

**Abstracts**

Lipidomics (MODam)

Time or Slot: 10:15

**Endogenous anti-inflammatory lipid mediators, resolvins and docosatrienes: LC-UV-MS-MS-based lipidomic analysis, databases, and searching algorithms**

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**Introduction**

Lipidomics holds the promise to decode the identity, structure-function relationships, and information held within biological systems where lipid domains and lipidic micro-compartments play essential roles in orchestrating complex physiologic and pathophysiologic events. Mediator-lipidomics is a subsection of metabolomics and lipidomics that focuses solely on the profiling of bioactive lipid mediators (LM). LM can be grouped into those that act extracellularly and those that are intracellular mediators. This lecture will review formation and actions of novel mediators of resolution of acute inflammatory events termed resolvins and protectins. These are examples of extracellular mediators derived from the omega-3 essential fatty acids. To also illustrate mediator lipidomics, we will present analysis of diacylglyceride (DAG) that serve as intracellular mediators in signal transduction.

**Methods**

LC-MS-MS methods for lipid mediator lipidomics include LC-tandem UV-MS-MS analyses together with recently constructed algorithms and databases for essential fatty acid-derived lipid mediators, resolvins, docosatrienes, protectins, and eicosanoids. In addition, LC-MS-MS analysis of diacylglyceride molecular species was carried out using LC-MS-MS together with synthetic standards for the major DAG species. Analyses were performed using a Finnigan LCQ-MAT quadrupole ion-trap mass spectrometer equipped with an electrospray ionization interface. Specific conditions for the chromatographic and mass spectral analysis of lipid mediators derived from essential fatty acids and those for the chromatographic analysis and identification of DAG will be presented.

**Preliminary Results**

Profiling of LM present within tissues and cells can provide a powerful diagnostic view of complex physiologic and pathologic events. Recent results obtained using our mediator-lipidomics approach with human diseases are examples. We shall discuss results obtained with leukocytes from localized aggressive periodontal disease as in "A molecular defect in intracellular lipid signaling in human neutrophils in localized aggressive periodontal tissue damage" (J. Immunol. 2004; 172:1856), and extracellular LM analysis of those formed from omega-3 pathways will be presented ("Stereochemical assignment, anti-inflammatory properties, and receptor for the omega-3 lipid mediator Resolvin E1," J. Exp. Med. 2005, in press). These provide examples of the powerful capabilities of lipid mediator lipidomics when carried out in tandem with in vivo and cellular-based systems.

Time or Slot: 10:55

**Lipidomics of cyclooxygenase-mediated oxidative stress**
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**Introduction**

The ability to resolve enantiomeric, regioisomeric, and stereoisomeric bioactive lipids is particularly important for cyclooxygenase (COX)- and lipoxygenase (LOX)-derived bioactive lipids as well as those arising non-enzymatically from reactive oxygen species (ROS). From a mechanistic perspective it is important to be able to distinguish these isomeric compounds with high specificity and sensitivity. It is relatively simple to derivatize bioactive lipids with an electron-capturing group such as the pentafluorobenzyl (PFB) moiety before LC analysis. In combination with LC/electron capture APCI/MS it is possible increase sensitivity by two orders of magnitude when compared with conventional LC/MS methodology and underivatized analytes.

**Methods**

A Finnigan TSQ 7000 triple stage quadrupole mass spectrometer (Thermo Electron, San Jose) equipped with an APCI source was used in the studies. For full-scan and MRM analyses, unit resolution was maintained for both parent and product ions. For the lipidomics profile, the instrument was operated in the negative ion mode. Operating conditions for the TSQ 7000 were as follows: vaporizer temperature at 500 °C, heated capillary temperature at 230 °C, with the corona discharge needle set at 16 •A. Nitrogen was used for the sheath and auxiliary gas. Collision-induced dissociation (CID) was performed using argon as the collision gas at 2.7 mTorr in the Rf-only quadrupole. Targeted chiral LC/electron capture APCI/MRM/MS analysis was conducted using pentafluorobenzyl (PFB) derivatives.

**Preliminary Results**

The targeted lipidomics approach showed that 15(S)-hydroxyeicosatetraenoic acid [15(S)-HETE] was the major hydroxylated non-esterified lipid formed in these cells. The corresponding hydroperoxide, 15(S)-hydroperoxyeicosatetraenoic acid [15(S)-HPETE] undergoes homolytic decomposition to the DNA-reactive bifunctional electrophile 4-oxo-2-nonenal, a precursor of heptanone-etheno-2-deoxyguanosine. This etheno-adduct was identified in DNA of RIES cells that expressed COX-2. A dose-dependent increase in adduct levels was observed in the presence of vitamin C. This suggested that vitamin C increased lipid hydroperoxide-mediated 4-oxo-2-nonenal formation in the cells. The selective cyclooxygenase-2 inhibitor NS-398 was protective against cellular DNA damage but was less effective if vitamin C was present. Prostaglandin E2 (PGE2) and 15(S)-HETE biosynthesis were completely inhibited by NS-398. 15(R)-HETE was detected in amounts that were slightly higher than the original 15(S)-HETE observed in the absence of aspirin, which suggested that significant amounts of 15(R)-HPETE had also been formed. Thus, the electron capture methodology provided an excellent means to analyze trace amounts of chiral lipids when chromatography was performed on a chiral column using normal phase solvents. Supported by NIH RO-1 CA91016.

