In vitro Characteristics of Whole Blood Stored in Novel Hypoxic Platform

Background

Whole blood (WB) has regained favor in treatment of massively bleeding patients in military and civilian settings. WB contains red blood cells (RBCs), platelets (PLTs) and plasma, from the same donor. Prior studies have demonstrated RBCs stored in hypoxic/hypocapnic conditions (HRBC) preserve high levels of 2,3-DPG while reducing storage lesions due to oxidative stress. PLT function and cytokine accumulation in hypoxic WB were examined.

Study Design/Methods

11 units of WB were leukoreduced using PLT-sparing filter (Terumo WB-S) then split into Control (C) and Hypoxic (H) WB. H-WB was processed by the O2-reduction bag (Hemanext, Lexington MA) to pO$_2$=5-15 mmHg and unit was stored in O$_2$-free bag. Aliquots were tested at day 1, weeks(W)1, 2, 3 for PLT counts, agonists (TRAP), ADP and collagen stimulated PLT aggregation, non-activated and agonists activated PLT surface phosphatidylserine, P-selectin, PAC-1 binding, and microvesicles (MV). Plasma samples were frozen for batch cytokine testing (RANTES, PDGF-BB, IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, TNF-a, TNF-b MIP-1a, MIP-1b, eotaxin, GMCSF, FGF-2) using single- and multiplex ELISA tests.

Results/Findings

H-PLT counts declined to ~60% by the O2-reduction process, while similar decline was observed after W1 in C, and thereafter remained steady. PLT activation (PS) increased over time (H >> C after processing; C increasing more rapidly during storage). P-selectin increased (H < C), while PAC-1 showed large increase after W1, then remained steady (H << C). PLT activation by TRAP or ADP declined modestly over 3W (~15%) while H-PLT showed additional ~10% reduction for all time points. Collagen activation for C-PLT increased after 1 W (74%) and gradually increased to 100% after 3W (~20% reduction with H compared to C). PLT-derived MV (CD61 and CD61/Annexin V) increased ~4-fold over storage time; Day 0 MV were higher for H, but subsequent increase rates were similar or lower. Total number of PLT-derived MV (CD42a) in WB supernatant increased 17-fold after 3W for C, while H suppressed increase to 7-fold (p<0.05). While other cytokines are being evaluated, RANTES showed higher levels (ng/mL) in C-WB W1–C 145 vs. H 113, p=0.321; W3–C...
PDGF-BB (ng/mL) showed a similar pattern: W1—C 19; H 17, p<0.19, and W3—C 3; H 26, p<0.001.

**Summary/Conclusions**

PLTs were activated over 3W when stored at 1-6°C in leukoreduced WB, accompanied by a modest loss of agonist-induced activation. Oxygen reduction treatment initially activated H-PLTs, while subsequent increase in activation rates were suppressed compared to C-PLTs. WB PLTs retained activatability, and hypoxic condition showed only modest further reduction on the activatability. HWB significantly reduced RANTES and PDF-BB accumulation, resulting in fewer transfusion reactions. Hypoxic WB may provide higher quality WB for trauma patients if the levels of initial PLT activation can be improved during oxygen reduction procedure.