NIH Public Access
Author Manuscript
Transfusion. Author manuscript; available in PMC 2013 July 02.

Published in final edited form as:
Transfusion. 2012 May ; 52(5): 1155–1158. doi:10.1111/j.1537-2995.2012.03564.x.

Bioactive lipids from stored cellular blood components: in vitro method is crucial for proper interpretation

Christopher C. Silliman, MD, PhD, **Marguerite Kelher, MS**, and **Daniel R. Ambruso, MD**
Bonfils Blood Center Denver, CO

Christopher C. Silliman: christopher.silliman@ucdenver.edu

We read with interest the recent publication of Vlaar and colleagues,[1] which detailed the accumulation of bio-active lipids during the storage of blood products and their findings that these lipids are not cell but plasma and temperature dependent. In this article the authors demonstrated that lyso-PCs did not accumulate during the routine storage of prestorage leukoreduced (buffy coat removal) red blood cells (LR-RBCs) in SAGM, 42 days at 2 to 4°C, and did not evidence priming activity using a 30-minute neutrophil (PMN) priming assay in which the respiratory burst was measured in the presence of the plasma or plasma fraction of the stored component. However, if plasma was added to the LR-RBCs then there was a significant increase in lyso-PCs on Day 1 that did not increase over the storage interval. In addition, platelet (PLT) concentrates, stored in plasma, did evidence lyso-PC accumulation during routine storage, 7 days at 20 to 24°C, and the plasma fraction primed the PMN oxidase after 30 minutes. The accumulation of lyso-PCs was inhibited; however, the priming activity was not abrogated when SSP was substituted as the storage solution $(65\%–95\%)_{Final}$. The authors concluded that the observed accumulation of lyso-PCs was time and temperature dependent and had little to do with the cellular constituents of the stored product.

The original article that described the accumulation of bioactive lipids demonstrated that two classes of lipids, nonpolar and lyso-PCs, accumulated in unmodified, stored RBCs, in AS-3 or AS-5 stored at 4°C for 42 days.[2] The priming assays employed used a 5-minute incubation time, which is maximal for most lipids, especially PLT-activating factor, which is the prototypic lipid-priming agent. Incubations (30 min) with the plasma fraction from two of the five unmodified RBC units induced PMN lysis and increased the baseline PMN priming activity of the Day 1 samples, such that the differences between the priming activity from Days 1 and 42 were decreased (Table 1). The assays included 5% to 10% (vol:vol) plasma optimized for the technique, for when PLT additive solutions were employed 5% plasma yielded more robust priming.[2,3] The inclusion of plasma during oxidase activation may inhibit the respiratory burst or its detection by various techniques because plasma proteins, for example albumin, effectively quench O_2^- production (Table 1). In addition, plasma quenching was observed when fluorescent detection for oxidase activity was measured with Diogenes and dihydrorhodamine (DHR): 1% to 10% plasma inhibited Diogenes by 30% to 90% and DHR by 23% to 60% (Table 1). Such quenching of the respiratory burst may explain why the lack of differences was seen, and even when present, they were very small.[2] Priming may sometimes be seen with samples added directly to the assay using cytochrome *c* reduction, but plasma clearly inhibits detection when fluorescent or chemiluminescent detection assays are employed. Because of these results, plasma should always be washed from PMNs before the priming activity is assayed, as previously

CONFLICT OF INTEREST

There are no conflicts of interest for any of the authors.

