Lysophospholipidomics Profiling of Media from Platelet Concentrates During Storage

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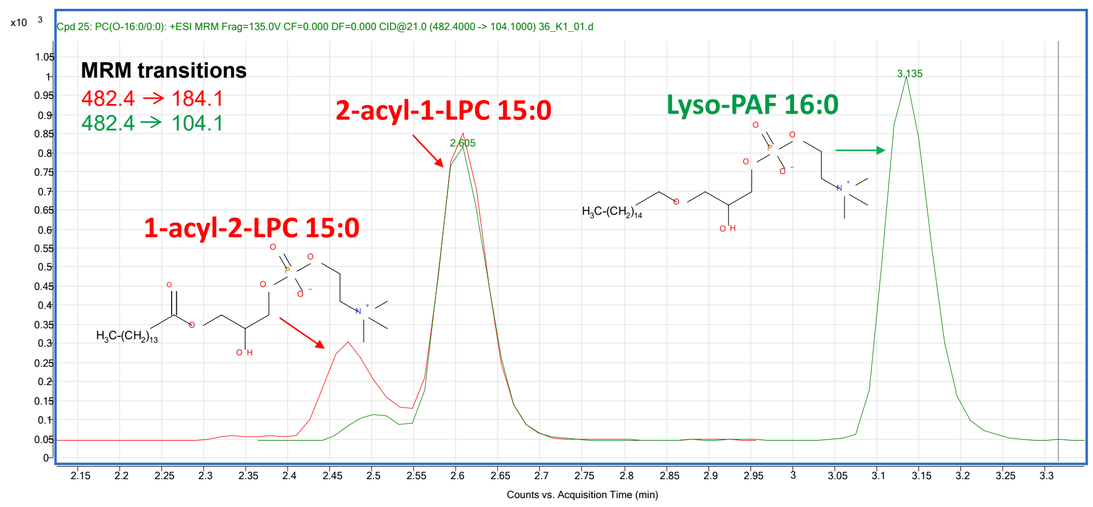
INTRODUCTION

Accumulation of specific lipid mediators in media of blood products have been identified as potential factors for side-effects of transfusions, including transfusion-related lung injury (TRALI) and immunomodulation (TRIM). Lysophosphatidylcholines (LPC), platelet-activating factors (PAF) and lyso-PAF have been shown to accumulate in platelet concentrates, to have immunomodulatory effects, and to prime neutrophil activation with implication in TRALI. [1-4] There are currently no data on initial PAF levels in platelet concentrate media, nor on the effects of storage on these levels. An accumulation of the lyso-PAF, a PAF precursor, in platelet concentrates has been reported, but no data are available for individual lyso-PAF species in human [2, 5]. The accumulation of other lysophospholipids such as lysophosphatidylethanolamines (LPE) has also not been investigated so far.

PROJECT AIM: To perform an in-depth targeted lipidomics profiling of media from apheresis platelet concentrates during storage and analyze lyso-PAF, LPC and LPE levels in detail.