Time or Slot: 11:15

**Analysis of Cell Membrane Aminophospholipids as Isotope-Tagged (iTRAQ) Derivatives**

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**Introduction**

Recently a set of four different isotopically enriched N-methylpiperazine acetic acid NHS (N-hydroxysuccinimide) ester reagents (iTRAQ) has been developed by Applied Biosystems that places isobaric mass labels at any primary amine group. The resulting derivatized products are isobaric and chromatographically indistinguishable, but yield reporter ions (m/z 114 or 117) during CID in the positive ion mode that can be used to identify and quantify individual members of a multiplex set. In this study, phospholipids that contain primary amine groups, such as glycerophosphatidylethanolamine (GPEtn) and glycerophosphatidylserine (GPSer), were modified using these reagents and it was established that this modification could aid in the mass spectrometric identification of phospholipid changes that occur during biological stimuli.

**Methods**

GPSer and GPEtn standards were labeled with 114 and 117 N-methylpiperazine acetic acid NHS ester reagents and the mass spectrometric, NP-HPLC, and RP-HPLC behavior was investigated using an electrospray triple quadrupole mass spectrometer. Once the mass spectrometric response of the N-methylpiperazine amide tagged aminophospholipids was determined, a precursor ion scan of m/z 114 or 117 scan was used to detect these species. The N-methylpiperazine acetic acid NHS ester reagents were used to assess the changes that occurred in the distribution of GPEtn lipids after exposure of liposomes made from phospholipids extracted from RAW 264.7 cells to Cu<sup>2+</sup>/H<sub>2</sub>O<sub>2</sub> in order to determine the feasibility of these reagents to track changes in the distribution of aminophospholipids after a stimulus.

**Preliminary Results**

The mass spectrometric response of N-methylpiperazine amide tagged aminophospholipids was probed using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine standards. In the positive ion full scan spectrum the [M+H]<sup>+</sup> of each of these N-methylpiperazine amide tagged aminophospholipids shifted 144 Da and during collision-induced dissociation the major fragmentation ion was at m/z 114 or 117 depending on the specific reagent used. The negative ion CID behavior of N-methylpiperazine amide tagged GPEtn and GPSer species remained the same compared to non-tagged GPEtn and GPSer species. In order to determine if these N-methylpiperazine acetic acid NHS ester reagents work well for complex biological samples, the GPEtn lipids extracted from RAW 264.7 cells were tagged and it was found that the [M+H]<sup>+</sup> distribution of GPEtn species was shifted by 144 Da and that the CID behavior in the positive ion mode of all subclasses of GPEtn is uniform with a major ion at m/z 114 or 117, which allows all subclasses of tagged GPEtn species to be detected using a precursors of m/z 114 or 117 scan. Finally, the N-methylpiperazine acetic acid NHS ester reagents were used to

assess the changes that occurred in the distribution of GPEtn lipids after exposure of liposomes made from phospholipids extracted from RAW 264.7 cells to  $\text{Cu}^{2+}/\text{H}_2\text{O}_2$ . The control liposomes were labeled with the 114 reagent and the  $\text{Cu}^{2+}/\text{H}_2\text{O}_2$  liposomes were labeled with the 117 reagent. Upon comparison of the precursors of m/z 114 scan to the precursors of m/z 117 scan it was found that the amount of lyso GPEtn increased in the oxidized sample. It has been found that the N-methylpiperazine amide isotope tag is a novel way to observe changes in the distribution of GPEtn and GPSer species, which has been difficult to achieve in the past.

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Time or Slot: 11:35

### Lipidomics of bacterial invasion and dormancy

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#### Introduction

Intracellular pathogens utilize a plethora of approaches to mimic host cell signaling during invasion, parasitic persistence and replication. Mycobacteria in metabolically inactive and non-replicative states for example pose an enormous problem in the fight against tuberculosis. A common emerging theme in a wide variety of host-pathogen interactions is the important regulatory role of lipids in pathogen entry and intracellular trafficking. Phosphoinositides (PIs, phosphorylated metabolites of phosphatidylinositol, PI) are an important class of membrane lipids and play a role in a wide variety of cellular processes including signaling, membrane trafficking and cytoskeletal dynamics. They have been implicated at various steps during bacterial invasion and they also act as ligands of CD1 receptors which present lipids to the immune system.