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

reported.[2,4] Furthermore, there is not an accumulation of lyso-PCs in prestorage leuko-reduced and PLT-reduced RBCs, via filtration, and the priming activity is composed of nonpolar lipids, namely, arachidonic acid and 5-, 12-, 15-hydroxyeicostetranoic acid (HETE), which also induced acute lung injury in vivo.[4] There is a minimal amount of plasma (5–7 mL, unpublished data) in LR-RBCs and unfortunately the methods employed by Vlaar and colleagues are not congruous to earlier work and likely missed the neutral lipid-priming activity, which occurs rapidly (5 min), lessens over prolonged (30 min) incubation times compared to the D1 plasma, and was dampened by their failure to remove the plasma before assaying the isolated PMNs. These nonpolar lipids are present in RBCs or LR-RBCs, and the lyso-PCs appear in PLT concentrates and those cellular blood components with significant PLT contamination (RBCs). Furthermore, soluble CD40 ligand accumulates in the plasma fraction during routine storage of PLTs and may represent the priming activity they observed in the PLT concentrates.[5] Lastly, the use of inhibitors of secretory and cytosolic phospholipase activity is not definitive because peroxiredoxins are released by RBCs and may accumulate during RBC storage, and some contain an inherent phospholipase activity.[6] Although further work is required to delineate the complete nature of bioactive lipids that accumulate during the routine storage of cellular components, the reported data by Vlaar and coworkers does not impart clarity to this issue due to the extensive differences in methodology from previous work.

References

1. Vlaar AP, Kulik W, Nieuwland R, Peters CP, Tool AT, van Bruggen R, Juffermans NP, de Korte D. Accumulation of bioactive lipids during storage of blood products is not cell but plasma derived and temperature dependent. Transfusion. 2011; 51:2358–66. [PubMed: 21575006]
2. Silliman CC, Clay KL, Thurman GW, Johnson CA, Ambruso DR. Partial characterization of lipids that develop during the routine storage of blood and prime the neutrophil NADPH oxidase. J Lab Clin Med. 1994; 124:684–94. [PubMed: 7964126]
3. Ambruso DR, Thurman G, Tran K, Marschner S, Gathof B, Janetzko K, Goodrich RP. Generation of neutrophil priming activity by cell-containing blood components treated with pathogen reduction technology and stored in platelet additive solutions. Transfusion. 2011; 51:1220–7. [PubMed: 21155831]
4. Silliman CC, Moore EE, Kelher MR, Khan SY, Gellar L, Elzi DJ. Identification of lipids that accumulate during the routine storage of prestorage leukoreduced red blood cells and cause acute lung injury. Transfusion. 2011; 51:2549–54. [PubMed: 21615744]
5. Khan SY, Kelher MR, Heal JM, Blumberg N, Boshkov LK, Phipps R, Gettings KF, McLaughlin NJ, Silliman CC. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. Blood. 2006; 108:2455–62. [PubMed: 16772606]
6. Antunes RF, Brandao C, Maia M, Arosa FA. Red blood cells release factors with growth and survival bioactivities for normal and leukemic T cells. Immunol Cell Biol. 2011; 89:111–21. [PubMed: 20440295]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

TABLE 1

Superoxide activity after 5 and 30 minutes of PRBC incubation*

		Superoxide activity (nmol O_2^-/min)	
Priming assays	**Plasma: storage stage/concentration**	**5 min**	**30 min**
Priming in tube			
Unmodified RBCs	D1	3.0 ± 0.7	3.6 ± 0.3
	D42	4.9 ± 0.5†	5.1 ± 1.6
LR-RBCs	D1	3.0 ± 0.5	3.1 ± 0.4
	D42	4.1 ± 0.5†	4.1 ± 0.1†
PLTs (5%)	D0	2.1 ± 0.2	
	D7	3.7 ± 0.6†	
Priming in plate			
Control	fMLP	2.7 ± 0.5	
Unmodified	D1	1.5 ± 0.4	
	D42	1.5 ± 0.3	
LR	D1	1.7 ± 0.7	
	D42	1.8 ± 0.6	
Diogenes (chemiluminescence)			
% Inhibition	1%	30	
	5%	80	
	10%	>90	
Dihydrorhodamine			
% Inhibition	1%	23	
	5%	46	
	10%	60	

*n = 5.

† $p < 0.05$ from D1 (analysis of variance, repeated-measures post hoc Newman-Keuls); D = day, % = $(\text{plasma})_{\text{FINAL}}$.