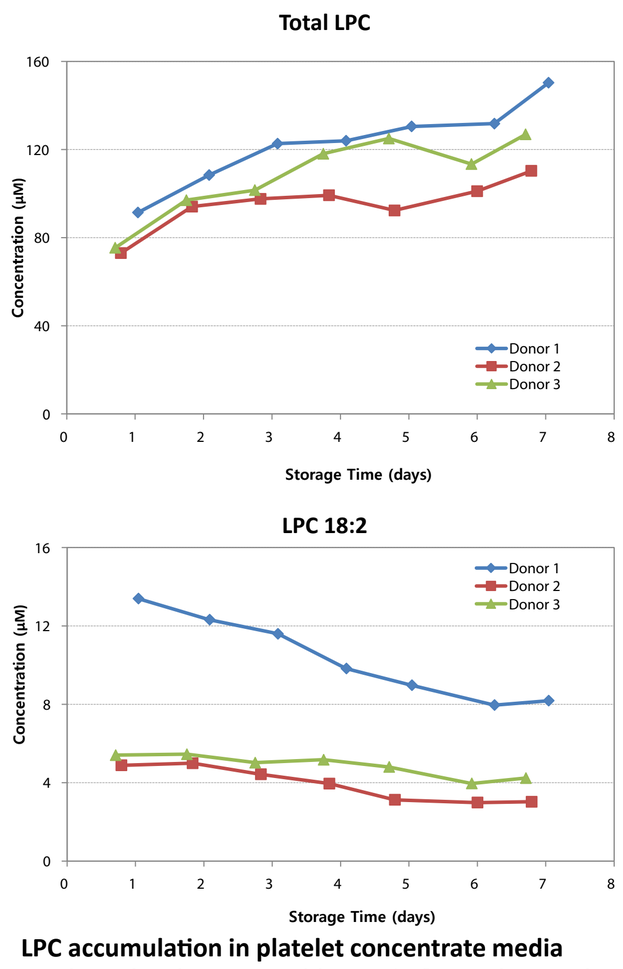
METHODS

- Leukoreduced apheresis platelets were collected from three male donors and 70% of the plasma was substituted by platelet additive solution (PAS). The platelet concentrates were stored for 7 days, and samples were taken daily. Media was separated from the platelets using a two-step centrifugation procedure to ensure platelet-free media.
- For lipidomics analyses, media samples were extracted using a one-step liquid extraction with butanol/methanol spiked with lipid class-specific internal standards [6]. Obtained extracts were analyzed by reversed-phase liquid chromatography mass spectrometry (LC-MS) [7] using Multiple Reaction Monitoring (MRM) on an Agilent 6460 triple quadrupole MS in positive ion mode. LPC and LPE species are reported as the sum of 1-acyl-2- and 2-acyl-1-regioisomers. The 104 and of 184 m/z product ions were monitored to distinguish isobaric lyso-PAF and LPC species, which were separated by retention time. Lipid species are designated as x:y, where x is the alkyl chain length, y is the number of C=C double bonds.



