NADPH OXIDASE-DEPENDENT GENERATION OF LYSO-PHOSPHATIDYLSERINE ENHANCES CLEARANCE OF ACTIVATED AND DYING NEUTROPHILS VIA G2A* S. Courtney Frasch¹, Karin Zemski Berry², Ruby Fernandez-Boyanapalli¹, Hyun-Sun Jin¹, Christina Leslie^{1,3}, Peter M. Henson^{1,3}, Robert C. Murphy² and Donna L. Bratton^{1,3} Department of Pediatrics¹ and Program in Cell Biology³, National Jewish Health, Denver, CO 80206 Department of Pharmacology², University of Colorado Denver, Aurora, CO 80045

Abbreviation	Structure of sn-2 side	MRM transition	Reference
	chain		
18:0a/OH-PS	-OH	524.5→437.5	This study
18:1a/OH-PS	-OH	522.5→435.5	This study
17:1a/OH-PS (IS)	-OH	508.5→421.5	This study
36a:2-PS + 2O	Linoleic acid + 2O	818.7→731.7	(1)
	Oleic acid + 2O		
36a:1-PS + 2O	Oleic acid + 2O	820.7→733.7	(1)
18:0a/9:0al (MOX)	9-oxononanoic acid	707.6→620.5	(2)
18:1a/9:0al (MOX)	9-oxononanoic acid	705.6→618.5	(2)
18:0a/5:0al (MOX)	5-oxovaleric acid	651.6→564.5	(2)
18:0a/9:0-COOH	azeleic acid	694.6→607.5	(2)
18:0a/KODA	9,12-dioxododec-10-enoic	790.7→703.5	(2)
(MOX)	acid		
18:0a/HODA	9-hydroxy-12-oxodedec-10-	763.7→676.5	(2)
(MOX)	enoic acid		
18:0a/KdiA	9-oxo-11-carboxyundec-6-	777.7→690.5	(2)
(MOX)	enoic acid		
18:0a/HdiA	9-hydroxy-11-	750.7→663.5	(2)
(MOX)	carboxyundec-6-enoic acid		

Supplemental Table 1: MRM transitions of oxPS species in negative ion mode

Supplemental Figure 1



Figure 1: Basic structures of representative diacyl-PS, oxidized PS and lyso-PS. *A*. Diacyl-PS contains 2 fatty acyl chains in the sn-1 and sn-2 position. The fatty acid in the sn-2 position is typically a monunsaturated or poly-unsaturated fatty acid. *B*. Oxidized PS typically contains one chain-shortened fatty acyl group in the sn-2 position often containing a reactive group (e.g. an aldehyde or a ketone). *C*. Lyso-PS contains 1 fatty acyl chain, usually in the sn-1 position with a hydroxyl group in the sn-2 position.

Supplemental Figure 2

Α

Non-oxidized SL-PS MOX derivatized



Figure 2: Multiple reaction monitoring (MRM) easily detects oxidized PS species resulting from the oxidation of SL-PS. *A*. Synthetic SL-PS was incubated in the absence of an oxidizing agent followed by derivatization with methoxylamine (MOX) and analyzed by MRM in the negative ion mode. Transitions monitored for all oxPS species previously characterized (1,2) are shown in the left panel (see also Supplementary talbe 1) demonstrating the presence of a minor amount of the aldehyde product (18:0/9-al-PS at m/z 707.7). The right panel is expanded 75X to view minor oxidized species . *B*. Synthetic SL-PS was oxidized *in vitro* with AAPH and all the transitions for oxPS species previously characterized (1,2) are shown in the left panel (see also Supplementary table 1) demonstrating the aldehyde product (m/z 707.7) as the most prominent species generated following oxidation. The right panel is expanded 25X demonstrating the presence of many predicted species previously identified indicating that analysis of MOX derivatized oxPS species by MRM is sensitive enough to detect even minor species.

References

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