Lipidomics Analysis Reveals Changes in the Regulation of Lipid Metabolism in a Surgical Bone Defect Model

Dilrukshika S.W. Palagama, Matthew J. Kwiatkowski, Phillip M. Rzeczycki, Thomas A. Owen, María I. Morano, Miguel A. Gijón, Paul D. Kennedy, and Stephen D. Barrett

Cayman Chemical Company, Ann Arbor, MI

KEY FINDING Lipidomics analysis provides rich data that can reveal novel mechanisms of metabolic regulation

INTRODUCTION

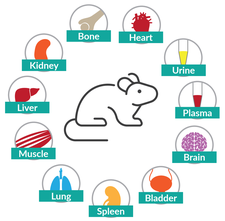
Lipids play critical roles in the physiology of biological systems. Comprehensive analysis of lipid profiles is being increasingly used to detect biomarkers and to generate hypotheses to help characterize potential mechanisms of disease progression that may offer novel preventive or therapeutic approaches. Advances in liquid chromatography and mass spectrometry instrumentation, and in specialized software, enable identification and relative quantitation of a wide variety of lipid species, providing powerful tools for the investigation of lipid profile changes over time.

The present study illustrates those advances by identifying large numbers of lipid molecular species in a variety of tissues collected at different times post-surgery from a femoral defect model in rats treated with a prostaglandin receptor agonist.

WORKFLOW

EXPERIMENTAL MODEL | **SAMPLE PREPARATION** | **LC-MS AND DATA ANALYSIS**

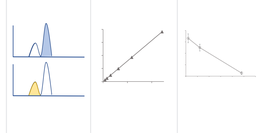
Rat femoral defect model: remove 5 mm portion of femur and replace with collagen-based matrix containing KMN-159 to locally stimulate bone formation and repair; collect tissues after 1, 6, and 24 h



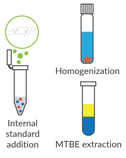
Biodistribution and Pharmacokinetics

RP-HPLC MRM MSMS (QqQ)

Quantitation | Statistics | Graphs

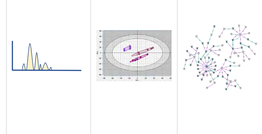


Untargeted Lipidomics



RP-HPLC HRMS ddMSMS (Orbitrap)

Quantitation | Statistics | Pathways

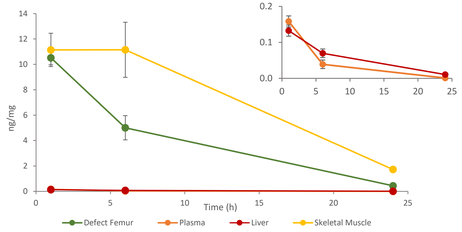


BIODISTRIBUTION AND PHARMACOKINETICS RESULTS

KMN-159 Distributes Mostly to the Defect Bone and Overlying Skeletal Muscle

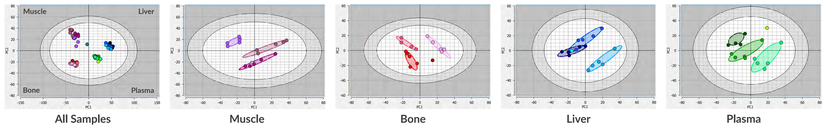
Tissue	C_{max} (ng/mg; avg ± SD)	T_{max} (h)
Skeletal muscle overlying defect femur	11.14 ± 2.94	1
Defect femur	10.52 ± 1.24	1
Lung	1.08 ± 0.10	1
Kidney	0.26 ± 0.06	1
Bladder	0.25 ± 0.16	1
Heart	0.22 ± 0.03	1
Spleen	0.21 ± 0.04	1
Plasma	0.16 ± 0.5	1
Liver	0.13 ± 0.04	1
Brain	0.03 ± 0.01	1
Urine	0.02 ± 0.02	1

Drug Concentration *vs.* Time Plots Show KMN-159 is Rapidly Cleared from Tissues



LIPID ANALYSIS RESULTS

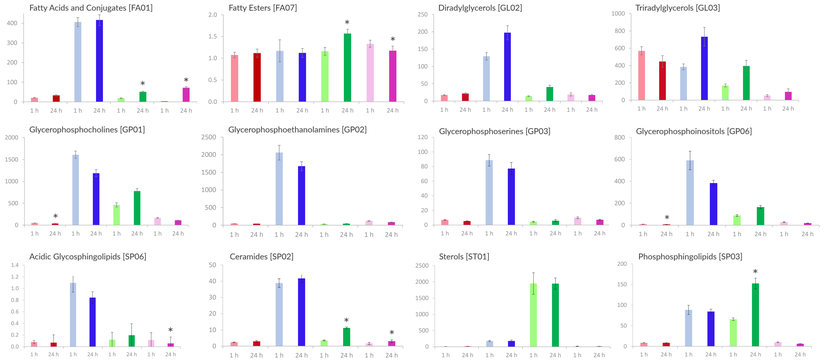
Principal Component Analysis Shows Tissue-Specific and Time-Dependent Lipid Changes