MRM chromatograms of the isobaric LPC 15:0 and Lyso-PAF 16:0 lipid species

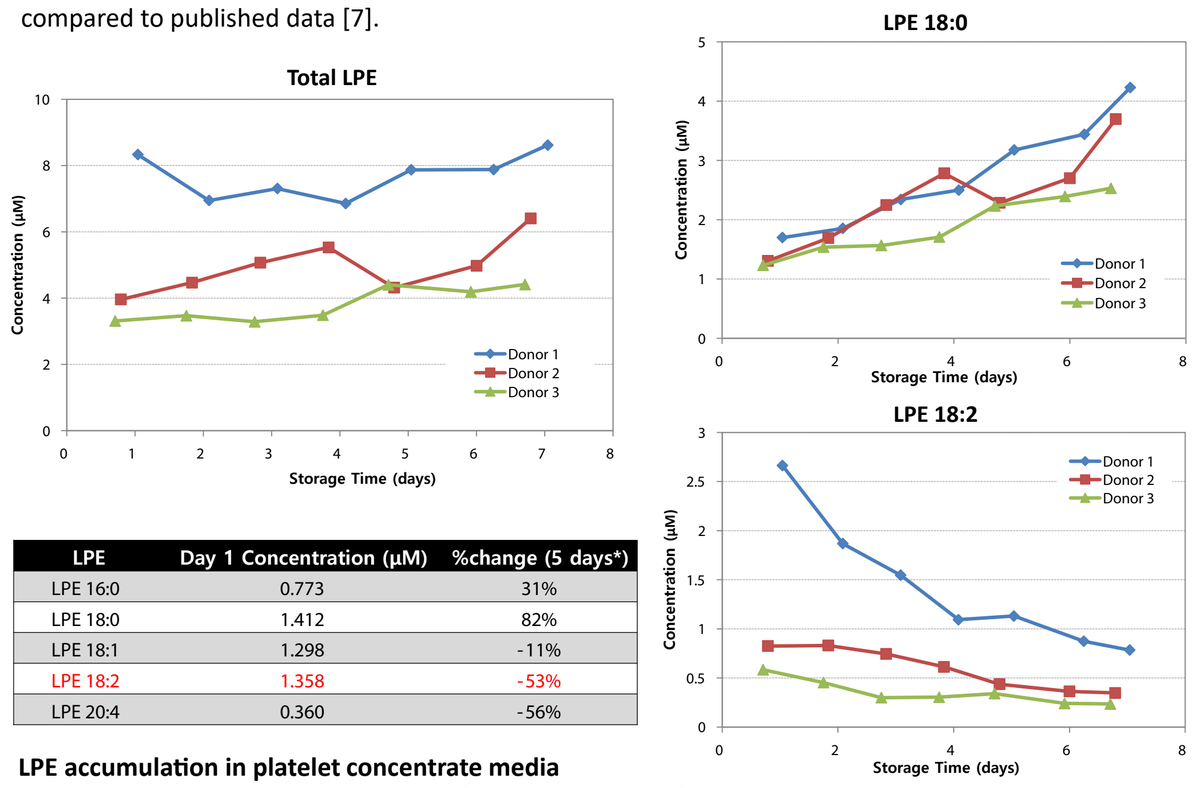
RESULTS



LPC	Day 1 Concentration (µM)	%change (5 days*)
LPC 14:0	0.625	17%
LPC 15:0	0.377	33%
LPC 16:0	45.185	58%
LPC 16:1	0.839	4%
LPC 17:0	0.697	54%
LPC 17:1	0.116	39%
LPC 18:0	13.146	72%
LPC 18:1	8.066	23%
LPC 18:2	7.895	-29%
LPC 18:3	0.162	-22%
LPC 19:0	0.053	55%
LPC 20:1	0.096	121%
LPC 20:2	0.088	30%
LPC 20:3	0.704	2%
LPC 20:4	1.356	-7%
LPC 20:5	0.176	-12%
LPC 22:0	0.010	46%
LPC 22:1	0.006	70%
LPC 22:5	0.120	8%
LPC 22:6	0.219	-5%

LPC accumulation in platelet concentrate media

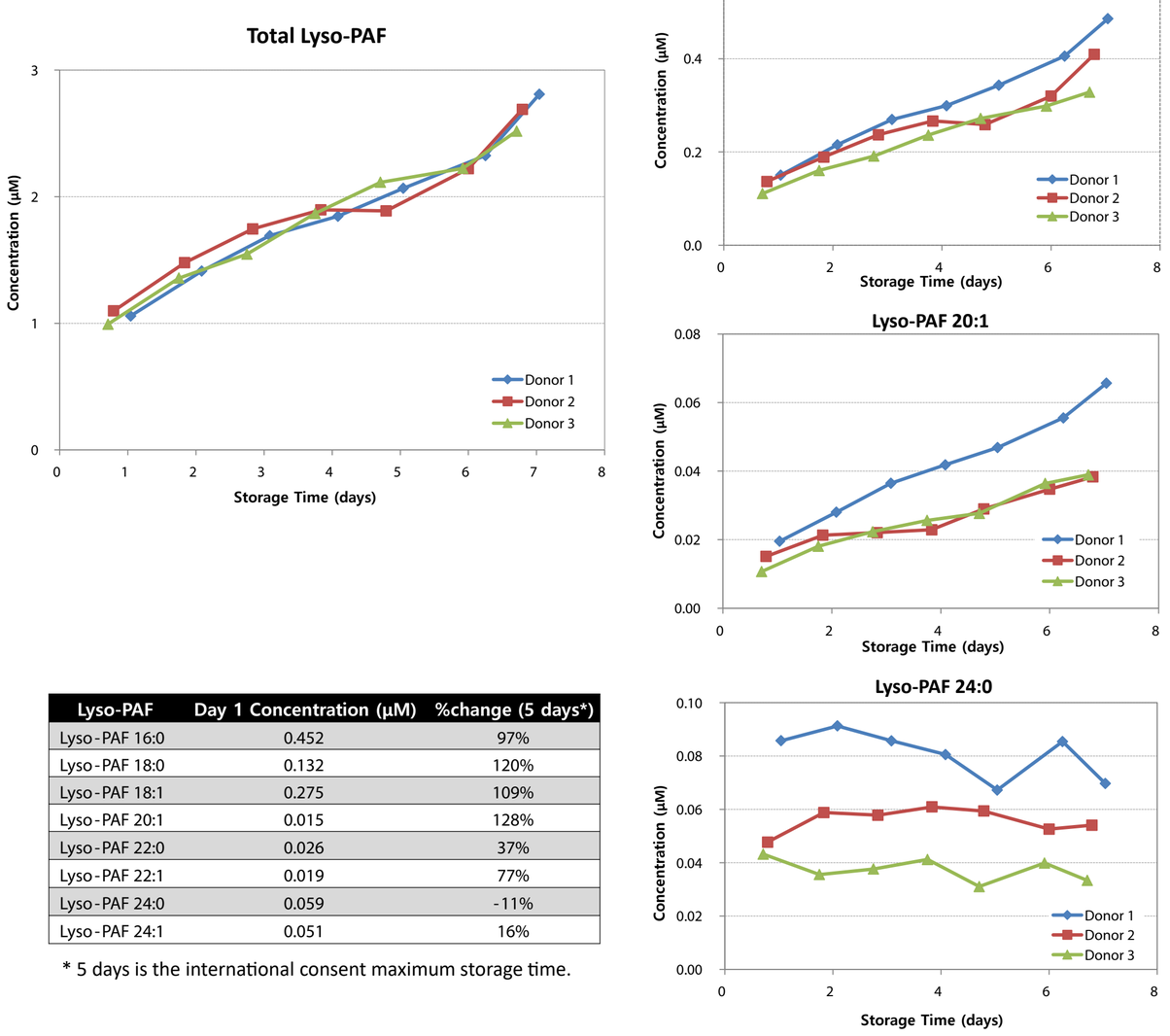
Total LPC levels increased by 55% after 5 days and 61% after 7 days. At species level, some LPC species were increased (i.e. LPC 18:0, LPC 20:1), decreased (e.g. LPC 18:2) or remained unchanged (e.g. LPC 20:3). These results are in agreement with published data [7]. However, absolute concentrations of some of the LPC species (e.g. LPC 18:1, 18:2 and 20:4) in our study were more than two times lower compared to published data [7].



