deficits in the brain structure in neurodegenerative Alzheimer's disease (AD) [19]. Peroxisomal dysfunction in AD leads to glycerophospholipid deficits. Numerous studies have also demonstrated that elevations of plasma fatty acids for e.g myristic acid, α-linoleic acid and eicosapentaenoic acids were associated with the risk of prostate cancer [20-21]. For instance, Zhou et al., demonstrated thatphosphatidylethanolamine, ether-linked phosphatidylethanolamine and ether-linked phosphatidylcholine could be considered as biomarkers for the diagnosis of prostate cancer [22]. The establishment of searchable and interactive databases of lipids and lipid-related genes/proteins is also an extremely important resource as a reference for the lipidomics community. Integration of these databases with MS and other experimental data, as well as with metabolic networksoffers an opportunity to devise therapeutic strategies to prevent or reverse these pathological states involving dysfunction of lipid-related processes. It is expected that software solutions will allow us to integrate lipidomics data with multi-disciplinary information, including genomic and proteomic data, for biological pathway analysis.

II. Conclusion

Recent developments in mass spectrometry have greatly improved the Lipidomics analysis. The standardization of various procedures for sample preparation and analysis will increase inter-laboratory reproducibility, allowing us to share and compare data across the world. The possibility for developing standard procedures for analyzing lipids could lead to the development of lipidomic kits, facilitating routine analysis for quality control and clinical research. Dynamic lipidomics for estimating lipid turnover kinetics and the effects of individual enzyme deactivation or activation on lipid homeostasis remains a challenge. Single cell lipidomics characterizes the unique chemical composition of individual cells, and is the new area of advancing lipidomics. Advances in mass spectrometry have greatly accelerated the lipidomics field. Chemical derivatization has shown its broad use in improving analytical sensitivity and specificity in lipidomics.

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