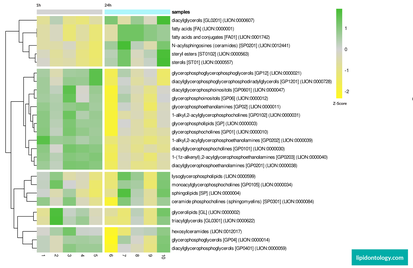
Statistically Significant Changes are Observed in Lipid Levels After Femur Surgery and Drug Administration

Bone | Liver | Plasma | Muscle

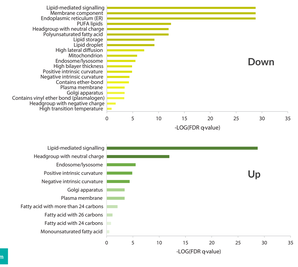
**Statistically significant (compared to 1 h, $p < 0.05$); area ratio (mean ± SEM)*



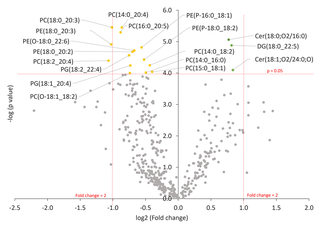
Heatmap Shows Upregulated Fatty Acyls, Sphingolipids, Sterols and Glycerolipids, and Downregulated Glycerophospholipids, in Bone

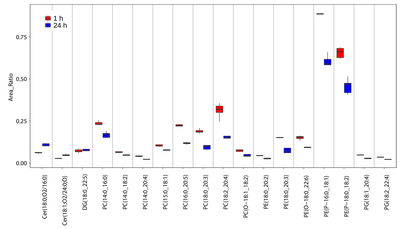


Enrichment Analysis Shows Regulation of Different Cellular Components, Functions, and Properties in Bone (24 h *vs.* 1 h)

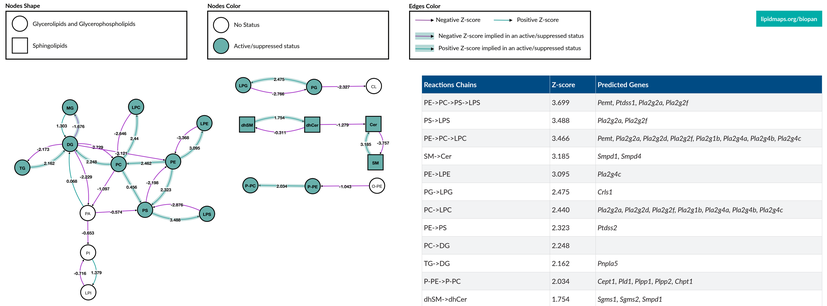


Volcano and Box-and-Whiskers Plots Show Changes in the Levels of Lipid Molecular Species in Bone





Pathway Analysis Indicates Regulation of Specific Enzymes Involved in Lipid Metabolism



Reactions Chains	Z-score	Predicted Genes
PE->PC->PS->LPS	3.699	*Pemt, Ptdss1, Pla2g2a, Pla2g2f*
PS->LPS	3.488	*Pla2g2a, Pla2g2f*
PE->PC->LPC	3.466	*Pemt, Pla2g2a, Pla2g2d, Pla2g2f, Pla2g1b, Pla2g4a, Pla2g4b, Pla2g4c*
SM->Cer	3.185	*Smpd1, Smpd4*
PE->LPE	3.095	*Pla2g4c*
PG->LPG	2.475	*Crls1*
PC->LPC	2.440	*Pla2g2a, Pla2g2d, Pla2g2f, Pla2g1b, Pla2g4a, Pla2g4b, Pla2g4c*
PE->PS	2.323	*Ptdss2*
PC->DG	2.248	
TG->DG	2.162	*Pnpla5*
P-PE->P-PC	2.034	*Cept1, Pld1, Plpp1, Plpp2, Chpt1*
dhSM->dhCer	1.754	*Sgms1, Sgms2, Smpd1*
PE->PC->DG->MG	1.752	*Pemt, Pnpla2, Pnpla3*

CONCLUSIONS

- KMN-159 localizes predominantly to the defect bone and adjacent muscle, decreasing to nearly undetectable levels in 24 h.
- Untargeted lipidomics analysis shows different spatial and temporal distribution of lipid molecular species.
- Changes in lipid signals occur at different rates in various organs, suggesting regulation at both local and systemic levels.
- Changes in the levels of lipid classes and individual molecular species are predicted to affect cellular properties and functions.
- Pathway analysis suggests regulation of specific enzymes that can be investigated to better understand the experimental model.
- This work demonstrates the unique ability of lipidomics studies to generate novel hypotheses to help drive research.

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