#### Methods

We use electrospray ionization mass spectrometry (ESI-MS) to qualitatively and quantitatively profile inositol lipids as well as other lipids present in complex mixtures derived from mycobacteria in different physiological states (hypoxic dormancy) or from mammalian cell cultures which were infected with salmonella. The goal of this study is to discover pathways that are induced or repressed during adaptation to different growth conditions or during infection. Chemometric and statistical analysis of mass spectra are used to align and analyze data from replicate experiments. Differences in lipid profiles are next characterized using tandem mass spectrometry and collision induced dissociation in order to identify underlying molecular lipid species.

#### Preliminary Results

SopB/SigD, a phosphoinositide phosphatase that is delivered into host cells by a type III secretion system, is essential for the establishment of Salmonella's intracellular replicative niche. SopB mediates the formation of spacious phagosomes following bacterial entry and is responsible for the maintenance of high levels of PI(3)P in the bacterial containing vacuoles. Recombinant SopB preferentially dephosphorylates PI(3,5)P<sub>2</sub> and PI(3,4,5)P<sub>3</sub> over other phosphoinositide lipids in vitro. It is attractive to speculate that SopB, through its PI(3,5)P<sub>2</sub> phosphatase activity, mimics the function of Fig4, a *Sacharomyces cerevisiae* phosphoinositide phosphatase that controls the size and maturation of the yeast degradative vacuole. Mycobacterial genomes encode for an unusually high number of lipid enzymes and as a consequence their lipid inventory is accordingly rich and diverse. Lipid extracts from logarithmically growing and metabolically inactive (dormant) cells were analyzed and compared by thin layer chromatography (TLC) and electrospray ionization mass spectrometry (ESI-MS). TLC profiles revealed striking differences in regions which co-migrated with standards for phosphatidylethanolamine (PE) and non-polar esters, such as triacylglycerols (TAG). ESI-MS indicated a 'switch' in abundance of molecular species of PE with different fatty acyl compositions. By tandem mass spectrometry these ions were identified as PE with palmitic acid (16:0)/oleic acid (18:1), and palmitic acid/tuberculostearic acid (10-methyl stearic acid), in their chains, respectively. A similar increase in tuberculostearic acid containing molecular species was observed for phosphatidylinositol (PI). Collectively, these results indicate that mycobacterium BCG specifically alters part of its lipid inventory upon entry into dormancy. Lipid profiling as described here is a powerful tool for identification of metabolic pathways, and hence potential enzymes, which are activated in bacteria that change their physiological states. In addition to promoting our understanding of molecular mechanisms of infection they also enhance various stages of drug and biomarker development.

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Time or Slot: 11:55

### LIPID MAPS Eicosanoid Lipidomics: LC-MS Methodologies Enabling the Comprehensive Identification and Accurate Quantitation of Eicosanoids in RAW 264.7 Cells

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**Introduction**

The LIPID MAPS (Lipid Metabolites And Pathways Strategy) Consortium is an ambitious five-year effort funded by the National Institute of General Medical Sciences (NIGMS) supporting numerous researchers at 18 universities, medical research institutes, and companies across the United States working together in a detailed analysis of the structure and function of lipids.

**Methods**

The LIPID MAPS Eicosanoid Lipidomics Core located at The University of California, San Diego is developing liquid chromatography-mass spectrometry (LC-MS) based methodologies enabling the comprehensive identification and accurate quantitation of different lipid eicosanoid classes in a single analysis.

**Preliminary Results**

We have compiled an in-house eicosanoid LC-MSMS library from a large number of standards and LIPID MAPS is making this web-based information freely available to the general lipids community. Our methods have allowed us to separate and identify thromboxanes, prostaglandins, leukotrienes and the HETE isomers in a single LC-MS analysis taking less than 16 minutes. Employing isotopic dilution methods, we have accurately quantitated a number of these eicosanoids in RAW 264.7 cells and have observed a 100 to 400-fold increase in LPS stimulated cells compared to their basal levels in resting cells. We have also quantitated the level of eicosanoid release as a function of LPS stimulation time. Chiral chromatography has enabled the determination of eicosanoids produced via enzymatic and non-enzymatic pathways. In an effort to explore the production of novel eicosanoid species by these cells, we have subjected them to incubation in deuterium-labeled arachidonic acid and have developed software that efficiently detects mass-offset analog pairs that indicates an arachidonic acid origin for the compound. During these studies we have also observed that acid supplementation in the cell growth media results in a two to three-fold increase in eicosanoid levels. This work is supported by the LIPID MAPS Large Scale Collaborative Grant number GM069338 from the National Institute of Health.

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