LPE	Day 1 Concentration (µM)	%change (5 days*)
LPE 16:0	0.773	31%
LPE 18:0	1.412	82%
LPE 18:1	1.298	-11%
LPE 18:2	1.358	-53%
LPE 20:4	0.360	-56%

LPE accumulation in platelet concentrate media

Total LPE levels remained unchanged after 5 and 7 days of storage. However, at species level, some LPE species were increased (e.g. LPE 16:0, LPE 18:0), while others were decreased (e.g. LPE 18:2). Interestingly, LPE 18:2 and LPC 18:2 levels both decreased, while LPE 18:0 and LPC 18:0 increased during storage. LPE 18:1 and LPC 18:1 showed minor or no change during storage.



Lyso-PAF	Day 1 Concentration (µM)	%change (5 days*)
Lyso-PAF 16:0	0.452	97%
Lyso-PAF 18:0	0.132	120%
Lyso-PAF 18:1	0.275	109%
Lyso-PAF 20:1	0.015	128%
Lyso-PAF 22:0	0.026	37%
Lyso-PAF 22:1	0.019	77%
Lyso-PAF 24:0	0.059	-11%
Lyso-PAF 24:1	0.051	16%

* 5 days is the international consent maximum storage time.

Lyso-PAF accumulation in platelet concentrate media

Lyso-PAF levels linearly increased during storage by 92% after 5 days and 155% after 7 days. All molecular species, except very long-chain (Lyso-PAF 24:0 and 24:1), increased during storage.

DISCUSSION AND CONCLUSIONS

- Lyso-PAF species significantly accumulate in medium of apheresis platelet concentrates during storage.
- Furthermore, our data extends published data on LPC accumulation [8] with additional species and quantitative results and with data on LPE levels.
- The difference in measured LPC levels compared to the literature [8] may be due to different platelet concentrate preparation methods.
- Our described rapid LC-MS workflow may be helpful in studying variability of lysophospholipids levels and their accumulation in platelet concentrates from a large number of donors.
- Our findings contribute to a better understanding of lysophospholipid accumulations in media of platelet concentrates, which are potential factors in transfusion-related side effects. However, the clinical significance of the observed accumulations remain to be elucidated.

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