Phospholipid Profile of Cerebral Thrombi in Acute Ischemic Stroke

Sarah R. Martha, PhD, RN,[1] Samuel H. Levy, BS[2], Emma Federico, BS[2], Michael R. Levitt, MD[2], and Melanie Walker, MD[2]
[1]Biobehavioral Nursing Science Department, College of Nursing, University of Illinois at Chicago USA and
[2]Department of Neurological Surgery and Stroke and Applied Neuroscience (SANS) Center, School of Medicine, University of Washington, Seattle, WA USA

UNIVERSITY OF ILLINOIS CHICAGO College of Nursing

UNIVERSITY of WASHINGTON

Introduction

- Ischemic stroke is the second leading cause of death and one of the leading causes of disability globally.
- There is limited research examining the effects of the physiological lipidomic changes associated with adults following AIS.
- Mechanical thrombectomy (MT) provides a novel opportunity to study lipid metabolism by allowing researchers to collect the aspirated thrombus and blood from the infarct zone.

The purpose of this exploratory study was to identify a signature lipid profile from cerebral thrombi in acute ischemic stroke (AIS) patients.

Methods

- Thrombi were collected for this investigation from May 2020 through December 2020.
- This study was a non-probability, convenience sampling of adult subjects (> 18 years of age) who underwent MT for large vessel occlusion (LVO).
- Specimens were retrieved during MT and preserved utilizing a tissue banking protocol optimized for omic analysis.

Thrombi Collection and Storage:
- Collected thrombi were washed with sterile saline, transferred into the sterile collection receptable, and frozen at -80℃.
- Specimens were collected within 24 hours of subjects' hospital admission for LVO.

Lipidomic Analyses:
- Untargeted lipidomic profiling was conducted at University of Washington's Northwest Metabolomics Research Center.
- Lipid species and concentrations were extracted from thawed thrombi and quantitative lipidomics with the SCIEX Lipidyzer™ platform profiling approach using standard protocol and quality controls.

Statistical and Pathway Analyses:
- Lipid species composition data were analyzed and processed using Analyst 1.6.3 and Lipidomics Workflow Manager 1.0.5.0.
- Lipid species were determined from the Human Metabolome Database (HMDB), LIPIDMAPS, KEGG, and NIST libraries.
- We carried out statistical and pathways analyses using MetaboAnalyst 5.0.
- Data were explored using Random Forest (RF) classification for machine learning analysis.

Results

Table 1: Demographic Characteristics of the Sample

				Common Chronic Comorbidities					Lipid Panel on Admission				NIHSS					
	Sex	Age	Race	Smokers (current)	Atrial Fibrillation	Hypertension	Carotid Stenosis	BMI	Total Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	Triglycerides (mg/dl)	Admission	Discharge	tPA Administration	Location	TICI Score	LKW to Reperfusion Time (hours)
Subject 1	F	74	White/ Caucasian	No	Yes	Yes	No	Morbidly Obese	160	32	106	108	33	27	Yes	L MCA, M1	3	7.5
Subject 2	M	30	Black/ African American	Yes	No	No	No	Under/ Normal Weight	101	33	---	505	8	6	No	R MCA, M1	3	11.5
Subject 3	M	20	White/ Caucasian	No	No	No	No	Overweight	152	48	66	190	40	35	Yes	R MCA, M1	3	9.0
Subject 4	F	78	Declined	Yes	Yes	No	Yes	Overweight	132	31	63	188	12	2	No	R MCA, M1	3	6.5
Subject 5	M	63	White/ Caucasian	Yes	No	Yes	No	Obese	126	30	61	176	6	2	No	L MCA, M1	3	12.0

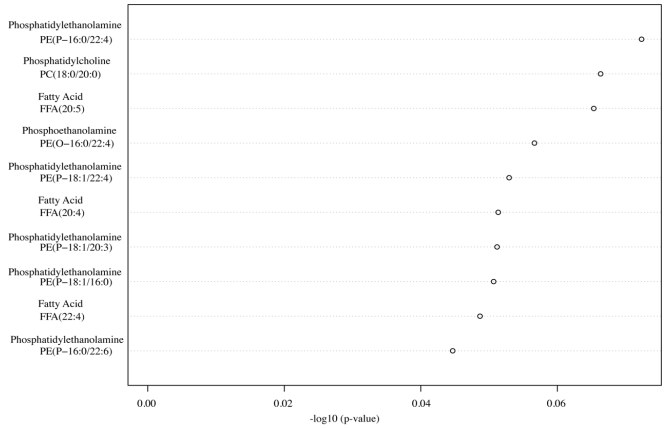
ACE-I: angiotensin-converting-enzyme inhibitors; ASA: aspirin; BMI: body mass index; HDL: high density lipoprotein; ICA: internal carotid artery; LDL: low density lipoprotein; LKW: last known well; MCA: middle cerebral artery; M1: proximal intracranial MCA segment; NIHSS: National Institutes of Health stroke scale; TICI: thrombolysis in cerebral infarction; tPA: tissue plasminogen activator

Table 2: Raw Data for Lipid Species Concentrations

	FFA(20:4)	FFA(20:5)	FFA(22:4)	PC(18:0/20:0)	PE(O-16:0/22:4)	PE(P-16:0/22:4)	PE(P-16:0/22:6)	PE(P-18:1/16:0)	PE(P-18:1/20:3)	PE(P-18:1/22:4)
QC-1	14.2997	3.1673	1.1075	1.6496	2.1273	0.7251	4.1341	0.3251	0.6173	2.3729
Clot 01	16.1132	2.0217	4.4697	1.3144	4.6155	39.4367	6.9589	2.9557	2.0340	8.1762
Clot 02	136.9770	2.9117	33.6422	0.8773	4.0203	40.7111	5.6932	2.1153	2.2618	7.3722
Clot 03	11.6972	1.6472	3.3567	5.0431	2.9843	23.6374	41.0937	1.3976	11.0247	5.4035
Clot 04	44.9278	2.1368	6.9260	2.7906	15.0771	142.6258	60.7007	13.0784	17.3120	34.0590
Clot 05	6.7635	0.8411	1.4271	5.8855	1.8031	16.1812	3.3681	0.8409	0.9992	1.9531
QC-2	14.2637	3.0693	1.0479	1.6831	2.8670	0.8104	4.3630	0.3265	0.9821	0.8721

QC: quality control; PC: phosphocholine; PE(P): phosphatidylethanolamine, PE(O): phosphoethanolamine; and FFA: fatty acids. 4 different lipid classes and their absolute concentrations. The concentrations are expressed in nmol/g of plasma (μM).

Figure 1: The top 10 lipids of interests from adults with acute ischemic stroke



Conclusions

- High throughput techniques to cerebral thrombi from AIS subjects for the purpose of testing feasibility of lipidomic analysis of cerebral thrombi.
- The top 10 metabolites identified from the RF analysis were of the glycerophospholipid and fatty acids species in cerebral thrombi.
- Thrombi from cerebral arteries of AIS patients are an untapped resource that allow for the direct evaluation of lipid biomarkers.
- Understanding of lipidomic changes in AIS may illuminate specific metabolite and lipid pathways involved and further the potential to develop personalized preventive strategies.

Support

This work was supported by Dr. Hilaire Thompson's Joanne Montgomery Endowed Professorship. Dr. Sarah Martha was supported by NIH/NINR T32NR016913 and is currently supported by NIH/NINR K23NR019864, STTI/WIN #17775.